PRELIMINARY STUDIES OF CHEMICAL REACTIONS AS INDICES OF HEAT LETHALITY

By

NEVILLE JOHN OPENSHAW

A THESIS

Presented to
the Faculty of the Department of Food Science and Biotechnology
Massey University

In Partial Fulfilment
of the Requirements for the Degree of
MASTER OF TECHNOLOGY (FOOD TECHNOLOGY)
(in Food Processing)

April, 1969

"The process by which wants are satisfied is also the process by which wants are created. The more wants that are satisfied, the more new ones are born".

- Galbraith

A C K N O W L E D G M E N T S

The author extends his sincere appreciation and gratitude to Mr H.A.L. Morris, the Supervisor of this thesis, whose experience, keen interest and untiring assistance proved invaluable.

Thanks are due to Dr J.W. Lyttleton and Dr R.D. Reeves for their willingness to discuss aspects of this work.

Financial assistance from J. Wattie Canneries Ltd., and Mauri Bros and Thompson Ltd., was appreciated and helped to make this work possible. The author is also grateful to J. Wattie Canneries Ltd., for the donation of samples and raw materials necessary for this study.

For the typing of the draft and Figures by Miss D.A. King and the final presentation by Mrs C.K. Pickett, the author is very thankful, and to the numerous other people who have contributed to this thesis, the author extends his thanks.

TABLE OF CONTENTS

TABLE OF FIGURES

INTRODUCTION

In commercial canning, food put into a container always contains micro-organisms that would cause spoilage if they were not subsequently destroyed. When the destruction of the spores of a certain organism in food has reached a certain pre-established point, the food is said to be sterile with respect to that organism and the object of heat processing canned foods is the attainment of sterility with respect to the most resistant micro-organisms present that would bring about spoilage.

Since the discovery of the principle of sterilisation by heat and the initial development of the art of heat processing by Appert at the beginning of the 19th century, considerable progress had been made in the fields of processing techniques and their evaluation. Standard methods have evolved for determining minimum processing conditions necessary to attain commercial sterility in products. However, as yet, there are no sensitive and reliable methods which do not involve the use of thermocouples for temperature measurement to check on under or over-processing during the routine sterilisation of canned products. The preparation of cans with inserted thermocouples and the recording of temperatures is too laborious and time consuming to become a routine check on processing and so the need for a more convenient method is obvious.

The purpose of this investigation was to carry out preliminary studies to ascertain the possibility of using chemical reactions occurring in model systems or food products as indices of heat lethality and so determine if correlations between a chemical index and heat lethality can be established and have commercial application.

In order to carry out this study, it is first necessary to briefly review theoretical aspects pertaining to the heat processing of canned foods.

THE HEAT PROCESSING OF CANNED FOODS

A can of heat processed "canned" food contains a "sterilised" product that at room temperature will remain microbiologically unspoiled indefinitely and depending on the type of food, will have a marketable quality shelf life of 6 months to 2 years. The majority of canned foods are heat treated to prevent microbiological and enzymatic spoilage and concurrent with the heat treatment, we may find loss of nutrients, e.g. thiamine destruction, and chemical reactions occurring, e.g. non enzymic browning.

Just as the rate of a chemical reaction depends on time, temperature and concentrations of reactants, so the lethal effect of heat on bacteria is a function of the time and temperature of heating and the bacterial population in the product. To design or evaluate an in-package heat process, it is necessary to know the heating characteristics of the system under study, the number of spoilage organisms present and the thermal resistance characteristics of the spoilage organisms.

HEATING CHARACTERISTICS OF CANNED FOODS

1. Conduction Packs

Products heating by conduction within a stationary container do not move or change their location during the process. During heating, heat from the surrounding medium, e.g. steam under pressure, is transferred almost immediately to the outermost layer of food in the container and is then conducted from particle to particle inward toward

the centre of the food mass from all points of the container wall. During the initial phase of heating there is a constant temperature portion of food near the centre of the container. The temperature of the food near the container wall rises during this lag period. Subsequent to the lag period the temperature from the centre to the can wall rises on a smooth curve. If heating is allowed to continue long enough the entire contents will eventually reach the temperature of the surrounding steam and the last portion of food to reach this temperature will be at the geometric centre of the container (the point of slowest heating or critical point).

If at the end of any given heating time the container is plunged into cooling water, heat transfer within the container is reversed in direction and the contents cool until equilibrium is reached with the surrounding medium. Because temperature rise during heating and temperature drop during cooling are logarithmic in order cooling to a non-lethal temperature is accomplished in considerably less time than is required to heat the food, (i.e. from the lowest temperature which is lethal, to the highest temperature attained during the process). Therefore, even though the temperature drops less rapidly at the centre than at any other points in the container during cooling, there is a small volume of food at the centre which receives a less severe heat treatment than any other food in the container and the severity of heat treatment increases progressively from the centre, in any direction, to the wall of the container.

2. Convection Packs

Foods which heat by convection exhibit much more rapid heating than do foods which heat by conduction. In the case of convection heating, transfer of heat in the food mass is aided by product movement within the container and for a condition of ideal convection heating, temperatures throughout the container of food during processing would be identical at all times, i.e. the temperature at any point in the container at any time is the same as the temperature at the critical point.

3. "Broken Heating" Packs

Certain foods exhibit "broken" heat penetration curves due to a change in consistency within the pack when a certain temperature is reached. An increased rate of heat penetration could result from the transformation of a conduction pack into a convection pack and similarly, a decreased rate of heat penetration could result from the transformation of a convection pack into a conduction pack. The critical point in a container of food of this type bears the same significance to the respective state the food is in as discussed above.

DEATH OF BACTERIAL SPORES

The first modern critical approach to the problem of death of bacterial spores under the influence of heat appears to be that of Bigelow and Esty (13) who define thermal death point in relation to time (i.e. thermal death time) as the time at different temperatures necessary to completely destroy a definite concentration of spores in a medium of known hydrogen ion concen-

tration. Using the concept of thermal death time, they demonstrated that with any given spore suspension, the thermal death time was a function of the spore concentration and that the thermal death time for a given spore concentration decreased with increase in temperature. Using the same data Bigelow (11) demonstrated the logarithmic relationship between temperature and thermal death time, that is, when the survival and destruction points for any given suspension and spore concentration were plotted upon a logarithmic scale against the corresponding temperatures on a linear scale, essentially straight lines were obtained.

Ball (5,6) on developing the mathematical methods used for the calculation of processes for canned foods pointed out that the thermal death time curves of Bigelow (11) could be characterised by a point and a slope. The reference point chosen was the time to destroy the organism at $250^{\circ}F$ and this was designated by the symbol F. The slope of the thermal death time curve symbolised by Z was defined as the number of degrees required for the curve to traverse one log cycle. This is the equivalent of the number of degrees the temperature must be raised or lowered from a given reference temperature to produce a tenfold decrease or increase in destruction time.

The lethal rate L was defined as the reciprocal of the thermal death time.

The above procedures are concerned with survival and destruction times for some given initial spore concentration, and the basic presupposition of this work is, that there is a time for a given temperature and suspension medium for any initial spore concentration of a given organism at which all of the organisms are dead.

Very early in the studies of the death of micro-organisms under adverse influences attention was given to the number of survivors remaining in relation to time, and attempts were made to measure the "rate of death" of bacterial suspensions, exposed to some adverse environmental factor such as chemicals or high temperature. In 1910 Chick (24) showed that the rate of death of micro-organisms was of logarithmic order, that is, the number of surviving organisms plotted on a logarithmic scale against time on a linear scale gave a straight line. The logarithmic order of death of micro-organisms under various influences has been extensively reviewed by Rahn (97,98) and considerable additional evidence substantiating logarithmic death has been presented.

Evidence for a non logarithmic order of death has been reviewed by Rahn (97,98) and a non logarithmic order of death of spores of food spoilage organisms has been reported (101,106).

It is evident that only in the case of straight line survivor curves can simple mathematical treatment, formulation and extrapolation of the results to other conditions or initial numbers be carried out. No satisfactory expression has been found to express resistance when the survivor curve is not a straight line. For this reason, death of bacterial spores is generally considered logarithmic (9,98,120) and this allows it to be described mathematically in the same manner as a unimolecular or first order bimolecular chemical reaction. In a unimolecular reaction only one substance reacts and its rate of decomposition is directly proportional to its concentration while in a first order bimolecular reaction one reactant is in such great excess that variation in its concentration is negligible and rate of decomposition of the second reactant is directly proportional to its

concentration.

The rate of a first order reaction is expressed by

$$\frac{dc}{dt} = -kc$$

$$\text{or} \quad -\frac{dc}{c} = k\,dt \qquad \ldots\ldots\ldots\ldots (1)$$

where c = concentration of reacting substance

t = time

dc/dt = rate of change of concentration of reactant

k = rate constant

Integrating equation (1) between the limits, concentration c_1 at time t_1 and c_2 at a later time t_2, we have

$$-\int_{c_1}^{c_2} \frac{dc}{c} = k \int_{t_1}^{t_2} dt$$

$$- \ln c_2 - (- \ln c_1) = k(t_2 - t_1)$$

$$k(t_2 - t_1) = \ln \frac{c_1}{c_2}$$

This can be modified to give

$$t = \frac{2.303}{k} \log \frac{c_o}{c} \qquad \ldots\ldots\ldots\ldots (2)$$

in which c_o = initial concentration of reactant

c = concentration after reaction time t

From the survivor curve let a represent the initial number of cells (comparable to c_o in equation (2)) and b represent the number of surviving

cells (comparable to c in equation (2)) after heating time t. Then

$$t = \frac{2.303}{k} \log \frac{a}{b}$$

The time required to destroy 90% of the cells is the time required for the survivor curve to traverse one log cycle. If this time is represented by D (decimal reduction time) the slope of the survivor curve may be expressed as

$$\frac{\log a - \log b}{D} = \frac{1}{D}$$

Substituting in the general equation of a straight line

$$y = mx$$

we obtain

$$\log a - \log b = \frac{1}{D} t$$

$$t = D(\log a - \log b) = D \log \frac{a}{b} \quad \ldots\ldots\ldots\ldots (3)$$

in which t = time of heating

D = time to destroy 90% of cells

a = initial number of cells

b = number of cells after heating time t

On comparing equations (2) and (3) the similarity between the expression for a first order chemical reaction and the expression for logarithmic destruction on bacterial spores can be seen.

PROCESS EVALUATION

1. The General Method

This method described by Bigelow, et al. (12) is essentially a graphical procedure for integrating the lethal effects of various time-

temperature relationships existent in a container of food during processing. The time-temperature relationships for which the lethal effects are integrated are those represented at the point of greatest temperature lag during heating and cooling of the product. This point was found to be at or near the geometric centre of the container.

The rate of destruction of an organism per minute at any given temperature (T) in a process is the reciprocal of the time in minutes (t) required to destroy the organism at that temperature. From the thermal death time curve, the following relationship can be obtained.

$$\frac{\log t - \log F}{\log 10} = \frac{250 - T}{Z}$$

$$\log \frac{t}{F} = \frac{250 - T}{Z}$$

$$\text{i.e.} \quad \frac{t}{F} = \text{antilog} \frac{250 - T}{Z} \qquad \ldots\ldots\ldots\ldots (4)$$

where Z = slope of thermal death time curve in ^{o}F

F = minutes to destroy the organism at $250^{o}F$

T = temperature under consideration (^{o}F)

t/F = time to destroy the organism at temperature (T) if $F = 1$

F/t = lethal rate at T

Since F and Z are known from the thermal death time curve, equation (4) can be solved and the lethal rate determined.

Since thermal reduction times were used to replace thermal death times, the Z concept was retained and so, if the values of thermal reduction time (for some constant reduction of spore numbers) or the decimal reduction time (D) are plotted against temperature (T), then the curve has the same slope

as the logarithm thermal death time versus temperature curve

$$\text{i.e. } \frac{1}{Z} = -\frac{d\ (\log d)}{T}$$

$$\text{or } \log (D_2/D_1) = -(T_2 - T_1)/Z$$

However, lethal rate L is defined as the reciprocal of the thermal reduction time.

$$\text{Hence } \frac{L_2}{L_1} = 10^{(T_2 - T_1)/Z} \qquad \ldots\ldots\ldots\ldots \quad (5)$$

Lethal rate values have been tabulated (15,120).

From the survivor curve, the destruction of spores can be represented

$$\log \frac{No}{N} = L\theta$$

$$\text{or } d \log \frac{No}{N} = Ld\theta$$

$$\text{whence } \log \frac{No}{N_1} = \int_{\theta_o}^{\theta_1} Ld\theta$$

The F value of a process can be defined as the time at $250^{o}F$ which accomplishes the same reduction in spore numbers as the process

$$\text{i.e.} \qquad F = D_{250} \log \frac{No}{N_1}$$

$$\text{therefore} \quad F = D_{250} \int_{\theta_o}^{\theta_1} L \, d\theta$$

$$= \int_{\theta_o}^{\theta_1} \frac{L}{L_{250}} \, d\theta$$

Substituting equation (5) we have

$$F = \int_{\theta_o}^{\theta_1} 10^{(T - 250)/Z} \, d\theta$$

Hence by plotting lethal rate against time, a lethality curve is obtained and the area under this curve represents the total lethal value of the process. This area is expressed in terms of equivalent time at 250°F by dividing into it a unit area representing the equivalent of one minute at 250°F (or Fo = 1 minute). The resulting value is the Fo value of the process in minutes. For example, the heat process required to destroy Clostridium botulinum in low acid products must have an Fo of not less than 2.78 minutes. That is, the area of the lethality curve must be greater than 2.78 times the unit

area to ensure adequate processing.

Various modifications have been made to the General Method. For example, Shultz and Olson (117) introduced specially ruled lethal paper on which the temperature - time data are plotted directly while Patashnik (92) used a simple arithmetic summation of lethal rate values at equal time intervals.

2. Formula Methods

Ball's "Formula Method", introduced in 1923 (5) uses heating and cooling curves of a standard form. The reciprocal slopes, f_h and f_c, of the heating and cooling curves are assumed equal and the cooling curve has a lag factor or j value incorporated. The lag portion of the cooling curve is approximated to by a hyperbolic expression.

The integration is carried out using the expression

$$F = f \text{ Lg } C$$

$$\text{where } Lg = {}_{10}(250 - Tg)/Z$$

and C is a tabulated function of g, Z and m + g

"g" is the difference between retort temperature and maximum centre temperature Tg at the end of the heating period, and Z is the slope of the thermal death time curve. "m + g" is the difference between retort and cooling water temperature.

Ball's technique was further improved in 1928 with the retabulating of the C : g tables as $f_h/_u$: g tables

$$\text{where } \frac{f_h}{u} = \frac{e^{2.303\ g/Z}}{C}$$

"u" is the number of minutes required to destroy an organism at retort temperatures. Further parameters and modifications have been introduced to the method (7) but discrepancies are still found (46, 121).

The above formula method treats only a single point and depending on whether thermal death time or thermal reduction time data is used, indicate whether a process will achieve "sterility" or reduce the population of spores by a given ratio. They do not indicate the probability of spore survival at any point other than that considered in the analysis. Ball, one of the chief exponents of the critical point method argues that the basic problem in sterilisation rests in determining when the food at the critical point has received a sufficient quantity of heat to destroy the organisms of the most resistant type that could spoil the food. Ball assumes that when sterilisation is accomplished at the critical point, sterilisation will already have been completed at all other points and hence the sterilisation of the entire mass is accomplished (7).

However, with the introduction of the thermal reduction time concept the critical point approach received considerable criticism. To calculate the survivors, Stumbo (119) considered the contents of the container to consist of a collection of portions that constitutes a series of nested shells, designated as iso-F value shells, which are defined by the condition that all material which constitutes any one shell receives equal lethal-heat treatment. As the series progresses from the outside surfaces of the container to the shell that contains the critical point, there is a progressive decrease in lethal value for the successive shells. Stumbo applied the survivor curve to each shell independently, basing the calculation of survival on the entire

volume of the food substance which constitutes the shell as the unit volume, that is, considering each shell as defining its own unit volume. No two shells have the same volume and it is visualised that the imaginery containers gradually change in shape from cylindrical to ellipsoidal or spherical from outside to centre of the real container.

Stumbo defined the region of greatest probability of survival as the region occupied by the shell in which the number of survivors would be greatest according to the calculation. His calculation showed that the shell occupying the region of the greatest probability of survival according to the above definition, is not the shell within which is the point of lowest degree of lethal treatment. He suggested that the latter point may not be critical in consideration of the effectiveness of a sterilising process and that the effect observed at that point may not be a true criterion of sufficiency of the sterilisation of the contents of the container.

Hicks (44) in a discussion of Stumbo's first two papers points out that with a uniform spore destruction in the container, the number of spores actually subjected to the conditions at the slowest heating point will be very small. Some regions in the container receiving slightly more heat treatment will be relatively large in volume and so contain more spores. The chance of survival of a spore in such a region may therefore be greater than at the slowest heating point.

The more recent concept above of integration of the lethality of the process in terms of the whole container is only applicable to conduction packs as in convection packs all bacteria are subject to essentially the same amount of lethal heat.

PRACTICAL SIGNIFICANCE OF PROCESS EVALUATION CONCEPTS

The practical significance of the newer concept of process evaluation does not appear very far reaching. Alstrand and Ecklund (2) remark in reference to this new approach,

> "all these workers have approached the problem from the premise that there is probability of survival of spoilage bacteria throughout the can contents and not just at the can centre. While the development of these methods will be followed with great interest by students of processing technology, there is but little likelihood that greatly different results will be obtained by application of the new methods as compared to the methods in common use".

The methods using the whole can as a basis for calculation require information on initial numbers of spores and their rate of destruction characteristics in order to give information on the numbers of spores per can that are likely to remain viable after processing. This data can then be related to the probability of spoilage. Hicks (45) pointed out that the values of these parameters are not well defined and much more data will be required in the canning industry on the initial number of spores and the value of the final tolerable spore level.

Stumbo (120) has commented that many of the formula methods (introduced to reduce the tedium of graphical integration, and to increase the versatility of the General Method) are complex and time consuming and data for one set of conditions usually cannot reliably be converted to apply to another set of conditions. These disadvantages of formula methods have been endorsed recently (56).

The General Method, with its variations, is still the most reliable method of calculating the lethal effect of heat for any given set of data (82). Hicks (47) points out that the General Method, in its original form involves graphical integration and the errors of calculation may be significant although Patashnik (92) claimed to be able to keep the error of calculation well within the experimental error or the inherent variation from can to can. Errors due to inaccuracy in the bacterial data affect calculation by the General Method in exactly the same way as calculation by formula methods but the General Method has the advantage that actual heating and cooling curves are used, whereas the formula methods involve simplifying assumptions about the forms of the curves that permit their specification by a small number of parameters. The theoretical cooling curves are not always good approximations of actual curves (14). On the basis of this, Hicks finds it preferable to use the General Method for conduction heating packs, although with convection heating packs, formula methods may be more convenient.

The General Method, which is based on the critical point concept is still used or implied in the canning industry although it is generally not used in basic investigations of process calculation methods. The critical point concept, which is implied if temperature data from the centre of the can only is considered, is probably quite adequate for industrial purposes, as there are approximate relationships between the residual spore concentration at the centre (that is, at the critical point) and the total number of spores surviving the process (90).

On the basis of the above, all sterilising values determined in this present study have been calculated by the General Method from heat measurements taken at the critical point.

However, it should be pointed out that although the General Method is used throughout this study for calculating sterilising values, one could equally justify the use of formula methods or integration over the whole can to attain this result.

Previous assessments of sterilising values attained in canned foods on heat processing are all based on direct temperature measurement. The basis of this study was to determine if indirect methods, e.g. the influence of heat on chemical reactions, could be used to supplement previous methods and to enable heat lethality values to be determined commercially by a simple and convenient technique.

P A R T "A"

CORRELATION OF CARBONYL-AMINO BROWNING

WITH HEAT LETHALITY

S E C T I O N I

BROWNING REACTIONS OCCURRING IN FOODS ON HEAT PROCESSING

(a brief review of browning reactions and factors affecting them)

Due to the diversity of browning reaction mechanisms and greatly varying compositions of different foodstuffs it is not surprising that in some foods "browning" is desirable while in other cases the converse holds. For example, browning reactions induce favourable flavours in roasted coffee, meat and yeast extract concentration but will impart bitter off flavours in dehydrated meat and fish.

Most of the carbohydrate constituents of foods will form brown caramelisation products by a complicated process of dehydration and polymerisation if heated sufficiently strongly, reducing sugars such as arabinose, xylose, glucose and fructose decomposing more rapidly than sucrose and starch, and glucuronic, galacturonic, ascorbic and reductic acids more easily still. These reactions can be accelerated by alkali or acid, and particularly by amino compounds, and then proceed extensively at much lower temperatures. In some foods, such as molasses, honey or maple syrup, the amount of non carbohydrate materials involved may be so small that the browning reaction appears to be little more than a catalysed caramelisation of sugar. In fruits and vegetables, organic acids, including ascorbic, can take part in complex interactions with reducing sugars and amino acids, while in protein rich foods such as fish and egg white, comparatively minor proportions of sugars are able to produce serious changes in the proteins which constitute the major part of the food.

In addition to proteins and amino acids, other nitrogenous food

constituents such as the B vitamins, thiamine, nicotinamide and p amino-benzoic acid can also undergo reactions of the browning type with reducing sugar and this is probably one of the routes by which losses occur during processing and storage of processed foods. Also, in dried egg yolk, traces of glucose are known to react with the lipid amino groups of cephalin, with a resulting serious deterioration in palatability and colour.

The main types of "browning" recognised in heat processed foods are:

1. Carbonyl-amino or Maillard reactions in which carbonyl or potential carbonyl groupings react with amino or amino compounds to form ultimately brown melanoidin pigments.
2. Caramelisation resulting from the action of acids or alkalies on sugars.
3. The decomposition of ascorbic acid under acid conditions.
4. The browning of oxidised oils on reacting with amines or proteins.
5. The degradation of imidazole and indole bodies.
6. Changes in the haem pigment in animal products.

Carbonyl-amino browning is the most thoroughly investigated and commonly encountered of the browning reactions and considerably less is known about the other forms of browning which occur on heating certain foods.

A. THE MAILLARD REACTION

Results of the many investigations into the mechanisms of the Maillard reaction support one of two main theories. The first (49) assumes the formation of glycosylamines which undergo the Amadori rearrangement. The

1-amino-1-deoxyketose derivative formed may be dehydrated and cyclised to form furan derivatives or it may enolise. In either case intermediates which are readily transformed into brown compounds are formed. A third possibility is for the deoxy sugar derivative to react with more amino acid to form coloured products. Optimum conditions for the Maillard reaction are:

(a) a fairly low water content,

(b) a pH of 7 to 10, and

(c) a high temperature.

These conditions are fairly flexible but no reaction proceeds in the absence of moisture.

The second theory (113) maintains that the browning reaction and the Maillard reaction are separate and distinct. In this theory browning is due to the effect of pH on the sugar and can occur over a wide range of pH, whereas the Maillard reaction occurs only in alkaline media. Hodge (49) pointed out the similarity between the browning reactions of sugars in amino acid and non amino systems and it has now been confirmed, by the isolation and characterisation of intermediates that similar mechanisms are involved in the degradation of sugars by acids and alkalies, and in the decomposition of ketosamines derived from primary amines. On the basis of this, the overall browning mechanism, which will be referred to as carbonyl-amino browning, will now be outlined as it is this form of browning which is of primary concern in this study.

However, since no attempt is made to distinguish and consider only the predominant form of browning occurring in any of the systems studied, the mechanisms of other important forms of browning will also be briefly discussed.

Mechanism of Carbonyl-amino Browning

The best known systematisation of the possible chemical reactions occurring in carbonyl-amino browning is that devised by Hodge (49) and a number of excellent reviews have since been written (29, 93, 103, 104).

In the first instance, aldoses react with primary or secondary amines to give aldosylamines (N substituted glycosylamines) and these, under the influence of a suitable catalyst, undergo the Amadori rearrangement giving 1-amino - 1-deoxyketoses. Kinetic studies indicate a 1 : 1 ratio of amino acid : sugar although at high temperature, more sugar reacts per mole of amino acid. It is generally agreed that the Amadori rearrangement proceeds via the cation of a schiff's base but final details of the mechanism have not been determined. This initial stage of browning which is colourless and gives no adsorption in the near ultraviolet region, proceeds particularly at low temperature and under low moisture conditions. Ketosamines have been identified in extracts of browned freeze dried apricots and peaches (3,4) and dried liver (17). Wager (139) has isolated these compounds from dehydrated carrots.

The second stage in carbonyl-amino browning as indicated by the appearance of colouration and adsorption in the near ultraviolet region, is

(a) sugar dehydration,

(b) sugar fragmentation, and

(c) either amino acid or amine degradation according to the compound combining with sugar.

(a) Two types of sugar dehydration reaction occur: in acidic systems furfurals are formed while in the presence of amines in anhydrous systems, 6 carbon and other reductones are formed. In the first case, three

moles of water are lost to form furfural or hydroxymethylfurfural (105). These compounds have been detected in heated apricot concentrates, in darkened dehydrated cabbage, and in orange and lemon concentrates (42) and in milk (93). Reductone formation resulting from the loss of two molecules of water from the sugar moiety has been demonstrated (123).

(b) During the latter part of the second stage of carbonyl-amino browning fission products from sugar fragmentation occur. Discolouration is greatly increased by the presence of amino groupings in the reaction mixture although fragments which retain the ∝ carbonyl grouping will brown on their own. The accepted mechanism of sugar fragmentation is dealdolisation (the reverse of aldol condensation) and amino acids in the anionic form catalyse this reaction.

(c) Strecker degradation of ∝ amino acids to aldehydes containing one carbon less than the amino acid, with the liberation of carbon dioxide (from the carbonyl group of the amino acid) in the presence of dicarbonyl compounds (reductones) could be important in the incorporation of nitrogen in the final brown product. The aldehydes formed by this degradation could be a source of browning due to the large number of compounds with which they may condense. Not much importance is attributed to them as a browning source, although the products formed by Strecker degradation are important flavour and odour precursors.

In the final stages of carbonyl amino reactions various intermediate polymeric and unsaturated, fluorescent and coloured polymers of varying molecular weight are formed. The main reactions are thought to be aldol condensation, aldehyde-amino polymerisation and the formation of heterocyclic nitrogen compounds (chromophoric compounds or aldimines) and finally the formation of melanoidins.

B. BROWNING REACTIONS OF LESSER IMPORTANCE OCCURRING IN FOODS

1. Caramelisation Action of Acids and Alkalies on Sugars

This method of non enzymatic browning is the dehydration of sugars with the resulting formation of very active aldehydes. Hexoses, pentoses and all uronic acids are broken down readily when heated in an acid medium, as is the case, for instance, during acid hydrolysis of starch for the manufacture of glucose syrups containing dextrins. These products are brown due to the "caramelisation" of sugars which can occur in the absence of protein or amino acids. Wolfrom (147) offered a scheme for such caramelisation phenomena, which are, in part, due to the formation of hydroxymethylfurfural (HMF).

HMF, as such, is capable of polymerising and, in addition, as a very active aldehyde, of readily combining with amino compounds if present even in very small quantities.

With increasing pH, the browning due to HMF is accompanied or replaced by the Maillard reaction or by other mechanisms not based on the presence of amino groups, such as enolisation or formation of reductones.

The degradation of reducing sugars to furfurals occurs in the presence of either mineral acids or organic acids. However, the formation of the 1, 2 enol, which is also the first step in the Lobry de Bruyn - Alberda van Ekenstein transformation, is subject to acid-base catalysis. It is likely that later stages of the reaction are also subject to acid base catalyses. It would be expected therefore, that the organic acids in foods would be good catalysts for the degradation of sugars.

Proctor and Goldblith (96) found evidence that furfurals are not the only intermediates in the formation of pigments when sugars are heated with acids. Bergdoll and Holmes (10) found that browning in heated sucrose solutions was increased by the replacement of part of the sucrose with invert sugar, but not by the addition of HMF. Also, it appears that oxygen has some affect on browning in relatively dilute, acidified, sugar solutions.

In all systems lacking amino groups, browning of the sugars, polysaccharides and polyhydroxy acids can proceed without catalysts at rather high temperatures. However, in the presence of phosphates and a number of carboxylic acids, acting as catalysts, these reactions proceed very rapidly. Malic acid had been found to be the most active of all carboxylic acids in accelerating this form of browning.

In alkaline media the 1, 2 enolisation of reducing sugars (the Lobry de Bruyn - Alberda van Ekenstein rearrangement) causes the formation of a very labile configuration capable of rapid oxidation and subsequent browning. The formation of 1, 2 enols of reducing sugars occurs more easily in the presence of alkalies than in acids and in this case the browning will follow the path of ascorbic acid and of other substances with dienolic configurations. Under anaerobic conditions, however, the action of alkalies on reducing sugars produces saccharinic acids with relatively little browning.

The acceleration of browning is appreciably greater through glycosylamine enolisation (the Amadori rearrangement) than through enolisation of the unsubstituted sugar (Lobry de Bruyn - Alberda van Ekenstein rearrangement).

It is now generally recognised that the sugar caramelisation process involves the following reaction steps (50): Enolisation of aldoses with

the production of the more reactive 2 - ketoses; dehydration of ketohexoses, without fission, to 5 - (hydroxy methyl) - 2 - furaldehyde, and dehydration of pentoses similarly to 2 - furaldehyde; hydrolytic fission of furaldehydes or intermediates leading to furaldehydes to yield, for example, formic acid and levulinic from hexoses; fission of 2 ketoses to yield dihydroxyacetone and glyceraldehyde, glycolaldehyde and four-carbon carbonyl compounds; dehydration of the trioses to yield acetol and pyruvaldehyde; dismutation of biose, trioses, tetroses, and their dehydration products to yield lactic aldehyde, pyruvic aldehyde, lactic acid, glycolic acid, acetaldehyde, acetic acid, formaldehyde, formic acid, acetoin, and diacetyl; self and cross-condensations of the aldehydes and ketones containing active hydrogen; reversion of aldoses and ketoses to di-, tri-, and higher oligosaccharides; dimerisation of fructose to difructose anhydrides; cyclodehydration of aldoses to glycosans, and then polymerisation; enolisation and dehydration of the synthetic oligosaccharides.

2. Browning Reactions of Ascorbic Acid

The decomposition of ascorbic acid, when heated with acid under aerobic or anaerobic conditions leads to the formation of brown pigments. Joslyn (64) has demonstrated that this kind of browning takes place only after the bulk of ascorbic acid has disappeared while the rate of loss of ascorbic acid in acid solution has been shown by Lamden and Harris (69) to be a monomolecular reaction. Joslyn (64) suggested that ascorbic acid was the most reactive component in browning in systems containing ascorbic acid, amino acids, and sugars. The rate of browning in orange juice was reduced to a low level by removing the anionic constituents; removal of the cationic constituents had less effect. The rate of browning of

ascorbic acid solutions, under oxidative conditions was decreased by the addition of glucose or fructose and the addition of amino acids decreased the rate of browning initially, but increased it in the later stages. Moore, et al. (81) have shown that additional quantities of ascorbic acid in the juices increased the formation of the brown pigment even more.

When solutions containing ascorbic acid and glycine (buffered at pH 3.7 or 7) were heated in a stream of oxygen, CO_2 was evolved. Less than 3% of the CO_2 was derived from glycine - C - 1, and less than 0.1% from glycine - C - 2. This finding showed that the glycine had not undergone a Strecker degradation, although ascorbic acid has been regarded as an active agent. Less than 4% of the CO_2 was derived from the buffer. These results showed that the evolution of CO_2 was due primarily to the multiple decarboxylation of ascorbic acid. The rate of production of CO_2 from ascorbic acid was linearly related to rate of browning at 37°C but not at 50°C, suggesting that a change in the reaction mechanism occurred in the range 30° - 50°C.

Furfural which has been shown to be formed when ascorbic acid is heated with acid is known to undergo polymerisation and browning. Moreover, as an active aldehyde it may easily undergo the Maillard reaction even with the very small quantities of proteins or amino acids found in citrus juices. Also various reductones possessing the same dienol configuration as ascorbic acid may follow the same scheme of browning.

The autoxidation of ascorbic acid follows a similar path to that outlined previously. Only small quantities of oxygen, normally acquired in any operations concerned with the preparation of foods, are able to start the chain of autoxidation with browning resulting, whether it is propagated

by flavanoids or otherwise. Ascorbic acid is not autoxidable in acid solution but the rate is increased with increasing pH above neutrality. Copper catalyses the reaction.

3. Browning of Oxidised Oils

Phospholipids and lipoproteins can react, through their amino groups, with aldehydes and reducing sugars; under oxidising conditions, reactive carbonyl groups formed in the lipid moiety can initiate browning reactions. The glucose-cephalin reaction in dried eggs is well known.

(a) Reaction with amines

Oxidised oils brown rapidly when treated with amines, but it is not certain that amines are involved in browning reactions of commercial significance such as the "rusting" of fish oils and, in another field, the yellowing of films of drying oils.

In an investigation primarily concerned with fishy flavours in oils, Davies and Gill (26) showed that the degree of fishiness was roughly proportional to the content of organically bound nitrogen and the degree of browning.

Nonaka (86) showed autoxidised oils browned in the presence of ammonia or amines, but various inorganic salts and organic compounds, including N-substituted thioureas, also caused browning.

Olcott (87) found that fish oil that had been carefully freed of nonglyceride impurities remained almost water-white even when it was very rancid. In the presence of added nitrogenous compounds the oils became yellow to dark brown during the development of rancidity.

(b) Reaction with proteins

Oxidised lipids emulsified in aqueous dispersions of proteins give brown copolymers or complexes. The results obtained by Tappel (126) suggested that an amino-carbonyl condensation had occurred, but this was not confirmed by Venolia and Tappel (136). The latter found that the apparent activation energy of browning was lower than expected for an amino-carbonyl reaction. Also, browning was not inhibited by means that should have been effective for a carbonyl-amino reaction, namely, the addition of bisulphite or acetylation of the protein. It is considered that the precipitates obtained from egg albumin and oxidised lipids were complexes in which the attachment of the lipid to the denatured protein did not involve covalent bonds.

4. Degradation of Imidazole and Indole Bodies

It is only recently that the importance of the degradation of naturally occurring, nitrogen containing, heterocyclic compounds as factors in the "browning" of foodstuffs has been realised. Japanese workers have demonstrated that tryptophan is readily degraded to brown pigment at physiological pH and high temperatures, and a considerable portion of the browning of some canned foodstuffs has been attributed to this source. 1 methylhistidine, liberated by muscle anserinase after death in fish muscle, has been shown to degrade very readily at low temperatures and near dry conditions. The reaction accounts for a considerable proportion of the discolouration of dehydrated cod (59).

These two types of browning are of considerable interest in relation to the carbonyl-amino reaction as 4 (5) methylimidazole and other nitrogen

containing heterocyclic compounds have been isolated from such reaction mixtures as glucose - glycine and glucose - ammonia but their exact significance, if any, in the production of brown pigment has not been appreciated.

5. Changes in the Haem Pigment in Many Animal Products

The pigments responsible for the colour of meats are the hematin compounds myoglobin and hemoglobin. Factors determining the colour of meat pigments are:

(a) The types of bonds of the central iron and compounds attached.

(b) The physical state of the globin.

(c) The oxidation state of the iron.

The abnormal brown, green and grey discolouration in processed meats are produced by oxidation of the ferrous to the ferric state to form brown "met" pigments, and of the porphyrin ring by oxygen to form grey or green products.

Tappel (127) reported on oxidation changes in hematin pigments of meat and fish and identified the non extractable brown pigment of cooked beef (and tuna) as mixed denatured globin nicotinamide hemichromes. This tan colouration is formed from a pink colour due to rapid oxidation of the hemochrome on contact with air. Results indicate that the hemochrome is derived from the reaction of heme of myoglobin and residual hemoglobin with either denatured globin or nicotinamide or both and on oxidation, is converted to the characteristic brown hemochrome.

C. FACTORS AFFECTING THE RATE OF CARBONYL-AMINO BROWNING IN HEAT PROCESSED FOODS

Since the carbonyl-amino browning reaction is the most frequent and important form of browning encountered during the heat processing of foods, the factors affecting this reaction will be considered.

1. Time

As might be expected, the manifestations of the Maillard reaction increase with time. While there is some discrepancy between the results of different workers, it has been reported that there is a lag phase, after which browning (as measured by optical density in the 430-490 mμ adsorption range) is linear for a period before tailing off (148). Other workers have reported the depth of colour increases with the square of the time (43). Observations on other characteristics of the reaction are largely qualitative but, in general, development of insolubility and reducing power, loss of amino nitrogen, and fall in pH increase with time. Fluorescence however, reaches a maximum and then decreases (89).

2. Temperature

The effect of temperature was first investigated by Maillard on the interaction of D-glucose and glycine and from visual observations on the rate of browning, gas release and increase in viscosity he was able to state the rate of the reaction increased with temperature. His general statement has since been confirmed by a number of workers in this field.

Katchalsky and Sharon (68) using a potentiometric method deduced that the observed rate constant for the interaction of the positive oxonium ion of D-glucose and the glycine anion bears a linear relation to the

temperature, while Lea and Hannan (71) showed that the rate of the D-glucose - casein reaction, as measured by the decrease in amino nitrogen content increased uniformly with temperature between 0 and 90°C and the reaction had an activation energy as determined from the Arrhenius equation of above 29 kcal.

The dependence of the rate of browning on temperature in food systems has also received considerable attention. The apparent activation energy of browning in apricots (122) and dried meat (116) is about 26 kcal while for other products, e.g. unsulphited onion, potato and carrot (73) and dehydrated potatoes (39) (108) values in the range of 36 to 42 kcal have been obtained. In milk systems, Burton (20) found the browning of evaporated milk had an activation energy of 29 kcal while Ward (140) obtained a value of 26 kcal for condensed milk.

It has been shown that different products may be formed at different temperatures as shown by a chromatographic study of the D-glucose - glycine reaction at 56.5 and 100°C (23). However, this is not surprising when one considers the complexity of the reaction.

3. pH

The rate of browning in mixtures of aldoses and amines increases with increasing pH over a pH range between about 4 and 11 (71). Barnes and Kaufman (8) found the rate of browning is doubled at pH 6 as compared with pH 4. Mohammad, et al. (80) showed the rate of browning of solutions of glucose and bovine serum albumin increased as the pH was increased and the plot of the logarithm of reaction rate against pH was linear suggesting specific hydroxyl ion catalysis.

However, Wolfrom, et al. (146) using xylose-glycine and xylose-alanine systems, found a significant change in slope appeared in a similar plot at pH 6.

Since the basic amino group disappears during the reaction, the pH of an aqueous solution of the reactants will decrease and so the initial pH of the solution or the presence of a buffer will have an important effect upon the progress of the reaction.

The magnitude of the pH depression has been shown to be dependent on the reactants and on the initial alkalinity of the solution (35).

Katchalsky and Sharon (68) studied the kinetics of the aldose amino acid interaction and found the rate of the reaction and the activation energy could be determined from the pH drop.

4. Moisture Content or Water Activity

It has been generally found (71) that browning in heterogeneous systems is maximal at a low critical moisture level, the exact value of which varies from system to system. Systems of carbonyl compounds combining with the amino function of compounds of high molecular weight have higher water activities (a_w) at the point of maximum browning than reaction mixtures with relatively simple compounds. Thus, for casein-glucose systems the maxima are of the order of 0.7 - 0.75 a_w (71) whereas a system of mixed extractives of low molecular weight from fish muscle had maxima of the order of 0.1 - 0.3 a_w (57), the exact value depending on time and temperature.

However, results have been obtained (107, 113) which demonstrate that browning is favoured by anhydrous conditions. Ward (140) using a

glycerol - skim milk powder system, showed browning was maximal at 0% moisture and postulated that browning in this system was dependent only on the mobility of the lactose and not on the presence of moisture.

5. Light and Radiation

It has been shown that light accelerates the browning of solutions of glucose and glycine in the absence of oxygen, but in aerobic conditions, partly browned solutions were bleached (16). Light is known to cause degradation and discolouration of ascorbic acid and other reductones and there is evidence that imidazole compounds such as are formed in the later stages of carbonyl-amino reactions are also unstable to light and other forms of radiation (59).

Ionising radiations have been shown to increase the tendency for non enzymic browning to occur in milk and also in a lactose-casein model system (142).

6. Metals

The addition of small amounts of metals can markedly effect the rate of the browning reaction. Iron and copper tend to accelerate the reaction while manganese and tin tend to retard it (16, 79). However, the presence of air, concentration of reactants etc can affect these results.

7. Carbonyl or Sugar Moiety

The reactivity of carbohydrates on browning parallels the general reactivity of the carbohydrate compounds (72, 95). The aldopentoses are more reactive than the hexoses which are in turn more reactive than the di-, tri- or polysaccharides. A free carbonyl group is essential for the

reaction to take place. An increase in the rate of browning has been observed for the hexose 6-phosphates as compared with the unsubstituted sugars (115, 129).

8. Amino Group

The greater the basicity of the amino group, the greater is the rate of the reaction. Hence primary amines are more reactive than secondary amines. In the case of cyclic amines, the presence of a substituent group in the benzene ring which increases the basicity of the amino group will increase the rate of reaction.

9. Oxygen

Oxygen has been variously described as increasing and eliminating carbonyl–amino browning according to the particular system involved (16, 75). It is likely to be of the greatest importance where the reductone intermediates are predominant in the reaction.

10. Ions

The effect of citrate, phosphate and calcium ions have been investigated by Burton (21). The effect of these ions on browning acts mainly through a change in pH although the ability of phosphates as buffers to accelerate the reaction when compared with other buffers has been observed by a number of workers (115).

D. THE CONSEQUENCES OF BROWNING REACTIONS OCCURRING ON HEAT PROCESSING

With the number of browning reactions possible and the complexity of

their mechanisms, it is not surprising that their net effects are in some cases beneficial while in others detrimental. Products of these reactions contribute to the flavour, colour and aroma of many cooked and processed foods, such as, toasted cereals, roasted coffee, baked goods and meats. The tailoring of synthetic meat flavours ranging from beef to roasted chicken is now carried out by reacting various reducing sugars and amino acids.

However, deleterious changes are also associated with browning (72) and these include:

1. Production of a stale, caramelised or otherwise unpleasant odour or taste;
2. development of a brown discolouration ranging from a pale cream or biscuit shade to almost black in extreme cases;
3. loss of solubility of the protein leading to a deterioration in texture and to failure of dried foods to reconstitute properly with water;
4. enhanced reducing properties;
5. an increased tendency to foam or froth;
6. the development of the property of fluorescing under ultra-violet light;
7. a decrease in pH and the production of carbon dioxide and water; and finally,
8. a loss of nutritive value of the protein owing to a reduced availability of certain of the essential amino acids and a destruction of ascorbic acid and some of the B vitamins when present.

The colour changes associated with browning reactions are often an accepted consequence of heat processing and only in extreme cases is there

concern. In the majority of heat processed products which show browning, the other deleterious changes associated with this reaction are generally very slight and unperceivable.

S E C T I O N II

INVESTIGATION OF FUNDAMENTAL FACTORS AFFECTING A MODEL CARBONYL-AMINO BROWNING SYSTEM

A. INTRODUCTION

Kinetic studies have shown that carbonyl-amino browning does not conform to a simple order reaction except at the initial stages during which the reaction is still colourless and no pH drop is detectable (4). At later stages it has been shown that the depth of colour increases with the square of the time (43) but this relation was not generally obtained in the present study.

Obviously the rate of browning is also going to depend on such factors as pH, reactivity of the carbonyl and amine compounds present, and presence of oxygen, metals, phosphates, and water but provided these factors are kept constant, then the degree of browning will be dependent on time and temperature.

Since the distribution of bacterial spores is dependent on time and temperature, the correlation of browning with heat input and hence spore destruction appeared a reasonable method of determining the degree of heat processing a canned product had received. An investigation of a suitable model system was carried out and factors affecting the rate of browning were studied.

B. PRELIMINARY INVESTIGATION

A large number of workers have carried out studies on the carbonyl-amino browning reaction using model systems but there is little data recorded showing degree of browning as a function of time. Ward (140)

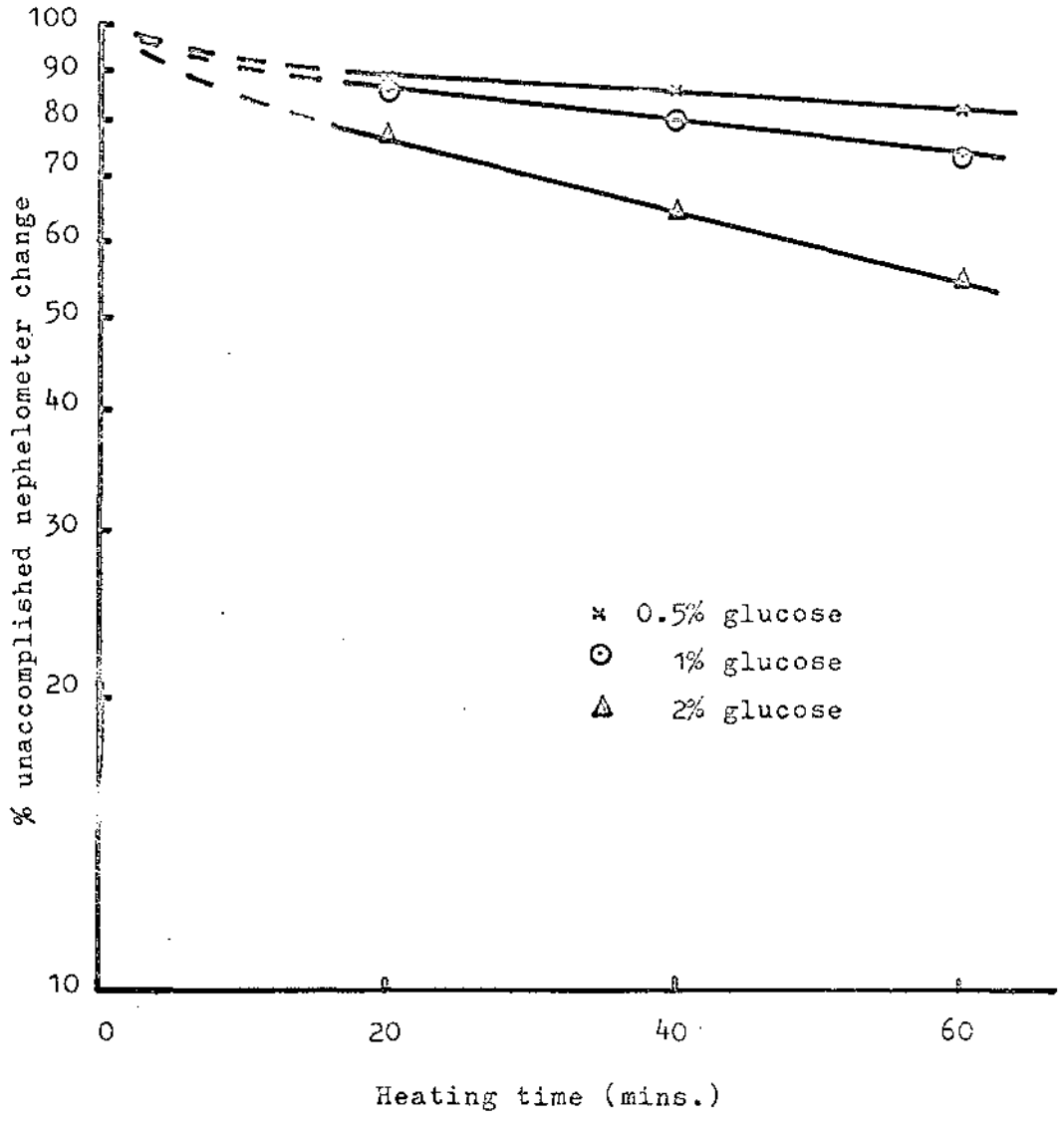


Fig. 1. BROWNING OF GLUCOSE-CASEIN SOLUTIONS ON HEATING

studied browning in sweetened condensed milk at various temperatures and was able to plot the logarithm of the percentage of unaccomplished change in browning (as measured by a reflectance spectro-photometer) against time. Although he was perhaps unjustified in calculating the activation energy from linear interpretations of his curves, the results he obtained do indicate a possible trend of browning as a function of time.

(a) Use of a glucose-casein model system

A preliminary investigation was carried out using a glucose-casein model system to investigate browning as this system has been used extensively to study mechanisms of carbonyl-amino browning.

Experimental method

211 x 400 code 3 cans (see appendix) containing solutions of 2% casein plus 0.5, 1.0, 2.0% glucose were steam flow closed with a net head of $\frac{1}{8}$ inch and heated by steam at 10 p.s.i. for times of 20, 40, and 60 minutes. The extent of the browning occurring in a sample was determined by means of a nephelometer after equilibrium in the solution had been reached (see appendix for details of method).

Results

The results obtained showing the effect of time of heating on the extent of browning are summarised in Fig.1. The extent of browning is expressed as the logarithm of the percentage unaccomplished change in nephelometer reading. In each case, the galvanometer was adjusted to give a scale reading of 100 by use of an unheated casein-glucose control. Readings were taken with filter B (a blank filter) in position in the nephelometer. A pH drop of 0.6 units (from 6.9 to 6.3) occurred in

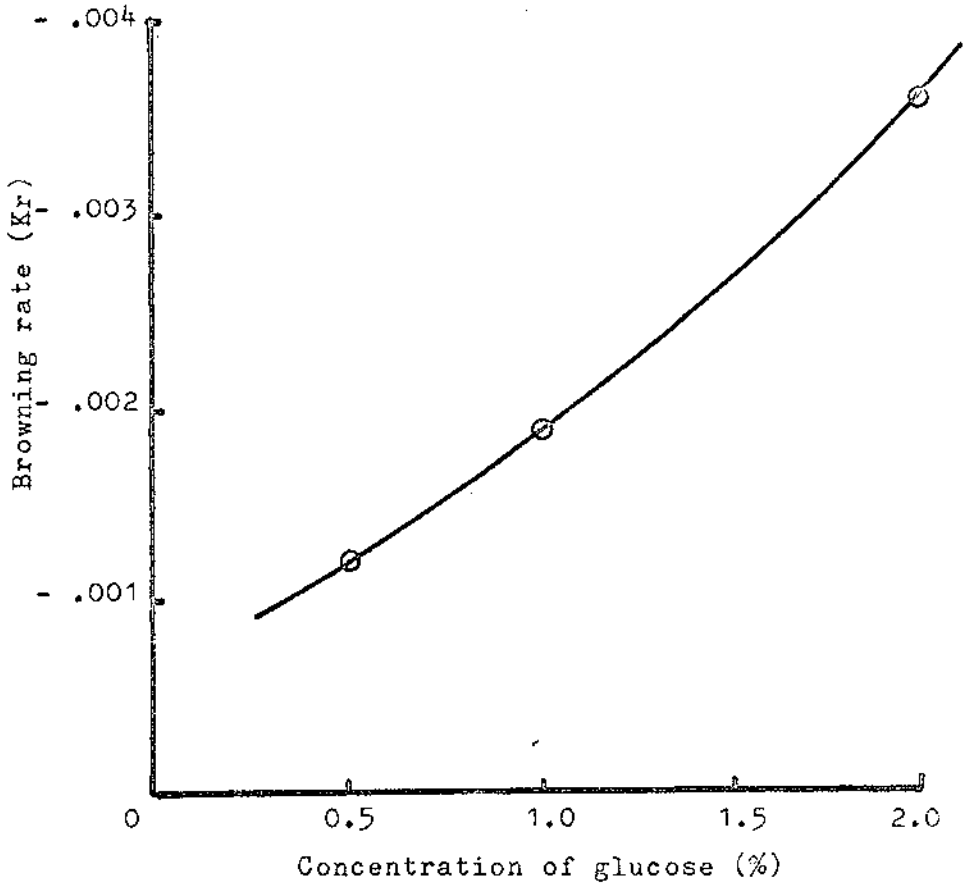


Fig. 2. EFFECT OF CONCENTRATION OF GLUCOSE ON THE RATE OF BROWNING OF 2% CASEIN SOLUTIONS

the 2% glucose-casein solution on heating for 60 minutes.

Discussion of results

It can be seen that the results show a similar trend to those obtained by Ward. There is an initial rapid rate of browning followed by the attainment of a steady rate of browning. It would be difficult to justify that the latter period of browning obeyed first order kinetics as the formation or loss of no single compound is followed and experimental inaccuracies in the results could be significant.

When the rates of browning for the three systems shown in Fig.1 are determined from the linear portions and plotted as a function of glucose concentration (Fig.2) it is seen that the relationship apparently existing between the rate of browning and concentration of reactants takes the form of a curve.

The pH drop encountered during the reaction is a characteristic of the browning reaction.

Casein-glucose as a model system has the drawback that the solution is turbid and this could interfere with the estimation of pigment formation. Photoelectric colorimeters were relatively insensitive in measuring colour development due to this turbidity and so a nephelometer was used.

It was found that the browned solutions had to be held under chilled conditions for at least 24 hours to allow the system to stabilise. This was possibly due to an equilibrium state having to be reached between the colloidal phase, brown pigment and aqueous phase.

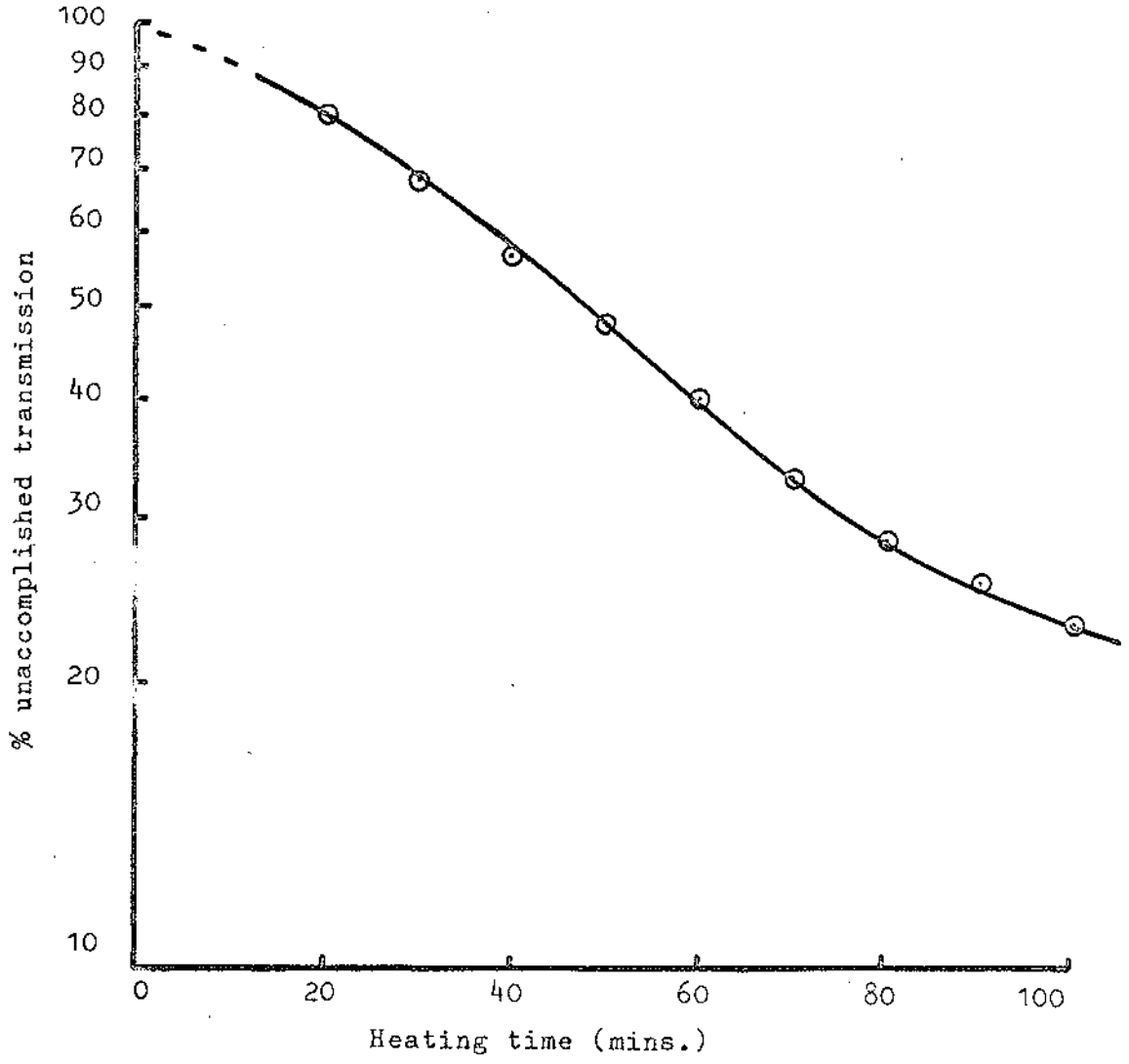


Fig. 3. BROWNING OF A GLUCOSE-GLYCINE SOLUTION ON HEATING

(b) A glucose-glycine model system

Much of the research on mechanisms of carbonyl-amino browning has been done using model systems incorporating glucose-glycine solutions and so a preliminary investigation was carried out to assess the feasibility of these as browning reactants. Of the readily available carbonyl reagents, glucose is one of the most reactive.

Experimental method

A solution of 2% glucose and 2% glycine was made up and filled into 211 x 400 code 3 cans. The resulting solution had a pH of about 7.0 - 7.1. As with the glucose-casein solutions the cans were steam flow closed with a net headspace of $\frac{1}{8}$ inch giving a vacuum in the can of about 5" Hg (see appendix). The cans were heated for varying times at a processing temperature of 238.0°F. In all runs, a standard procedure was adhered to in venting the retort. After venting, the retort was heated to the desired process temperature (which was measured with a mercury in glass thermometer) and timing was commenced at this point. After the required process time had been reached, cooling of the cans to room temperature by flooding the retort with cold water was carried out using a standard procedure. The cans were then opened and the degree of browning determined using a photoelectric colorimeter (see appendix for details of method).

Results

The results obtained are summarised in Fig.3 in a plot of the logarithm of the percentage unaccomplished transmission against time. The pH of the solution decreased steadily with increased time of

heating (and increased browning) and after 100 minutes, had reached a value of about 5.9. There was no detectable decrease in the vacuum within the can after processing for 100 minutes.

Discussion of results

The results obtained for the degree of browning with time indicate a simple linear relationship does not exist. The pH drop occurring during the reaction could be expected because as the reaction proceeds, the glycine amine groups are utilised, giving a decrease in basicity.

Glucose and glycine are both stable compounds, can be obtained in pure form and are readily soluble giving clear colourless solutions. On heating, the resulting browned solution is clear and this enables readings of the degree of browning to be taken directly with a photo-electric colorimeter. No equilibrium period is required as for the glucose - casein solution. Also, the reaction rate of a glucose-glycine system of a concentration of about 2% is such that it gives a convenient degree of browning over the desired time intervals such as are found in commercial heat processing and so, on the basis of the above factors, it was decided to use this system to investigate further the factors affecting carbonyl-amino browning.

Conclusions

The preliminary investigation indicated the feasibility of correlating carbonyl-amino browning with time. Of the two systems investigated, the glucose-glycine appeared to have distinct advantages over the glucose-casein system.

C. A STUDY OF THE FACTORS AFFECTING THE RATE OF COLOUR DEVELOPMENT IN A GLUCOSE-GLYCINE SYSTEM ON HEAT PROCESSING

Many of the factors affecting the rate of carbonyl-amino browning have not been studied quantitatively and since this fundamental knowledge is of prime concern in this work, it was necessary to study their effect in more detail.

(a) A study of the effect of metals on the rate of browning

Workers have shown that under varying conditions, the presence of small amounts of metals can have marked effects on the rate of carbonyl-amino browning (16, 79). Also, between different metals the effect is not consistent. Since in this study tinplate cans were used as containers for the reaction system, metal ions could be present in quite large concentrations and so the purpose of this study was to determine the effect of metals likely to be encountered in such a container and their effect, if any, on the reaction system.

Experimental method

To test the effect of metal ions, the following reaction systems were set up.

(a) A 211 x 400 code 3 can was filled with a 2% glucose-glycine solution and seamed.

(b) A 211 x 400 code 3 can with its interior badly scratched and marked, was filled with a 2% glucose-glycine solution and seamed.

(c) A 211 x 400 code 2 (plain tinplate) can was filled with a 2% glucose-glycine solution and seamed.

(d) A 211 x 400 code 6 can was filled with 2% glucose-glycine solution and seamed.

(e) 211 x 400 Code 3 cans were filled with 2% glucose-glycine solutions containing 20ppm, 50ppm, 100ppm of Fe (as $FeCl_2 4H_2O$) and seamed.

(f) A 211 x 400 can was filled with 2% glucose-glycine solution containing 0.1% E.D.T.A. and seamed. It was calculated that 0.1% E.D.T.A. would sequester up to about 150ppm of iron.

In all cases, the glucose-glycine solution was prepared using tap water, at room temperature. All cans were steam flow closed with a net headspace of $\frac{1}{8}$ inch and then heat processed for 60 minutes at 238.0°F. After cooling samples were removed from the cans and the degree of browning determined with a photoelectric colorimeter. The pH of the initial and processed solutions was also noted.

Results

The results obtained are tabulated below:

	Condition	Initial pH	Final pH	Degree of browning (% unaccomplished transmission)
a.	Code 3 can (control)	7.0-7.1	6.1	40.1
b.	Code 3 scratched	7.0-7.1	6.1	38
c.	Code 2 (tinplate)	7.0-7.1	6.1	39.2
d.	Code 6	7.0-7.1	6.1	40.1
e.	Code 3 + 20ppm Fe	6.8	5.8	45.0
	Code 3 + 50 " "	6.5	5.5	54
	Code 3 + 100 " "	6.19	5.2	64
f.	Code 3 + .1% EDTA	5.58	5.32	79.8

Discussion of results

There was no difference in the degree of browning between the contents of the cans with Code 3 and Code 6 lacquers indicating that the different lacquers probably had no effect on the rate of the reaction.

The Code 3 can that was scratched was very badly marked. The lacquer coating was extensively broken and removed exposing the tinplate and in many places, the tinplate layer was damaged exposing the steel base. It can be seen that the contents of this can showed slightly greater browning than the control which was an unmarked Code 3 can.

The degree of browning in the Code 2 can was in between that of the control and the badly marked can. It can be deduced from this that tin only very slightly increases the rate of browning in this system and that the reaction is more sensitive to traces of iron present, since the marked can (with small areas of iron exposed) caused more browning than the plain tinplate can.

The contents of the cans containing the added iron browned to a lesser extent than did the control and as the content of iron increased, so the degree of browning decreased. However, the added iron had a marked effect upon the initial pH of the solution and it is thought that it was the decreased initial pH of the solution that decreased the rate of the reaction rather than the presence of iron.

The initial pH of the contents of the can containing the added 0.1% E.D.T.A. was considerably lower than that of any of the other systems and this is reflected in the degree of browning in this system being less than any other. It appears that the pH effect of the E.D.T.A. in the system

over-rode any consequences from the sequestering of any iron present.

Conclusions

1. The area of tin which one could expect to be exposed in a lacquered can has a negligible effect on the rate of browning in a glucose-glycine system.

2. The reaction is much more sensitive to any exposed iron and so only cans which do not show excessive damage to the interior coatings should be used.

3. pH has a marked effect on the rate of the reaction.

4. Since the lacquered cans tested had no effect on the rate of the reaction, all subsequent trials were carried out using Code 3 cans in which the coating was in good condition.

(b) A study of the effect of processing time on the rate of browning at a specific temperature

Workers in this field have expressed rate of browning as a function of time but there is little uniformity between the correlations they have developed. For example, some have found the curve is of sigmoid form (148) while others found colour increases with the square of the time (43).

The purpose of this study was to determine the effect of processing time on the formation of pigment under conditions typically employed for commercial canning.

Experimental method

211 x 400 Code 3 cans were filled to $\frac{1}{8}$ inch net headspace with a 2%

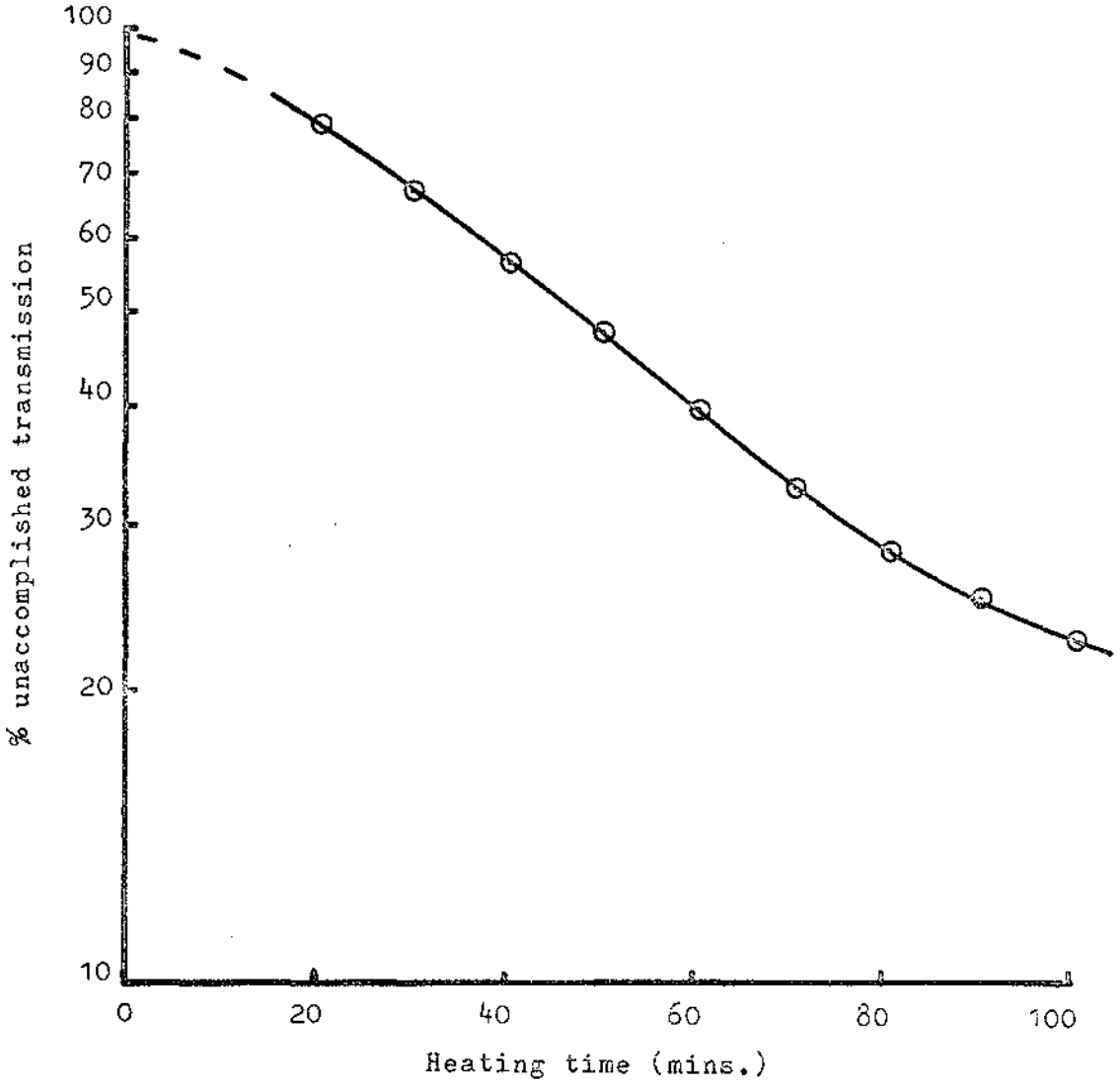


Fig. 4. THE EFFECT OF PROCESSING TIME ON THE RATE OF BROWNING AT A SPECIFIC TEMPERATURE

glucose-glycine solution made with tap water and steam flow closed. The cans were retorted for varying times at 238.0°F, cooled to room temperature and samples of their contents were removed and the degree of browning determined with a photoelectric colorimeter (see appendix).

Results

The results obtained are summarised in Fig.4 as a plot of the logarithm of the percentage unaccomplished transmission against time of heating.

Discussion of results

The curve obtained is similar in form to that observed by Wolfrom, et al. (148), i.e. there is an initial lag period during which the formation of brown pigments is very slow. It is well documented that the initial reaction products of the carbonyl-amine reaction are colourless. The initial lag period is followed by a linear phase during which there is a steady formation of pigments and this, in turn, is followed by a "tailing off" during which the apparent rate of pigment formation slows. Hence the overall shape of the curve is sigmoid. The "tailing off" of the curve could be due to:

1. The effect of pH on reaction rate. As the reaction proceeds, the pH drops and so the rate of browning decreases. However, results later indicated that this is not a probable cause.
2. A decrease in concentration of available initial reactants. It is well known that with some chemical reactions, when the concentration of the initial reactants is reduced to a certain amount, the kinetics of the reaction change.
3. An increase in reaction products causing a suppression of the rate of the browning reaction.

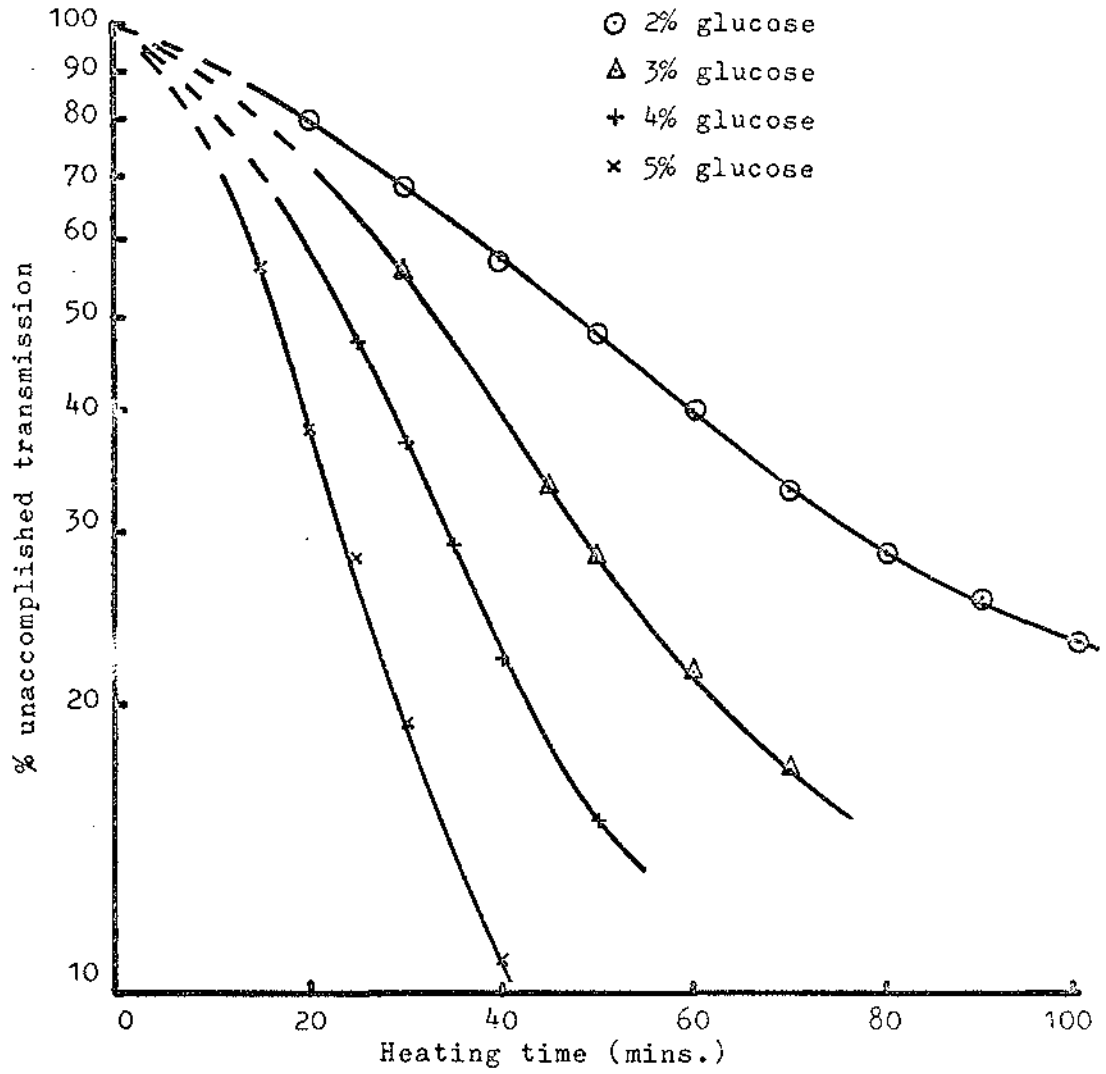


Fig. 5. THE EFFECT OF CONCENTRATION OF REACTANTS ON THE RATE OF BROWNING

4. As the reaction proceeds polymeric compounds of high molecular weight are formed in greater concentrations and since these have a different effect on the transmission of light in the 375 - 515 mμ wavelength range compared with intermediates formed during the reaction, the rate of the reaction as measured by transmission, apparently decreases.

(c) A study of the effect of concentration of the reactants on the rate of browning

From previous work it is obvious that the concentration of the reactants plays a major part in the degree of browning attained in a carbonyl-amino system. The preliminary survey using a glucose-casein system indicated that a non linear relationship could exist between the degree of browning as measured by the percentage of unaccomplished transmission and the concentration of the reactants.

The purpose of this study was to determine the affect of concentration of reactants on the degree of browning in a glucose-glycine system.

Experimental method

211 x 400 Code 3 cans were filled to $\frac{1}{8}$ inch headspace with solutions of 2%, 3%, 4% and 5% glucose-glycine in tap water at room temperature. The cans were steam flow closed and heat processed at 238.0°F for varying times. The cans were then cooled and the degree of browning of the contents determined with a photoelectric colorimeter.

Results

The results obtained are summarised in Fig.5 as a plot of the logarithm of the percentage unaccomplished transmission against time of heating. As the glucose-glycine solutions were made more concentrated,

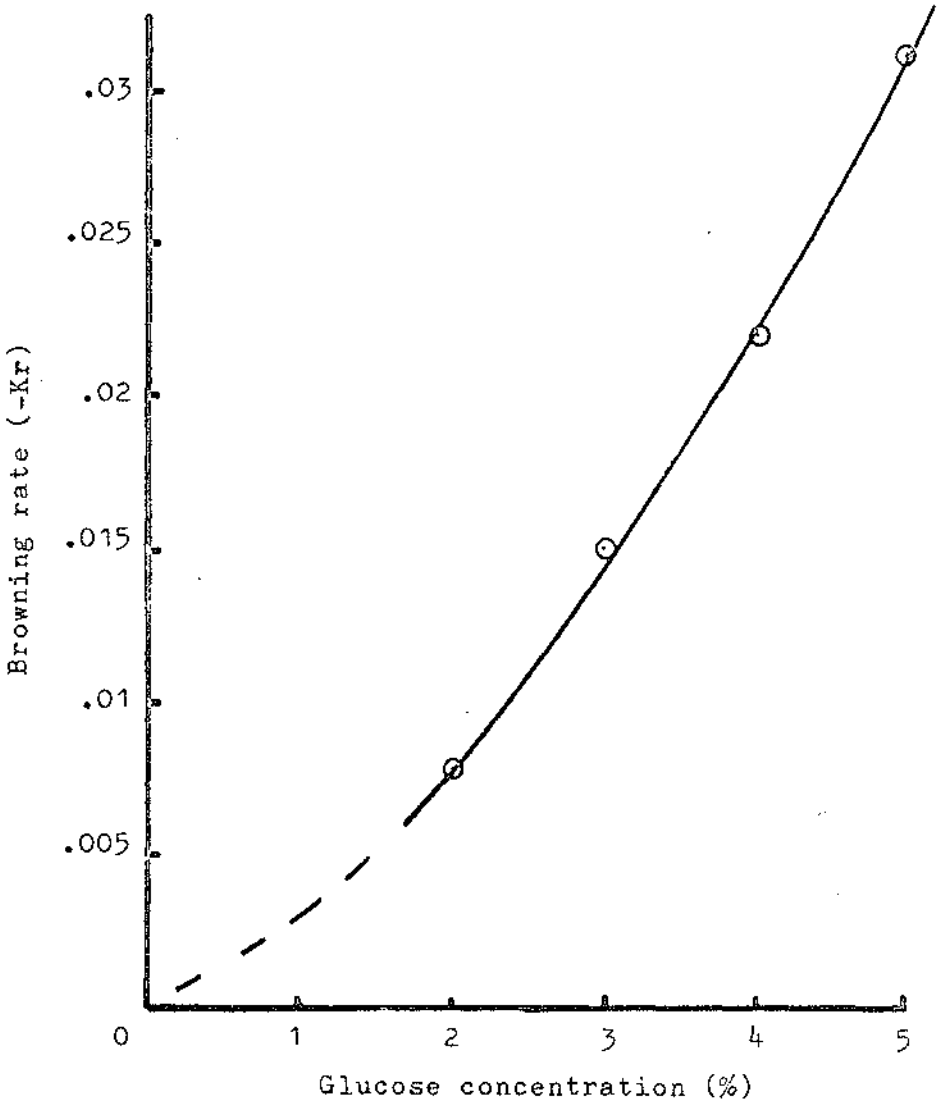


Fig. 6. THE RELATIONSHIP BETWEEN BROWNING RATE AND CONCENTRATION OF REACTANTS

so the heating time had to be reduced in order to keep the pigment concentration at a measurable level.

Discussion of results

It can be seen from Fig.5 that as the concentration of reactants increases, so the rate of browning increases. This is in accordance with the Law of Mass Action which may be written: The rate of chemical change varies directly as the active concentrations of the reactants.

For each concentration plotted, the graph of the logarithm of the percentage unaccomplished transmission against time is not linear but is sigmoid in form with an initial lag period, a linear period and a tailing off stage. These curves are similar in shape to that shown in Fig.4.

Due to the consistency of the trend shown by these curves it was felt justified to assume the linear portion of each curve was representative of the reaction for that particular system and that reaction rates could be calculated from the slopes of these portions of the curves.

If the slopes of the linear portions of the curves shown in Fig.5 are determined and plotted against the concentration of the glucose-glycine system, then the curve shown in Fig.6 is obtained. It is seen that Fig.6 is similar in form to Fig.2, i.e. when browning rate versus concentration of reactants was plotted from results obtained in the preliminary study using a glucose-casein system. Hence, the curve resulting from this type of plot is probably typical of a carbonyl-amino type reaction.

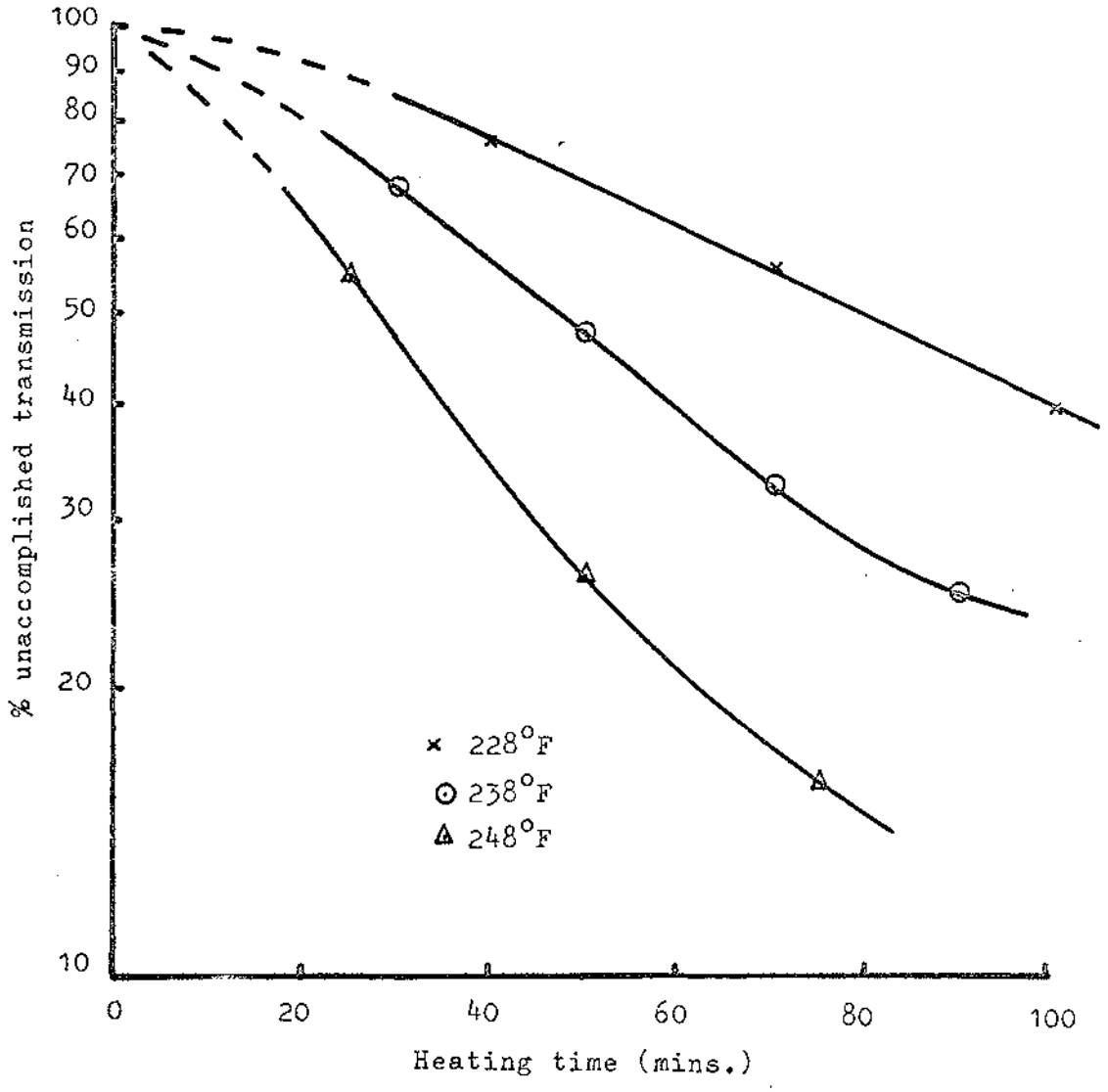


Fig. 7. THE EFFECT OF PROCESSING TEMPERATURE ON THE RATE OF BROWNING

is held constant is of the same form as when the pH drops during the reaction. Hence pH depression is not the predominant cause of the "tailing off" of the rate of browning. Possible causes for this apparent reduction in reaction rate are discussed earlier in this section under the effect of time of browning.

Conclusions

1. With increasing initial pH, the rate of browning in a carbonyl-amino system increases.

2. Where more browning occurs, e.g. under conditions of higher pH or temperature, and longer heating time, the pH depression occurring during the reaction is greater. The extent of pH depression could perhaps be correlated with the degree of browning and be used as a check on the reaction.

3. The "tailing off" of the reaction rate with time is not due to a reduced reaction rate resulting from pH depression.

D. SUMMARY

Salient results arising from work done in this section, are listed below:

1. Under the conditions of study, when browning is plotted against heating time on semi-logarithmic paper, the curve is sigmoid in form with an initial lag, a linear and a "tailing off" stage.

2. When reaction rates are calculated from the linear portion of the log browning versus time curves, the reaction is found to obey the Arrhenius

equation and have an activation energy of about 48000 cal/mole.

3. The rate of the reaction is very sensitive to the initial pH of the system. With increasing pH, the reaction rate increases.

4. The reaction is sensitive to the presence of trace amounts of iron and to a lesser extent, tin.

5. The reaction is sensitive to small amounts of air occluded in the system.

6. The rate of the reaction increases with increasing concentration of reactants.

S E C T I O N III

INVESTIGATION OF THE BROWNING REACTION IN SELECTED CANNED FOODS SUBJECTED TO TYPICAL COMMERCIAL HEAT TREATMENTS

The carbonyl-amino browning reaction and other less important browning reactions are often accepted consequences of heat processing in many canned foods. The purpose of this investigation was to demonstrate in certain foods, that these reactions could be followed quantitatively using relatively simple analytical techniques.

A. BROWNING OCCURRING IN BAKED BEANS IN TOMATO SAUCE ON HEAT PROCESSING

There is considerable evidence indicating that tomato products undergo non enzymic browning when processed. Spray dried tomato powders show a marked colour change resulting from this reaction, as do various tomato purees, pulps and sauces added to fish packs, spaghetti, etc.

There is also some indication that in baked beans, the beans themselves darken in colour on heat processing and it has been suggested that this is due to selective adsorption by the beans of carotenoid pigments from the added tomato.

The purpose of this study was to determine:

(a) if browning in the pack on heat processing could be followed, and

(b) to ascertain the predominant colour change mechanism.

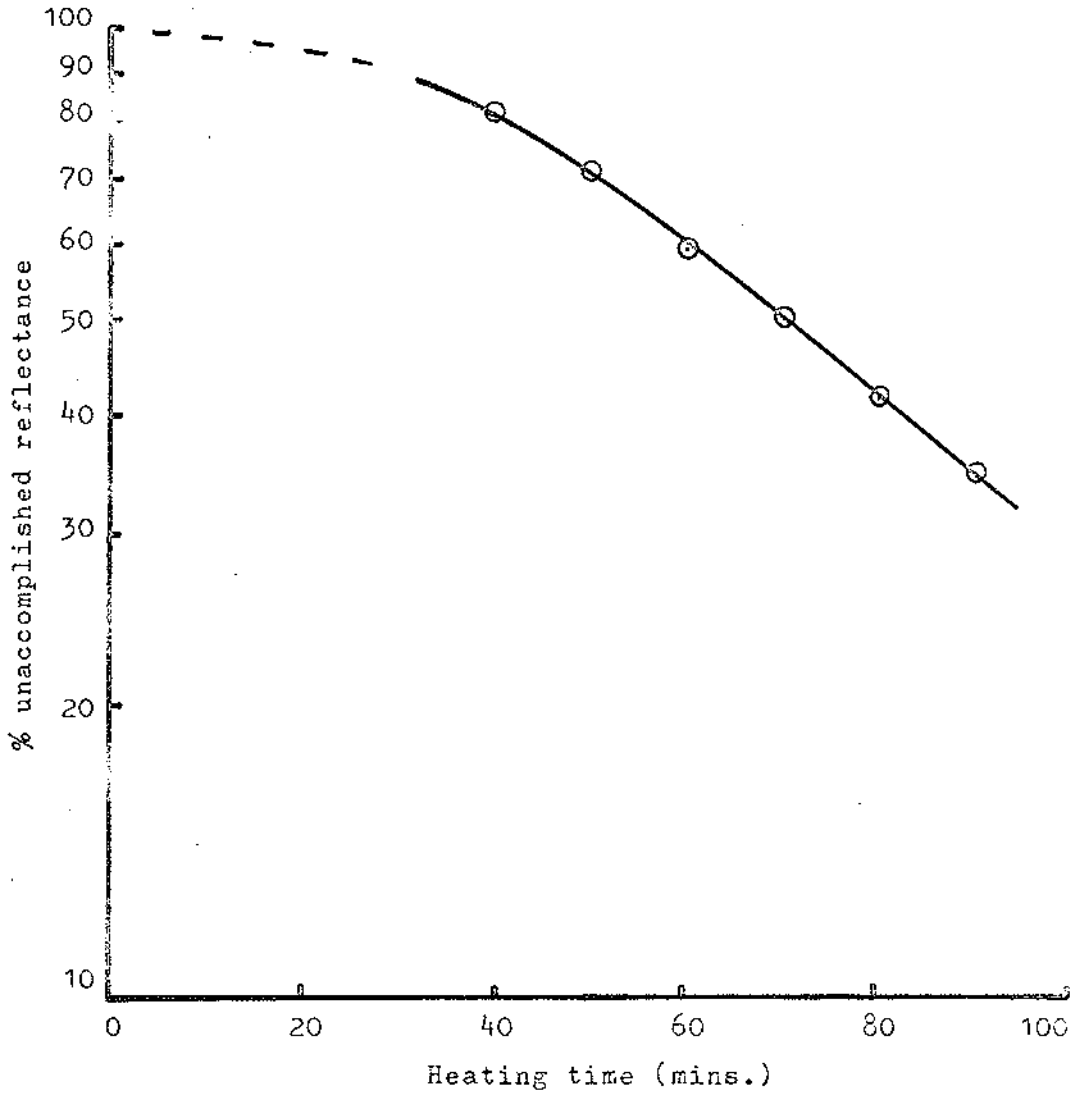


Fig. 13. BROWNING OF BAKED BEANS IN TOMATO SAUCE ON HEATING

(a) Following browning changes on heat processing

Preliminary investigations indicated that only a negligible degree of browning was taking place within the tomato sauce component on sterilising and that it would be very difficult to follow this colour change using only simple analytical techniques. However, a marked colour change was observed within the beans themselves when a pack of baked beans in tomato sauce was heat processed and, since the degree of colour change increased with the severity of the heat process, it was decided to follow pigment formation in the bean.

Experimental method

211 x 400 Code 3 cans were filled with 160 gm of soaked and blanched haricot beans (see appendix) and 160 gm of tomato sauce. This gave a net headspace of about 3/16 inch. The cans were steam flow closed, their contents thoroughly mixed by inverting the can a number of times and then heat processed for varying times at a retort temperature of 238.0°F. The cans were then cooled to room temperature, and a sample of the beans removed and their degree of browning determined using a reflectance spectrophotometer (see appendix).

Results

The results are summarised in Fig.13 as a plot of the logarithm of the percentage unaccomplished reflectance against time of heating.

Discussion of results

It can be seen from Fig.13 that after an initial lag period, a linear semi-logarithmic plot exists. The graph is, in effect, a plot

of the logarithm of the concentration of pigment formed against time and it appears that the reaction conveniently follows first order kinetics after the initial lag stage. However it would be very difficult to justify any statement suggesting that this reaction obeys first order kinetics as the development of no one pigment is followed, but perhaps the formation of a large number of different pigments by an indeterminable number of different chemical mechanisms.

Fig.13 does indicate that pigment formation in the beans increases with severity of heat treatment and that the technique developed to measure the colour change is relatively sensitive although perhaps too insensitive to detect an initial lag in the rate of browning as was observed in the model glucose-glycine system.

(b) The nature of the mechanism of colour change in the bean

An investigation was carried out to determine the nature and origin of the reactants involved in the browning of the bean on heat processing as the colour change is quite marked.

Experimental method

211 x 400 Code 3 cans were used in the trials below. In each case, the can was filled to a net headspace of 3/16 inch, steam flow closed, heat processed for 70 minutes at a retort temperature of 238.0°F and then cooled and the extent of browning of the beans determined (see appendix).

Trial A. 160 gm of prepared (soaked and blanched) haricot beans plus 160 gm tomato sauce were mixed and filled into the can and processed as outlined above.

Trial B. 160 gm of prepared haricot beans plus 145 gm water were heat processed.

Trial C. 160 gm of prepared haricot beans plus 145 gm water were heat processed as above and then retorted for a further period of 70 minutes.

Trial D. 160 gm of prepared haricot beans plus 145 gm water were heat processed for 70 minutes, drained, reseamed with 125 gm of tomato sauce and heat processed for a further 70 minutes.

Trial E. Cans containing prepared haricot beans were heat processed for 70 minutes with 3% and 6% glucose solutions.

Results

The results obtained are tabulated below:

Trial	% Unaccomplished reflectance after processing	% Unaccomplished reflectance after second heating period
A	47	-
B	84	-
C	84	79
D	84	60
E	54 (3% glucose)	-
	36 (6% glucose)	-

During Trial D it was observed that during the first heat processing stage, the extent of hydration of the beans increased and they took up a further 30 gm of liquid. Hence, during the second processing stage only 125 gm of tomato sauce were added to give the desired volume.

Discussion of results

On taking browned beans which had been heated with tomato sauce and

washing and extracting with a number of organic solvents it was established that the pigmentation in the bean was not due to absorption of carotenoids from the tomato sauce as no colour could be extracted.

Trial B indicates that the bean itself has some capacity to brown on heat processing. However, since there was little further browning on prolonged heating (Trial C) it is evident that the reducing sugar content of the bean is limited and exhausted very readily. That it is the carbonyl content that is limiting the extent of the browning reaction is shown (Trial E) by the browning being promoted when the beans are processed in the presence of reducing sugar (glucose). As the concentration of glucose was increased so the extent of browning increased. The acceleration of the browning by the presence of reducing sugar confirms it is a carbonyl-amino reaction taking place in the bean.

Tomatoes contain up to about 3% reducing sugar and on comparing trials A and E, it is seen that the degree of browning obtained in A is equivalent to about a 4% solution of glucose. However, with the large number of factors which can affect the rate of the browning reaction the difference in magnitude of the results of Trials A and E is very small.

On the basis of the results obtained, it is evident that most of the colour formed in baked beans in tomato sauce on heat processing is due to:

1. On heat processing, the beans undergo further hydration. In Trial D, a weight gain of 30 gm occurred during the first heating period and little further hydration took place during the second heating period and the tomato sauce maintained a thin consistency. In the case of a typical baked bean in tomato sauce pack (Trial A),

the sauce becomes thick due to absorption of liquid by the beans. Reducing sugars present in the tomato sauce are hence absorbed into the bean where they react with amino groups to form brown pigments.

2. Browning occurs as a result of reaction of carbonyl and amino constituents in the tomato sauce and some of the soluble brown pigments formed are absorbed by the bean on its hydration.

3. Carbonyl-amino browning occurring within the bean itself.

Evidence in support of these conclusions is:

(i) When haricot beans browned by processing in tomato sauce were put into water, some leaching of brown pigments from the beans took place, showing that some brown pigments had probably been absorbed on the hydration of the bean.

(ii) Leaching of pigments from the browned beans soaked in water was very slow and so it is evident that most of the pigment must be firmly bound in the bean. This endorses that reducing sugars have been absorbed by the bean and undergone browning with amino groups present in the bean and hence the pigments remain firmly bound in the bean.

Beans heated in water 70 minutes and then tomato sauce (Trial D) would undergo further browning due to reducing sugars being absorbed into the beans by an equilibrium being set up between the liquid in the already fully hydrated beans and the tomato sauce. However, the same extent of browning as for baked beans in tomato sauce (Trial A) is not reached due to the position of the equilibrium and dilution effects.

Conclusions

1. The predominant form of pigment formation in haricot beans on the sterilisation of baked beans in tomato sauce is carbonyl-amino browning.

2. The degree of browning increases progressively with increased processing time.

3. Reducing sugars are absorbed by the beans from the tomato sauce and are available as major browning reactants in the beans.

4. Soluble brown pigments formed by carbonyl-amino browning taking place in the tomato sauce are absorbed by the beans during heat processing.

B. BROWNING OCCURRING IN CREAM STYLE CORN ON HEAT PROCESSING

Non enzymic browning has been a phenomenon associated with the heat processing of cream style corn for many years and on occasions has lead to economic loss. It would be very difficult to eliminate this brown pigment formation completely and so commercially, conditions are kept such that only minimal browning takes place. New techniques, e.g. the Cremogevac Process, have been introduced to reduce the extent of browning taking place.

The purpose of this investigation was to develop a suitable technique for measuring the extent of browning and to determine the relationship between pigment formation and time.

Throughout this study commercial cream style corn which had been frozen immediately prior to filling into cans and heat processing was

used. The corn was donated by a large food processing company.

Development of an analytical technique

The brown pigment formed on heat processing cream style corn is readily discernible by the eye and this suggested that the differentiation in colour could be followed using reflectance techniques. However, on homogenising samples and testing for colour differences with a reflectance spectrophotometer the method proved to be relatively insensitive. Deaeration of the sample prior to testing had little effect.

It has been shown that where a carbonyl group reacts with the amino group of a protein, the pigment is bound chemically to the protein moiety (103). Using solutions of browned sweetened condensed milk, it was observed that by precipitating the protein with chemical agents such as trichloracetic acid, urea, ammonium sulphate, and lead acetate, the brown pigment precipitated with the protein and so concentrated the brown colouration. However, as a technique, this proved unsuccessful in following pigment formation in cream style corn.

Treatment of the cream style corn with protein sequestering agents, e.g. phosphates also proved unsuccessful, as did treatment with chemicals used by other workers, e.g. dilute nitric acid, sodium hydroxide, acetone, alcohol, potassium ferricyanide and combinations of these.

On homogenising a sample of heat processed cream style corn with acetone and then filtering and washing with acetone until the filtrate was colourless, a whitish powder was obtained. On drying and grinding to a fine powder, a faint brown pigmentation was observed but was relatively insensitive to reflectance measurements. The procedure was repeated

using alcohol but there was little improvement in the results.

Concentration of the brown pigmentation could be carried out, not by separation of the brown pigment, but by removal of any extraneous colouration such as carotenoid pigments. On the basis of this, a sample of heat processed cream style corn was homogenised with an equal volume of carbon tetrachloride and centrifuged. It was found that the carbon tetrachloride extracted the carotenoids and this solution collected at the bottom of the centrifuge tube. Immediately above this was a thin layer of corn husks, shells and pieces of unhomogenised kernel. The contents of the upper half of the tube consisted of a uniform slurry, the colour of which varied with the degree of browning. It was found that the brown pigments had been sufficiently concentrated in the slurry to enable sufficiently sensitive reflectance measurements to be recorded. This technique was repeated using petroleum ether to extract the carotenoids and a double extraction using carbon tetrachloride followed by petroleum ether was also tested, but it was found in both cases that a single extraction with carbon tetrachloride gave superior results.

With the development of a suitable analytical technique the relationship between brown pigment formation and time of retorting could be studied.

Experimental method

The frozen commercial cream style corn was thawed, heated to 160°F and seamed by steam flow closing in 211 x 400 Code 3 cans with a net headspace of about 3/16 inch. The cans were then heated for varying times at a retort temperature of 238.0°F. After the desired process

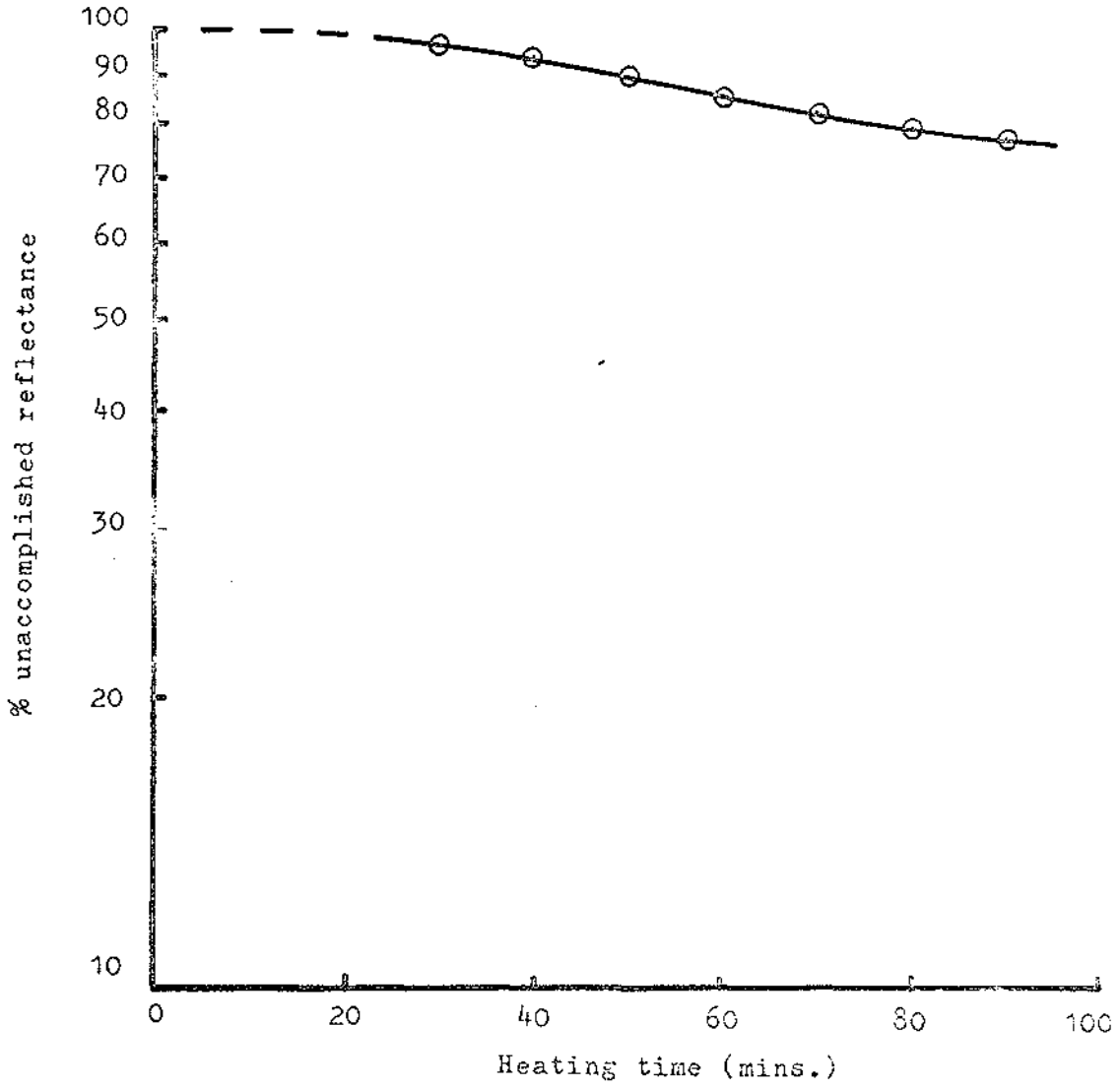


Fig. 14. BROWNING OF CREAM STYLE CORN ON HEATING

time had been reached, the cans were cooled to room temperature and the degree of browning of a sample of the contents determined using a reflectance spectrophotometer (see appendix for details of method).

Results

The results obtained are summarised in Fig.14 as a plot of the logarithm of the percentage of unaccomplished reflectance against time of heating.

Discussion of results

It can be seen from Fig.14 that the curve obtained is sigmoid in form. In the initial stages of the reaction there is a lag period and this is followed by a linear portion which is in turn followed by a tailing off of the reaction rate. This form of curve was obtained in Part "A" Section II, and has also been obtained by other workers (148). Since Fig.14 is similar in form to curves obtained in the model browning system it appears that the analytical procedure devised for following the pigment formation in cream style corn is in fact measuring the desired reaction and is very sensitive. The concentration of reducing sugars in a sample of the cream style corn was determined before processing by Lane and Eynon's method (94) and was found to be 0.25%. Hence, although the general form of the curve conforms to that obtained in a model carbonyl-amino browning system the overall extent of pigment formation is relatively small due to the low initial concentration of reducing sugars present.

From the results, it is evident that the degree of browning taking place in cream style corn on heat sterilisation is a function of the

time of heating and the processing temperature.

C. CARBONYL-AMINO BROWNING IN HEAT PROCESSED CANNED FISH

(a brief review of the formation of browning reactants and factors affecting the reaction followed by a study extending the factors influencing the reaction, and a discussion on the browning reaction in fish, resulting from typical commercial heat treatments)

Carbonyl-amino browning has been recognised as occurring in heat processed canned fish for a number of years, and a large amount of research has been done on the reactants involved and factors affecting the rate of the reaction.

The carbonyl-amino browning occurring in fish is an excellent and unique example of this chemical system in a food as the predominant browning reactants are formed after the death of the fish due to autolytic changes occurring in the flesh and the degree of browning can be related to the extent to which autolysis has proceeded.

1. Post-mortem formation of browning reactants

Since the early studies, there have been further investigations concerning glycogen in fish muscles and its post-mortem degradation, and these have been reviewed by Tomlinson and Geiger (135) and by Partmann (91).

There has been a tendency to assume that glycogen content is considerably lower in fish muscles than in mammalian muscles. However, as Tomlinson and Geiger (135) have pointed out, many species of fish have a muscle glycogen content which compares favourably with that of warm-blooded mammals. Since the procedures used to capture fish almost invariably involve excessive struggling, the glycogen content of the flesh of marketed fish is usually very low. However, the products of

post-mortem degradation of glycogen are present and undoubtedly contribute to both the flavour and texture of fish. Glycogen may be broken down post-mortem by two routes, discussed below.

It has long been known that lactic acid accumulates in muscle of living fish as a result of exercise or struggling and may also increase after death. Several investigators have observed that there is not always a parallel between glycogen disappearance and lactic acid formation. However, there is now abundant evidence that lactic acid is formed in fish muscles by the same sequence of enzymes that is operative in mammalian muscles - the Embden-Meyerhof (glycolytic) pathway. On the other hand, glycogen is also broken down by an amylolytic route.

Glucose occurs in quite variable amounts in fish muscles. Glucose 6-phosphate is hydrolyzed to glucose only very slowly in fish muscles after death, and available evidence indicates that glucose arises largely or entirely by hydrolysis of glycogen. Ghanekar, et al. (36) first suggested that glucose was formed in fish muscles post-mortem by direct hydrolysis of glycogen.

During studies of sugars and sugar phosphates in fish muscles, Jones (60), Burt and Jones (18), and Tarr and Leroux (132) observed maltose and dextrins in addition to glucose. Thus, the latter investigators observed that radioactive glycogen when introduced into ground fish muscle post-mortem yielded radioactive glucose, maltose (identified only by its R_f value) and what was presumably dextrin(s).

Burt (19) has studied some of the glycogenolytic enzymes of cod muscle, and concluded that the amylolytic route accounted for the greater

proportion of glycogen degradation post-mortem. This view is also held by other investigators (84, 133).

The presence of hexose and pentose phosphate esters in fish muscles was first recorded some eighteen years ago (128), and several years elapsed before active studies on the occurrence of these compounds were resumed. The more recent work has been greatly facilitated by better methods of separation and identification. Burt and Jones (18), using ion-exchange chromatography, identified a number of sugar phosphates in cod muscle including glucose 1-phosphate, glucose 6-phosphate, fructose 1-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, ribose 1-phosphate, and ribose 5-phosphate. They found that the total concentration of hexose phosphates was about 220 μ moles per 100 g in fresh-trawled cod muscle, and about double this value in rested cod muscle. These values were of the same order as those recorded for several species of fish previously (128). In general the amounts of hexose phosphates decreased post-mortem, though the concentrations of the pentose phosphates increased for several days and subsequently decreased. Tarr and Leroux (132) studied acid-soluble phosphorous compounds and free sugars in muscles of several fish species using a combination of radioactive tracer techniques and a sensitive ion-exchange chromatographic method of separation. The results with salmon species, cod and halibut indicated that extreme quantitative variations can be expected in the hexose phosphates. Glucose 6-phosphate usually predominated, and fructose 6-phosphate and fructose 1,6-diphosphate were present in lower concentrations. Examination of fructose 6-phosphate fractions indicated that if fructose 1-phosphate were present, it accounted for less than 5% of the total

fructose monophosphate fraction. It was concluded that it was almost certainly absent from the fish examined. Ribose 5-phosphate was rarely found, and ribose 1-phosphate did not occur in any fish muscle studied. Since fish muscles usually possess a fairly strong phosphoribomutase enzyme which promotes a reaction which is about 90-95% in favour of ribose 5-phosphate, it is not surprising that ribose 1-phosphate is rarely found.

Work carried out some 18 years ago with a barium salt-alcohol fractionation procedure showed that muscles of several species of fish examined contained adenine nucleotides in concentrations rather similar to those occurring in rat muscle. Subsequent research by several investigators showed that the ATP content of rested fish muscles averages about 500-800 μ moles per 100 g of muscle (63). Except in unusual circumstances ATP is rapidly degraded post-mortem by a series of enzyme reactions which give in turn ADP, AMP, IMP, inosine, and finally hypoxanthine plus ribose or ribose 1-phosphate. As mentioned above, any ribose 1-phosphate formed is generally converted to ribose 5-phosphate (61,91,135). The enzymes responsible have been studied to some extent. It appears that hydrolysis to the stage of inosine monophosphate (IMP) is quite rapid, and that the comparative rate of hydrolysis of IMP to inosine is slower. Thus, IMP tends to accumulate in fish muscles. The inosine that is formed is split by one or both of two different enzymes, a nucleoside hydrolase or a nucleoside phosphorylase. The comparative activities of these two enzymes post-mortem has never been accurately assessed for any fish muscle. The fact that free ribose occurs much more frequently and in much higher concentrations than does

pentose phosphate in fish muscles post-mortem, would indicate that the hydrolytic mechanism is much more active. In addition to ATP and related nucleotides, fish muscles contain di- and triphosphopyridine nucleotides. These occur in comparatively small concentrations, however, and are probably of little technological significance in comparison with other nucleotides. Jones and Murray (63) found rather small concentrations of a number of other nucleotides in rested cod muscles.

Fish muscles also contain ribonucleic acid (RNA) (42-142 mg/100 g) and deoxyribonucleic acid in very low concentrations (0.2-2.5 mg/100 g).

At one time the possibility that RNA and ATP might both be precursors of free ribose in fish muscles was considered. However, experiments indicated that indigenous or added RNA was not degraded appreciably in lingcod muscle held at 0°C (134). The possibility cannot be entirely discounted that RNA is degraded post-mortem to yield nucleotides and free ribose in other species of fish. It is now believed that the free ribose in fish muscles arises largely from post-mortem degradation of ATP. Thus, when generally labelled (^{14}C) ATP was introduced into fish muscle, both radioactive inosinic acid and ribose were isolated after it was held 2 days at 0°C. Since uniformly labelled glucose did not cause formation of radioactive ribose 5-phosphate or ribose under conditions, it was inferred that ribose does not arise from glucose by post-mortem operation of the hexosemonophosphate shunt pathway (132). Very small amounts of IMP and ribose may arise through degradation of nicotinamide adenine dinucleotide (67). The general course of ATP breakdown in fish muscles post-mortem has therefore been established, and many of the enzymes concerned identified. It was originally stated

that inosine triphosphate and inosine diphosphate might be intermediates in formation of ribose and hypoxanthine from ATP, but this has not been borne out by subsequent investigations.

2. Factors affecting carbonyl-amino browning in heat processed fish

In order to correlate the extent of carbonyl-amino browning in heat sterilised fish with the time of heating at a known processing temperature, it is necessary to know what factors influence the rate of the reaction. These factors and their effects are now outlined.

(a) Freshness of the raw material

From the preceding account of post-mortem changes occurring in fish muscle it is evident that freshness of the fish is going to play a major part in determining the extent of subsequent browning on heat processing. The period of holding prior to processing is going to influence the ultimate pH of the flesh and the concentration of browning reactants and both these factors markedly affect browning (see Part "A" Section I, 'Factors affecting the rate of browning'). Ono and Nagayama (88) showed the degree of browning grew in parallel with the length of storage of fish.

(b) pH of the flesh

The accumulation of lactic acid in fish muscle after death is of considerable importance as it is almost certainly the principal factor in determining post-mortem muscle acidity. While in livestock the post-mortem lactic acid concentration may be controlled to a significant extent by feeding and by slaughtering techniques, this is obviously difficult or impossible with fish. Certain fish

such as tuna and halibut may exhibit high lactic acid concentration post-mortem and correspondingly low pH values, while in Atlantic cod the pH of the muscle may be as high as 6.8 or 7.0. The carbonyl-amino browning reaction is accelerated by increasing pH and this has been demonstrated in fish (118,129). Also magnesium ammonium phosphate (struvite) formation in canned fish occurs more frequently if the muscle is above pH6.

Low pH values, while reducing the rate of browning, also cause muscle proteins to approach their isolectric zones and consequently they tend to lose their water holding ability. This generally results in a loss of free liquid (drip) on thawing frozen fish and this would help reduce browning by removal of soluble reactants. Also, low pH values tend to inhibit bacterial spoilage of fish and this reduces ultimate colour by limiting further formation of browning reactants.

(c) Concentration and nature of the carbonyl moiety

Figures illustrating the typical changes taking place in potential browning reactants on chill storage of cod muscle are shown by Jones (62). Although variations are present between different varieties of fish, the overall trend is similar. Due to the variability in the concentration of hexose phosphate in fish flesh, it is difficult to assess the importance of these in browning reactions. Addition of substances possessing a free aldehyde group, for example, hexose phosphates, reducing sugars, aldehydes, reductones, etc., to leached fish flesh that was subsequently heated caused browning (129). Ribose 5-phosphate was

shown to be a little more reactive than glucose in causing browning (138) while ribose is about five times as reactive as glucose (131). Certain of the hexose phosphates could be significant in causing carbonyl-amino browning in heated fish flesh but pentose phosphates usually occur in very low concentrations and so are probably of little importance.

The ribose and glucose concentrations present in a large number of fish have been determined (130) and the browning potential of a variety of reducing sugars, organic reducing substances and amino acids in fish flesh (leached ling cod) has been studied (129). Quite large amounts of glucose were found in muscles of "fresh" fish of different post-mortem age, while others contained negligible amounts. Thus, several fish examined had between 100 and 400 μ moles of glucose per gm of muscle (131).

An approximate relationship between the ribose content of fish muscle and the degree of browning that occurs on heating has been demonstrated by a number of workers (88,130). However, Nagayama (83) found no linear correlation between browning of heated fish flesh and ribose or glucose content. He believed that concentrations of amino compounds were important and agreed that the browning was caused by sugar-amino reactions.

Removal of free sugars from fish flesh has been found to be quite effective in decreasing browning. Tarr (129) showed that the browning that usually occurred on heating muscle of white fleshed fish 1 hour at 120^{o}C was largely eliminated by prior leaching of the flesh in water.

(d) Nature of the amine moiety

Fish flesh contains a high concentration of protein which can readily enter into carbonyl-amino reactions. Jones (58) has presented evidence that free amino acids in fish flesh are also very important adjuncts to this reaction and that significant amounts of α methyl histidine and β alanine are liberated in codling skeletal muscle post-mortem at $0^{\circ}C$ by the enzyme "anserinase".

(e) Variety of fish

The amounts of potential browning reactants have been shown to vary in concentration depending on the variety of fish. Free ribose concentrations in various fish have been determined by a number of workers (88,130) and Tarr (131) has also found the per cent glucose present.

(f) Time and temperature of heating

The reaction rate of browning is enhanced by increased temperature. Ono and Nagayama (88) have shown the effect of temperature on colour and sugar content of flounder flesh with and without added ribose. With increased temperature there was increased browning and this was enhanced when ribose was added. Colour development was correlated with reducing sugar loss. Spilde (118) has presented graphs showing the browning of fish paste as a function of temperature and time at various pH values.

(g) Presence of vacuum

Tarr (129) has shown that the presence of vacuum may slightly increase the degree of browning but that this may be dependent of the species of fish.

(h) Effect of metal ions

Levels of 5 ppm of ferrous, ferric and copper ions in fish flesh have been shown to slightly decrease the extent of browning while aluminium, cobalt, nickel, silver, tin, and zinc ions had no effect (129).

(i) Season of year

Jones and Murray (61) compared the course of catabolism in fish of poor nutritional condition and about to spawn with that of fish of good nutritional condition caught at a different season. They found that after 3 days in ice, levels of AMP, ADP, ATP and NAD remained roughly constant in the poor condition fish whereas in the fish caught at other seasons, a slight rise in ADP and loss of ATP, and AMP was found.

(j) Added ingredients

i. Tomato sauce

Fish are frequently heat processed in the presence of tomato sauce and this could increase the degree of browning as in the case of baked beans in tomato sauce (see Part "A" Section III) due to selective absorption of the reducing sugars in the sauce by the fish, so enabling these to enter into browning reactions with amino compounds in the flesh.

ii. Brine

It would be expected that fish heat processed in the presence of brine would undergo less browning due to leaching of potential browning reactants and brown pigments, from the flesh.

iii. Oil

It has been shown that under oxidising conditions, reactive carbonyl groups formed in the lipid moiety can react with amine groups to initiate browning reactions (26).

(k) Uniformity of sample for analysis

Most species of fish, contain a region down each side of the body of highly pigmented flesh called "blood tissue". Just as the factors discussed above would affect the concentration of reactants and rate of the reaction, so the incorporation of blood tissue in a sample would affect the final estimate of the degree of browning. An investigation was carried out to demonstrate the effect of blood tissue on the estimation of browning in a sample.

Experimental method

A preliminary investigation was carried out to establish a method of obtaining a uniform sample of blood tissue. The following techniques were studied.

1. Skin fillet of fish, cut away blood tissue, and heat this in steam at atmospheric pressure for 5 minutes. Mince blood tissue to give uniform sample.

2. Heat fillet in atmospheric steam for 10 minutes, cut away blood tissue and mince it.

3. Skin fillet, cut away blood tissue and heat it in steam at atmospheric pressure for 5 minutes. Remince tissue to give uniform sample.

It was found method 2 was the most convenient technique for removing blood tissue and gave the most uniform samples.

To establish the effect of blood tissue on the estimation of browning fillets of trevalli with the blood tissue removed as above, were packed

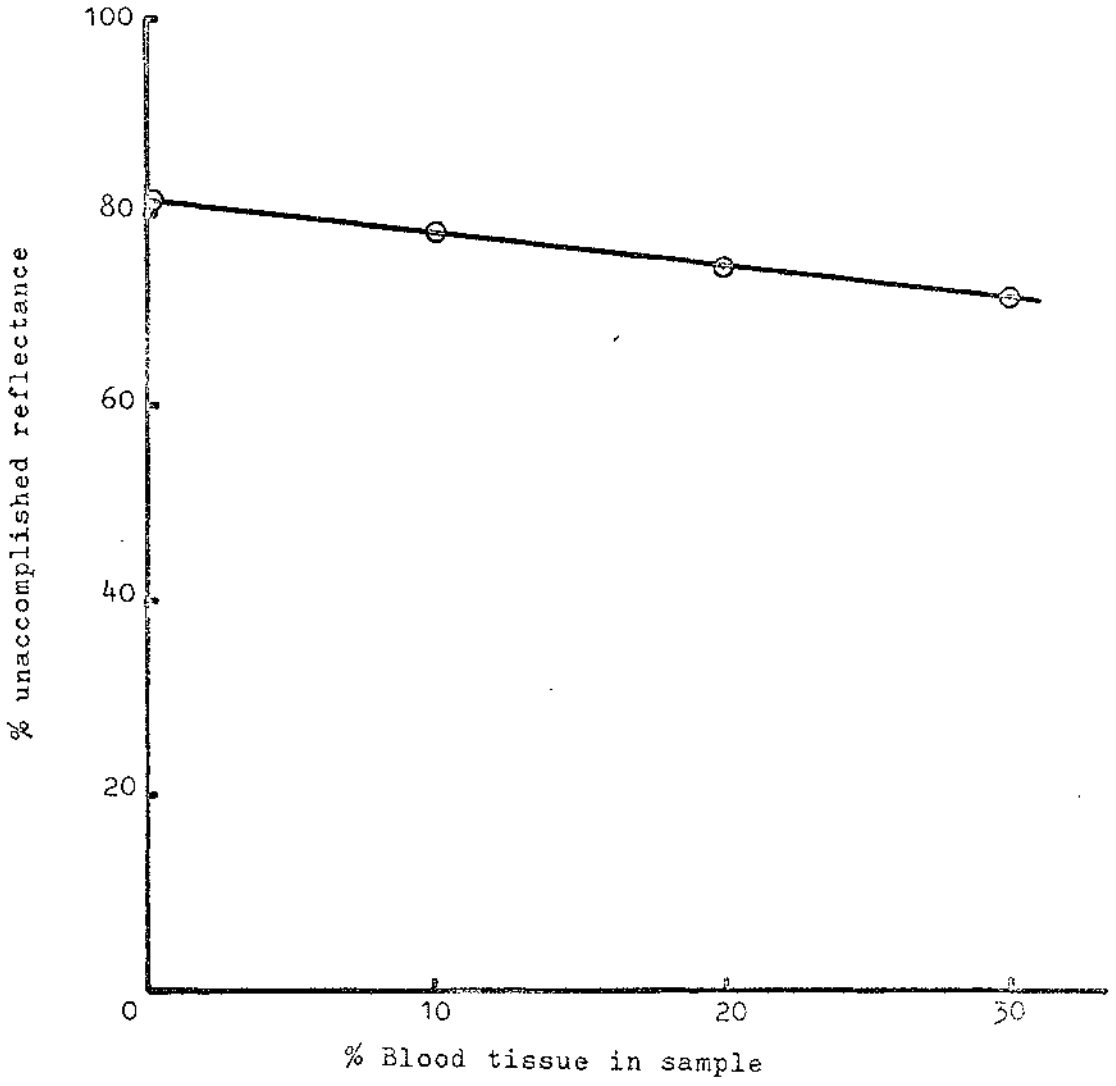


Fig. 15. THE EFFECT OF BLOOD TISSUE ON BROWNING INDEX

into Code 3 cans, seamed under vacuum and heat processed for 75 minutes at 238.0°F. After cooling, a sample in the form of a plug was removed from the centre of each can and to these were added 10%, 20% and 30% blood tissue prepared by method 2 above, and heat processed in an identical manner to the fillets. The heated fillet plus blood tissue samples were then homogenised with a solution of trichloracetic acid and their colour determined by reflectance spectrophotometry (see appendix for details of method).

Results

The results obtained are summarised in Fig.15 as a plot of percentage blood tissue in sample against percentage unaccomplished reflectance.

Discussion of results

The results obtained indicate that the presence of any blood tissue in a sample could markedly affect the estimation of the degree of browning taking place on heat processing. Where no blood tissue was incorporated in the sample a reflectance value of 82 was obtained. This value would represent the colour change due to carbonyl-amino browning in the sample after heating for 75 minutes at 238°F as the reflectance spectrophotometer had been standardised against the reflectance of a sample of fish flesh which had been homogenised after heating in atmospheric steam for 10 minutes. (This small amount of heat was sufficient to denature the protein but did not cause significant browning and hence the flesh was suitable as a control).

Conclusions

There are a large number of factors which can affect the degree of carbonyl-amine browning taking place in heat processed fish and many of these would be very difficult to control. It has been shown that the degree of browning is dependent on time of heating and processing temperature and consistent results should be able to be obtained if all other influencing factors are kept constant.

Results obtained by Farr (130) indicate that many species of fish undergo extensive browning under conditions of a typical commercial heat treatment and so the following of this reaction quantitatively should be relatively simple.

D. SUMMARY

In the products selected, i.e. baked beans in tomato sauce, cream style corn, and canned fish, it has been shown that the degree of carbonyl-amine browning occurring on heat sterilising can be related to the time of heating at retort temperature. This correlation could be applied to other products which exhibit colour changes of this type on heat processing, e.g. various tomato products such as spaghetti; fish in tomato sauce; pet foods; and many fish products.

On heat sterilising baked beans in tomato sauce, the beans themselves have an inherent potential to brown but the extent of this is limited by their low concentration of carbonyl reactants. It was found that during processing the beans absorb reducing sugars from the added tomato sauce and this increases their browning potential.

The rate of carbonyl-amino browning occurring in heat processed fish can be related to the autolytic changes which have taken place in the raw material and so any factor which influences these changes will influence the degree of browning in the product. It would be very difficult to keep the extent of the autolytic changes constant and so, although the degree of browning occurring in a sample of heat sterilised fish can be related to processing time and temperature little significance could be attached to variability between results unless treatment of the raw material had been carefully controlled.

It has been shown (130) that the extent of browning occurring in some species of fish on undergoing a typical commercial heat treatment is quite marked and so a correlation of carbonyl-amino browning in heated fish with time of heating is feasible if other factors influencing the reaction are not dismissed.

S E C T I O N IV

DEVELOPMENT OF A METHOD BASED ON CARBONYL-AMINO BROWNING FOR USE AS AN INDEX OF HEAT STERILISATION IN COMMERCIAL PRACTICE

Kinetic studies have shown that carbonyl-amino browning does not conform to a simple order reaction except at the initial stages during which the reaction is still colourless and no pH drop is detectable (4). At later stages it has been shown that the depth of colour increases with the square of the time (43) but this relation was not generally obtained in the present study. Indeed, it was found, along with other workers (148), that during the initial stages of the browning reaction there was a lag period, followed by a period in which browning increased logarithmically with time, and finally a "tailing off" of the reaction rate with time.

Obviously the rate of browning is also going to depend on such factors as pH, reactivity of the carbonyl and amine moieties, presence of oxygen, metals, and phosphates, and moisture content but provided these factors are kept constant then the degree of browning will be dependent on time and temperature.

It has been shown (see Introduction) that the thermal destruction of bacterial spores is also dependent on time and temperature and hence it should be possible to use carbonyl-amino browning as an index of heat sterilisation.

The carbonyl-amino browning occurring in a model system in a tracer can or brown pigment formation resulting from the sterilising of a food

could both be used as an index of the heat lethality of a process and are considered in more detail below.

A. CORRELATION OF HEAT LETHALITY WITH CARBONYL-AMINO BROWNING OCCURRING IN A MODEL SYSTEM

Carbonyl-amino browning occurring in a model system is a reaction which can be studied quantitatively and this makes it ideal as a system for correlating with heat lethality. With respect to its practical application, a model browning system could be placed in a "tracer can" alongside a product during processing and the amount of effective heat received by the product could be determined from the degree of browning taking place in the tracer can. The tracer can technique has obvious advantages in that correlation should be possible with a large number of processes for different products.

The glucose-glycine carbonyl-amino model browning system has been studied extensively under conditions typically encountered during the heat sterilisation of canned foods and the quantitative effects of factors affecting this reaction have been investigated. In order to correlate the degree of browning in this system with time at a standard temperature (250°F) it is necessary to keep all other factors which can affect the reaction constant. The previous investigation on a glucose-glycine model browning system established the effect of metals, concentration of reactants, oxygen, sampling technique, temperature and pH on the reaction and from this it was found that the optimum conditions to be maintained constant were:

1. Use a Code 3 lacquered can.

2. Seam the contents at atmospheric pressure with a net headspace of ½ inch.

3. Prepare samples immediately prior to use and readings should be taken immediately can is opened for analysis.

4. Concentration of reactants.

5. Initial pH of system.

6. Processing temperature.

In order to correlate the degree of browning occurring in a system with heat lethality, it is necessary to determine the amount of effective heat received by the system. In this study this was carried out by inserting a thermocouple into the geometric centre of the system as shown in Fig.33 and then following the temperature change with time using a Cambridge workshop potentiometer (see appendix). The thermocouple wire, of gauge 24, had been calibrated by a previous worker. The lethality of the process was calculated by the General Method, i.e. temperatures attained at the geometric centre of the system were converted to lethality values from tables (15) and these values were then plotted against time. The area of this plot, as determined by planimeter, divided by unit area gave the sterilising value (Fo) of the process.

Using a glucose-glycine carbonyl-amino browning system, the method of temperature measurement in the can outlined above, and the General Method for calculating sterilising values, correlation of the degree of browning with Fo was examined.

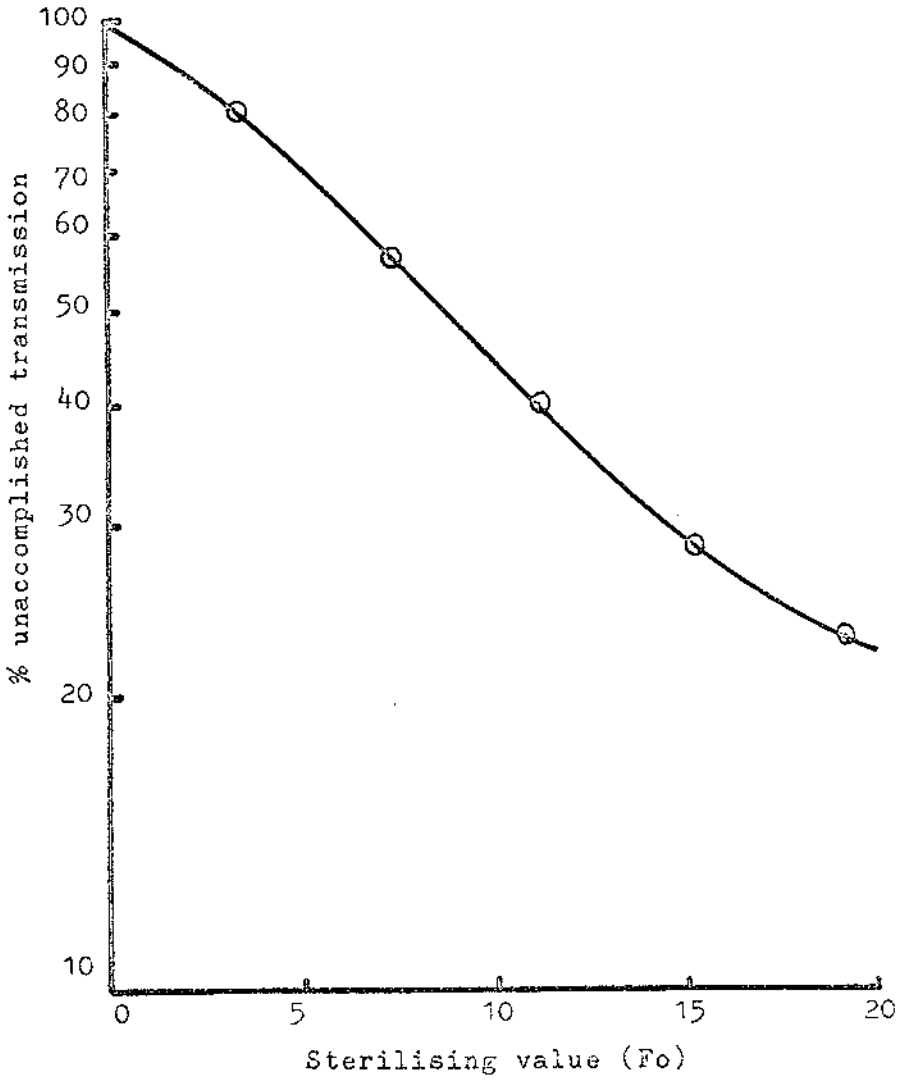


Fig. 16. THE RELATIONSHIP BETWEEN BROWNING OF A GLUCOSE-GLYCINE SOLUTION AND STERILISING VALUE

Experimental Method

211 x 400 Code 3 cans were filled to a net headspace of ½ inch with a 2% glucose-glycine solution prepared with tap water (this gave a constant initial pH of about 7.1) and seamed at atmospheric pressure. The cans had been previously fitted with a thermocouple positioned at their geometric centre. The cans, in turn, were placed in a retort and the thermocouples connected to the potentiometer. They were then heat processed for varying times at 238°F after a standard venting procedure had been carried out. Throughout each run (heating and cooling) the temperatures within the can were recorded at 2 minute intervals. After cooling the degree of browning of a sample of the can contents was determined by the colorimetric method outlined in the appendix. For each run the Fo value of the process was determined by the General Method from the temperature/time values recorded.

Results

The results obtained are summarised in Fig.16 as a plot of the logarithm of the percentage unaccomplished transmission against the sterilising value (Fo).

Discussion of Results

It can be seen that the curve follows the same trend as the plot of the logarithm of the percentage unaccomplished transmission versus time curve (Fig.4), i.e. the curve is sigmoid in form. This is not unexpected when one considers the relationship between Fo and time of processing at retort temperature.

From Fig.16 it is evident that the degree of browning occurring in

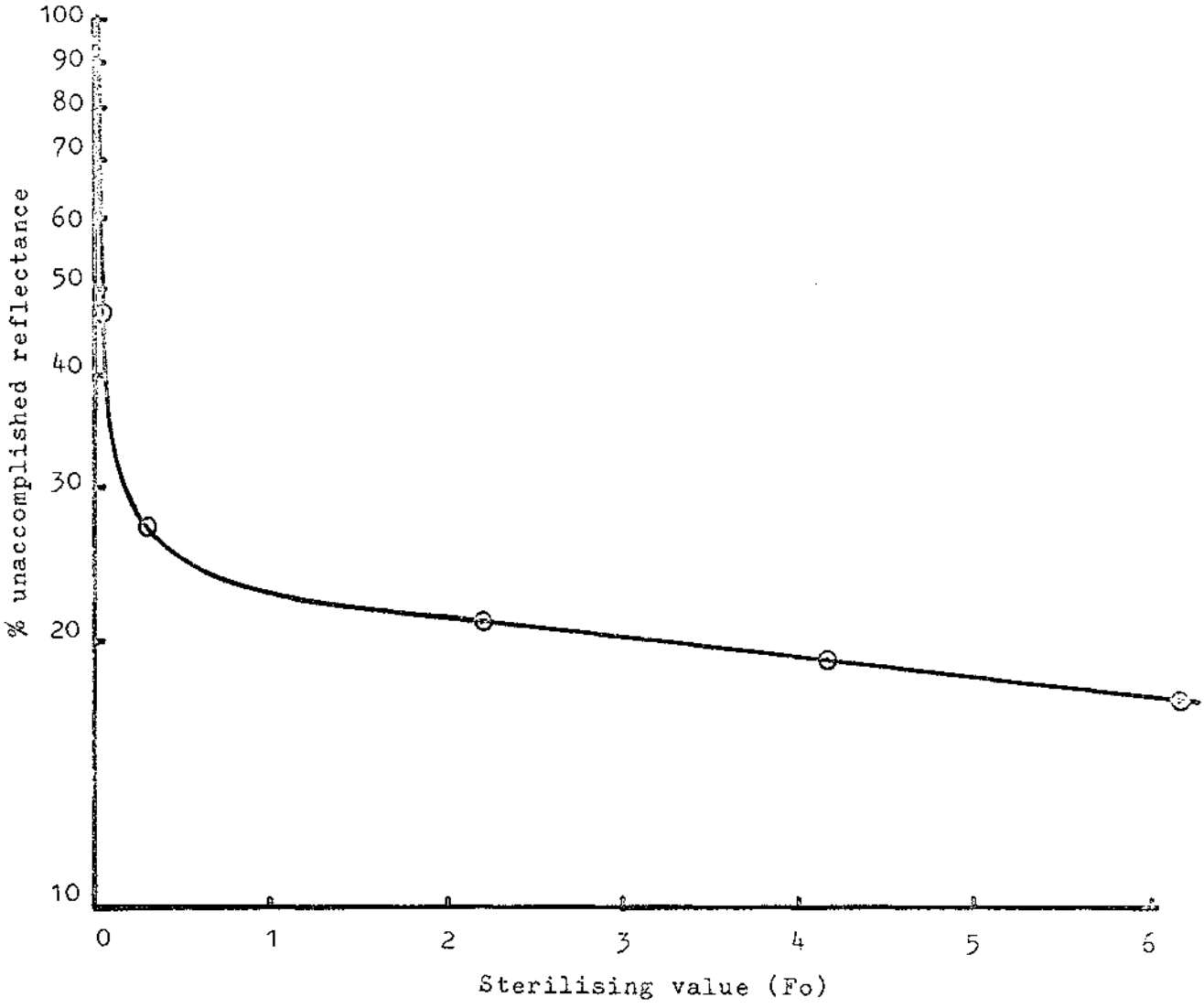


Fig. 17. THE RELATIONSHIP BETWEEN BROWNING OF A SWEETENED CONDENSED MILK SYSTEM AND STERILISING VALUE

a glucose-glycine carbonyl-amino model system can be correlated with the sterilising value of the process and that this technique perhaps offers a feasible method of determining the heat lethality of a process. It is estimated that the error in the Fo value, as determined from the degree of browning in a sample, would be about Fo $\pm$ 1. However, the insignificance of this error is realised later.

Ward (140) studied browning in sweetened condensed milk at various temperatures and was able to plot the logarithm of the percentage unaccomplished change in reflectance against time of heating. He also gave a typical heat penetration plot for the system and so from these results it was possible to calculate sterilising values for various times during the process. Using the results he obtained at 110°C, a graph of the logarithm of the percentage unaccomplished change in reflectance against the sterilising value of the process (Fo) was plotted (Fig.17).

The graph obtained from Ward's results is not sigmoid in form like that obtained using a glucose-glycine model system but portrays an initial rapid rate of colour change which abruptly decreases to give a linear semi-logarithmic plot. The different form of curve to that given by the model system is probably because:

(a) The initial lag stage of the reaction as indicated by the model system is not shown by the sweetened condensed milk system because the method of following colour development is much less sensitive and hence the curve obtained from Ward's results corresponds to the "linear" and "tailing off" portions of the curve given by the model system.

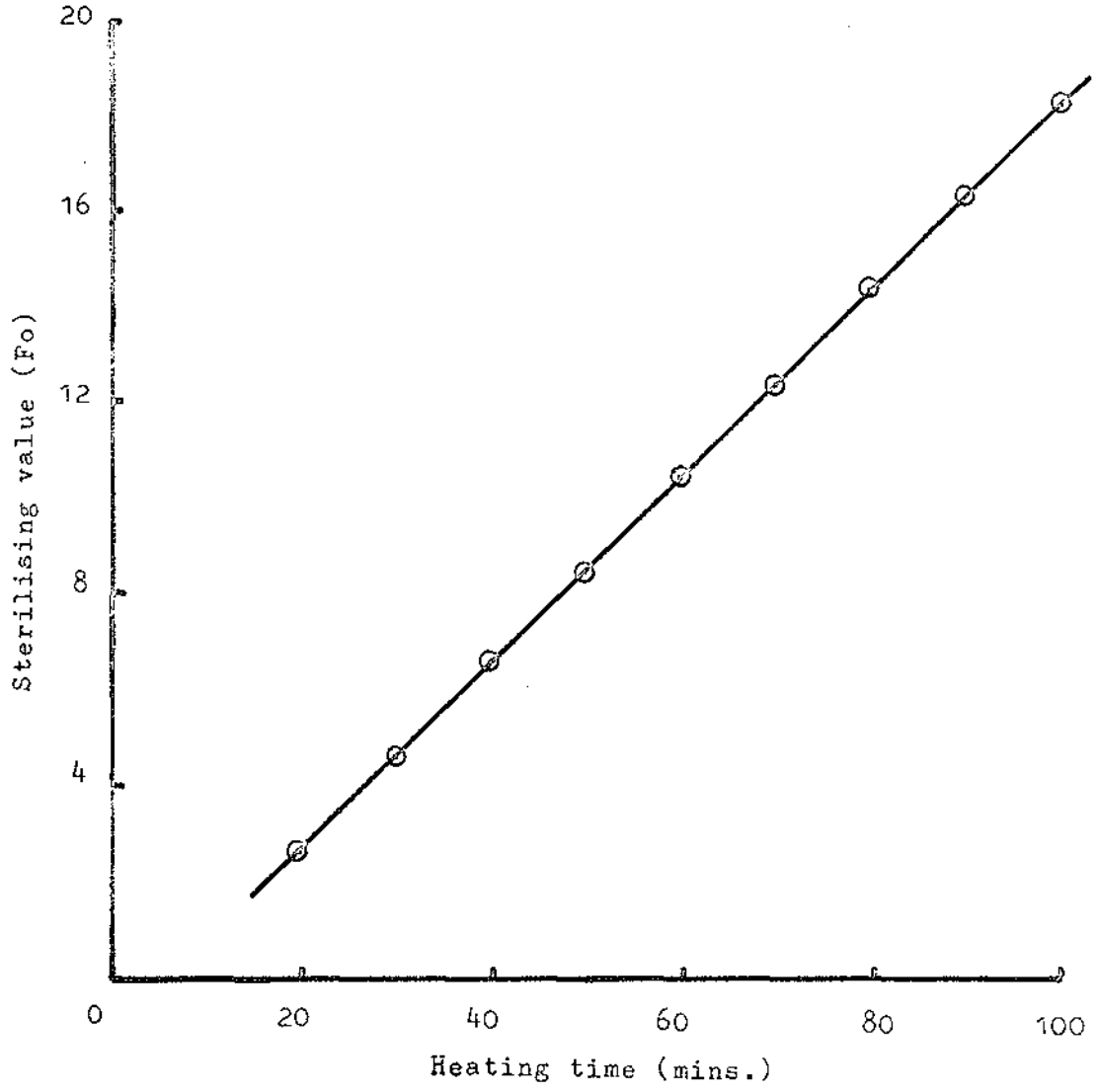


Fig. 18. RELATIONSHIP BETWEEN STERILISING VALUE AND HEATING TIME FOR A CONVECTION TYPE PACK

(b) The sweetened condensed milk system exhibits a broken heating curve and so the rate of heat penetration would be high initially until the pack became of conduction form, after which it would decrease. All of the effective lethal heat is received by this pack when it is in the conduction state and so considerable pigment formation could take place before the heat adsorbed had a significant lethal effect. In contrast a convection system would heat quickly and the lethal effect of the heat would be more marked than the increase in browning.

(c) In natural systems, e.g. sweetened condensed milk, compounds which catalyse the carbonyl-amino browning reaction or other browning mechanisms could be present.

Fig.16 and Fig.17 apply only to the type of pack in which the temperature/time measurements were recorded and hence their application is limited. It is necessary that the concept of correlating browning with heat lethality be extended to apply to packs with all types of heating characteristics.

Extension of the Browning versus Fo Correlation for Practical Application

Using experimental results obtained from glucose/glycine model systems, curves of the logarithm of the percentage unaccomplished transmission against time (Fig.4) and Fo (Fig.16) have been plotted. Since, in both cases, a convection type pack was used, a plot of Fo against time can be drawn (Fig.18) from these two curves. Fig.18 would only apply to a pack of identical heating characteristics to those used experimentally to obtain results to enable this curve to be drawn.

In order to extend the concept of correlation of browning with Fo, it was necessary to prepare packs with heating characteristics ranging from convection through to conduction so that these packs could be used as standards with which to correlate browning and Fo. The heat penetration characteristics of a wide range of systems were studied before packs giving the desired results were found. This involved taking a can fitted with a thermocouple as shown in Fig.33 and filling with the system to be tested. The can was then seamed, placed in a retort, and with the thermocouple connected to the potentiometer, heat processed at 238.0°F after venting. During processing readings of the temperature within the can were taken at known time intervals and this data was used to plot a heat penetration curve. The curve is plotted by taking a sheet of semilogarithmic graph paper and with the logarithmic scale forming the Y axis, turn the paper through 180° and from the top, starting at 1°F below the retort temperature write the temperatures according to the scale. The linear x axis is the time scale. i.e. The curve is obtained by plotting (Retort temperature - temperature of product) versus time on semi-logarithmic paper. The majority of heating curves when plotted on this semi-logarithmic scale will be linear although some will exhibit broken heating curves (see Introduction). In this study only systems giving linear curves were selected.

The slope (f_h) of the linear portion of the heating curve is expressed as the number of minutes required for the curve to transverse one logarithmic cycle. Typical commercial conduction packs, e.g. cream style corn and baked beans in 211 x 400 cans have an f_h of about 40 minutes while a convection pack, e.g. peas would have an f_h of 2 - 3.

f_h is obviously dependent on rate of heat penetration and so is affected by such factors as consistency of pack, size of can, and retort temperature and equations have been formulated to enable f_h values to be determined for different conditions (38).

The systems with linear heat penetration curves chosen to represent packs with various heating characteristics were:

1. Convection, $f_h = 2 \pm 0.5$

   A 301 x 409 can (16 oz) filled with water to 3/16 inch net headspace and seamed at atmospheric pressure.

2. Semi convection $f_h = 9 \pm 1$

   A 211 x 400 can was filled with 107 gm water plus 198 gm of 200 lb breaking strain nylon fishing line cut into lengths of about $\frac{1}{2}$ inch. This gave a net headspace of about 3/16 inch. In this pack there was insufficient water for heating to be by true convection and too much water for heating to be by conduction alone. The nylon, cut into small pieces, was suitable because it packed uniformly into the can, it had a similar density to water, and was heat stable and non hygroscopic.

3. Semi conduction $f_h = 26 \pm 1$

   A 211 x 301 can (8 oz) was filled with 986 gm of $\frac{3}{8}$ inch steel ball bearings and seamed under a vacuum of about 22" Hg. The absence of air in the can would ensure more ideal conduction conditions and the relatively high rate of heat conduction by steel gives this pack a comparatively low f_h value.

4. Conduction $f_h = 33 \pm 1$

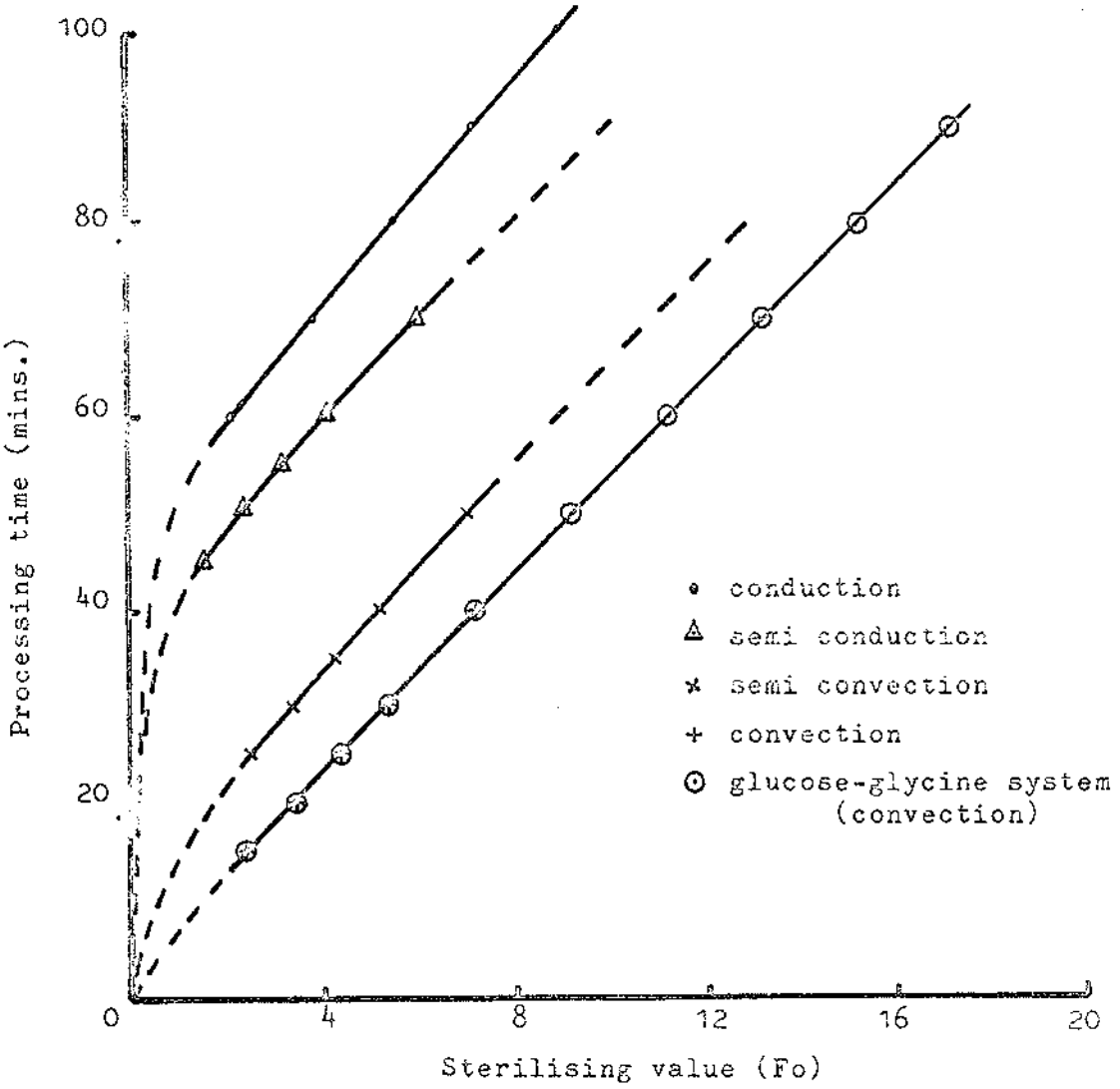


Fig. 19. RELATIONSHIP BETWEEN PROCESSING TIME AND STERILISING VALUE FOR PACKS WITH DIFFERENT HEATING CHARACTERISTICS

A 211 x 400 can was filled with 500 gm of 1/5 inch diameter glass balls and seamed under a vacuum of about 22" Hg. The lower rate of heat conduction by glass gives this pack a higher f_h than that of steel above.

It should be pointed out that only f_h values suitable for correlation with the carbonyl-amino system chosen for this study were selected and that some commercial packs have f_h values considerably greater in magnitude than those considered here.

The systems selected with the different heating characteristics were fitted with thermocouples and in turn these packs were retorted at 238°F for various times and during the heat processing temperature - time measurements were recorded. From these results, Fo values were calculated for the times at which the packs were processed and this enabled a plot of processing time versus Fo to be drawn for the different packs (Fig.19).

Commercial packs of neutral or low acid foods are generally processed to give a heat lethality of about Fo = 6 and so in this study processing times were used to give, for the various systems selected, Fo values ranging from about 3 to 7 to enable underprocessing (and overprocessing) to be detected on subsequent correlation with browning.

In the time intervals for which these packs were studied, the contents of the slower heating systems had not reached retort temperature and so their processing time versus Fo curves were not straight. However when the contents of all the systems reach retort temperature then their Fo's will increase constantly with time of heating and so the curves of processing time versus Fo will be straight and parallel.

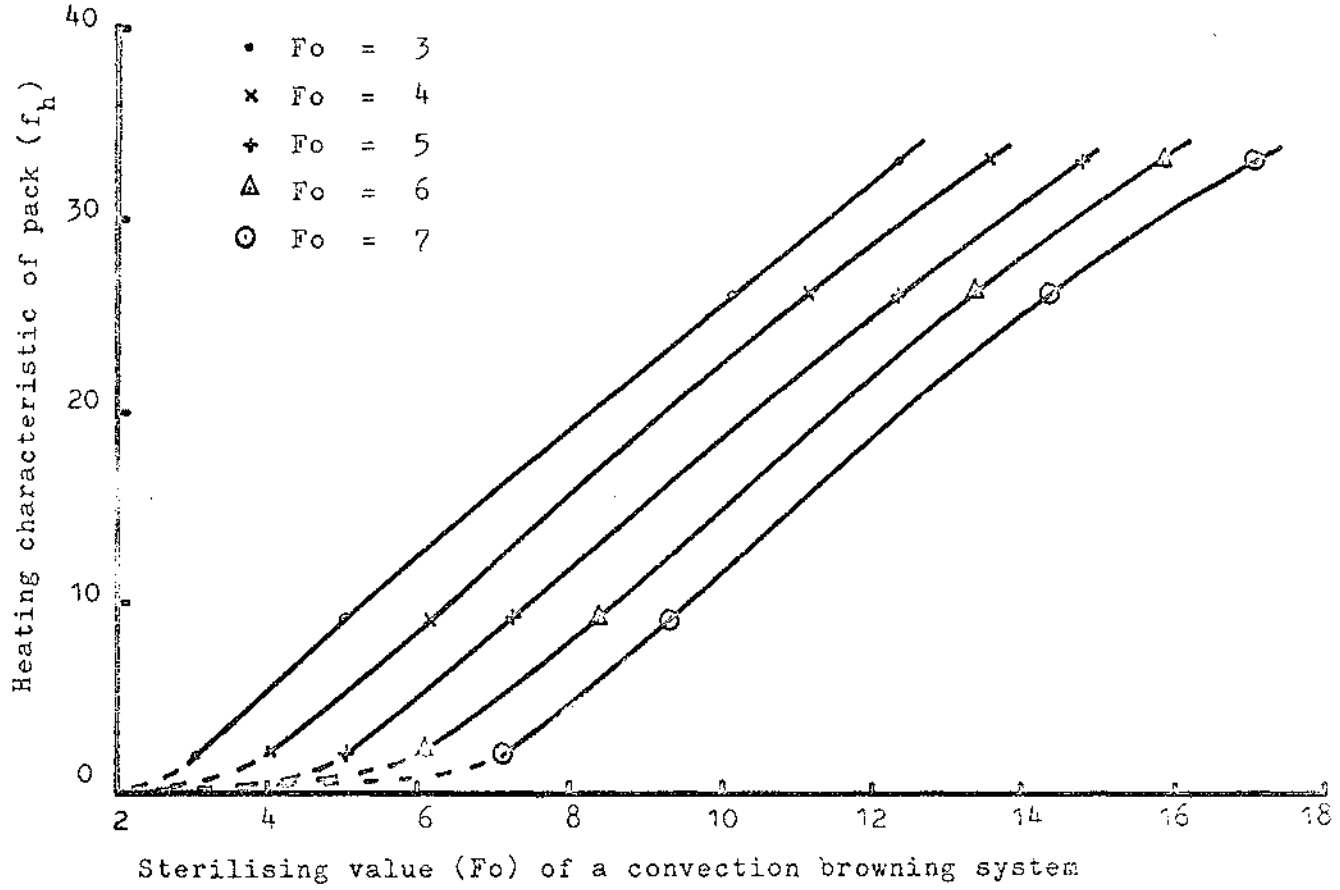


Fig. 20. RELATIONSHIP BETWEEN STERILISING VALUES OF DIFFERENT PACKS WITH THE STERILISING VALUE OF A CONVECTION BROWNING SYSTEM

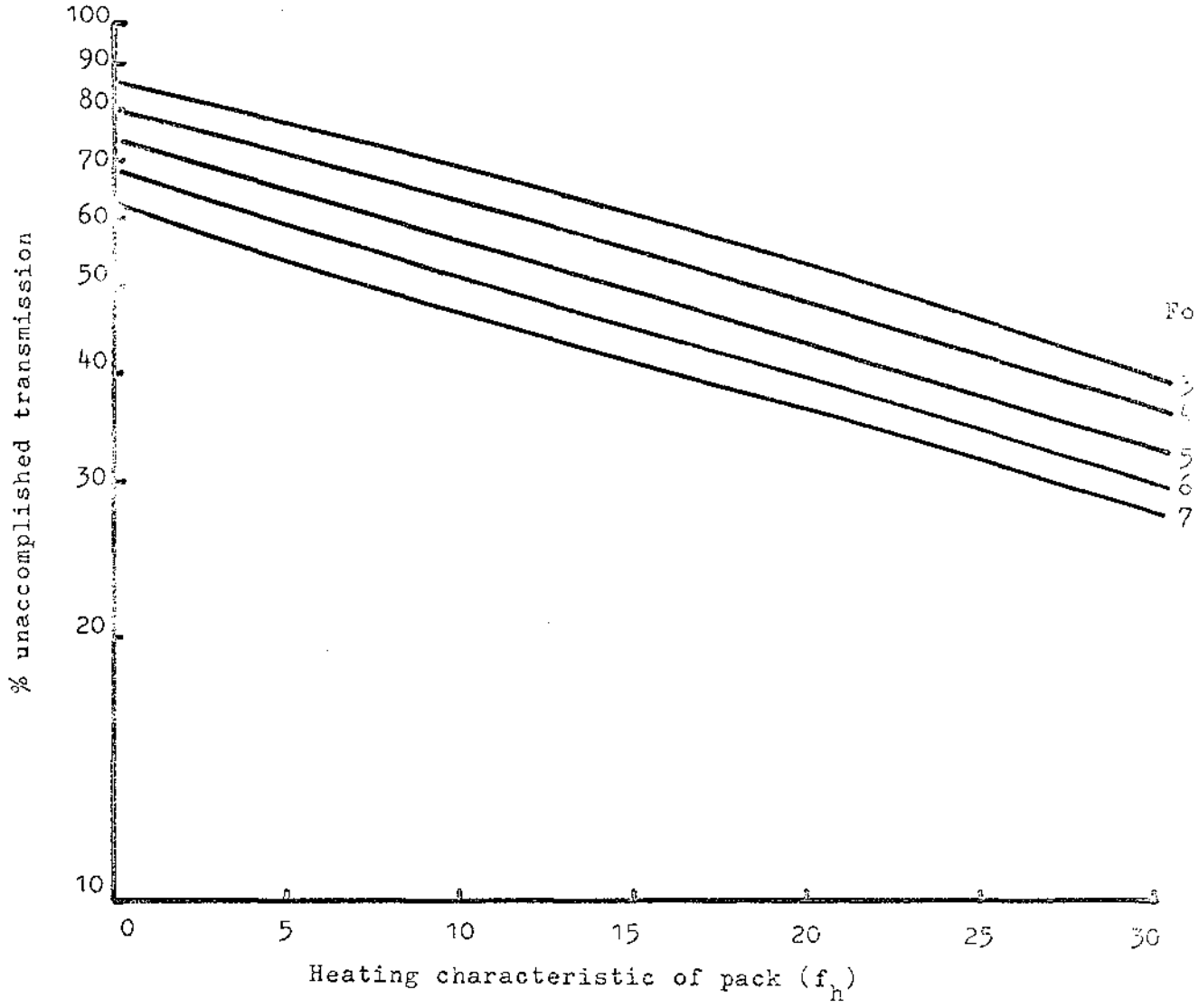


Fig. 21. RELATIONSHIP BETWEEN BROWNING AND STERILISING VALUES OF PACKS WITH DIFFERENT HEATING CHARACTERISTICS

The processing time versus Fo curve for a 211 x 400 can containing a 2% carbonyl-amino convection system is also shown in Fig.19 and in this study all correlations of browning and Fo are done with respect to this pack. Hence from Fig.19 if Fo values from 3 to 7 are considered for the various packs selected with different f_h values and related to the Fo value of the standard 211 x 400 browning system, then Fig.20 can be plotted. Fig.20 is a plot of the f_h of the pack against Fo of the standard convection browning system. From Fig.20 for any pack which has a linear heat penetration curve and an f_h of less than about 40, then its Fo can be determined between the values of Fo = 3 to 7 if the Fo of a 211 x 400 carbonyl-amino convection browning system under identical process conditions is known. However, it is known from Fig.16 that the percentage unaccomplished browning can be related to the Fo of the carbonyl-amino convection system, i.e. from the percentage unaccomplished browning which results from heat processing the heat lethality of the process can be determined.

Hence from Fig.16 and Fig.20 a plot of the percentage unaccomplished browning against the f_h of the pack for Fo's ranging from 3 to 7 can be drawn (Fig.21).

It is estimated that the accuracy of the Fo values obtained from the curves is about $\pm$ 0.5.

From Fig.21 for any pack which has a linear heat penetration curve and an f_h of less than 40, then the Fo of the process between the values of Fo = 3 to Fo = 7 can be determined from the per cent unaccomplished browning taking place in a 2% glucose-glycine solution in a 211 x 400 Code 3 can (and in accordance with other conditions outlined previously)

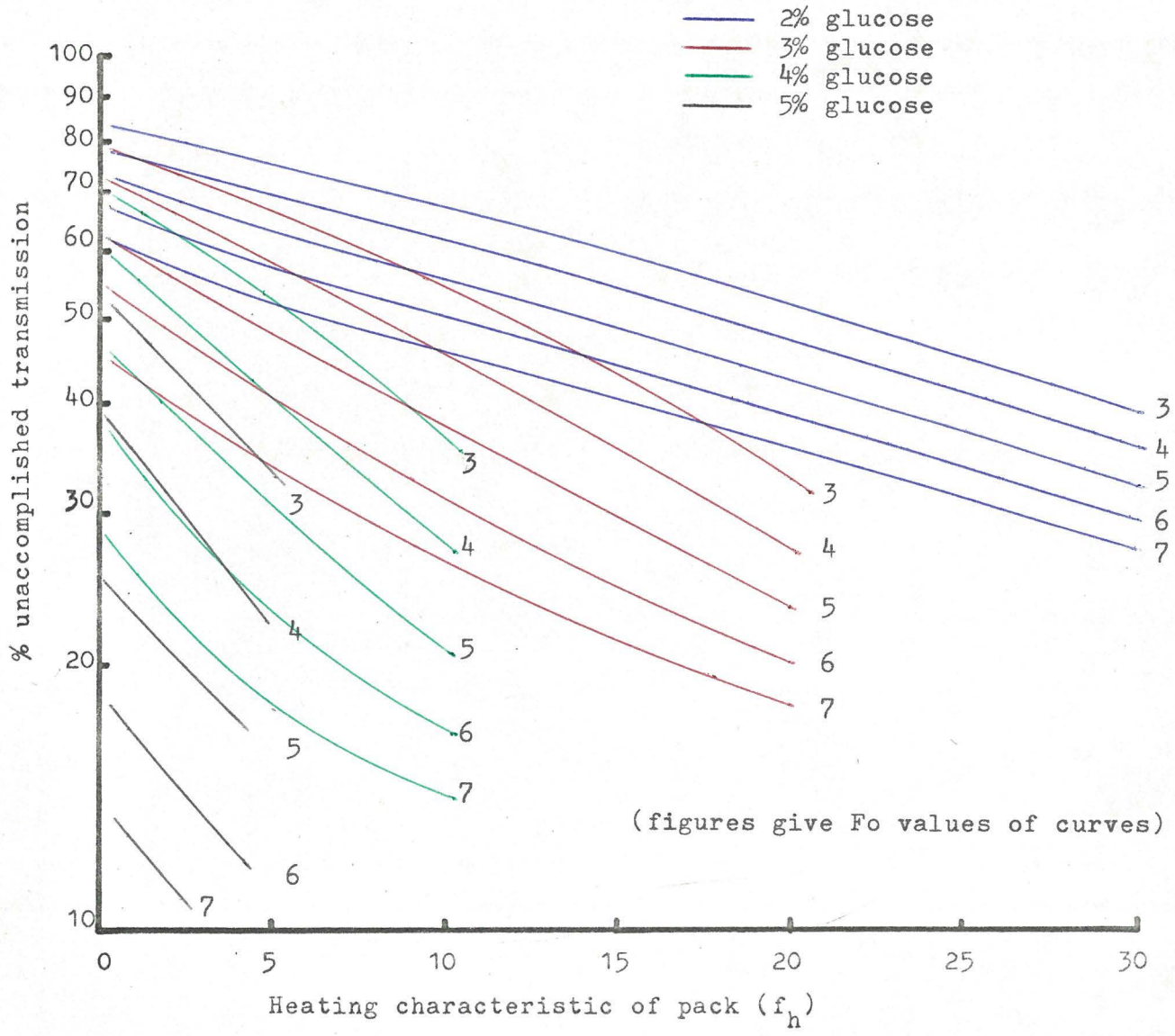


Fig. 22. USE OF REACTANT CONCENTRATION TO INCREASE SENSITIVITY OF RELATIONSHIP BETWEEN BROWNING AND STERILISING VALUE FOR DIFFERENT PACKS

subjected to an identical heat process, i.e. placed in the same retort and hence present as a tracer can.

Fig.21 is relatively insensitive for estimating the Fo's of products which are sterilised quickly, e.g. convection packs, as these packs have low f_h values and only a small degree of browning has taken place in a tracer can containing a 2% glucose-glycine solution before a sufficiently high heat lethality is reached. However, it can be seen from Fig.5 that with increasing concentration of reactants, the rate of browning also increases. Hence, by increasing the concentration of the reactants in the tracer can, curves of increasing sensitivity for products which require shorter processing times are obtained. Fig.22 is a plot of the percentage of unaccomplished browning in the tracer can versus f_h of the pack for Fo values from 3 to 7 for concentrations of 2%, 3%, 4%, 5% glucose-glycine in the tracer can. It can be seen that depending on the f_h of the product of which the heat lethality is to be determined, the concentration of the reactants in the browning system in the tracer can may be varied to give maximum sensitivity for the process under consideration. Hence, for a conduction pack one would choose a 2% tracer browning system while for a convection pack, a 5% browning system would be used. Similarly, for packs not wholly convection or conduction, i.e. f_h's between 5 and 30 say, browning systems between 2% and 5% would be selected.

A disadvantage of the above correlation is that it is not applicable to products which do not have linear heat penetration curves. However, browning can still be used as an index for heat sterility for products exhibiting broken heating curves if a plot of the percentage unaccomplished transmission against time (Fig.4) is used. For this to have practical

application, it would first be necessary to carry out trial heat processes with a thermocouple inserted into a can of the product to determine at what time the desired Fo is reached. From the degree of browning taking place in a tracer can containing a glucose-glycine solution one could then determine if the product had been heated for the desired time. Depending on the concentration of the glucose-glycine solution in the tracer can, so the degree of browning occurring with time can be varied, e.g. the sensitivity of the correlation is increased for products with high rates of heat penetration by increasing the concentration of the glucose-glycine solution in the tracer can. A plot of the percentage of unaccomplished transmission against time for concentrations of 2%, 3%, 4%, 5% solutions of glucose-glycine is shown in Fig.5.

The correlations developed above do not allow for any change in retort temperature during the process and so to determine if they would be suitable for a continuous type process in which the processing temperature was not constant throughout the run the following investigation was carried out.

Experimental Method

211 x 400 Code 3 cans were fitted with thermocouples and filled with a 2% glucose-glycine solution to $\frac{1}{2}$ inch net headspace before steam flow closing. The cans were then heat processed at 228°, 238°, 248°F for varying times and during the processing, temperatures within the cans were determined at known times with a potentiometer. After processing, the cans were cooled and the degree of browning of their contents esti-

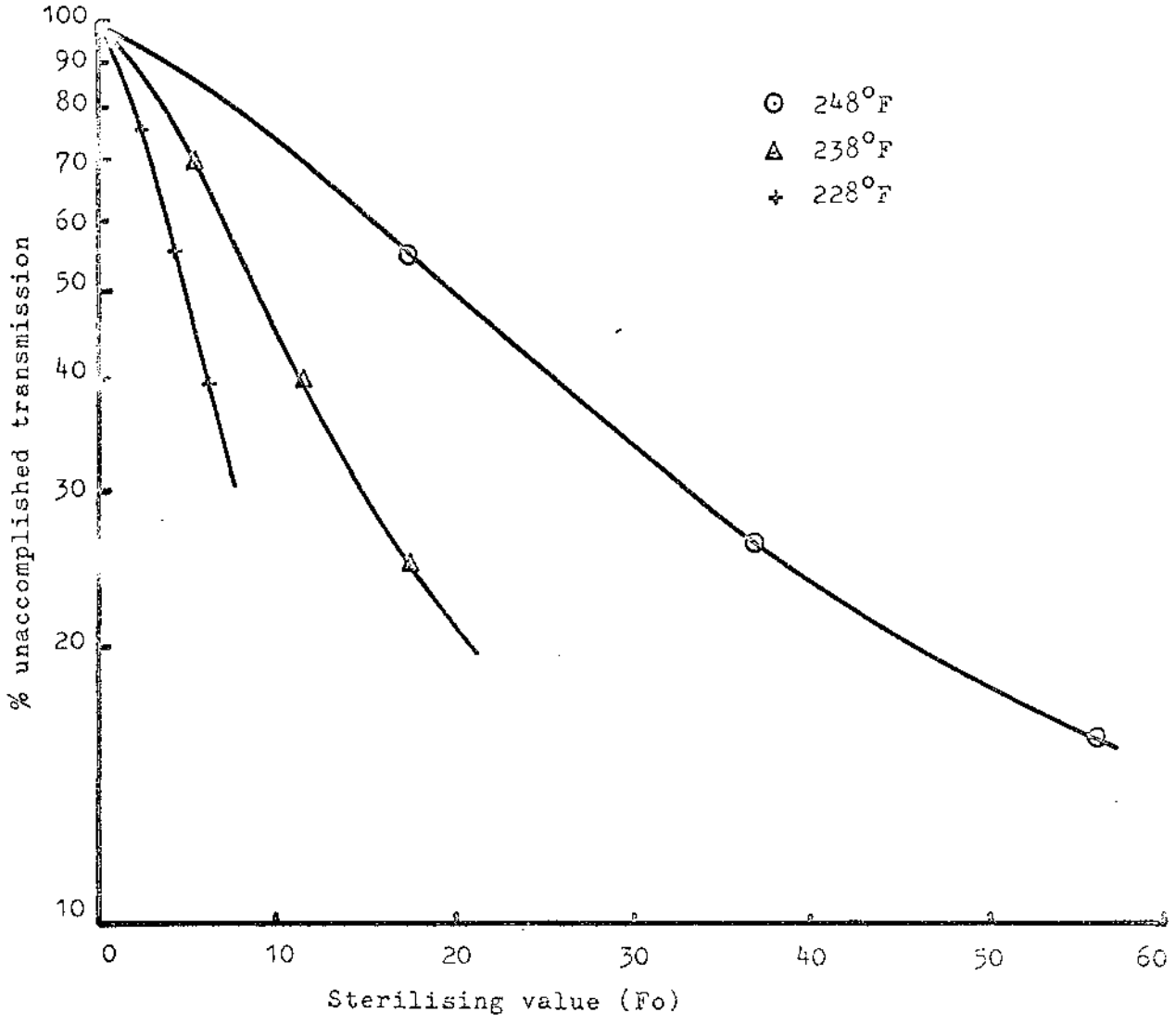


Fig. 23. THE EFFECT OF TEMPERATURE ON THE RELATIONSHIP BETWEEN BROWNING AND STERILISING VALUE

mated with a photometric colorimeter. Sterilising values were calculated for the processes.

Results

The results obtained are summarised in Fig.23 as a plot of the logarithm of the per cent unaccomplished transmission against the sterilising value for the three temperatures studied.

The sterilising value plotted and shown in Fig.23 is the heat lethality attained within the glucose-glycine convection system.

Discussion of Results

At first glances the graph appears to be misleading as it tends to indicate that the rate of browning is more rapid at lower temperatures. However, it is known from Fig.7 that this is not the case.

The results in Fig.23 show that the relationship between a glucose-glycine browning system and time is more complex than it is with temperature.

To absorb a comparable amount of effective heat, the browning system at a lower temperature must be heated for a longer period, and so we find that the system which must be heated longer to attain a similar Fo value shows greater browning due to the longer heating period. It has been shown that the browning reaction under study obeys the Arrhenius equation and that the order of the reaction, i.e. its relation to time, is not simple. However, the exact correlation between browning and time is unknown.

The results shown in Fig.23 indicate that the glucose-glycine

browning system adopted would be unsuitable as an index of heat lethality if the process temperature fluctuates more than a few degrees, because for similar processes, i.e. processes with the same sterilising value (Fo value) the amount of browning taking place varies with the processing temperature even though the systems may absorb the same amount of effective heat.

In an ideal case, a plot of the type shown in Fig.23 should display only one curve applicable to all processing temperatures so that the results are unaffected by changes in processing temperature.

Conclusions

It has been shown that carbonyl-amino browning occurring in a tracer can may be effectively correlated with the heat lethality attained in a product during processing.

Where a product has a linear heat penetration curve a correlation of the type shown in Fig.22 could be used. In this case, the f_h of the product is determined previously by experiment.

To determine the heat lethality attained within the product on processing, a can containing a solution of glucose-glycine of optimum concentration (for greatest sensitivity) is positioned alongside the product during the heat treatment. After retorting, from Fig.22 the Fo value of the process (between the values of 3 and 7) with respect to the product is determined from its f_h value and the degree of browning taking place in the tracer can.

Where a product does not have a linear heat penetration curve, it is necessary by experimentation, to first establish the time necessary

for the desired heat lethality to be reached in the product on processing. Underprocessing (and/or overprocessing) can then be determined in the product from the degree of browning taking place in a tracer can containing a glucose-glycine solution of optimum concentration by referring to the plot of the percentage unaccomplished transmission against time for varying concentrations of reactants (Fig.5).

Although the correlation developed between the percentage unaccomplished transmission and f_h value (Fig.22) is plotted for f_h values less than about 40 and for Fo values between 3 and 7, the graph could be extended to give much wider application. Also, the curves shown in Fig.5 and Fig.22 are applicable only when a retort temperature of 238°F is used. However, it has been shown that the glucose-glycine system does obey the Arrhenius equation and so it should be possible to extend this correlation curve to enable it to be used over a wide temperature range as long as the processing temperature remains constant during the process.

B. CORRELATION OF HEAT LETHALITY WITH BROWNING OCCURRING IN SELECTED CANNED FOOD PRODUCTS

In Part "A" Section III techniques were developed to show that the carbonyl-amino browning occurring on heat processing certain canned foods could be followed quantitatively and that the degree of browning taking place could be correlated with the time of processing. It is important that processes given to canned foods should be able to be checked to ensure underprocessing (or overprocessing) is not occurring and it is much more convenient if some test can be carried out on the product itself to determine if the desired sterilising value has been reached.

The purpose of this study was to show that the degree of browning occurring in a canned food on heat processing could be correlated with the heat lethality of the process in selected products, i.e. baked beans in tomato sauce, cream style corn, and canned fish.

(a) Browning as an index of heat lethality in baked beans in tomato sauce

A correlation of the percentage unaccomplished reflectance (browning) with processing time is shown for baked beans in tomato sauce in Fig.13. Since a relationship exists between processing time and minutes at 250°F (Fo value) it should be possible to correlate the degree of browning occurring in baked beans in tomato sauce on processing with the sterilising value (Fo) of the process.

Experimental method

211 x 400 Code 3 cans were fitted with thermocouples as shown in Fig.33 and filled with 160 gm of soaked and blanched haricot beans (see appendix) and 160 gm of tomato sauce. The cans were steam flow closed and the contents mixed. After the thermocouples had been connected to the potentiometer, the cans, in turn, were heat processed for varying times at a retort temperature of 230°F. During retorting, the temperature within the cans was recorded at known time intervals. When the desired processing time had been reached the cans were cooled and a sample of the contents removed and their degree of browning determined using a reflectance spectrophotometer. From the time-temperature measurements recorded sterilising values for each of the processing times were calculated.

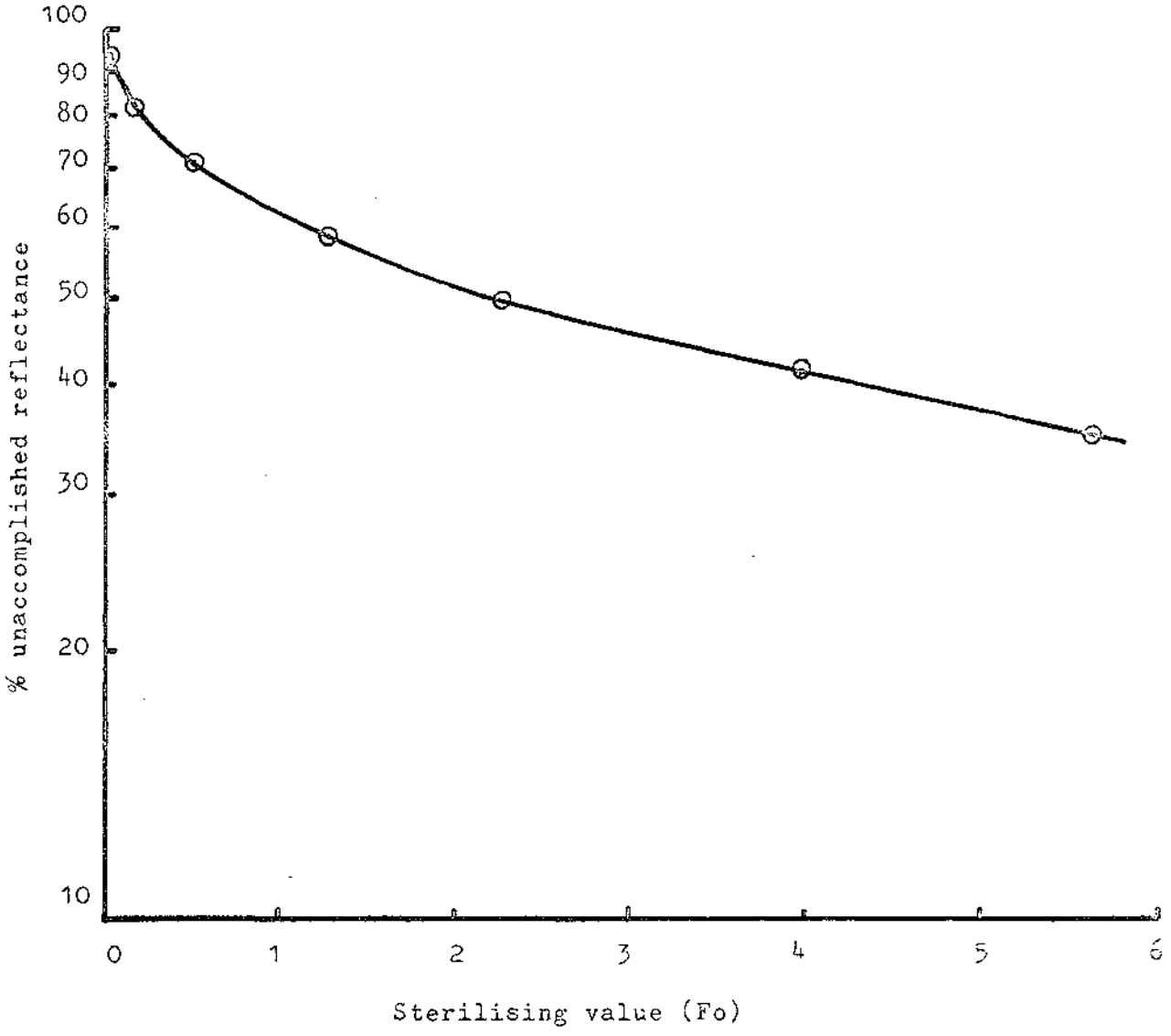


Fig. 24. THE RELATIONSHIP BETWEEN BROWNING IN BAKED BEANS IN TOMATO SAUCE AND STERILISING VALUE

Results

The results obtained are summarised in Fig.24 as a plot of the percentage unaccomplished reflectance against the Fo value of the process.

Discussion of results

It can be seen that the curve obtained is similar in form to when the percentage unaccomplished reflectance was plotted against process time (Fig.13), i.e. there is an initial lag period followed by a linear semi-logarithmic plot except the lag portion in this case is with respect to the time axis due to the different units employed. The linear portion of the plot occurs when the Fo value of the process is greater than about 2 and it is over this linear region of the curve that this plot would have most application commercially as an index of heat lethality. The curve shown in Fig.24 is identical in form to that determined from Ward's results (140) who used a sweetened condensed milk system (Fig.17), and as with that graph, is probably not sigmoid in form due to the method of following the reaction being too insensitive to detect the initial browning phase with the particular time scale (minutes at 250°F) used.

The results indicate that this would be a relatively sensitive technique for detecting variations in sterilising values in baked beans and tomato sauce and that application of this method commercially for checking on under or overprocessing could be feasible. It is estimated that Fo values could be determined to an accuracy of about $\pm$ 0.5 from the correlation shown in Fig.24.

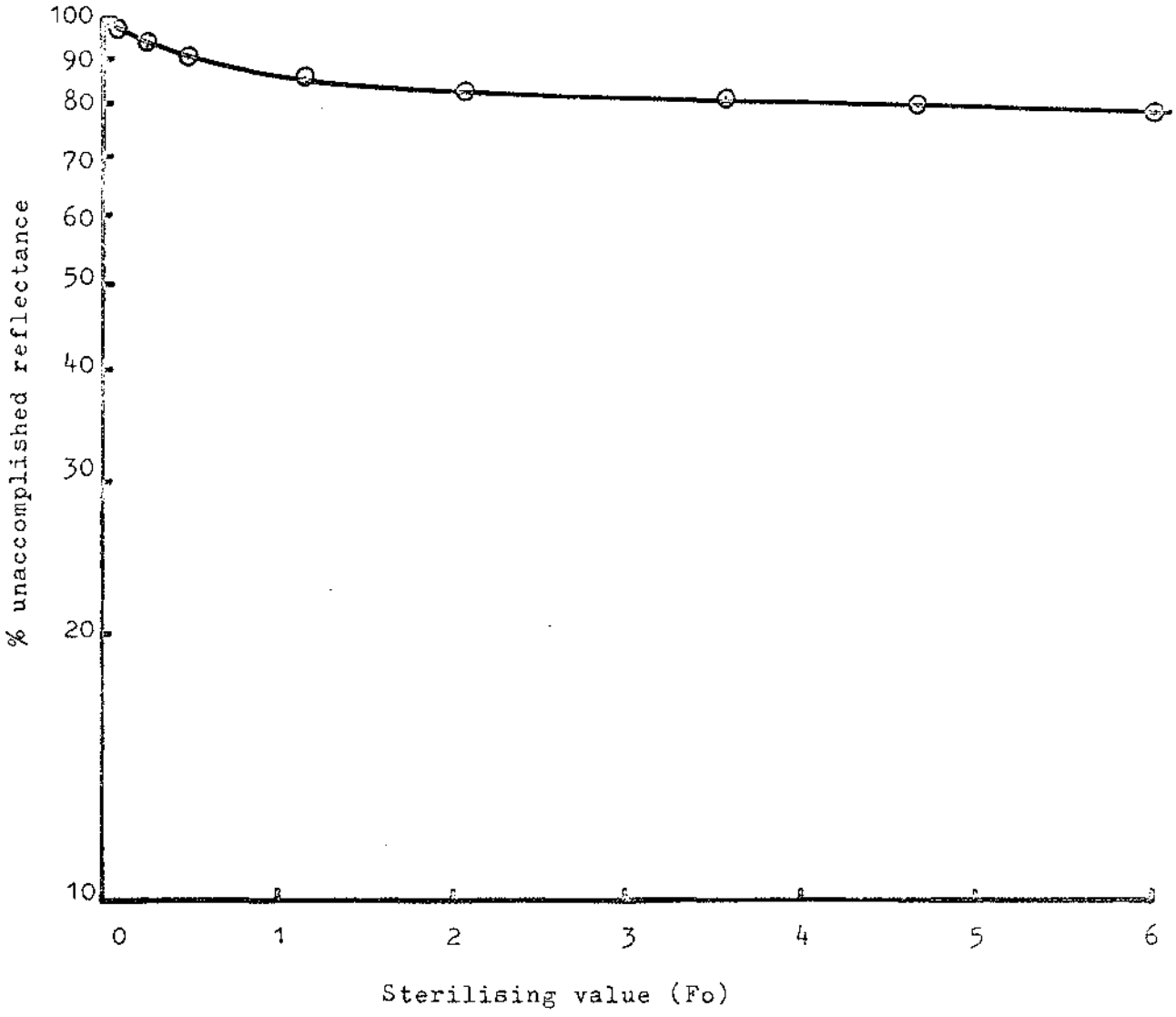


Fig. 25. THE RELATIONSHIP BETWEEN BROWNING IN CREAM STYLE CORN AND STERILISING VALUE

(b) Browning as an index of heat lethality in cream style corn

As with baked beans in tomato sauce, it was shown that the degree of browning occurring in cream style corn on heat processing could be correlated with the processing time. The purpose of this study was to show that the browning occurring on heat processing cream style corn could be used as an index of heat lethality.

Experimental method

211 x 400 Code 3 cans fitted with thermocouples as in Fig.33 were filled with cream style corn at 160°F to a net headspace of 3/16 inch and steam flow closed. The cans were processed for varying times at 238°F and temperatures within the cans were recorded at known time intervals with a potentiometer. After processing and cooling the degree of browning of a sample of the contents was determined using a reflectance spectrophotometer (see appendix). From the time - temperature measurements recorded, sterilising values for the times at which the samples were processed were calculated.

Results

The results obtained are summarised in Fig.25 as a plot of the percentage unaccomplished reflectance against the sterilising value (Fo) of the process.

Discussion of results

The results obtained are similar in form to those shown in Fig.24, i.e. the plot of the percentage unaccomplished reflectance against the sterilising value for baked beans in tomato sauce. The initial rate of

browning is relatively rapid but gradually tails off and when an Fo value of about 2 is reached, the curve becomes linear. The chance that this curve would be important commercially below an Fo of 2 is doubtful although it could be used in this region as an index of gross under-processing. The curve shown in Fig.25 is relatively insensitive compared with that obtained for baked beans in tomato sauce due to the low concentration of carbonyl reactants present in the raw material and hence the relatively small overall colour change. The curve obtained for cream style corn when the percentage of unaccomplished change in reflectance was plotted against time of heating would lose its sigmoid form on replotting as a function of the sterilising values because of the change in time units and the small colour difference to be measured. The method employed in this study for following browning in cream style corn could only be utilised commercially for detecting marked underprocessing although by developing a more sensitive method for following the colour change than that developed for this study, this technique, as an index of heat lethality could be feasible and have commercial application.

(c) Browning as an index of heat lethality in canned fish

As discussed previously, the degree of carbonyl-amino browning occurring in a system on heat processing can be related to the time and temperature of heating. That this system can be applied to fish flesh has been demonstrated by Spilde (113) who showed the effect of time and temperature on the rate of browning at different pHs.

However due to the nature of the factors influencing the rate of browning in fish flesh and the inability, in many cases, to control these,

for example, the ultimate pH of the flesh, difficulty could be experienced in establishing a general correlation between browning and heat lethality. Factors affecting the rate of browning are discussed in Part "A" Section III, and from this, the difficulties which could be encountered are apparent. It would be possible to standardise such factors as the variety of fish used, canning technique (i.e. vacuum applied, metal ions present, and ingredients added to the pack) and sampling procedure, but factors such as freshness of the raw material (and hence, concentration and nature of the carbonyl moiety present) and the pH of the flesh could be difficult to control. The pH of the flesh will depend on how the fish is caught and the state of the fish, e.g. if it is exhausted when it is caught, while the freshness of the raw material will depend on when it is caught, treatment prior to and during storage, how it was stored, and length of storage. The season of the year will also affect the ultimate extent of browning in the heat processed product.

Hence, it can be seen that it would be necessary to determine quantitatively the effect of these factors upon browning and incorporate them into any correlation developed or to standardise the raw material in some manner prior to heat processing before a realistic correlation between browning of the flesh and heat lethality could be established.

C. SUMMARY

It has been demonstrated that carbonyl-amino browning whether occurring in a product or model system can be correlated with the amount of effective heat received and hence be used as an index of the heat lethality of the process.

A tracer can technique developed using a model browning system could have wide application commercially as it is not restricted to any one product. In this case, the degree of browning is related to either the heating time of the process (which is, in turn, related to Fo) or to the heating characteristics of the product and hence Fo value. It is envisaged that this correlation could be used as a routine quality control technique to maintain a check on heat processes given to canned foods. However, due to the complex nature of the carbonyl-amino reaction, the correlations presented are invalidated if the processing temperature is allowed to fluctuate more than a few degrees from the temperature for which the correlation was determined.

A correlation between the degree of browning taking place in selected foods on heat processing and the heat lethality of the process has been demonstrated. As with the model carbonyl-amino system, the correlation is invalidated by fluctuations of more than a few degrees in temperature during the process but this should not detract from the commercial acceptancy of the technique for batch type processes which generally maintain reasonably constant temperatures.

Where the correlation was sufficiently sensitive, it could be used as a routine quality control technique but otherwise it could only be used as a check against marked under or over processing. This correlation between the inherent browning in foods and heat lethality could be extended to include a large number of products and become an important commercial technique. It has the advantages over the tracer can method in that a model browning system does not have to be prepared and any number of cans of product from any position in the retort can be tested.

However, the raw material of products used for this type of correlation must consistently contain the same concentrations of reactants and these must not be subject to change by such influences as maturity, climate, etc., if constant and reliable results are to be obtained.

P A R T "B"

CORRELATION OF THE THERMAL DEGRADATION OF CHLOROPHYLL IN CANNED GARDEN PEAS WITH THE HEAT LETHALITY OF THE PROCESS

S E C T I O N I

THE CHEMISTRY OF CHLOROPHYLL AND FACTORS AFFECTING ITS DEGRADATION

(a brief review of the chemistry of chlorophyll, factors affecting its degradation and the kinetics of this reaction).

Before it is possible to establish if a correlation exists between the thermal degradation of chlorophyll in garden peas and the heat lethality of the process, it is necessary to study the chemistry of chlorophyll and the factors affecting its degradation.

(a) Location of chlorophyll in the plant

The green plant pigment, the magnesium complex chlorophyll, occurs together with the red or yellow pigments carotene and xanthophyll in the chloroplasts of plant cells and its biological function lies in photosynthesis. The chloroplasts in the mature leaves of green plants are generally flattened ellipsoid-shaped bodies of a circumference of 2 to 5 μ. They contain 60 to 70% protein and 30 to 40% lipid. This lipid portion also includes chlorophyll and carotenoids. A portion of the chloroplast proteins, together with chlorophyll, can be separated as a complex compound (chloroplastin). The proportion of pigment to protein appears to disclose differences according to plant species, and differences due to nutrition, age and to quality of the protein itself. The combined chlorophyll content of the leaves varies from 0.13 to 0.35% and chlorophyll a. is nearly always present in an amount three times as high as that of chlorophyll b. The quantity of carotenoids and xanthophylls present is generally about one third of that of chlorophyll. The breakdown of chloroplastin into chloro-

phyll and protein occurs under mild conditions, e.g. by drying freshly cut leaves or by precipitation with salts. The leaf pigments chlorophyll, carotene and xanthophyll, and a lipid fraction of complex nature can be separated with organic solvents while the protein fraction plastin separates as flocks.

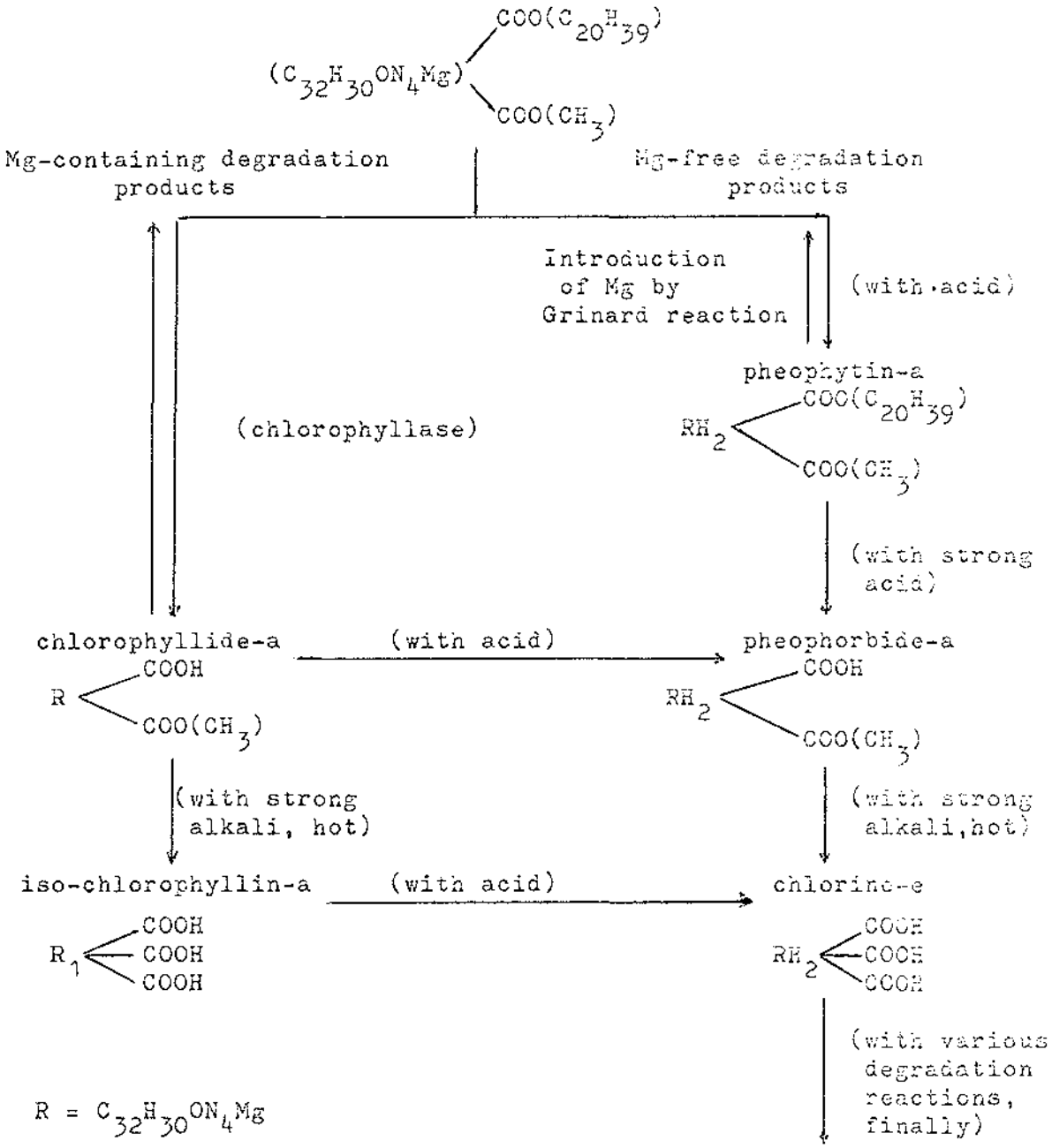
(b) Structure and properties

Clarification of the structures of chlorophylls a. and b. involved the work of many investigators. Willstätter and Stoll (145) showed natural chlorophyll consisted of two components: chlorophyll a. and chlorophyll b. of formula $C_{55}H_{72}O_5N_4Mg$ and $C_{55}H_{70}O_6N_4Mg$ respectively. By 1940 Hans Fischer (33) had established the essential structures of chlorophylls a. and b. and bacteriochlorophyll a. Chlorophylls may be regarded as magnesium complexes of compounds derived from phorbin which in turn, may be regarded as the dihydro derivative of porphin but with the addition of the isocyclic ring V.

Chlorophyll a. crystallises from ether - petroleum ether in a spear-shaped form and is tinted a greenish blue-black colour. It is soluble in alcohols, ether, benzene and acetone and insoluble in water. When it is pure it is only slightly soluble in petroleum ether. Its ethanolic solution is blue-green in incident light with red fluorescence, while disclosing a dark red tint in transmitted light.

Chlorophyll b. is dark green or blue-black and its ethanolic solution is green with a slight yellowish tint. Its solubility characteristics are similar to chlorophyll a.

In addition to chlorophylls a. and b, chlorophylls c, d, and e



$R = C_{32}H_{30}ON_4Mg$

$R_1 = C_{31}H_{30}N_4Mg$ (owing to the saponification of OCH_3, the number of C atoms is decreased by one)

$RH_2 = C_{32}H_{32}ON_4$ (owing to the cleavage of Mg, two pyrrole rings each contain one additional hydrogen atom. In the porphyrins (with the exception of pheophorphyrin) the 5-membered carbon ring, linked to pyrrole ring III of chlorophyll and characteristic of chlorophyll and of its Mg-containing precursors is split.

Fig. 26. SCHEME OF DEGRADATION OF CHLOROPHYLL-A - from (145)

have been distinguished although the structures of d. and e. are uncertain.

Both chlorophylls a. and b. are optically active: laevorotatory. The spectrum of pure chlorophyll a. shows strong adsorption in the red and blue-violet range and very slight adsorption in the yellow range. The spectrum of chlorophyll b. shifts slightly to the centre in both culminating parts. Their spectrum in the leaf is shifted to the red.

(c) Chlorophyll degradation products

The nomenclature of chlorophyll degradation products is as follows: Porphyrins are substituted porphins. Phyllins, phyllides and chlorophylls contain magnesium whereas phorbins, phorbides and phytins are magnesium free compounds, the magnesium atom having been removed and replaced by 2 Hydrogen atoms. When magnesium is present, the compound is green but the colour of the magnesium free compounds is generally grey or brown. 7 : 8-Dihydroporphin is the nucleus of the chlorin series of compounds (tricarboxylic derivatives) which are derived from chlorophyll a; rhodins are the corresponding compounds derived from chlorophyll b. The introduction of the extra ring - two methylene groups across the 6 : 8 positions give rise to the phorbins. The prefix phaeo designates those compounds which have the same substituents that occur in chlorophyll. Chlorin itself is dihydroporphin and the natural red porphyrin pigments are derivatives of porphin, whereas the green chlorophylls and their derivatives are derivatives of chlorin.

A scheme outlining the degradation of chlorophyll a. is shown (Fig.26).

Treatment of the chlorophylls, even with very weak acids, removes the magnesium from the structure and replaces it by 2 hydrogen atoms, thus from

each of the chlorophylls the corresponding pheophytin a. or b. is obtained and the colour undergoes a considerable change. With chlorophyll a, the colour change is from blue-green to olive green and for chlorophyll b, it is from yellow green to magenta. The 2 hydrogen atoms in the position originally occupied by magnesium, can be replaced very easily by a number of other metals (111) and in each case the corresponding metal complex of intense colour and characteristic spectral properties is formed. These complexes are usually very stable, especially to light, while the magnesium complex chlorophylls bleach in light very readily while in solution.

The conversion of chlorophyll to pheophytin in plant material occurs relatively rapidly under the influence of heat and this is accelerated by acid conditions. Mackinney and Weast (78) showed 100% conversion of chlorophyll to pheophytin occurred in 60 minutes at 100°C.

It has been suggested (78) that owing to the severe conditions employed in heat processing, degradation products other than pheophytins occur. Westcott, et al. (143) found pheophytin and small quantities of pheophorbide in commercially canned green bean puree.

Jones, et al. (54) showed that during the brining and brine storage of cucumbers, the chlorophylls were converted to the pheophytins and the pheophorbides upon development of acidic brines following acid fermentation.

The formation of chlorophyllides and pheophorbides as well as pheophytins has been reported as occurring during blanching (55).

Work has been carried out, mainly with spinach, to determine the optimum conditions for the conversion of chlorophylls to chlorophyllides

by the enzyme chlorophyllase with the hope of maintaining the desirable green colour (25). The chlorophyllides formed will readily lose magnesium in an acid medium to form the yellowish brown pheophorbides.

In the spring, chlorophyllase remains active at 65 to 75°C, a temperature at which most other enzymes are inactivated, while in the summer, the same enzyme appears to be relatively inactive. Blanching by the Thomas process can be very successfully applied to spinach, which preserves its good green colour if blanched by steam at exactly 77°C notwithstanding the fact that after such blanching this product is processed at 120°C. However, if canned directly at boiling temperature without previous blanching, it will not retain its green colour. It is thought that the enzyme chlorophyllase is not inactivated at the temperature of 77°C and therefore continues to convert chlorophyll into green chlorophyllin.

Some vegetables, such as peas, string beans, and asparagus apparently do not contain the chlorophyllase enzyme at all.

(d) Factors affecting chlorophyll degradation

1. Temperature

As is the case with the majority of chemical reactions, the thermal degradation of chlorophyll to pheophytin is accelerated with increased temperature, and it has been shown that the rate of reaction of chlorophyll a is 7 - 9 times that of chlorophyll b (77). The kinetics of this reaction have been studied and are considered in more detail later. Since the degradation of chlorophyll exhibits a "Z" value of the order of 8 - 10 times that used to calculate sterilisation values for processed foods (41) it is apparent that high temperature/short time sterilisation

will result in less thermal degradation of chlorophyll. Gold and Weckel (37) assuming instanteous heating and cooling calculated that on sterilising a pea puree (pH : 6) at 240°F, 74.1% of the chlorophyll would be degradated while at 280°F, 1.09% would be converted to pheophytin. However, as the pH of conventionally canned peas is approximately 6.5, the conversion of chlorophyll to pheophytin proceeds rapidly during storage at room temperature and is practically complete after a few weeks. When high temperature/short time sterilisation is employed the risk of enzyme regeneration and subsequent development of off flavours is present (see Part "C").

2. Time

Time is obviously going to be a major consideration in the conversion of chlorophyll to pheophytin. Mackinney and Weast (78) showed that heating fresh string beans at 100°C for 60 minutes converted all the chlorophyll to pheophytin resulting in the formation of a yellowish colour. The effect of cooking time on retention of chlorophyll a. has been demonstrated by other workers (124, 125) and the results indicate that destruction of chlorophyll a. was the principal factor responsible for the loss of colour in cooked green vegetables.

3. pH

As mentioned previously, acidic conditions accelerate the formation of pheophytin from chlorophyll and pheophorbide from chlorophyllide. The pH range 6 - 7 appears to be critical with respect to the stability of chlorophyll in vegetables while the use of buffers of pH greater than 7 results in marked deterioration in flavour and little further improvement

in colour (124). Gold and Weckel (37) showed that raising the pH of peas will have a definite protective effect upon chlorophyll. They found on processing pea puree at 240°F to a state of commercial sterility, 74.1% of the chlorophyll would be degraded at pH 6, 21.0% at pH 7 and 7.43% at pH 8. Several patents have been issued which involve elevation of pH conditions in order to reduce pheophytin formation on heat processing.

4. Blanching

From the basic effect of time and temperature on chlorophyll degradation, one would expect increased formation of pheophytin with increased time and temperature of blanching. Legault, et al. (74) investigated chlorophyll conversion to pheophytin in peas during blanching in steam at 190°F and 212°F for various time intervals. They found no practical differences when the chlorophyll losses at the two temperatures were expressed on the basis of "adequacy of blanch" (as determined by tests for peroxidase inactivation). The loss of chlorophyll increased with blanch intensity and reached a maximum of approximately 8.5%.

Jones, et al. (55) presented evidence that blanching treatments are in many cases responsible for the formation of the chlorophyllides and pheophorbides as well as pheophytin and that pheophorbides may in fact represent a greater proportion of the converted chlorophyll than pheophytins in certain plant tissue receiving blanching treatment.

5. Reducing agents

These have no influence on the formation of pheophytin but may render the chlorophyll more resistant to oxidation (70).

6. Oxidising agents and occluded air

These have no influence on the formation of pheophytin although, as in the case of photo oxidation by light, they will induce the formation of compounds with adsorption spectra different to the chlorophylls and pheophytins (70).

7. Metal ions

Some metal ions react with the chlorophylls to form compounds with bright green colours. Ferric, zinc and cupric ions will replace the magnesium in chlorophyll and under favourable conditions, pheophytins, and in some foods, pheophorbides form green complexes with very small amounts of copper and zinc in stored vegetable products. These pigments are the cause of the so-called re-greening and are very stable. It has been shown (111, 112) that 1 - 2 ppm of copper is sufficient to give re-greening while about 25 ppm of zinc are required. If sufficient metal ions are present complete conversion of pheophytin to the complex is possible.

(e) Kinetics of chlorophyll degradation

The rates of conversion of chlorophylls a. and b. to their respective pheophytins were first studied by Joslyn and Mackinney (65) and their findings have since been examined in more detail.

Schanderl, et al. (110) studied the kinetics of the reaction of chlorophylls a. and b, and the ethyl, methyl and free chlorophyllides in acid solution. Using highly purified freshly prepared pigments they were able to demonstrate the conversion reaction apparently followed 1st order kinetics. By plotting the logarithm of the reaction rate against the

reciprocal of the absolute temperature, they were able to show that the reaction conformed to the Arrhenius equation. The data obtained gave a series of approximately parallel sloping lines with activation energies of 10.4, 10.4, 10.6, 10.8 x 10^3 cal/mole for chlorophyll a, ethyl chlorophyllide a, methyl chlorophyllide a, and free chlorophyllide a, respectively. These results indicate chlorophyll a. has the slowest rate and ethyl, methyl and free chlorophyllides are slightly faster. The temperature quotient (Q_{10}) for this conversion varied from 1.73 to 1.77. The data obtained for chlorophyll b. were generally similar to those obtained for chlorophyll a. The two compounds had about the same activation energies and temperature quotient although chlorophyll b. was found to react 5.5 times slower than chlorophyll a.

Cho and Chichester (48) showed chlorophylls a. and b. obeyed general acid catalysts in their conversion to pheophytins a. and b. The rate law indicated 2nd order dependence on hydrogen ions and 1st order dependence on chlorophyll. A mechanism was proposed in which two hydrogen ions in rapid equilibrium replaced Mg^{++} in its attachment to the pyrroles. This fast step was followed by the rate determining step in which pheophytin was produced. The slower rate of production of pheophytin b. was due to a smaller equilibrium constant in the first reaction step due to resonance contribution in the pyrroles.

Gupte, et al. (41) studied the kinetics of thermal degradation of chlorophyll in spinach puree over the temperature range 260° - 300°F. Linear curves were obtained when log. residual chlorophyll was plotted against heating time indicating a 1st order reaction. In the initial stages of the reaction, however, pigment degradation was not 1st order.

A linear Arrhenius plot was also obtained. Values for temperature quotient, free energy, entropy and enthalpy of the reaction were calculated and indicated chlorophyll a. reacted at a greater rate than chlorophyll b. However, the values calculated were not absoluted values since a pure system was not used.

Gold and Weckel (37) studied the degradation of chlorophyll to pheophytin during sterilisation of canned green peas by heat and deduced pseudo 1st order kinetics from the straight line relationship they found to exist. On plotting the logarithm of the per cent unchanged chlorophyll versus time of heat processing at a given temperature, they calculated activation energies for the reaction from the Arrhenius plot.

S E C T I O N II

A STUDY OF THE THERMAL DEGRADATION OF CHLOROPHYLL IN PEAS ON HEAT PROCESSING

During commercial processing conditions, the chlorophyll is degraded to a number of products and these products, due to loss of magnesium, are olive green/brown in colour. Kinetic studies of chlorophyll degradation have established the relationship of this reaction with time.

Since the thermal destruction of bacterial spores has also been related to time it should be possible to correlate directly chlorophyll degradation and spore destruction and so obtain an index of heat lethality from the percentage of residual chlorophyll present.

The purpose of this study was to develop a simple technique for following chlorophyll degradation and to investigate some of the factors affecting the rate of this reaction. Throughout this entire study frozen garden peas were used as fresh peas were unavailable.

(a) Development of a technique for following chlorophyll degradation

A number of techniques are available for following the degradation of chlorophyll or determining the percentage residual chlorophyll, but invariably they require the use of expensive equipment or are analytically involved and time consuming (40). It was necessary in this study to develop a technique which was simple and which only required the use of relatively inexpensive equipment normally encountered in food processing establishments.

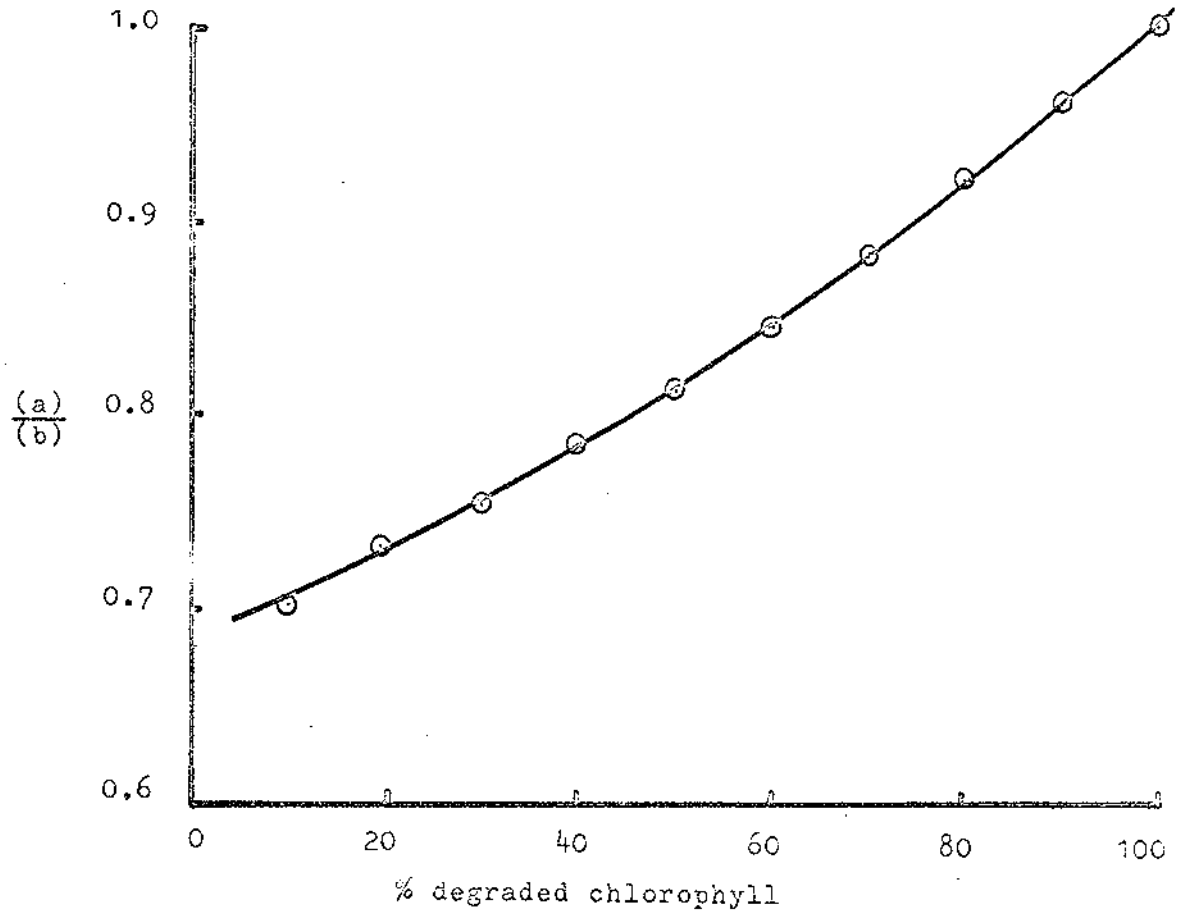


Fig. 27. STANDARD CURVE FOR CHLOROPHYLL DEGRADATION

The method developed required the use of a photo-electric colorimeter and readings of light transmission from this were referred to a standard curve from which the percentage of degraded chlorophyll was determined. The standard curve was prepared in the following manner: Chlorophyll pigments were extracted from a sample of frozen garden peas by the method outlined in the appendix. Degraded pigments were also extracted using the same method from a sample of peas which had been seamed together with water in a Code 3 can and heat processed for $2\frac{1}{2}$ hours at $240^{\circ}F$. It was assumed that all chlorophyll in the retorted peas had been degraded as there was only 0.2% difference in the light transmission values when the extract from the heated peas was compared with an extract of chlorophyll which had been degraded with excess oxalic acid. Light transmission values were determined for solutions of (100 - x)% undegraded chlorophyll plus x% degraded chlorophyll where x = 0,10,20....100 and the standard curve was prepared by plotting:

$$\frac{\text{transmission of } (100 - x)\% \text{ undegraded chlorophyll} + x\% \text{ degraded chlorophyll}}{\text{transmission of } 100\% \text{ degraded chlorophyll}}$$

against % degraded chlorophyll (x).

The curve is shown in Fig.27.

Hence for any sample of heated peas, the percentage of degraded chlorophyll could be determined from the standard curve by:

(i) Determine the transmission of the extract from the sample of peas (details of the extraction method used throughout this study are given in the appendix). This result is equivalent to transmission of (100 - x)% undegraded chlorophyll + x% degraded chlorophyll. (a)

(ii) Degrade any chlorophyll present in the extract with oxalic acid and determine the transmission of this solution. This results in the value of the transmission of a 100% degraded chlorophyll solution. (b)

Hence the value of $\frac{(a)}{(b)}$ represents the abscissa and the percentage of degraded chlorophyll in the sample is given by where this point intercepts the standard curve. Details of the analytical technique are given in the appendix. It should be pointed out that this technique using a standard curve can only be used for peas that have similar initial chlorophyll contents at the time of processing.

(b) Chlorophyll degradation in heat processed peas

Most quantitative techniques for following chlorophyll degradation involve measurement of a purified chlorophyll compound at particular wavelengths with spectrophotometers etc., and it has been found by methods of this type that the reaction obeys first order kinetics. However, in the technique developed in this study, the degradation of no single chlorophyll, e.g. chlorophyll a, b, etc., but the degradation of the chlorophyll pigment as a whole is followed, and so it was necessary to investigate the effect of time on chlorophyll degradation under typical commercial heat processing conditions using the method of analysis developed.

Experimental method

211 x 400 Code 3 cans were filled with 200 gm of thawed frozen peas and 105 gm distilled water (in accordance with the New Zealand Standard Specification NZSS 1911 : 1965 for minimum drained weights), steam flow seamed and heat processed at 238°F for varying times. The cans were then cooled and during the hours of darkness the degree of chlorophyll degrada-

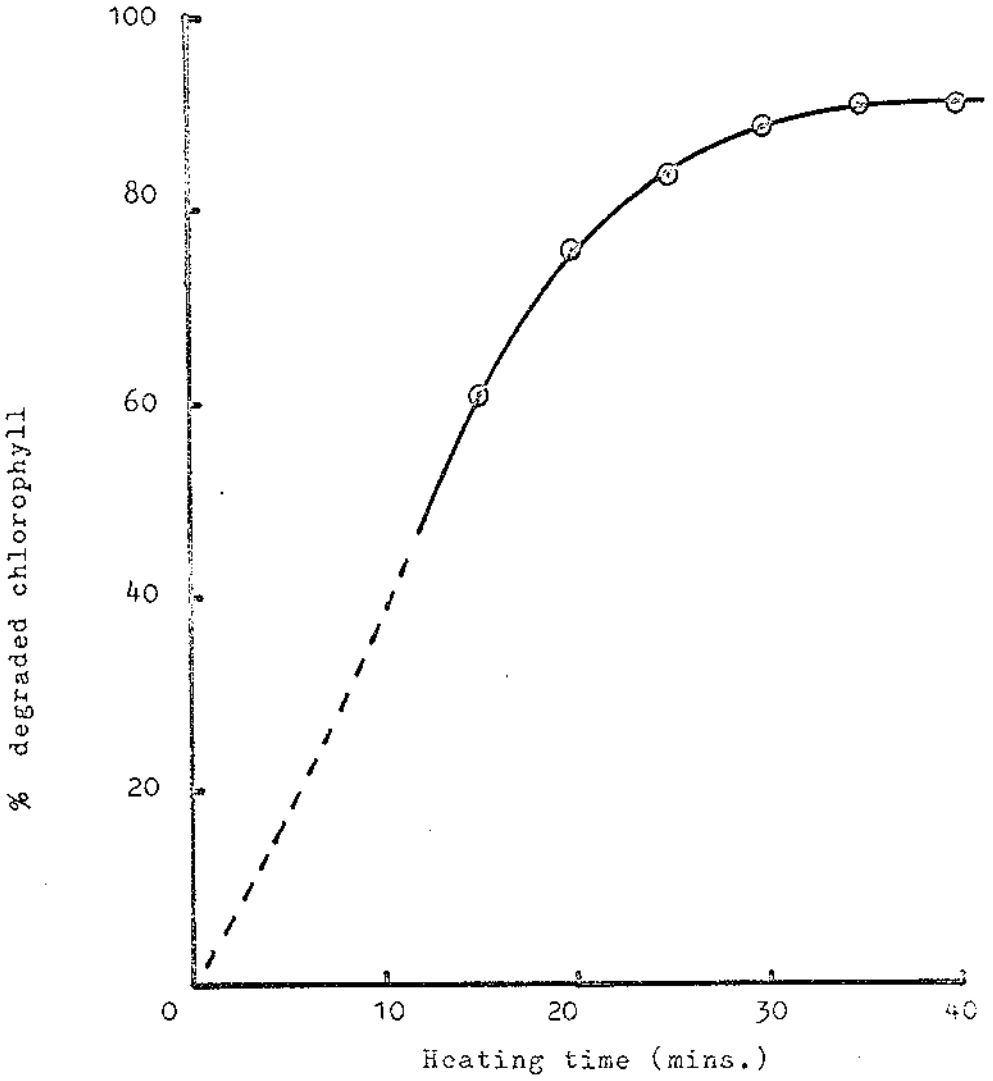


Fig. 28. THE DEGRADATION OF CHLOROPHYLL ON HEATING

tion determined by the method outlined in the appendix.

Results

The results obtained are summarised in Fig.28 as a plot of the percentage of degraded chlorophyll against time of heating at retort temperature.

Discussion of results

The results obtained do not conform to either first or second order kinetics but this is not surprising as the degradation of no single purified compound is followed. Within the first 20 minutes the greatest colour change takes place but since not only chlorophyll pigments but also carotenoids and xanthophylls etc., are present in the extract and the analysis is made over a wide band of wavelengths it could be difficult to justify any conclusion as to rates of chlorophyll degradation. It is evident that the technique developed does give a measure of the colour change occurring with time of heat processing and although it is impossible to give a kinetic interpretation of the results obtained the method is suitable for the purposes of this study.

(c) A study of the factors affecting chlorophyll degradation

Before chlorophyll degradation can be used as an index of heat lethality, it is necessary to study the effect of factors encountered as a result of commercial methods. These factors would include pH, the concentration of sugar, salt and metal ions present in the added fluid, and oxygen in the headspace of the can.

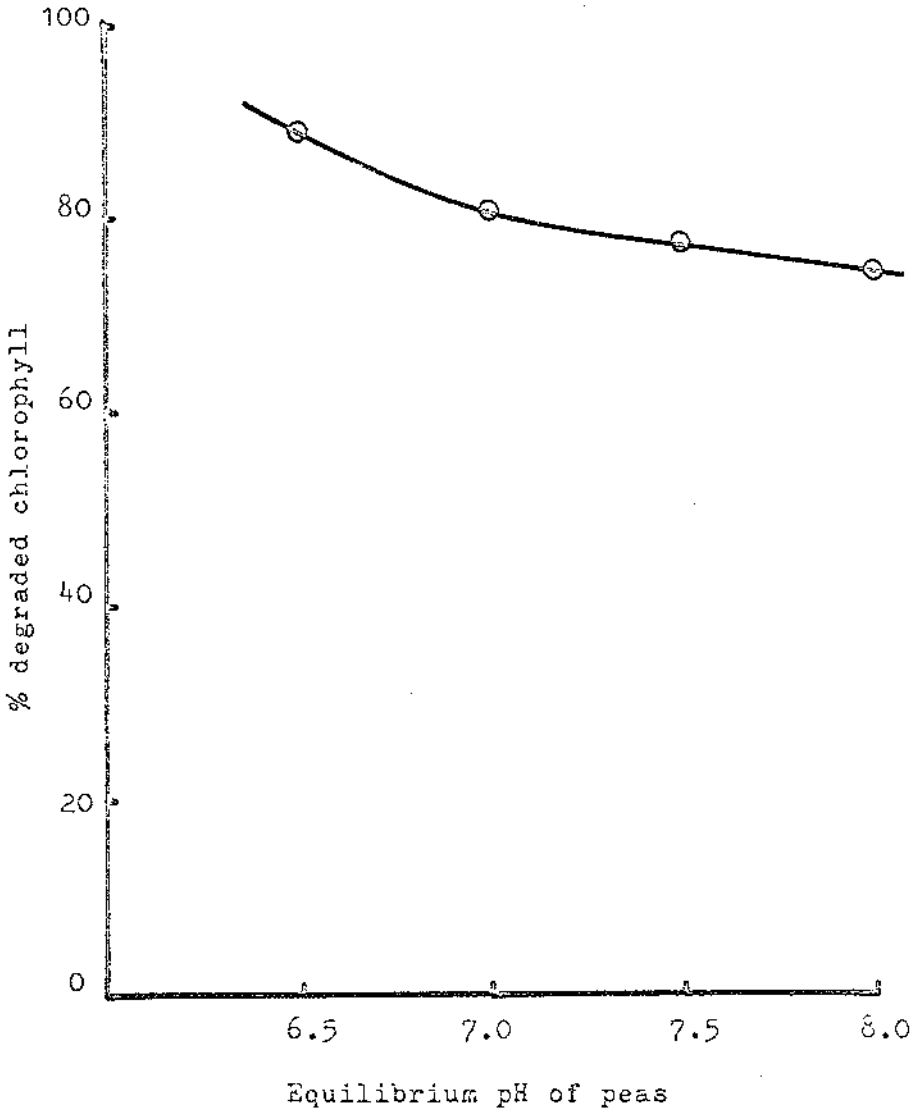


Fig. 29. THE EFFECT OF pH ON CHLOROPHYLL DEGRADATION

(i) The effect of pH on chlorophyll degradation

Some workers have found that chlorophyll degradation in acid conditions obeys first order kinetics (110) while others have found it conforms to second order kinetics (48). It has been found that the critical pH with respect to chlorophyll stability lies between pH 6 - 7 and there are a number of patents outlining methods of retaining a desirable colour by using alkaline conditions.

The purpose of this study was to examine the effect of pH under typical canning conditions over the range pH 6.5 (the pH of peas used in the test) to pH 8.

Experimental method

To 200 gms of peas in 211 x 400 Code 3 cans were added 105 gm of water or trisodium phosphate solutions of varying concentration to give equilibrium pHs of 6.5, 7.0, 7.5 and 8.0. The equilibrium pH of peas in water was 6.5. The cans were steam flow seamed and heat processed for 30 minutes at 238^{o}F. They were then cooled and the percentage of degraded chlorophyll determined from a sample of the contents.

Results

The results obtained and summarised in Fig.29 as a plot of the percentage of degraded chlorophyll against the equilibrium pH of the peas.

The differences in colour between peas processed at pH 6.5 and those at pH 8 was quite marked, the peas in the alkaline conditions being distinctly greener in colour. There was also a change in texture of the peas as a result of processing at the higher pHs.

With increasing pH above 7.0, there was an increased tendency for collapse of cellular structure and a complete loss of firmness. At higher pHs the fluid in the can tended to gel and the peas had a nutty smell.

Discussion of results

From Fig.29 it can be seen that above pH 7 there is a marked change in the effect of pH on chlorophyll degradation. This is in agreement with results obtained by other workers. At pHs above 7 the colour of the peas was very acceptable but changes in flavour and texture were unfavourable. The change in texture, which was evident by a loss of firmness and appearance of slime was probably due to hydrolysis of cellulose on heating under alkaline conditions. Breakdown of the cellular structure and release of starch from the peas were indicated in the pH 8 sample by the surrounding fluid (gel) turning blue-violet on treatment with iodine. It can be concluded that raising the pH of the system is an effective means of preventing degradation of chlorophyll but care would have to be taken to prevent unfavourable texture and flavour changes.

(ii) The effect of salt concentration on chlorophyll degradation

Brine is added to peas on canning to give an acceptable flavour. The concentration of salt added may vary slightly from manufacturer to manufacturer and the purpose of this study was to determine if this variation in concentration has any effect on chlorophyll degradation.

Experimental method

211 x 400 Code 3 cans containing 200 gms of peas were filled to a net headspace of 3/16 inch with solutions containing 0%, 1%, 2% and

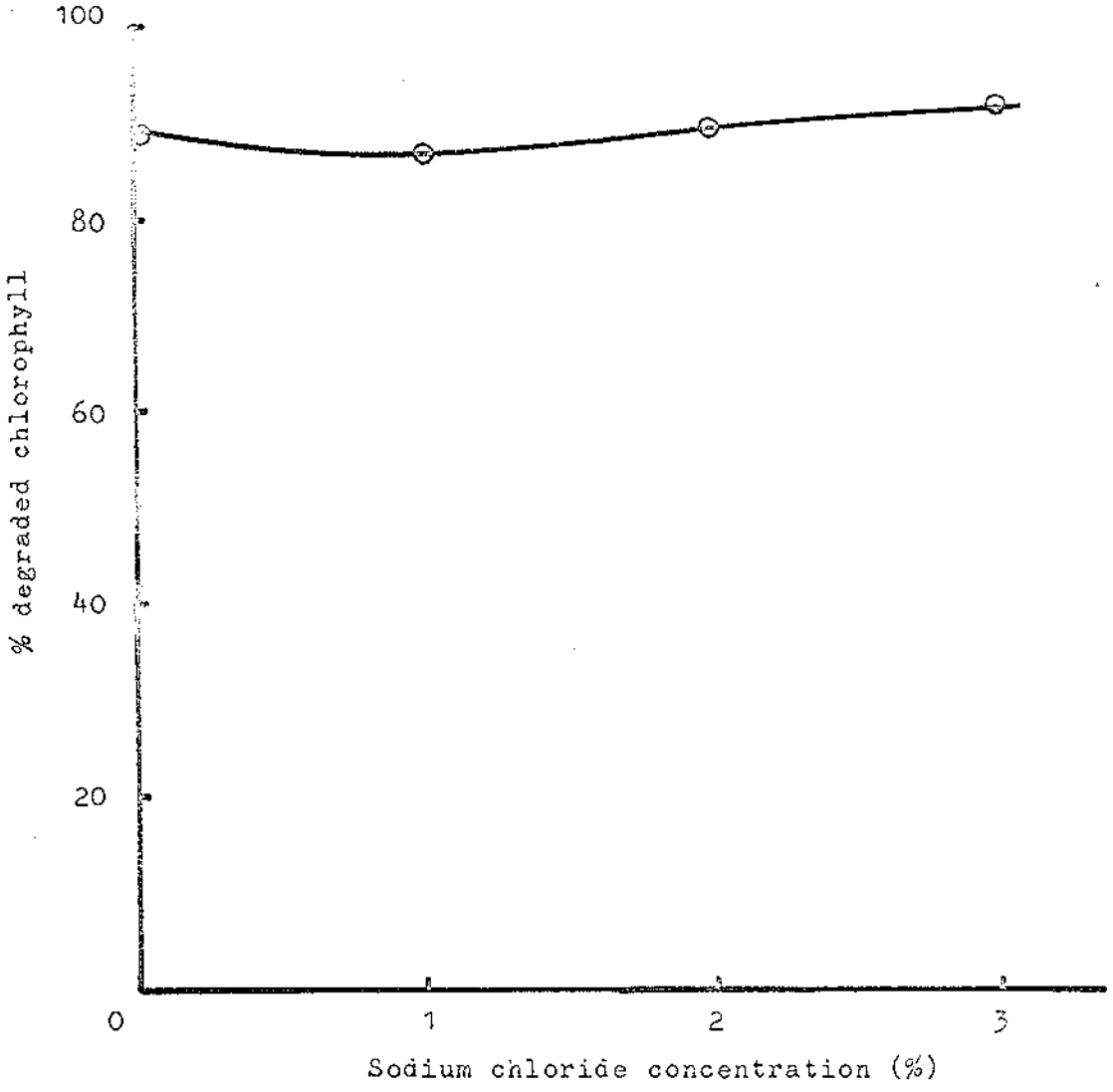


Fig. 30. THE EFFECT OF SALT CONCENTRATION ON CHLOROPHYLL DEGRADATION

3% sodium chloride and steam flow closed. The cans were then retorted at 238°F for 30 minutes, cooled and the per cent degraded chlorophyll in the peas determined.

Results

The results obtained are summarised in Fig.30 as a plot of percentage of degraded chlorophyll against concentration of sodium chloride in the added fluid.

Discussion of results

The results indicate that the system under study had the most acceptable colour when 0.5 - 1% sodium chloride was present. It would be difficult to ascertain the cause of this phenomenon but it could perhaps be due to:

(1) An ionic effect by the sodium chloride on the replacement of the magnesium atom by two hydrogen atoms in the chlorophyll molecule.

(2) Compounds, e.g. carotenoids and xanthophylls, which are extracted with the chlorophyll, being influenced by the presence of a critical amount of salt and affecting the transmission of a certain wavelength of light through the solution.

(iii) The effect of sugar concentration on chlorophyll degradation

A concentration of about 1 - 2% sucrose is generally added to the brine in canned garden peas to improve the flavour. The purpose of this study was to determine if this added sugar has any effect on the degradation of chlorophyll in the pea.

Experimental method

211 x 400 Code 3 cans containing 200 gm peas were filled with solutions containing 0%, 1% and 2% sucrose to a net headspace of about

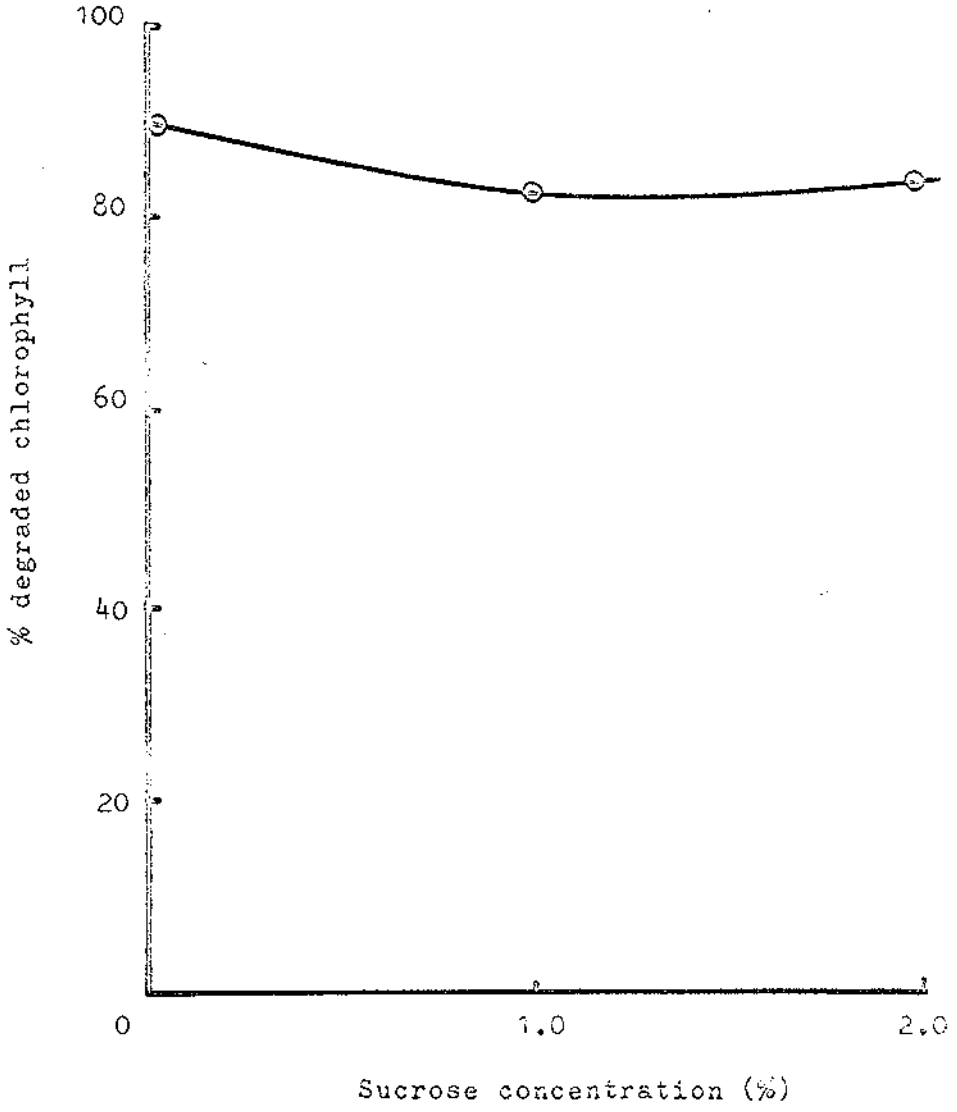


Fig. 31. THE EFFECT OF SUGAR CONCENTRATION ON CHLOROPHYLL DEGRADATION

3/16 inch. The cans were steam flow closed and heat processed at 238°F for 30 minutes before cooling and determining the percentage of degraded chlorophyll.

Results

The results obtained are summarised in Fig.31 as a plot of percentage of degraded chlorophyll against concentration of sucrose added to the fluid.

Discussion of results

It can be seen the results give a curve similar in form to that obtained when the percentage of degraded chlorophyll was plotted against the concentration of sodium chloride added. In this case, (Fig.31) the optimum colour occurs when 1 - 1.5% sucrose is added.

It is difficult to explain why this curve should be of the same form as that shown in Fig.30 and it is thought the same explanations apply for both cases, i.e. it is due to an ionic effect and/or a certain concentration of salt or sugar affecting the extraction and transmission of extraneous compounds in the chlorophyll extract.

(iv) The effect of headspace oxygen on chlorophyll degradation

Workers have shown that the presence of oxygen has no effect on chlorophyll degradation unless light is present (70). The purpose of this study was to determine if the percentage of degraded chlorophyll in a sample which had been in contact with the headspace during retorting was different to that of a sample taken from a portion of the can remote from the headspace influence.

Experimental method

A 211 x 400 Code 3 can containing 200 gms of peas and 105 gm of

water was steam flow closed and retorted for 30 minutes at 238°F. The can was then cooled and samples of the contents removed from the top and bottom of the container. Throughout the preparation and processing the can was maintained in an upright position to ensure that any influence from the headspace was restricted to the peas in the top of the can.

Results

The percentage of chlorophyl degraded was the same for peas which had been in contact with the headspace during processing and those taken from the bottom of the can remote from the headspace. The headspace vacuum of the can after processing was about 8 inches mercury.

Discussion of results

Although peas canned in fluid, e.g. brine is a pack which has heat penetration characteristics typical of a convection pack, it is only movement of the fluid portion and not the peas which takes place on heating. Hence, peas which were at the top of the can prior to processing would maintain their position in the can throughout the retorting period. Other workers have shown that oxygen in the absence of light, has no effect on chlorophyll degradation, and so the results obtained are in agreement with their findings.

The vacuum in the can was 8 ins. Hg and in commercial practice, it would be considered to be slightly too low, i.e. more air was in the experimental can than would be typically encountered commercially. Since no difference in chlorophyll degradation was detected in the experimental pack, there would be even less chance of there being a

detectable difference in a commercial pack where less air was present in the headspace.

The results obtained indicate that samples could be taken from any position in a pack and no difference in the percentage of degraded chlorophyll would be detected.

(v) The effect of metal ions on chlorophyll degradation

The effect of metal ions has been studied by a number of workers and the phenomena of "regreening" has been examined using model systems (112).

The purpose of this study was to determine if one could expect any significant change in colour on processing peas in the presence of iron.

Experimental method

The following systems were set up using 211 x 400 cans containing 200 gms of peas.

1. A Code 3 can containing peas plus 105 gm of water.
2. A Code 3 can containing peas plus 20 ppm of iron (as $FeCl_2 4H_2O$) in solution.
3. A Code 3 can containing peas plus 100 ppm of iron in solution.
4. a Code 2 can containing peas plus 100 ppm of iron in solution.
5. To a Code 3 can containing peas was added 20 ppm of iron and 20 ppm of phosphate (as tetra sodium pyrophosphate) in solution.
6. The peas, in a Code 3 can, were soaked in 20 ppm of iron in solution for 1 hour and then 20 ppm of phosphate was added.
7. 200 gm of peas were treated with 10% acetic acid to degrade the chlorophyll, washed thoroughly with water, and placed in a

Code 3 can. 20 ppm of iron and 20 ppm of phosphate in solution were then added.

8. 200 gm of peas were treated with 10% acetic acid, thoroughly washed, placed in a Code 3 can and soaked in 20 ppm of iron in solution for 1 hour before adding 20 ppm of phosphate.

A net headspace of about 3/16 inch was present in each can. The cans were steam flow closed and heat processed for 30 minutes at 238°F. On cooling the cans were opened and examined for any marked difference in colour.

Results

There was no significant colour difference between the first six systems. Systems 7 and 8 showed even more pronounced degradation and were the typical olive green/brown colour of pheophytin and pheophorbide.

Discussion of results

It is evident from the results that "regreening" due to the presence of iron occurs over a period of time and the phenomenon is not apparent if the cans are tested immediately after processing. Other workers have indicated that the presence of iron in the concentration used in this study should be sufficient to cause regreening and that the reaction occurs during storage in warm conditions.

The results indicate that there is no immediate acceleration of the regreening reaction if the central magnesium atom of the chlorophyll molecule has already been replaced by two hydrogen atoms. Also, the reaction is not significantly accelerated by the chlorophyll being maintained in a more stabilised state by the presence of phosphate

raising the pH or by a short preliminary soaking of the peas in a solution containing iron.

(d) Summary

The thermal degradation of chlorophyll with time can be satisfactorily followed using the technique developed. Since this technique does not follow the loss of a pure homogeneous compound, e.g. chlorophyll a. or formation of a pure homogeneous compound, e.g. pheophytin a, any kinetic interpretation of the reaction with respect to the order would be difficult to justify.

The rate of chlorophyll degradation increases with decreasing pH. The concentration of sucrose or sodium chloride added to the pack affects the rate of chlorophyll degradation or influences the extraneous compounds also extracted by the acetone, and these in turn affect the transmission of the chlorophyll extract. Since the presence of oxygen in the headspace of the can does not affect chlorophyll degradation, samples for analysis can be taken from any point in the container.

S E C T I O N III

EXAMINATION OF CHLOROPHYLL DEGRADATION IN FOODS AS AN INDEX OF HEAT STERILISATION

It has been established by a number of workers (41, 110) that the degradation of a pure homogeneous chlorophyll to its corresponding pheophytin is a first order reaction and hence it can be expressed in a manner identical to the destruction of spores. This enables a direct correlation to be made between these two first order reaction systems and similar terms and symbols may be used in each case.

Q_{10} values (also known as temperature quotients and defined as the increase in reaction rate for a 10°C rise in temperature) are significantly higher for the thermal death of bacterial endospores and denaturation of proteins than for chemical reactions. For example, Q_{10} values for the destruction of spores are of the order of 10 while values for chlorophyll degradation are only of the order of about 1.5. This trend is also reflected in the Z values (the degrees fahrenheit required to give logarithmic reduction in the thermal degradation time of chlorophyll). For example, the Z value for spore destruction is generally taken as 18°F while in the case of chlorophyll degradation it may be 5 - 10 times this order.

The lower Q_{10} values (and higher Z values) for chlorophylls compared with bacterial spores explain the higher residual pigment retention and hence the colour, in a high temperature-short time sterilisation method than in a conventional thermoprocess of equivalent F value.

Thermal degradation rates for chlorophyll in spinach puree were

determined by Gupte, et al. (41) over the temperature range 260 - 300°F using thermal death-time tubes heated in a constant temperature oil bath. D values (the decimal reduction time in seconds) calculated from the slope of the thermal degradation rate curves, were plotted against temperature on semilogarithmic paper in order to obtain thermal reduction time curves. The Z values computed from the slopes of the thermal reduction time curves were 92° and 177°F for chlorophylls a. and b. respectively. The Fo values of the respective processes were 38.4 and 42.9 minutes.

On extending results obtained by Gold and Weckel (37) it is seen that on blanching peas at 190°F and heat processing at 240°F, an Fo value of 13.6 is required to degrade 99% of the chlorophyll present. This is neglecting any chlorophyll degradation during the "come up" period during which time up to 50% of the chlorophyll may be degraded.

The technique developed in this present study to follow the thermal degradation of chlorophyll does not enable the order of this reaction to be determined. However, it was demonstrated in Part "B" Section II that this method could be used to follow pigment degradation and the purpose of this study was to determine if the colour change occurring on heat processing garden peas could be used as an index of heat lethality.

Experimental method

211 x 400 Code 3 cans fitted with thermocouples were filled with 200 gms of thawed "frozen peas", plus 105 gms of a solution containing 2% sodium chloride and 2% sucrose. The cans which were then steam flow closed with a net headspace of 3/16 inch, contained ingredients with a formulation typical of those found in commercial packs. The cans were

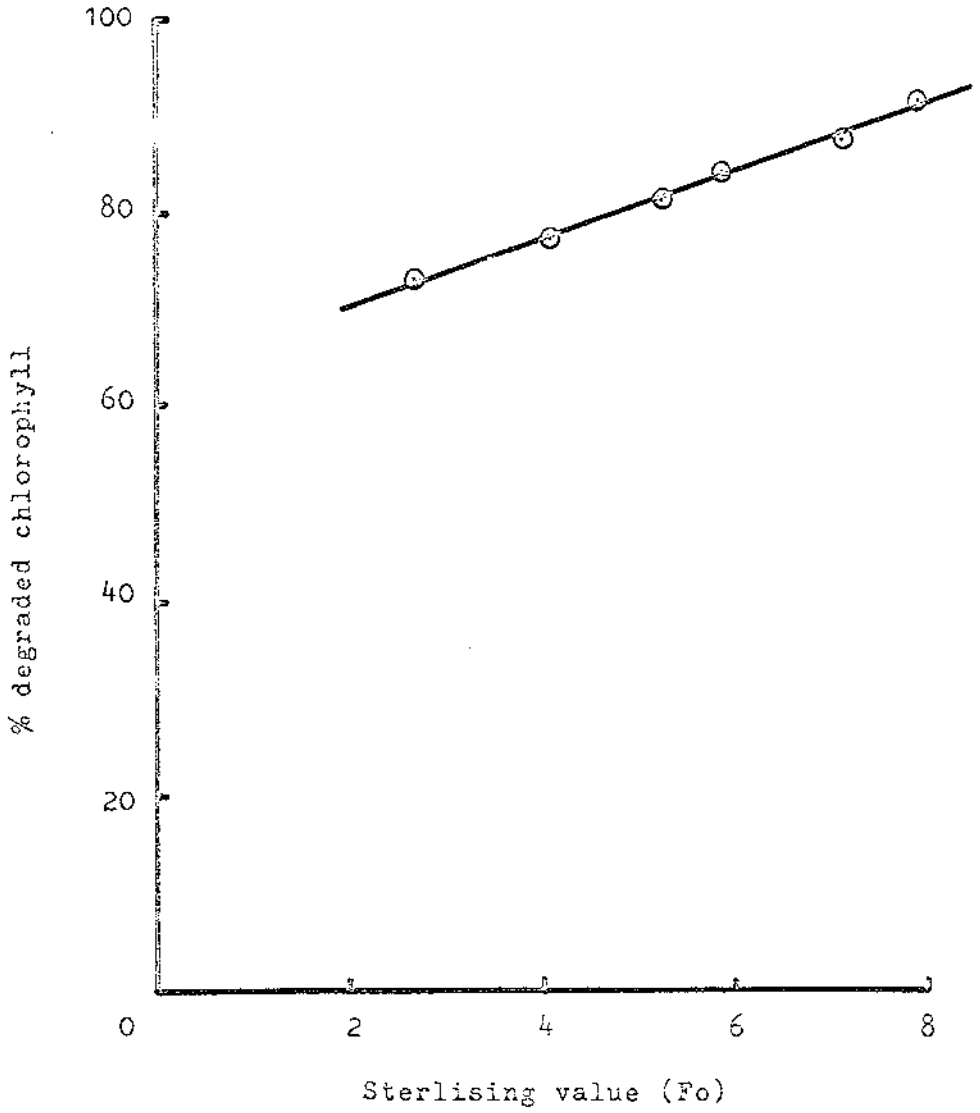


Fig. 32. THE RELATIONSHIP BETWEEN CHLOROPHYLL DEGRADATION AND STERILISING VALUE

then heat processed for varying times at a retort temperature of 238°F and after processing, they were cooled and the percentage of degraded chlorophyll determined in a sample of the contents (see appendix). During retorting, the temperatures within the cans were measured with a potentiometer at known time intervals and from these results Fo values for the processes were calculated.

Results

The results obtained are summarised in Fig.32 as a plot of the percentage of degraded chlorophyll against the Fo value of the process.

Discussion of results

Fig.32 shows a linear correlation exists between the percentage of degraded chlorophyll in a "commercial" pack of canned peas and the sterilising value of the process when degraded chlorophyll is determined by the technique developed in this study. The straight line plot shown in Fig. 32 differs from the curve shown in Fig.28 (a plot of the percentage degraded chlorophyll in a peas in water system against time of heating at retort temperature) due to the accumulative effect of a number of factors. These include the different time scales used on plotting the graphs, and the presence of salt and sugar in the system from which Fig.32 was determined. Salt and sugar have been shown to affect chlorophyll degradation and it is assumed these are the predominant factors responsible for the linear form of Fig.32 as when the "time at retort temperature" scale in Fig.28 is re-interpreted in terms of Fo values, the form of the plot is unchanged.

The linear nature of the plot shown in Fig.32 would enable this

correlation to be used much more conveniently as an index of heat lethality and the application of this technique to determine sterilising values in products of this nature would have distinct advantages commercially, e.g. the simplicity of the method, relatively inexpensive equipment required and reasonable accuracy (Fo's are able to be determined to about $\pm$ 0.5).

Conclusions

The thermal degradation of chlorophyll occurring in peas on heat processing can be used as an index of the heat lethality of the process. This method of determining sterilising values by following chlorophyll degradation could be applied to any product containing chlorophyll, e.g. broccoli, asparagus, green beans, etc., and hence be developed as a useful commercial technique.

However, it should be pointed out that since the analytical technique developed in this study does not enable the kinetics of the reaction to be deduced due to the heterogeneous nature of the system, the correlation of chlorophyll degradation with heat lethality would not be reliable if temperature and time both varied during the process. An indication of under or over processing could only be obtained from the results if either processing time or temperature (or both) were maintained constant during the process (see Conclusions, Part "D").

P A R T "C"

CORRELATION OF ENZYME ACTIVITY WITH HEAT LETHALITY

(a brief review of heat inactivation of enzymes, factors affecting this reaction and on the basis of previous work in this study, the feasibility of its utilisation as an index of heat lethality in typical commercial canning processes)

S E C T I O N I

THE HEAT INACTIVATION (DENATURATION) OF ENZYMES AND THE PHENOMENON OF REGENERATION

A considerable amount of work has been published on the theory of the thermal inactivation of enzymes, factors affecting this reaction and its kinetics. Much of the terminology used for describing the kinetics of this reaction is similar to that adopted to describe the thermal destruction of spores and hence it is possible to re-interpret many of the results to enable a correlation of the per cent residual enzyme activity present in a product with the heat lethality of the process to be established.

The theory of heat denaturation and regeneration and factors affecting these reactions are briefly reviewed before discussing the feasibility of using enzyme inactivation as an index of heat lethality.

A. THEORY OF HEAT DENATURATION

The irreversible coagulation of egg white on heating is a phenomenon familiar to all. Similar changes can be brought about not only by other physical means, such as vigorous shaking or stirring, and irradiation with ultra violet light or ultrasonic waves, but also by the action of acids, basis, organic solvents, salts of heavy metals, by urea, guanidine, salicylates, detergents, and other compounds. In all these reactions the proteins lose their original solubility; in most instances they become insoluble at their isoelectric range. Collagen, however, becomes soluble if heated

with water. We call the changed protein "denatured" in contrast to the original "native" protein. Denaturation is frequently accompanied by a loss of biological activity of the protein; enzymes lose their catalytic activity, hormones their physiological action, and antibodies their ability to combine with antigens.

It has been suggested that most enzymes are globular proteins and their action seems to depend on the specific internal structure of their molecules. This structure is stabilised by a great number of weak secondary intra-molecular bonds.

It was first proposed that heat causes a dehydration of the protein molecule or an establishment of peptide linkages between some of the free amino and carboxyl groups. In addition, the reverse reaction, the cleavage of peptide bonds by heat was also considered possible. Dilatometric measurements show, however, that denaturation by heat is not accompanied by any noticeable change in volume. The hydration of denatured proteins in humid air is only slightly lower than that of native proteins; their water binding, however, is of the same order of magnitude.

The first reasonable theory of denaturation was advanced by Wu (1925). In this theory it was proposed that denaturation consists of a rearrangement of the peptide chains in the protein molecule due to rupture by the denaturing agent of the weak bonds which hold these chains together. Applying these concepts of Wu it can be said that denaturation consists of an alteration of the chain conformation. The closely folded peptide chains are unfolded and/or refolded. The particular mode of denaturation will determine whether the disrupted peptide chains remain in the unfolded state, whether they are refolded to give the original specific pattern,

or whether there will result some other pattern different from the original internal structure. Obviously, the extent of denaturation can vary from slight structural changes to complete rearrangement of the peptide chains.

That denaturation is accompanied by unfolding of the peptide chains is indicated by the more intensive colour reactions given by denatured proteins than by the same protein in the native state. The higher reactivity of the denatured protein shows that some of the reacting groups are either buried inside the native globular protein or screened off in another manner, e.g. by neighbouring groups which repel the molecules of the reagent used or bind them without giving a colour reaction. The higher reactivity of the denatured proteins was first shown for the sulphydryl groups of cysteine and the dithio groups of cystine. By the nitroprusside test, by titration with ferricyanide, iodine, and by polarography, more sulphydryl and disulphide groups are found in the denatured than in the native protein. Similarly, the denatured protein gives more intensive colour reactions for tyrosine and arginine. The resistance of many native proteins to trypsin and other proteolytic enzymes as compared with the rapid proteolytic degradation of the same proteins in the denatured state may be explained on a similar basis.

All these observations support the view that some of the reactive groups of the native protein molecule are inaccessible to the different reagents but become accessible through the unfolding of the peptide chains. A rearrangement of the peptide chains upon denaturation is also indicated by an increase in flow birefringence and viscosity. Denaturation is usually accompanied by an increase in levorotation which is correlated with the change in chain conformation. Since changes in conformation

involve numerous hydrogen bonds, it is not surprising that denaturation is accompanied by a shift of the infra red absorption maximum of the co band, and also by a shift of the isoelectric point towards higher pH values. For the same reasons, the rate of denaturation depends, to a high extent, on pH and temperature. The rate of denaturation is low at the isoelectric point of the protein and increases in acid or alkaline solutions.

The complicated specific arrangement of the peptide chains in the native protein molecule can be disturbed by agents of different types. Almost any physical or chemical agent will alter the labile structure of the native protein. Mineral acids convert the negative - coo^- groups into - cooH groups, whereas the positively charged ammonium groups remain unchanged. The mutual electrostatic repulsion of these NH_3^+ groups causes subsequent unfolding, expansion of the molecules, and changes in the specific rotation. Similarly, treatment of the protein with alkali causes unfolding due to the mutual repulsion of the negatively charged groups. If the added acid or alkali is neutralised the protein is reconverted to its amphoteric state, although some of the original chain conformation may be changed.

If denaturation is brought about by heat, the protein remains in the zwitterion state. Hydrogen bonds between the peptide chains are cleaved by the thermal motion of the peptide chains, and bonds between hydrophobic groups may "melt". The insolubility of the heat-treated protein is probably caused by the s-s interchange reaction and the resulting formation of new intermolecular s-s bonds. This view is in agreement with the fact that collagen, which is free of cystine residues, is converted into soluble gelatin after heating. As long as the s-s bonds

of the native protein are intact, denaturation seems to be reversible.

An alteration of molecular weight is by no means an invariable consequence of denaturation. The coagulation of denatured protein is a distinctly secondary effect involving the formation of intermolecular linkages by groups which are displaced to the surface of the protein by the unfolding process. Interaction of denatured molecules has been variously ascribed to salt linkages between ionic groups of the protein, interaction of non polar amino acid residues, hydrogen bond formation, and sulphide linkages. It is thought that the characteristic insolubility of denatured proteins is derived primarily from intermolecular salt bridges between ionic groups. The significance of non polar group interactions of denatured proteins was advanced by Wu and supported by a number of investigations. Eilers (28) attributes the lower stability of denatured milk protein dispersions to the non polar surface groups, devoid of charge and hydration, which provide available sites for aggregation through van der Waal's attractive forces.

B. REGENERATION

With the advent of high temperature short time sterilisation (HTST), peroxidase has received attention primarily because of its high thermal death time coefficient (Z value) and apparent regeneration under HTST conditions (31, 150). Joffe and Ball (53) found there was a lag period of approximately 20 hours in establishing regeneration. This was attributed to a definite relaxation time of certain bonds critical to the partial renaturation necessary before the protein-porphyrin bonds could be reformed.

They found maximum regeneration occurred in 2-10 days of storage after inactivation, the longer time (10 days) being required following the higher temperature process. After maximum regeneration, there was a decrease in activity that was particularly noticeable for samples with the most extensive regeneration. Thus it appeared that the regenerated molecule was less stable than the native molecule.

The reason for the high initial regeneration rate followed by the decreased rate is not known, but a possible explanation based on the theory of Fischer (34) is: Upon heating, the enzyme is partially denatured, the protein fraction being precipitated. This precipitated protein absorbs and protects from heat some of the remaining undenatured enzyme. During the first few days of storage, the undenatured enzyme is eluted from the denatured protein and again becomes active in solution. Then the denatured protein, according to the postulation of Schwimmer (114) regenerates at a slow rate to give an increase in peroxidase activity. The longer the heat treatment, the greater the denaturation and the less possible is the generation of the undenatured enzyme during the first few days of storage as the repair of the enzyme molecules needs more time in samples heated for longer times at higher temperatures than samples heated for shorter times at lower temperatures.

Esselen and Anderson (30) found that in the temperature range of 215-290^{o}F the degree of heat required to prevent regeneration of peroxidase activity in vegetable tissues was 2 to 4 times greater than that required to destroy the enzyme on the basis of tests made immediately after heating. Zoueil and Esselen (150) reported 5 to 6 times the amount of heat was required.

S E C T I O N II

FACTORS AFFECTING HEAT INACTIVATION OF ENZYMES

1. Temperature

It has long been realised that enzyme reactions increase in rate with temperature and reach a maximum at the "optimum temperature". Above the optimum the rate decreases with further rise in temperature. The optimum temperature is not a constant for a given enzyme as it varies widely with such factors as enzyme and substract purity, presence of activators or inhibitors, and the method used in measuring the rate of the catalysed reaction. Also, the stability of different enzymes to thermal denaturation varies considerably and in general plant enzymes are more resistant to higher temperatures than those from animals. The apparent optimum temperature of enzyme reaction is explained by two independent processes being simultaneously accelerated by temperature; the catalysed reaction and the thermal inactivation of the enzyme. At temperatures lower than the optimum it is the catalysed reaction which is chiefly affected, while at temperatures higher than the optimum the inactivation of the enzyme by heat is the predominant factor.

The rate of inactivation of enzymes in solution increases rapidly with the temperature and in nearly all cases inactivation becomes virtually instantaneous at temperatures well below 100^{o}C, in the majority of cases below 70^{o}C.

The thermal inactivation of enzymes is usually unimolecular and increases exponentially with temperature in accordance with the Arrhenius equation over a fairly wide range, indicating that the activation energy

is independent of temperature changes.

As mentioned above, the inactivation of enzymes by heat is nearly always due to the inactivation of the enzyme protein. The temperature coefficient of inactivation is considerably higher than that of any other known process, with the exception of protein denaturation, and an exact proportionately between inactivation and denaturation has been shown for pepsin and trypsin. In these cases denaturation can be reversed under certain conditions and a recovery of activity parallel with the renaturation of the protein is obtained. The high coefficient of inactivation implies a high heat of activation for denaturation which makes the rate sharply dependent on temperature. This temperature at which extensive denaturation occurs is fairly closely defined. By applying the theory of absolute reaction rates it is possible to calculate the free energy and entropy of activation for denaturation; it is found that the value of the free energy is not exceptional and the high heat of activation is due to exceptionally high positive entropies of activation. This has been interpreted as indicating the breaking of a large number of weak bonds, such as hydrogen bonds, in the denaturation of proteins and enzyme. This is consistent with the accepted picture of denaturation as an opening up of the molecule by unfolding or separation of adjacent portions of peptide chains.

2. Time

If a series of progress curves for different temperatures is plotted it will be seen that each curve has its apparent optimum temperature and that this optimum temperature is not constant but falls as the time interval increases. That is, the optimum temperature, which heralds the onset of enzyme inactivation is dependent on time. The significance of

time is also shown by thermal inactivation of enzymes generally following first order kinetics.

3. pH

The rate of inactivation of enzymes, like other protein denaturations, is in most cases greatly dependent on the pH of the solution. The effect of pH varies greatly from one enzyme to another. In general there is a zone of maximum stability, not necessarily around the isoelectric point and the inactivation increases on the acid and alkaline sides. Many enzymes are inactivated even at room temperature at pH 4-5 and 8-10. Dimick, et al. (27) studied the heat inactivation of polyphenolase in fruit purees and found the pH of maximum stability to be: pears, 6.0; apricots, 3.9; applies, 6.2; grapes, 4.5. Only with apricots did the pH of maximum stability nearly coincide with that of the fresh fruit. Wilder (144) using peroxidase purified from horse radish, showed the pH of maximum activity to be 7, while lipoxidase was found to have maximum stability between pHs 5-7 (32). In acid products, the Z value (degress F required to give logarithmic reduction in the inactivation time) is generally smaller than that found in low acid foods.

4. Concentration

The rate of denaturation is also dependent on the concentration of enzyme. In the inactivation of pepsin by heat (22), the reaction was found to vary from 5th order in dilute solutions to 1st order in concentrated solutions and simultaneously, the activation energy decreased from approximately 150 kcal to 60 kcal/mole. The reduction in activation energy with increase in concentration implies the existence of strong

repulsive forces between the pepsin molecules. In concentrated solutions the molecules are in states of higher potential energy, with a consequent reduction in the activation energy needed for denaturation. On studying the effect of the concentration of apple and cucumber peroxidase on its thermal stability, Nebesky, et al. (85) showed that an increase in concentration of peroxidase increased the resistance to heat inactivation.

5. Type of enzyme

As indicated previously different enzymes are affected to different extents by the above factors, e.g. temperature. For example, adenylate kinase will withstand prolonged heating at 100°C at pH 1 and crystalline ∝ amylase isolated from B. stearothermophilus was shown to retain 90% of its activity after 1 hour at 90°C while certain other enzymes are denatured at room temperature. Of the plant enzymes, which are generally more heat resistant than enzymes of animal origin, peroxidase is one of the most heat resistant. The relative heat stabilities of peroxidase, catecholase and ascorbase have been examined (141).

Peroxidase from pea filtrates was shown to require 1 minute at 284°F for complete inactivation (31). Schwimmer (114) showed that different vegetables contain more than one peroxidase and that varieties vary in their activities towards substrates. Jermyn (52) isolated 4 different enzymes showing peroxidase activity from horse radish and Vetter, et al. (137) and later Yamamoto, et al. (149) have shown that sweet corn peroxidase is composed of 2 enzymes differing in their inactivation resistance.

Peroxidase from different sources have been shown to vary in their

heat inactivation properties (30).

6. Effect of test substrate

Work by Nebesky, et al. (85) indicated that the apparent thermal destruction times for peroxidase in certain fruit and pickle products varied according to the substrate used in testing for the presence of the active enzyme. Also, Keddi, et al. (99) showed the peroxidase activity of apple tissue varied with the method of analysis.

7. Moisture content

Herrlinger and Kiermeier (76) showed at constant temperature the peroxidase in sunflower seeds was inactivated quicker when the seeds had been soaked in water. Kiermeier and Koberlein (76) showed with crystallised catalase that at constant temperature, the inactivation increased with increased water content.

8. Ionic strength

Using phosphate buffer solutions, Wilder (144) demonstrated that the lower the ionic strength, the greater the activity of the enzyme (peroxidase).

9. Ingredients added to canned food packs

(a) Sugar

Studies (66, 85) on the effect of sugar concentration on the heat inactivation of apple and pear peroxidase show that an increase in sugar concentration increased the resistance of the peroxidase to inactivation although it had the opposite effect on peach peroxidase. Sapers and Nickerson (109) found the addition of 3.6% sucrose or 3.6% starch to spinach catalase had no effect on the

stability of the enzyme at 55°C.

(b) Salt

Studies (85, 109) show that the addition of small amounts of salt (2%) had no effect on the thermal stability of pickle peroxidase or catalase.

(c) Vinegar

It has been shown (66, 85) that the addition of 2.5 to 5% of vinegar solution markedly decreased the resistance of pickle peroxidase to inactivation by heat. This would be due to primarily a pH effect.

S E C T I O N III

CORRELATION OF ENZYME INACTIVATION WITH DESTRUCTION OF SPORES

A considerable amount of work has been done on peroxidase inactivation as it appears that the advantages of high temperature short time processing resulting from the rapid inactivation of bacteria by high temperatures are offset to a considerable extent by the relatively lower rate of peroxidase destruction at high temperatures. Also regeneration of peroxidase is undesirable as it leads to the production of off flavours.

It is generally agreed that the thermal destruction of enzymes follows 1st order kinetics (31,32,53,149), although some anomalies are present (109,149).

Enzyme inactivation has also been shown to obey the Arrhenius equation. Hence the kinetics of this reaction indicated a dependence on time and temperature in a manner analogous to the thermal destruction of bacterial spores. This has lead to the adoption of terminology used in spore destruction to describe enzyme inactivation. For example, Farkas, et al. (31) found between 100 - 150°C, the heat inactivation curve for peroxidase indicated an Fo of 6 and a Z value of 48°F.

Z values, F values and D values have been determined for a number of enzymes in a variety of fruits and vegetables under varying conditions (1,30,31,150).

The Z values are generally considerably higher than those encountered with spore destruction, and values of over 80°F have been determined (53).

Due to their similar destruction characteristics, enzyme inactivation

and spore destruction can be directly related. Hence by determining the per cent residual enzyme activity in a sample, the number or per cent of spores surviving can be ascertained. Adams and Yawger (1) found the heat inactivation of peroxidase in peas had a Z value of 67°F. They then reinterpreted this data and plotted as log lethality value (F_{250}^{18}), versus retort temperature (°F) and so determined the process conditions necessary to destroy peroxidase. From their results they found that to destroy the peroxidase at a retort temperature of 240°F, an Fo value of about 3.3 was required; at 250°F, Fo = 9; and at 280°F, Fo = 100.

The results of Adams and Yawger indicate that at high temperatures, bacterial sterility will be achieved before significant enzyme destruction has occurred and this could lead to problems of off flavours etc., in the product due to enzyme action. In the temperature range 240 - 250°F bacterial sterility is achieved at approximately the same time as total enzyme destruction, while at temperatures less than this, all the enzyme is destroyed before the required heat lethality is reached. Hence at high temperatures enzyme destruction would be the overriding factor while at low temperatures, total enzyme destruction would indicate a certain percentage of bacterial sterility had been achieved.

In acid conditions, the enzyme is inactivated more readily and so in this case also, total enzyme destruction could only be used as an index of partial bacterial sterility.

Resende, et al. (100) studied the thermal destruction and regeneration of enzymes in green bean and spinach puree at pH 8.3 and assays for peroxidase showed a Z value of 88° and 29°F respectively. Spinach chlorophyllase had a Z value of 22°F. They found an Fo process value of

5.0 was sufficient to prevent regeneration of peroxidase in spinach at temperatures up to 290°F, while for green beans at temperatures of 270° and 290°F, process values of 31 and 245 respectively were required to prevent peroxidase regeneration.

These results indicate that although peroxidase inactivation in spinach at pH 8.3 could be a convenient method of determining heat lethality, it would not be suitable for beans because the desired bacterial sterility would be achieved before significant enzyme inactivation had occurred and so the process would have to be extended to prevent the development of off flavours in the product.

Hence, it can be seen that by following enzyme inactivation, an indication of the sterilising value of the process can be obtained at any retort temperature although its application as a feasible commercial technique could have limitations because:

1. Most enzymes are destroyed well before the desired bacterial sterility is achieved. This is especially so in low temperature or high acid conditions.
2. In high temperature conditions, enzyme destruction and not bacterial sterility governs the process.
3. The temperature range over which enzyme destruction could be correlated conveniently with heat lethality is critical and small.
4. The rate of enzyme destruction is influenced by a number of factors which could be difficult to control, e.g. concentration of enzyme and pH could depend on the variety of raw material etc.

P A R T "D"

CONCLUSIONS AND SUGGESTIONS FOR FURTHER STUDY

CONCLUSIONS

The object of this study was to carry out preliminary studies to ascertain whether chemical reactions occurring in a selected model system or food could be used as indices of heat lethality. The results obtained indicate that the chemical reactions selected could be correlated with sterilising values but since simple kinetics were not demonstrated due to the selection of the reaction to be followed or the method of analysis, the correlations could not be extended to show the integrated effect of both processing time and temperature on the degree of browning.

However, the results obtained could have commercial application within the limitations listed below:

1. The technique could be used to determine with reasonable accuracy the sterilising value of a process where the processing temperature varied not more than a few degrees throughout the process. The degree of under or overprocessing could be estimated from the extent of the chemical change occurring in the reference system.

2. Where a standard processing time is adhered to, any variability in the results given by the chemical system would indicate that the processing temperature had fluctuated during the process and from the extent of the chemical change in the reference system, an indication of whether under or over processing had resulted could be obtained.

   No significance can be attached to a variability in the reference system if the processing time and temperature are both allowed to deviate.

3. In the case of a continuous type cooker, if the temperature profile

and hence amount of effective heat is known, then the degree of under or overprocessing resulting from inconsistencies in processing time can be estimated from the extent of the change in the reference system.

4. In a continuous cooker, if the processing time is constant, an indication of under or overprocessing and hence conditions within the cooker, can be obtained from the reference system.

It should be pointed out that the technique of correlating a chemical change with heat lethality is not envisaged as a method of determining desirable processes for new products and the standard methods should be strictly adhered to for this estimation. However, the usefulness of the technique as a quality control check on fluctuations in time and temperature in static and continuous cookers is obvious. Zones of poor heat contact in a retort could also be checked for by this method.

SUGGESTIONS FOR FURTHER STUDY

The aim of this study was to investigate and establish if the heat lethality attained in a system could be determined from an associated change, such as colour or enzyme activity, taking place in the system or in a tracer can subjected to the same conditions.

Although it is submitted that this aim has been fulfilled, there are a number of suggestions arising from this study which warranted further investigation and would enable the concept of correlating a chemical change taking place in a product with the heat lethality of the process to become commercially a more acceptable technique.

It is generally accepted that the thermal destruction of bacterial

spores obeys first order kinetics and so if the chemical system adopted to follow spore survival is also a first order reaction and obeys the Arrhenius equation, then the correlation is made so much more convenient. A suitable chemical system could be more readily selected where a tracer can technique is used, but where this correlation is to be made in a food product it may be very difficult to find a suitable chemical change taking place, let alone find a simple, quick method of analysing the change. Carbonyl-amino browning is a relatively common chemical reaction taking place in heat processed foods although in some cases the colour change may be unnoticeable. In this study products were selected in which the colour change could be detected and followed relatively easily but the reaction could also be followed by other means such as changes in fluorescence, reducing power, foaming properties, production of carbon dioxide or water, formation of intermediate compounds, e.g. hydroxymethylfurfural, and so on.

Hence it can be seen that there are a large number of methods available to follow this and other reactions and depending on the situation some are more suitable than others. For example, carbonyl-amino browning undoubtedly occurs in heat processed peas but in this case any colour change is marked by other pigments present, and in this case, the degradation of the chlorophyll pigments serves as a very sensitive index of heat input.

Considerable further work could be carried out on the selection of a tracer can system with simple kinetics and a suitable reaction rate constant and activation energy to enable a convenient relationship between chemical index, temperature and time to be developed and used commercially

as a check on heat processing.

The glucose-glycine system selected in this study does not follow simple kinetics and this limits its usefulness where processing temperatures may change during a process unless involved mathematics are introduced. An index or system which was not affected by changing temperatures would be very convenient for determining sterilising values commercially and so an investigation for such a system would be warranted.

Where analysis of a food product is carried out, similar difficulties in correlating the chemical index with time and temperature would also be encountered unless care was taken to select a reaction with simple kinetics.

Other browning mechanisms, for example, the degradation of ascorbic acid or the oxidation of polyhydroxy compounds could be investigated to determine if more convenient "colour change" systems are available. However, the application of this technique is not limited to "colour change" systems although this method does often enable measurements to be made very readily. Many organic reactions proceed at the desired rates under conditions of commercial processing and could perhaps be followed and correlated with heat lethality. A reaction of this type followed by a simple and quick titration method could be suitable.

A considerable amount of work has been carried out on the thermal destruction of thiamine and other heat sensitive compounds found in foods (51) and this type of reaction could warrant further investigation with a view to correlating it with heat lethality in tracer systems or food products.

Reactions occurring in tracer cans give a result which is directly related to the conditions of heat penetration present in the tracer can and for these results to be of value, they must be correlated with processing time or a range of heat penetration characteristics. f_h values can only be introduced where linear heat penetration curves exist and a correlation should be determined over a wide range of these values, i.e. to much higher values than those used in this study. Where linear heat penetration curves do not exist, the reaction occurring in the tracer can must be correlated with processing time and a preliminary investigation is required to determine at what time the desired sterilising value in a product is reached. This inconvenience could be overcome by having a variety of tracer can systems with differing heat penetration characteristics or by having a standard tracer can system in which the rate of heat penetration could be adjusted to be identical to that of the product to be tested. This could be extended to include tracer systems with "broken" heating curves so the results could be correlated directly with products with similar heating characteristics.

However, where heat penetration characteristics are used in a correlation, the f_h value in question must be determined accurately and if it is calculated from a product, it must be consistent or else serious errors could be introduced. Where the correlation between some index and heat lethality is made in a product, i.e. a tracer can is not involved, changes in the f_h will not affect the result as long as the concentration of reactants remains constant and other factors affecting the reaction are not altered.

Where an index system is influenced by a large number of factors care must be taken to keep as many factors as possible constant. In some cases, e.g. fish, this may be almost impossible and this could apply to many natural products. For this type of product it would be best to develop some index in which the reactants were not influenced by variables such as climate etc. However, this drawback is overcome by using a tracer can index system.

A P P E N D I X

A P P E N D I X

I. CAN NOMENCLATURE

A. Can sizes

The system used to describe can sizes is: the first group of figures gives the diameter, the second the height. Within the groups of figures, the first figure represents whole inches, and the following two figures represent fractions of an inch in sixteenths. e.g. a 211 x 400 can would have a diameter of two 11/16 inches and a height of four inches.

B. Can lacquering codes

The code system for can coatings used in this study is:

Code 2 - tinplate (no body or end lacquers)

Code 3 - sulphur resistant lacquer (phenolic meat lacquer on can body and a zinc oxide pigmented anti-sulphur oleoresinous type lacquer on the ends)

Code 6 - acid resistant lacquer (double epoxy phenolic lacquer on body and double oleoresinous acid resisting lacquer on the ends)

C. Net headspace

In accordance with the New Zealand Standard Specification NZSS 1715: 1963, 3/16 inches is allowed for the lid so in a can with a 3/16 inch net headspace, before seaming, the surface of the contents would be $\frac{3}{8}$ of an inch below the top surface of the can.

D. Drained weights (peas)

The New Zealand Standard Specification NZSS 1911: 1965 states that

for a 30 oz can, the minimum drained weight should be not less than 19 oz of peas and that this relative proportion should be constant for all sized cans.

E. Can vacuum

Throughout this study, can vacuums were determined with a B.F.M.R.A. patent vacuum gauge, giving results in "inches of mercury". The results obtained were true readings in the sense that the technique of measuring the vacuum compensates for air present in the gauge.

II. METHOD FOR DETERMINING THE DEGREE OF BROWNING IN CASEIN-GLUCOSE SOLUTIONS

After processing and cooling to room temperature, the contents of the cans were poured into beakers and kept under chilled conditions (40-50°F) for 24 hours to allow equilibrium to be reached. Readings of the degree of browning were determined using filter B in an EEL Nephelometer head coupled to an EEL Unigalvo type 20 galvanometer standardised on 100 with a control casein-glucose solution which had undergone no browning.

III. METHOD FOR DETERMINING THE DEGREE OF BROWNING IN GLUCOSE-GLYCINE SOLUTIONS

The degree of browning of a sample was given by the transmission obtained by using filter 1 (370-515 mμ violet spectrum) in a Unicam SP 1300 photoelectric colorimeter. The colorimeter was standardised (transmission = 100) against an unheated glucose-glycine solution of similar formulation to that being tested.

It was found that the contents of the can could be tested up to 3 hours after processing and cooling to room temperature. On opening a can, the degree of browning of the contents had to be determined immediately.

IV. PREPARATION OF BEANS AND ANALYSIS OF BROWNING IN BAKED BEANS IN TOMATO SAUCE

The dry haricot beans were prepared for canning as below:

1. Soaked in excess water at 90°F for 4 hours.
2. Drained, soaked one hour in excess cool water.
3. Drained, soaked overnight in cold water.
4. Drained, blanched in water at 210°F for 12 minutes.
5. Cooled immediately.

The beans, on preparing, absorbed about an equal volume of water. The tomato sauce added to the beans was donated by a large food processing company. The degree of browning taking place in the beans on heat processing was determined as below:

1. A representative sample of about 50 gms of beans was taken from the can to be tested and washed thoroughly in water to remove all visible traces of tomato. It was very important that a representative sample be taken as some beans had split skins and hence browned more readily.
2. Excess water adhering to the beans was removed.
3. 25 gm beans was homogenised for 5 minutes with 40 ml petroleum ether using a MSE homogeniser.
4. The petroleum ether was decanted from the blended beans.

The petroleum ether, as well as being present as a fluid to enable the homogenisation to proceed, removed any carotenoid pigments which were present and could interfere with results.

5. The reflectance porcelain square was filled with a portion of the blended beans and the surface smoothed.

6. The reflectance from the beans was measured by placing the EEL reflectance spectrophotometer head on the sample and reading the result indicated on the attached galvanometer (EEL Unigalvo Type 20) using filter 603. (This filter gave maximum sensitivity).

The galvanometer was standardised by adjusting the reflectance given by placing the spectrophotometer (with filter 603 in position) on an N7 Munsell grey card to 100. Although magnesium carbonate is the accepted standard, in this case the Munsell card could be adopted satisfactorily as it is a standard colour and reflecting surface, and since only one filter was used throughout the trial, all readings were directly related to the adopted standard. The reflectance given by the Munsell standard was the same as that given by a control sample of beans, i.e. beans which had not been heated but treated identically to beans which had been processed.

V. ESTIMATION OF THE DEGREE OF BROWNING IN HEAT PROCESSED CREAM STYLE CORN

To determine the degree of browning taking place on heat processing cream style corn, the following procedure was adhered to.

1. The can to be examined was opened and a plug about $\frac{3}{4}$ inch in diameter was removed from the centre of the contents. A plug from the region

immediately adjacent to the central vertical axis gave a sample in which a correlation between browning and heat lethality was consistent with the General Method of process evaluation.

2. 65 gm of corn was taken from the plug sample and homogenised with a Silverson laboratory model mixer-emulsfier until a uniform slurry was obtained.

3. To this slurry was added 35 ml of carbon tetrachloride and this was homogenised for 5 mimutes.

4. A centrifuge tube about $\frac{3}{4}$ inch in diameter was filled with the slurry and centrifuged at 2,700 r.p.m. for 15 minutes using an IEC International centrifuge, universal model UV. This centrifuge had a head diameter of $19\frac{1}{2}$ inches where the "head diameter" is the distance between the inside bottoms of opposite cups measured through the centre of rotation of the centrifuge while the cups are horizontally extended.

5. After centrifuging all the surface skin was carefully removed from the tube and the top slurry layer poured into a square porcelain reflectance dish to a depth of 2mm from the top.

6. With filter 604 in position, the reflectance of the sample was determined using an EEL reflectance spectrophotometer head coupled to an EEL Unigalvo Type 20 galvanometer. The galvanometer was standardised previously to give a reading of 100 when the reflectant spectrophotometer was placed on an N7 Munsell grey card.

VI. TO DETERMINE THE DEGREE OF BROWNING IN HEAT PROCESSED FISH

Although in this study the sample had already been removed from the can and mixed with blood tissue, the technique used below could be applied for determining the degree of browning in any fish sample.

1. To 30 gm of fish sample was added 30 ml of a 12% solution of trichloracetic acid.

2. The fish plus acid mixture was homogenised with a Silverson laboratory model mixer-emulsifier until a uniform slurry was obtained.

3. The slurry was centrifuged for 5 minutes at 2,700 r.p.m. using an IEC International centrifuge, Universal model UV.

4. The precipitate was removed from the bottom of the centrifuge tube and packed firmly in a square porcelain reflectance dish to 2 mm below the surface.

5. The reflectance was determined immediately using an EEL reflectance spectrophotometer head fitted with filter 603, coupled to an EEL Unigalvo Type 20 galvanometer. The spectrophotometer was previously standardised on an N7 Munsell card to give a reading of 95 on the galvanometer.

VII. THE METHOD OF CHLOROPHYLL EXTRACTION AND ESTIMATION

A. Extraction of chlorophyll

1. A representative 20 gm sample of peas was removed from the can to be tested and together with 70 ml of 85% acetone and a small quantity (about 0.1 gms) of calcium carbonate, disintegrated to a uniform

slurry by blending for 10 minutes with an MSE homogeniser.

2. The slurry was washed with 85% acetone into a buchner funnel and filtered under suction through a Whatman No.30 filter paper. The washing was continued with 85% acetone until all the colour was extracted from the material, i.e. until the filtrate remained colourless on passing through the filter.

3. A buchner funnel fitted with a Whatman No.32 filter paper and a slurry of Celite 545 and filtrate was prepared and poured into the newly prepared buchner funnel to form a filter pad.

4. The filtrate was passed twice through the filter pad using suction to ensure the filtrate became clear.

5. The filtrate was transferred to a 250 ml volumetric flask and made up to volume with 85% acetone.

It was important to maintain all steps as quantitative as possible. This extraction had to be carried out in the absence of sunlight to prevent degradation of the extracted chlorophy..

B. Estimation of the per cent degraded chlorophyll

1. To 9 ml of the chlorophyll extract prepared above was added 1 ml of 85% acetone and the contents thoroughly mixed.

2. The transmission of this solution was determined using filter 1 positioned in a Unicam SP 1300 photoelectric colorimeter. Filter 1 had a wavelength of 370 - 515 mμ. The colorimeter was standardised to give 100% transmission with 85% acetone.

The transmission value obtained above was reading (a).

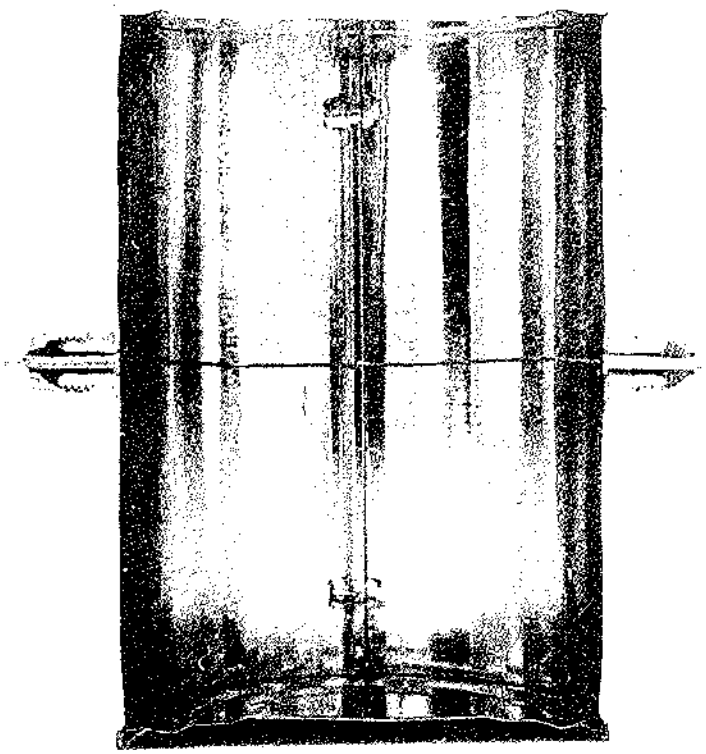


Fig. 33. SECTIONED CAN SHOWING POSITION OF THERMOCOUPLE AND GLAND FITTING

3. To 9 ml of the chlorophyll extract was added about 0.2 gm of oxalic acid and this was then made up to 10 mls with 85% acetone. If insufficient oxalic acid was used, all the chlorophyll would not be degraded in the required time, while if too much oxalic acid was used the solution would become saturated and turbid due to undissolved crystals.

4. The contents were mixed to ensure all the oxalic acid dissolved.

5. The solution was allowed to stand for at least 5 minutes to ensure complete degradation of chlorophy..

6. The transmission of this solution was determined using the Unicam colorimeter standardised as above. The solution had to be tested within 15 minutes of preparation. The value obtained for the transmission was reading (b) and from the standard curve, the percentage of degraded chlorophyll was found from $\frac{(a)}{(b)}$.

VIII. TEMPERATURE MEASUREMENT

1. A Cambridge workshop potentiometer (Type 44228, with built-in potential source) was used in this study to determine temperatures within cans.

2. The thermocouples were positioned in the geometric centre of the can as shown in Fig.33 by soldering to the can wall 1/16 inch ID copper pipe-olive compression fittings cut in two, with a gland of $\frac{1}{8}$ inch insertion rubber making the seal.

3. Calibrated copper-constantan thermocouple wire of gauge 24 was used. Precautions taken to prevent errors in temperature measurement were:

(i) Thermocouple wires were kept separated in the retort.

(ii) Care was taken to ensure insulation on the thermocouple wires in the cans was intact.

(iii) All joints were soldered.

(iv) All soldered joints were kept as small as possible.

(v) All thermocouple wire was calibrated (90).

B I B L I O G R A P H Y

Ref. No.

1. ADAMS, H.W. and YAWGER, E.S. Enzyme inactivation and colour of processed peas. Food Technol. 15, 314 (1961).

2. ALSTEAND, D.V. and ECKLUND, O.F. The mechanics and interpretation of heat penetration tests in canned foods. Food Technol. 6, 185 (1952).

3. ANET, E.F.L.J. and REYNOLDS, T.M. Reaction between amino acids, organic acids and sugars in freeze dried apricots. Nature, 177, 1082 (1956).

4. ANET, E.F.L.J. and REYNOLDS, T.M. Chemistry of non enzymic browning. I. Reactions between amino acids, organic acids and sugars in freeze dried apricots and peaches. Aust. J. Chem. 10, 182 (1957).

5. BALL, C.O. "Thermal processes for canned foods". Nat. Res. Council Bull. Part I. No.37, 76 (1923).

6. BALL, C.O. Mathematical solution of problems on thermal processing of canned foods. University of California (Berkeley) Publication, Public Health 1, 15 (1928).

7. BALL, C.O. and OLSON, F.C.W. Sterilisation in Food Technology. McGraw Hill, 1957.

8. BARNES, H.M., and KAUFMAN, C.W. Industrial aspects of browning reaction. Ind. Eng. Chem. 39, 1167 (1947).

9. BAUMGARTNER, J.G. Canned foods; an introduction to their microbiology. 3rd Edition, 1949 Churchill.

10. BENGDOLL, M.S. and HOLMES, E. The heating of sucrose solutions. I. The relationship of 5-(hydroxymethyl) - furfural to colour formation. Food Res. 16, 50 (1951).

11. BIGELOW, W.D. The logarithmic nature of TDT curves. J. Infectious Diseases 29, 528 (1921).

12. BIGELOW, W.D., BOHART, G.S., RICHARDSON, A.C. and BALL, C.O. Heat penetration in processing canned foods. Nat. Canners Assoc. Bull. No. 16L (1920).

13. BIGELOW, W.D. and ESTY, J.R. "Thermal death point in relation to time of typical thermophilic organisms". J. Infectious Diseases 27, 602 (1920).

Ref. No.

14. BOARD, P.W., COWELL, N.D. AND HICKS, E.W. Studies in canning processes. III. The cooling phase of processes for products heating by conduction. Food Res. 25, 449 (1960).

15. BOARD, P.W. Determination of thermal processes for canned foods. C.S.I.R.O. Div. of Food Res. Circ. No.7-P, (1965).

16. BOHART, G.S. and CARSON, J.F. Effects of trace metals, oxygen and light on the glucose-glycine browning reaction. Nature 175, 470 (1955).

17. BORSOOK, H., ABRAMS, A. and LOWY, P.H. Fructose-amino acids in liver; stimuli of amino acid incorporation in vitro. J. Biol. Chem. 215, 111 (1955).

18. BURT, J.R. and JONES, N.R. Changes in sugar phosphates of chilled codling (Gadus callarias) muscle. J. Sci. Food Agr. 12, 344 (1961).

19. BURT, J.R. Glycogenolytic enzymes of cod (Gadus callarias) muscle. J. Fisheries Res. Board. Can. 23, 527 (1966).

20. BURTON, H. Colour changes in heated and unheated milk. I. The browning of milk on heating. J. Dairy Res. 21, 194 (1954).

21. BURTON, H. Colour changes in heated and unheated milk. III. The effect of variation in milk composition on the whitening and browning of separated milk on heating. J. Dairy Res. 22, 82 (1955).

22. CASEY, E.J. and LAIDLER, K.J. The mechanism of the heat inactivation of pepsin. Science 111, 110 (1950).

23. CHICHESTER, C.O., STADTMAN, F.H. and MACKINNEY, G. On the products of the Maillard reaction. J. Amer. Chem. Soc. 74, 3418 (1952).

24. CHICK, H. The process of disinfection by chemical agencies and hot water. J. Hygiene 10, 237 (1910).

25. CLYDESDALE, F.M. and FRANCIS, F.J. Chlorophyll changes in thermally processed spinach as influenced by enzyme conversion and pH adjustment. Food Technol. 22, 793 (1968).

26. DAVIS, W.L. and GILL, E. Investigations on fishy flavour. J. Soc. Chem. Ind. (London) 55, 141 T (1936).

27. DIMICK, K.P., PONTING, J.D. and MAKOWER, B. Heat inactivation of polyphenolase in fruit purees. Food Technol. 5, 237 (1951).

28. EILERS, H., SAAL, R.N.J. and VAARDEN, M. VAN DER. "Chemical and physical investigations on dairy products". Elsevier Book Co. New York, 1947.

Ref. No.

29. ELLIS, G.P. The Maillard reaction. Adv. Carbohyd. Chem. 14, 63 (1959).

30. ESSELEN, W.D. and ANDERSON, E.E. Thermal destruction of peroxidase at high temperatures. Food Res. 21, 322 (1956).

31. FARKAS, D.F., GOLDBITH, S.A. and PROCTOR, B.E. Stopping off flavours by curbing peroxidase. Food Eng. 28, 52 (1956).

32. FARKAS, D.F. and GOLDBLITH, S.A. Studies on the kinetics of lipoxidase inactivation using thermal and ionising energy. J. Food Sci. 27, 262 (1962).

33. FISCHER, H. and ORTH, H. "Die chemie des pyrrols" Akadem Verlagsgesellschaft, Leipzig, 1934 - 1940.

34. FISCHER, O. Milk peroxidase. Milchw Forsch 21, 285 (1943).

35. FRANKEL, M. and KATCHALSKY, A. The interaction of α amino acids and peptides with sugars in aqueous solution. Biochem. J. 31, 1595 (1937).

36. GHANEKAR, D.S., BAL, D.V. and KAMALA, S. Enzymes of some elasmobranches from Bombay III Analyses of Scoliodon sorrakowah and Sphyrna blochii. Proc. Indian Acad. Sci. 43B, 134 (1956).

37. GOLD, H.J. and WECKEL, K.G. Degradation of chlorophyll to pheophytin during sterilisation of canned green peas by heat. Food Technol. 13, 281 (1959).

38. GOLDBLITH, S.A., JOSLYN, M.A. and NICHOLSON, J.J.C. "An introduction to the thermal processing of foods". A.V.I. Connecticut, (1961).

39. GOODING, E.G.B. and HOCHWORTH, R.B. An accelerated storage test for dehydrated vegetables. J. Sci. Food Agric. 8, 498 (1957).

40. GOODWIN, T.W. "Chemistry and biochemistry of plant pigments" Academic Press, 1965.

41. GUPTE, S.M., EL BISI, H.M. and FRANCIS, F.J. Kinetics of thermal degradation of chlorophyll in spinach puree. J. Food Sci. 29, 379 (1964).

42. HAAS, V.A., STADTMAN, E.R., STADTMAN, F.H. and MACKINNEY, G. Deterioration of dried fruits. I. The effect of sugars and furfurals. J. Amer. Chem. Soc. 70, 3576 (1948).

43. HAUGAARD, G., TUREMAN, L. and SILVESTRI, H. A study on the reaction of aldoses and amino acids. J. Amer. Chem. Soc. 73, 4594 (1951).

Ref.No.

44. HICKS, E.W. On the evaluation of canning processes. Food Technol. 5, 134 (1951).

45. HICKS, E.W. Some implications of recent theoretical work on canning processes. Food Technol. 6 (5), 175 (1952).

46. HICKS, E.W. Revised table of the Fh function of Ball and Olson. Food Res. 23, 396 (1958).

47. HICKS, E.W. Uncertainties in canning process calculations. J. Food Sci. 26, 218 (1961).

48. HIXON, C&O D. and CHICHESTER, C.O. Kinetics of the pheophytinisation of chlorophylls. Paper B 2.17 presented at the 2nd International Congress of Food Science and Technology, Warszawa, Poland, 1966.

49. HODGE, J.E. Dehydrated foods. Chemistry of browning reactions in model systems. J. Agr. Food Chem. 1, 928 (1953).

50. HODGE, J.E. Non-enzymic browning reactions. In "Chemistry and physiology of flavours". H.W. Schultz ed. A.V.I. Connecticut, 1967.

51. JACKSON, J.M., WEAST, J.F. and PILCHER, K.V. The effect of canning procedures on vitamins in foods. Proc. Inst. Food Technol. p.81, (1945).

52. JENCKS, W.A. Horse radish peroxidase. Nature 169, 488 (1952).

53. JOHNS, R.H. and BAILEY, C.O. Kinetics and energetics of thermal inactivation and the regeneration rates of a peroxidase system. J. Food Sci. 27, 587 (1962).

54. JONES, I.D., WHITE, R.C. and GIBBS, E. Some pigment changes in cucumbers during brining and brine storage. Food Technol. 16, (3), 96 (1962).

55. JONES, I.D., WHITE, R.C. and GIBBS, E. Influence of blanching and brining treatments on the formation of chlorophyllides, pheophytins and pheophorbides in green plant tissue. J. Food Sci. 28, 437 (1963).

56. JONES, M.G. The temperature dependence of the lethal rate in sterilisation calculations. J. Food Technol. 3, 31 (1968).

57. JONES, N.R. "Browning" reactions in freeze-dried extractions from the skeletal muscle of codling (Gadus callarias). Nature 174, 605 (1954).

58. JONES, N.R. The free amino acids of fish. 1 methylhistidine and β-alanine liberation by skeletal muscle anserinase of codling (Gadus callarias). Biochem. J. 60, 81 (1955).

Ref. No.

59. JONES, N.R. Discolouration of muscle preparations from codling (Gadus callarias) by degradation products of 1-methylhistidine. Nature 177, 748 (1956).

60. JONES, N.R. Free sugars in chill-stored trawled codling (Gadus callarias) muscle. J. Sci. Food Agr. 9, 672 (1960).

61. JONES, N.R. and MURRAY, J. Degradation of adenine- and hypoxanthine-nucleotide in the muscle of chill-stored trawled cod (Gadus callarias). J. Sci. Food Agr. 13, 475 (1962).

62. JONES, N.R. Observations on the relation of flavour and texture to mononucleotide breakdown and glycolysis in fish muscle. p.109 from "Proc. 2nd International Congress Food Science and Technology" Warszawa, Poland, 1966 ed. by D.J. Tilgner and A. Borys.

63. JONES, N.R. and MURRAY, J. The acid soluble nucleotides of codling (Gadus callarias) muscle. Biochem. J. 77, 567 (1960).

64. JOSLYN, M.A. Role of amino acids in the browning of orange juice. Food Res. 22, 1 (1957).

65. JOSLYN, M. and MACKINNEY, G. The rate of conversion of chlorophyll to pheophytin. J. Am. Chem. Soc. 60, 1132 (1938).

66. KAPLAN, A.M., ESSELEN, W.B. and JELINEK, C.F. Enzyme systems of canned acid foods. Ind. Eng. Chem. 41, 2017 (1949).

67. KASSEMSEN, B.O., SAN MIGUEL, B., MURRAY, J. and JONES, N.R. Nucleotide degradation in the muscle of iced haddock (Gadus aeglefines), lemon sole (Pleuronectes microcephalus) and plaice (Pleuronectes platessa). J. Food Sci. 28, 28 (1963).

68. KATCHALSKY, A. and SHLAON, N. Kinetics of aldose-amino acid interaction. Biochim et Biophys acta 10, 290 (1953).

69. LANDER, H.F. and HARRIS, R.S. Browning of ascorbic acid in pure solution. Food Res. 15, 79 (1950).

70. LAMOTT, C. Etude spectrophotometrique des modifications des chlorophylles a et b sous l'influence de divers agents physiques et chimiques. Rev. Ferment. Industr. aliment 11, 34 (1956).

71. LEA, C.H. and HANNAN, R.S. Studies of the reaction between proteins and reducing sugars in the "dry" state. I. The effect of activity of water, of pH and of temperature on the primary reaction between casein and glucose. Biochim. et Biophys. acta 3, 313 (1949).

72. LEA, C.H. The role of amino acids in the deterioration of food: the browning reaction. Chem. and Ind. 155, (1950).

Ref. No.

73. LEGAULT, R.R., TALBURT, W.F., MYLNE, M. and BRYAN, L.A. Browning of dehydrated vegetables during storage. Ind. Eng. Chem. 39, 1294 (1947).

74. LEGAULT, R.R., TALBURT, W.F., HANSON, H.L. and LEINBACH, L.R. Effect of steam blanching on quality of frozen peas. Food Technol. 4, 194 (1950).

75. LEWIS, V.M., ESSELEN, W.B. and FELLERS, C.R. Nonenzymatic browning of foodstuffs. Nitrogen-free carboxylic acids in the browning reaction. Ind. Eng. Chem. 41, 2591 (1949).

76. LUBIENIECKA-VON SCHELHORN, M. Beziehungen zwischen dem absterben von mikroorganismen und der inaktivierung von enzymen unter der wirkung einer thermischen behandlung. Zeitschrift fur Lebensmittel untersuchung 116, 349 (1962).

77. MACKINNEY, G. and JUSEN, M. Chlorophyll-pheophytin: Temperature coefficient of the rate of pheophytin formation. J. Am. Chem. Soc. 63, 2530 (1941).

78. MACKINNEY, G. and WEAST, C.A. Colour changes in green vegetables. Ind. Eng. Chem. 32, 392 (1940).

79. MARKUZE, Z. Effects of traces of metals in the browning of glucose-lysine solutions. Chem. Abstr. 59, 4980 (1963).

80. MOHAMAD, A., HRASNEL-COHEAT, M. and OLCOTT, H.S. The "browning" reaction of proteins with glucose. Arch. Biochem. 24, 157 (1949).

81. MOORE, E.L., ESSELEN, W.B. and FELLERS, C.R. Factors responsible for the darkening of packaged orange juice. Fruit Products J. 22, 100 (1942).

82. MORRIS, H.A.L. In "The development of a chemical analogue of thermal destruction of bacterial spores". G.J.K. Packer, Ph.D. Thesis, Massey University, 1967.

83. NAGAYAMA, F. Studies on the browning of fish flesh II. Change in sugar content by heat process and browning. Bull. Japan. Soc. Sci. Fisheries 26, 1026 (1960).

84. NAGAYAMA, F. Mechanisms of breakdown and synthesis of glycogen in tissues of marine animals. Bull. Japan. Soc. Sci. Fisheries 32, 188, 229 (1966).

85. NEISKY, E.A., ESSELEN, W.B., KAPLAN, A.M. and FELLERS, C.R. Thermal destruction and stability of peroxidase in acid foods. Food Res. 15, 114 (1950).

86. NONAKA, J. Studies on the chemical essentials to the discussion of fish oil. J. Tokyo Univ. Fisheries 43, 127 (1957).

Ref. No.

87. OLCOTT, H.S. Marine Products. In "Symposium on foods: lipids and their oxidation". H.W. Schultz, ed. A.V.I. 1962.

88. ONO, T. and NAKAMURA, F. Browning in fish meat I. Browning of white meat by heat. Bull. Japan. Soc. Sci. Fisheries 24, 833 (1958-1959).

89. OVERBY, L.R. and FROST, D.V. The effects of heat on the nutritive value of protein hydrolysates with dextrose. J. Nutrition 46, 539 (1952).

90. PARISH, G.J.K. The development of a chemical analogue of thermal destruction of bacterial spores. Ph.D. Thesis, Massey University 1967.

91. PARTMAN, W. In "The technology of fish utilisation". R. Kreuzer ed. Fishing News (Books) Inc. Lond, 1965, pp.4-13.

92. PATASHNIK, M. A simplified procedure for thermal process evaluation. Food Technol. 7, 1 (1953).

93. PATTON, S. Browning in milk: a review. J. Dairy Sci. 38, 457 (1955).

94. PEARSON, D. "The chemical analysis of foods". Churchill 5th ed. 1962.

95. POMERANZ, Y., JOHNSON, J.A. and SHELLENBERGER, J.A. Effect of various sugars on browning. J. Food Sci. 27, 350 (1962).

96. PROCTOR, B.E. and GOLDBLITH, S.A. Electromagnetic radiation fundamentals and their application in food technology. Adv. Food Res. 3, 119 (1951).

97. RAHN, O. The problem of the logarithmic order of death in bacteria. Biodynamica 4, 41 (1943).

98. RAHN, O. Physical methods of sterilisation of micro-organisms. Bact. Review. 9, 1 (1945).

99. REMDI, D.A., ESSELEN, W.B. and FELLERS, C.R. Peroxidase activity of apple tissue. Food Technol. 4, 63 (1950).

100. RESENDE, R., FRANCIS, F.J. and STUEDO, C.R. Thermal destruction and regeneration of enzymes in green bean and spinach puree. Food Technol. 23, 63 (1969).

101. REYNOLDS, H. and LICHTENSTEIN, H. Symposium on the biology of bacterial spores. Part VIII Evaluation of heat resistance data for bacterial spores. Bact. Review 16, 126 (1952).

102. REYNOLDS, T.M. Chemistry of non-enzymic browning III. Effect of bisulphite, phosphate and malate on the reaction of glycine and glucose. Aust. J. Chem. 12, 265 (1959).

Ref. No.

103. REYNOLDS, T.M. Chemistry of non-enzymic browning I. Adv. Food Res. 12, 1 (1963).

104. REYNOLDS, T.M. Chemistry of non-enzymic browning II. Adv. Food Res. 14, 167 (1965).

105. RICHARDS, E.L. Non-enzymic browning: the reaction between D glucose and glycine in the "dry" state. Biochem. J. 64, 639 (1956).

106. RIED, J.M., BOERER, C.W. and CAMERON, E.J. Spore destruction rate studies on organisms of significance in the processing of canned foods. Food Res. 16, 383 (1951).

107. ROSEN, L., JOHNSON, K.C. and PIGMAN, W.W. Reactions of carbohydrates with nitrogenous substances II. Factors affecting the darkening of N-D-glycosylaniline. J. Amer. Chem. Soc. 75, 3460 (1953).

108. ROSS, A.F. Deterioration of processed potatoes. Adv. Food Res. 1, 257 (1948).

109. SAPERS, G.M. and NICKERSON, J.T.R. Stability of spinach catalase. II. Inactivation by heat. J. Food Sci. 27, 282 (1962).

110. SCHAEDEL, S.M., CHICHESTER, C.O. and MACK, G.L. Degradation of chlorophylls and several derivatives in acid solution. J. Org. Chem. 27, 3865 (1962).

111. SCHAEDEL, S.M., MACK, G.L. and CHICHESTER, C.O. Colour reversion in processed vegetables I. Studies in regreened pea purees J. Food Sci. 30, 312 (1965).

112. SCHAEDEL, S.M., MACK, G.L. and CHICHESTER, C.O. Colour reversion in processed vegetables II. Model system studies. J. Food Sci. 30, 317 (1965).

113. SCHROEDER, L.J., IACOBELLIS, N. and SMITH, A.M. The influence of water and pH on the reaction between amino compounds and carbohydrates. J. Biol. Chem. 212, 973 (1955).

114. SCHULZ, S. Regeneration of heat inactivated peroxidase. J. Biol. Chem. 154, 487 (1944).

115. SCHWIMMER, S. and OLCOTT, H.S. Reaction between glycine and the hexose phosphates. J. Amer. Chem. Soc. 75, 4855 (1953).

116. SHARP, J.G. Deterioration of dehydrated meat during storage II. Effect of pH and temperature on browning changes in dehydrated aqueous extracts. J. Sci. Food Agric. 8, 21 (1957).

117. SHULTZ, O.T. and OLSON, F.C.W. Thermal processing of canned foods in tin containers. XII Recent improvements in the General Method of thermal process calculation - a special co-ordinate paper and methods of converting initial and retort temperatures. Food Res. 5, 399 (1940).

Ref. No.

118. SPILDE, O. The fundamental principles of heat sterilisation. Tidsskr Hermetikkind 37, 451 (1951).

119. STUMBO, C.R. Further considerations relating to evaluation of thermal processes for foods. Food Technol. 3, 126 (1949).

120. STUMBO, C.R. Thermobacteriology in food processing. Academic Press, 1965.

121. STUMBO, C.R. and LONGLEY, R.E. New parameters for process calculations. Food Technol. 20, 341 (1966).

122. STADTMAN, E.R., BARKER, H.A., HAAS, V. and MAAK, E.K. Storage of dried fruit. Influence of temperature on deterioration of apricots. Ind. Eng. Chem. 38, 541 (1946).

123. STADTMAN, E.R. The chemical deterioration of dried fruit during storage. III. Chromatographic separation of carbonyl compounds as 2, 4-dinitrophenylhydrazones. J. Amer. Chem. Soc. 70, 3583 (1948).

124. SWEENEY, J.P. and MARTIN, M.E. Stability of chlorophyll in vegetables as affected by pH. Food Technol. 15, 263 (1961).

125. TAN, C.T. and FRANCIS, F.J. Effect of processing temperature on pigments and colour of spinach. J. Food Sci. 27, 232 (1962).

126. TAPPEL, A.L. Studies of the mechanism of vitamin E action. III. In vitro copolymerisation of oxidised fats with protein. Arch. Biochem. Biophys. 54, 266 (1955).

127. TAPPEL, A.L. Reflectance spectral studies of the hematin pigments of cooked beef. Food Res. 22, 404 (1957).

128. TARR, H.L.A. The acid-soluble phosphorus compounds of fish skeletal muscle. J. Fisheries Res. Bd. Can. 7, 608 (1950).

129. TARR, H.L.A. The Maillard reaction in fish products. J. Fisheries Res. Bd. Can. 8, (2), 74 (1950).

130. TARR, H.L.A. Cause of the browning of certain heat processed fish products. Fisheries Res. Bd. Can. Progr. Reports. Pacific Coast Stas. No. 92, 23 (1952).

131. TARR, H.L.A. The Maillard reaction in flesh foods. Food Technol. 8, 15 (1954).

132. TARR, H.L.A. and LEBOUX, M. Acid soluble compounds and free sugars in fish muscle and their origin. Can. J. Biochem. Physiol. 40, 571 (1962).

Ref. No.

133. TARR, H.L.A. Pathways of glycogen degradation. In "The technology of fish utilisation" R. Kreuzer, ed. Fishing News (Books) Inc. London 1965, p.34.

134. TOMLINSON, N. and CREELMAN, V.M. On the source of free ribose formed post-mortem in muscle of lingcod (Ophiodon elongatus). J. Fisheries Res. Bd. Can. 17, 603 (1960).

135. TOMLINSON, N. and GEIGER, S.E. Glycogen concentration and post-mortem loss of adenosine triphosphate in fish and mammalian skeletal muscle. A review. J. Fisheries Res. Bd. Can. 19, 997 (1962).

136. VENOLIA, A.W. and TAPPEL, A.L. Brown coloured oxypolymers of unsaturated fats. J. Amer. Oil Chemists' Soc. 35, 135 (1958).

137. VETTEL, J.L., NELSON, A.I. and STEINBERG, M.P. Heat inactivation of peroxidase in HTST processed whole kernel corn. Food Technol. 13, 410 (1959).

138. VON TIGERSTROM, R. and TARR, H.L.A. Enzymic removal of carbohydrates from fish muscle. In "The technology of fish utilisation" R. Kreuzer ed. Fishing News (Books) Inc. London 1965, p.256.

139. WAGER, H.G. The browning reaction in dehydrated carrot and potato; its initiation and the separation and partial characterisation of an intermediate from dehydrated carrot. J. Sci. Food Agric. 6, 57 (1955).

140. WANG, Y.D. Promotion of browning in the development of new dairy products. M. Tech. (Food) Thesis, Massey University, 1967.

141. WATTS, J.O. and GRISWOLD, R.M. Enzyme inactivation - relation of rates of inactivation of peroxidase, catecholase, and ascorbase to oxidation of ascorbic acid in potatoes and parsnips. J. Agr. Food Chem. 1, 569 (1953).

142. WEETMAN, J.H., PROCTOR, B.E. and GOLDBLITH, S.A. Radiation preservation of milk and milk products. IV. Radiation-induced browning and some related chemical changes in milk. J. Dairy Sci. 39, 1236 (1956).

143. WESTCOTT, D., LIVINGSTON, G., ROSEMAN, V. and MELLERS, C. Non-enzymatic discolouration in green bean puree. Food Res. 20, 149 (1955).

144. WILDER, C.J. Factors affecting heat inactivation and partial reactivation of peroxidase purified by ion-exchange chromatography. J. Food Sci. 27, 567 (1962).

145. WILLSTATTER, R. and STOLL, A. Investigations of chlorophyll. Transl. by F.M. Schertz and A.R. Mirz. Science Press 1928.

Ref. No.

146. WOLFROM, M.L. KOLB, D.K. and LANGER, A.V. Chemical interactions of amino compounds and sugars. VII. pH dependency. J. Amer. Chem. Soc. 75, 3471 (1953).

147. WOLFROM, M.L., SCHUETZ, R.D. and CAVALIERI, L.F. Chemical interactions of amino compounds and sugars. III. The conversion of D-glucose to 5-(hydroxymethyl)-2-furaldehyde. J. Amer. Chem. Soc. 70, 514 (1948).

148. WOLFROM, M.L., SCHUETZ, R.D. and CAVALIERI, L.F. Chemical interactions of amino compounds and sugars. IV. Significance of furon derivatives in colour formation. J. Amer. Chem. Soc. 71, 3518 (1949).

149. YAMAMOTO, H.Y., STEINBERG, M.P. and NELSON, A.I. Kinetic studies on the heat inactivation of peroxidase in sweet corn. J. Food. Sci. 27, 113 (1962).

150. ZOUEIL, M.E. and ESSELEN, W.B. Thermal destruction rates and regeneration of peroxidase in green beans and turnips. Food Res. 24, 119 (1954).