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ACCELERATED SHELF-LIFE TESTING OF A READY-TO-EAT CEREAL

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ACCELERATED SHELF-LIFE TESTING OF A READY-TO-EAT CEREAL

By

Jeffrey Douglass Feneley

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ABSTRACT

ACCELERATED SHELF-LIFE TESTING OF A READY-TO-EAT CEREAL

By

Jeffrey Douglass Feneley

The shelf-life of ready-to-eat cereal is often limited by susceptibility to lipid oxidation. Lipid oxidation forms a number of compounds (e.g., hexanal) which contribute objectionable off-odors to the food. In order to assess the degree of lipid oxidation, an assay was validated that quantitates the concentration of hexanal in the headspace of packaged cereal. After the validation of the assay was completed, an accelerated shelf-life study was performed in order to determine the oxidative stability of a packaged cereal at room temperature. This task was carried out by analyzing cereal stored at temperatures above 23 °C (i.e., above room temperature) with the hexanal assay. A mathematical model consisting of data from the accelerated shelf-life studies was then successfully used to predict the shelf-life of the cereal stored at room temperature.

To the memory of Douglass Feneley, for his continuous support, interest, and appreciation for life.

Acknowledgments

First and foremost, a heartfelt thank you for the never ending patience and support of my family. I cannot begin to express how their tolerance, love, and strength have helped me while pursuing my graduate degree.

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Introduction

Lipids are essential nutrients in the human diet. They provide a number of functions including serving as sources of energy, carriers for fat-soluble vitamins, and suppliers of essential fatty acids (Loliger, 1990). Next to microbial spoilage, the oxidation of lipids is the second largest limiting factor in the shelf-life of most types of food, even when their fat content is very low (Loliger, 1990; Frankel, 1993). During lipid oxidation, oxygen reacts with unsaturated fatty acids to form hydroperoxides. These compounds undergo decomposition to produce a number of other compounds, e.g., hydrocarbons, ketones, aldehydes, and smaller amounts of epoxides and alcohols that contribute to the development of rancid off-odors and flavors (Frankel, 1993).

Many studies have addressed the factors which influence the shelf-life of food products during storage. Factors include the presence of metals, moisture level, heat, enzymes, exposure to light, and the fatty acid composition of food products (Farrer, 1955; De Ritter, 1976; Fritsch and Gale, 1976; Singh et al., 1976; Labuza and Riboh, 1982; Labuza and Schmidl, 1985). It is generally believed that these factors increase the rates of lipid oxidation by lowering energies of activation, raising the potential energies of products and reactants, or a combination of both.

Because of the ever changing demographics between production sites and where foods are finally consumed, the time between the production of foods and when these foods are consumed may increase. Therefore, the limited shelf-life of lipid-containing food products has received much attention. Determining the shelf-life of these food products

under normal conditions can be a relatively lengthy process. In the corporate environment, the shelf-life of a product must be known before it can be introduced to the market. A delay in introducing a products to market may cost a company its competitive advantage. Therefore, there is a need for an expedient procedure for determining the oxidative stability of food products, i.e., an accelerated shelf-life test. However, it is important to note that conclusions drawn from past studies may not be valid because inappropriate methods may have been used for evaluating product stability (Frankel, 1993). It is imperative that an appropriate method be selected that accurately assesses the degree of lipid oxidation for a given product.

The objectives of this research were two-fold:

1. To evaluate and / or improve existing gas chromatographic assays to quantitate hexanal, a volatile oxidation product, in the headspace of packaged food products.
2. To develop a model based on the quantitation of hexanal from which the shelf-life of a cereal product stored at room temperature can be accurately predicted.

Literature Review

Accelerated shelf-life testing

Marketing, research, and development managers of food companies will often ask product development scientists for information concerning the shelf-life of a product. It generally takes a long time for a product to reach a level of unacceptability to the consumer under normal conditions of storage. This would conceivably delay the introduction of the product to market. Consequently, product development scientists will often perform an array of tests using accelerated conditions to enhance the rate at which a product becomes unacceptable to the consumer (Labuza and Schmidl, 1985). By obtaining this quicker determination of the length of the shelf-life for a product, the product development cycle is shortened. This process ultimately allows for a quicker introduction of the product to the market. Although this task appears simple in concept, the product development scientist is faced with many questions such as how to perform an accelerated shelf-life test and how to handle the data gathered from such testing.

Execution of accelerated shelf-life testing

The task of providing an estimate for the shelf-life of a food product is complicated as there are a number of factors that must be considered when determining the shelf-life. Fuller (1994) stated that the first task to consider is the selection of appropriate criteria to assess the shelf-life of a product. The general idea is to monitor any change in the proposed criteria over time. Typically, research scientists will select the

growth of microorganisms, the loss of nutrients, the onset of staleness, changes in the functional properties, or the progressive gain of an undesirable attribute as criteria to monitor. However, food products are complex systems and it is rare that only one characteristic flaw will appear throughout the shelf-life of a product (Labuza and Schmidl, 1985; Fuller, 1994).

There are a number of ways to determine the criteria that could be used to define the shelf-life of a product. An initial step is to review scientific literature concerning similar products (Labuza and Schmidl, 1985). Often, magazines and television commercials regarding competitive products will allude to areas of interest. If a product of interest is being developed in an established business, there is often in-house information pertaining to similar products. Raw material specifications will have limits on microbial loads which may be of concern to the food manufacturer. These specifications can serve as indicators of the amount of stress to which the ingredients have been subjected. Finally, if the product is already being manufactured, incoming consumer complaints can give some valuable insight into the loss of a particular quality factor such as flavor or taste.

Another task to consider when assessing the shelf-life of a product is how much of a change in the chosen criterion is acceptable (Labuza and Schmidl, 1985; Fuller, 1994). The question might be asked "Has the microbial load exceeded a hazardous level?" or "Has the taste or aroma of the product deteriorated to a point where it is unacceptable?" These questions bring up another concern (Labuza and Schmidl, 1985; Fuller, 1994)---- who or what determines when changes in the selected criteria render the product

unacceptable? Is it the consumer? Has the product deteriorated to a point that it no longer holds true to its label, i.e., has it lost essential nutrients?

Finally, research scientists must determine the altered conditions under which the accelerated shelf-life test will be carried out. For example, variations in temperature and moisture contents are the driving forces behind most shelf-life studies (Anderson et al., 1976; Labuza and Schmidl, 1985; Fuller, 1994). For temperature studies, carrying out this procedure at a number of different elevated temperatures gives kinetic information that can be used to predict the shelf-life of a product stored under normal conditions.

Data modeling

Researchers will often fit data to modeled equations to generate important information about degradation reactions. Typically, the general rate law can be used to describe these reactions:

$$-\frac{\delta A}{\delta t} = k[A]^n \qquad (1)$$

where t is time, δ represents a change in the assessed parameter, A is the measured criterion, k is the rate constant, and n is the reaction order. It is important to be able to apply general rate law to modeled data so there can be a better understanding of the extent of the observed reaction at any time (Labuza and Riboh, 1982). Labuza and Schmidl (1985) estimated that approximately 99% of all food "quality issue" reactions can be fitted to zero- and first-order reactions. A rare example of a reaction that did not fit either zero- or first-order reaction parameters was the degradation of vitamin C in packaged food

systems in which the oxygen content was found to be the limiting factor (Singh et al., 1976).

Integrated forms of the general rate law for zero-, first- and second-order reactions are as follows:

$[A] = [A_0] - kt$	zero-order	(2)
$\ln[A] = \ln[A_0] - kt$	first-order	(3)
$1/[A] = 1/[A_0] + kt$	second-order	(4)

The reaction order can then be determined by plotting the concentrations or the natural logarithm of the concentrations as a function of time (Fritsch and Gale, 1976). In addition, the rate constant of that reaction can also be determined.

Application of modeled data

Useful information can be obtained by monitoring the rate of change for a reaction as a result of exposure to different temperatures. The kinetic modeling of information can be used to describe chemical, physical, or microbiological changes in a food product over time (Van Boekel, 1996). The most common ways of presenting this type of information are via Arrhenius and Q_{10} models.

Arrhenius models: Plotting the rate constants at which a product expires as functions of the temperatures at which it is held, is one of the ways of displaying data. This type of diagram, known as an Arrhenius plot, can be extrapolated to either higher or lower

temperatures, thereby providing an estimate of product shelf-life (Fritsch and Gale, 1976; Frankel, 1993).

$$k = A \exp(-D/T) \qquad (5)$$

where D is a constant, A is the pre-exponential factor, k is the rate constant (as determined by the general rate law), and T is temperature in Kelvin. By plotting the natural logarithm of several reaction rates as a function of their respective temperatures (in inverse Kelvin), a straight line can be obtained.

The energy of activation (Ea) for the reaction can be determined from the following equation:

$$Ea/R = D \qquad (6)$$

where Ea is the energy of activation in units of $Kcal*mol^{-1}$, R is the molar gas constant ($1.98*10^{-3}$ $Kcal*mol^{-1}*K^{-1}$, and D as in equation 5 is in units of Kelvin. As a caution, it should be kept in mind that food systems are very complex. Bromberg (1984) stated that large deviations from exponential Arrhenius plots often mean that the observed rate constant is a combination of several reactions with different energies of activation. This implies that a different step in the overall reaction has become the rate limiting step.

Q_{10} factors: Another convenient way to characterize the influence of temperature on the shelf-life of a product is to determine its Q_{10} factor. This factor gives the rate of increase for an observed reaction resulting from an increase in temperature of 10°C. The general

Q_{10} equation is:

$$Q_{10} = \frac{\text{rate to unacceptability at temperature (T+10)}}{\text{rate to unacceptability at temperature (T)}} \quad (7)$$

$$= \frac{\text{shelf life at temperature (T)}}{\text{shelf life at temperature (T+10)}} \quad (8)$$

If the Q_{10} factor is known and the shelf-life is known at a particular temperature, then the shelf-life at different temperatures can be calculated. Bromberg (1984) reported that as a rule of thumb, reaction rates double per 10°C rise in temperature. Simply stated, most reactions have Q_{10} values around 2. As a caution, kinetic model values should only be applied to a temperature range of 30 to 40 °C (Labuza and Schmidl, 1985; Frankel, 1993). This avoids gross discrepancies between predicted shelf-lives and actual shelf-lives.

Researchers will often interrelate energies of activation (Ea) with Q_{10} values. Caution should be exercised when converting between the two relationships as the conversion is temperature dependent. The mathematical relationship between the energy of activation (Ea) and Q_{10} is:

$$\log Q_{10} = \frac{2.2\,Ea}{(T)\,(T+10)} \quad (9)$$

where Ea is in units of cal/ mole, T is temperature in units Kelvin (Labuza and Riboh, 1982).

Errors encountered in accelerated shelf-life testing

Literature evidence indicates that researchers should be cautious when designing temperature-driven accelerated shelf-life tests. Labuza and Schmidl (1985) reported that relatively high temperatures may cause a change in the phase of products. They pointed out that phase changes can cause large changes in certain reaction rates that may cause error in prediction models. Examples of phase changes occurring would be fats changing from semi-solid to liquid, or crystalline structure melting (i.e., glass phase transitions).

Exposure to temperatures and humidities that are higher than typically encountered during the storage of food products can cause an increase in the rates of protein denaturation within the product (Labuza and Schmidl, 1985; St. Angelo, 1996). These denatured products are then available for a number of side-reactions, including the Maillard browning reaction. Some Maillard browning products exhibit antioxidant properties (Sato et al., 1973). Although the existence of antioxidants is favorable, their uncontrolled production can affect modeling.

Water activity (A_w) is another factor to consider if temperature is used as the driving force to accelerate shelf-life testing in a closed system. The water activity of a food product increases with an increase in temperature (Saravacos and Stinchfield, 1965; Labuza and Schmidl, 1985). If a food product is sealed at an initial water activity, an increase in temperature will result in a corresponding increase in water activity. This increase in water activity can influence a number of reaction rates (Figure 1) even more than if temperature alone was the variable. This unexpected factor can result in an overestimation of the shelf-life of the product at lower temperatures (Labuza and Schmidl,

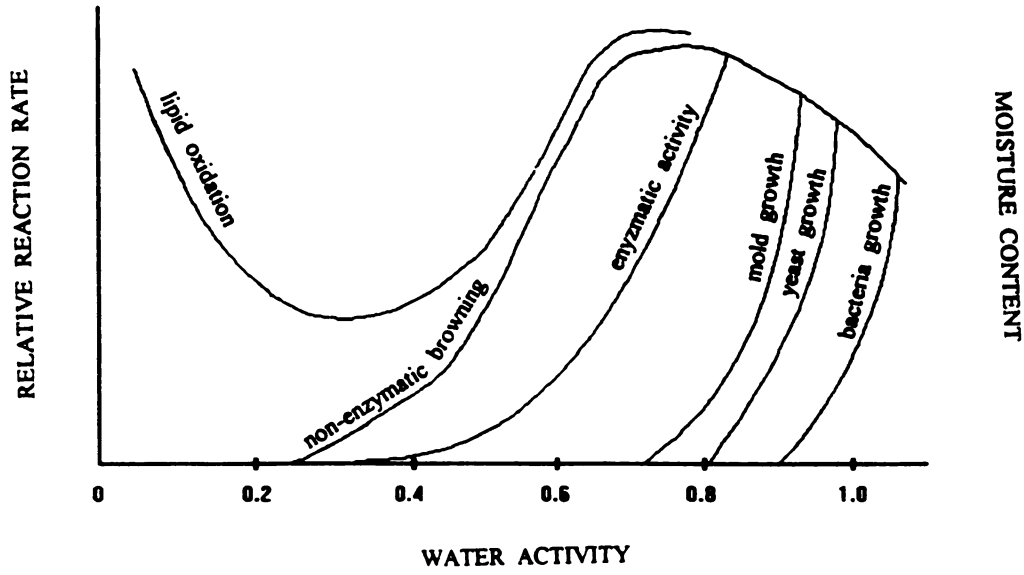

Figure 1. Stability of foods as a function of water activity (Labuza, 1971).

1985).

Through the duration of accelerated shelf-life tests, analytical methods are generally used to quantitate changes in the degree of acceptability for the products. Labuza and Schmidl (1985) recommended that instrumental methods have a variability of 10% or less to minimize shelf-life prediction errors. It is important to have a validated assay to use.

The problems described above are only a few of those that may be encountered during accelerated shelf-life testing. However, if these issues are properly addressed before conducting any testing, then they should be of little concern when analyzing data generated during testing. It should be kept in mind that each product is unique. Therefore, it is unlikely that any two products will encounter the same issues.

Validation of analytical procedures

Analytical techniques are often used to quantify degrees of product acceptability. Examples of such assays include: vitamin quantification, moisture analysis for specifications, and the degree of lipid oxidation for product acceptability. Frequently, the performance of the technique used is unknown. In these cases, a method verification program should be used to account for the different types of variability within the assay. According to Dols and Armbretch (1977), method verification programs need to be developed to the point that:

-The method satisfies the defined needs of the assay.

-A linear calibration function is obtained where the slope of the line defines the

sensitivity of the method.

-The method has been optimized.

-The method is in statistical control (i.e., all the causes of error remain the same).

-A set of adequate instructions defining the method has been developed.

A number of factors must be determined in order to satisfy the aforementioned criteria. Recently, several studies with guidelines for assay evaluations have been published (Dols and Armbretch 1977; Cardone, 1983; Hewlett Packard G.L.P., 1993). The most frequently mentioned aspects that need to be known in order to have a validated method include (as defined by Hewlett Packard G.L.P., 1993):

-*Selectivity* refers to a method which provides responses for a number of chemical entities which may or may not be distinguished.

-*Precision* of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings. Repeatability is obtained if the analysis is carried out in one laboratory by one operator, using one piece of equipment over a relatively short time span.

-The *accuracy* of an analytical method is the degree of agreement of test results generated by the method to the true value.

-The *linearity* of an analytical method is its ability to elicit test results that are directly, or by means of well defined mathematical transformations, proportional to the concentrations of analytes in samples with a given range.

-*Limit of quantification* is the smallest amount which results in a reproducible measurement of peak areas.

Factors that determine food quality

Moisture content

Food matrices are comprised of solids and liquids, e.g., water. Many fresh foods

have high moisture contents which increase their susceptibility to microbiological decay (Labuza and Riboh, 1982). Microorganism proliferation becomes apparent at water activities of 0.7 and higher. However, in lower-moisture foods (water activity ≤ 0.7), corresponding lower water activities decrease rates of proliferation of molds, yeasts, and bacteria (Figures 1 and 2). Other chemical modes of deteoriation, such as lipid oxidation, proceed at these lower water activities. As seen in Figure 1, the water activity of food affects the rates of a number of reactions including non-enzymatic browning and lipid oxidation. Non-enzymatic browning activity greatly increases at water activities between 0.4 to 0.6 (Martinez and Labuza, 1968).

Water activity also has a great influence on rates of lipid oxidation (Labuza et al., 1966; Labuza et al., 1969; Heidelbaugh and Karel, 1970). Initial amounts of water will bind to polysaccharides which will not affect rates of lipid oxidation (Heidelbaugh and Karel, 1970). The addition of more water acts as an antioxidant, because water hydrates metal catalysts and forms hydrogen bonds with hydroperoxides (Labuza et al., 1966; Heidelbaugh and Karel, 1970). At an optimal water content, a minimal rate of lipid oxidation occurs. This level of moisture in the food matrix is known as the monolayer. Eventually, as higher water contents are encountered, water acts as a solvent and gives mobility to catalysts (Labuza et al., 1969; Heidelbaugh and Karel, 1970). From Figure 1, it appears that the optimal water activity of foods to minimize these reactions is around 0.35. This would correlate to a 35% relative humidity, assuming that the product is in equilibrium with its surroundings.

The apparent moisture within a product can also influence the onset of staleness.

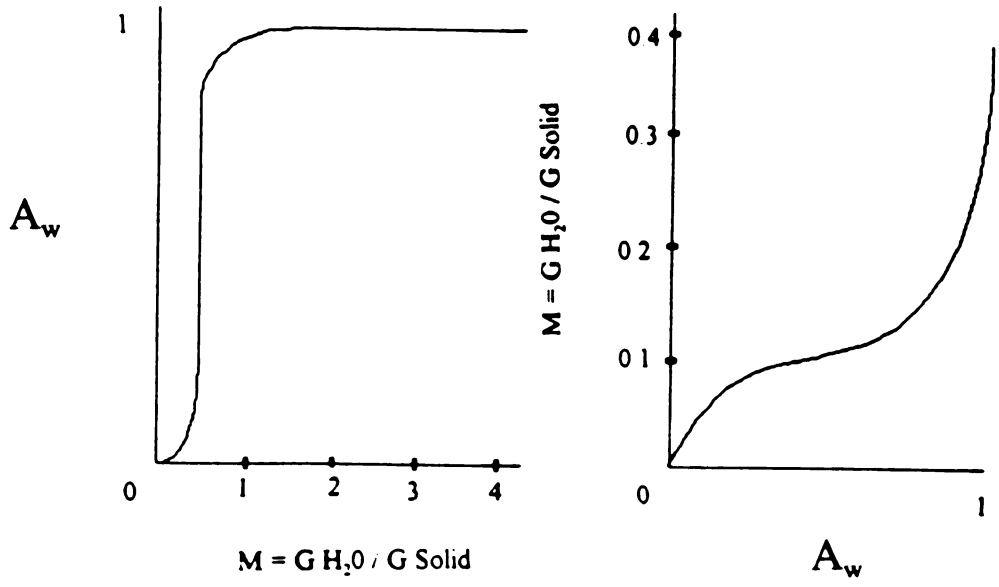

Figure 2. Moisture sorption isotherm for a food (Labuza, 1971).

For example, commercial cereals are packaged to protect the products from exposure to high humidities, as many cereals will become 'stale' in flavor and tough in texture if the moisture contents exceed 5 to 6 % (Anderson et al., 1976). Moisture contents of cereals are typically around 3% (Anderson et al., 1976). Therefore, as long as cereals are maintained below moisturecontents of approximately 4%, then the effect of moisture on staling is minimal.

Degradation of vitamins

The degradation of vitamins during the storage of food products is often a major concern when making label claims. Vitamin claims for cereals can be based upon the amount present at the end of the shelf-life for the product. Therefore, there is a need to know the relative stability of the vitamins in a food product. Some factors that affect the stability of vitamins in ready-to-eat cereals are temperature, moisture, metal ions, and pH (Farrer, 1955; De Ritter, 1976). The effects of temperature and moisture are the two most common issues that have been studied relative to vitamin degradation.

In general, vitamins are more stable in breakfast cereals than in foods of higher moisture contents. It has been demonstrated that vitamins A and C are adversely affected in ready-to-eat cereals as a result of exposure to moisture contents above 6%. However, Anderson et al. (1976) stated that cereals are unacceptable from an organoleptic standpoint when moisture contents exceed 6%.

Interactions between vitamins and other compounds found in foods can cause vitamins to degrade (Figure 3). The breakdown products of vitamins contribute to off-

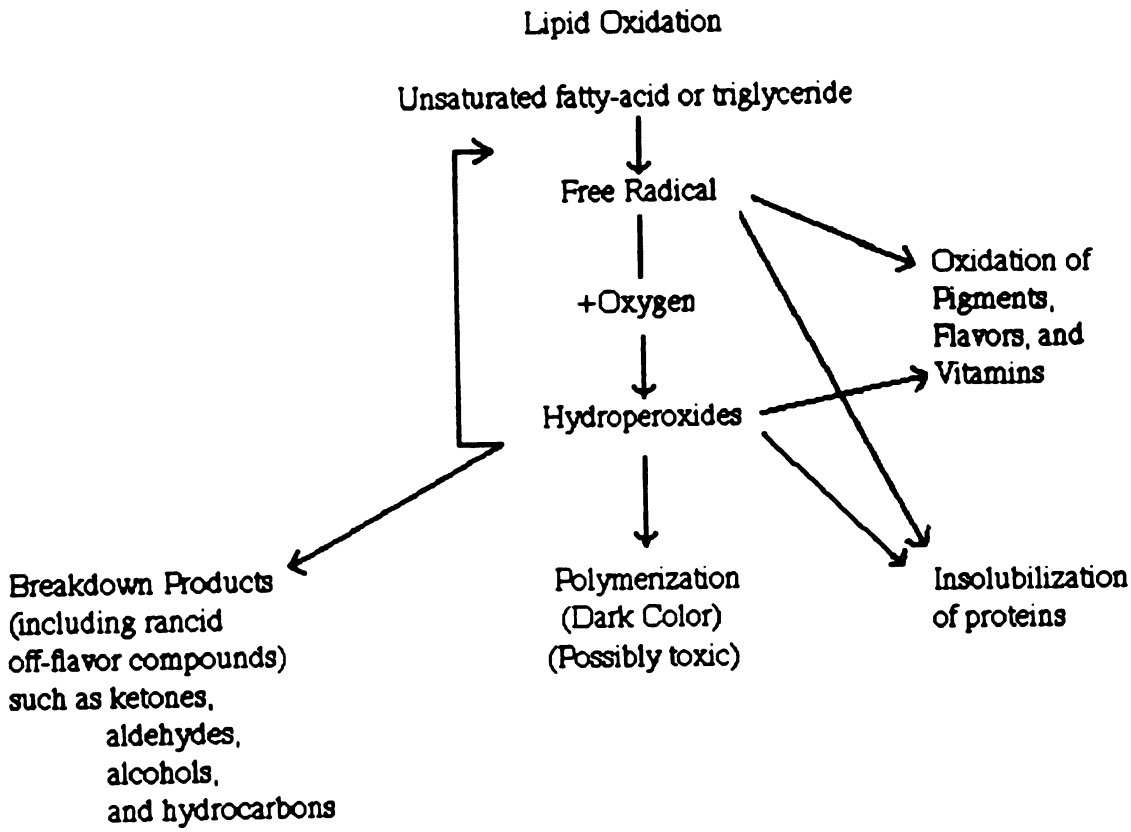

Figure 3. Overall mechanism of lipid oxidation (Labuza, 1971).

odors and tastes that can be attributed to the consumer's dislike of the product. As an example, Sarama et al. (1995) stated that the primary breakdown product of vitamin A is β-ionone. This compound imparts a cedar wood or raspberry-like aroma to foods and has a very low threshold level. They also pointed out that other off-odors produced during vitamin A degradation are similar to those generated via lipid oxidation (e.g., hexanal).

The effects of temperature abuse on vitamins has also been studied. It has been found that upon exposure to heat, trans-isomers of vitamin A can convert to less bioavailable cis-isomers. The cis-isomers are more susceptible to oxidation and form deleterious by-products as described above (De Ritter, 1976; Sarama et. al., 1995). Therefore, if the previously mentioned conditions are addressed, then vitamin deterioration is kept at a minimum while the food is on the shelf.

Lipid Oxidation

Mechanism: One of the primary limiting factors affecting the shelf-life of food products is oxidative degradation due to the autoxidation of polyunsaturated lipids (Loliger, 1990). The interaction between oxygen and lipid structures is known as lipid oxidation. Oxidation causes losses in organoleptic acceptability of a product, as well as losses in vitamins and nutrients (Figure 3). The most noticeable consequence of lipid oxidation is the formation of volatile compounds that contribute to off-flavors in foods.

Basically, lipid oxidation involves a free radical chain mechanism. This reaction is influenced by several factors including fatty acid profile, oxygen content, temperature, light, enzymes, and catalysts. St. Angelo (1996), citing Farmer et. al. (1942), presented

the overall sequence of lipid oxidation in three events:

(1) initiation- period during which free radicals are formed

(2) propagation-period during which there is a further increase in the number of free radicals and the formation of hydroperoxides (which are colorless, tasteless, and odorless); and

(3) termination-period during which the formation of non-radical products brings about the conclusion of the reaction.

The following scheme summarizes the free-radical chain mechanism of lipid oxidation:

Initiation :	RH + initiator	---> $R^{\bullet} + H^{\bullet}$
	ROOH	---> ROO* + $H^{\bullet}$
Propagation:	$R^{\bullet} + O_2$	---> $ROO^{\bullet}$
	$ROO^{\bullet}$ + RH	---> ROOH + $R^{\bullet}$
Termination:	$R^{\bullet} + R^{\bullet}$	---> RR
	$ROO^{\bullet} + ROO^{\bullet}$	---> ROOR + O_2
	$R^{\bullet}$ + ROO	---> ROOR
	$2RO^{\bullet} + 2ROO^{\bullet}$	---> 2ROOR + O_2

where RH is an unsaturated fatty acid
ROO* is a peroxyl free radical
RO* is an alkoxy radical

The initiation step is considered the rate limiting step to the overall reaction mechanism. In an uncatalyzed reaction system, the formation of radicals is unfavorable due to the high energy of activation (35 to 65 Kcal/ mole) (Privett and Blank, 1962).

However, transition metals with multiple valence states that have a suitable oxidation/ reduction potential between them are often present in sufficient concentration in most foods for lipid oxidation to proceed through a catalyzed reaction mechanism. Using the data of Fritsch and Gale (1976), Labuza and Riboh (1982) calculated the energy of activation for the formation of hexanal in cereals to be 15 and 20 Kcal / mole. Frankel (1993) reported energies of hexanal formation of 19 to 50 Kcal / mole for fish and vegetable oils.

In addition to external factors, lipid oxidation is also controlled by the relative reactivity of the reacting substrate. Nawar (1985), in a review on lipid oxidation, indicated the relative oxidative reactivities of several fatty acid substrates (in parenthesis) are a function of the degree of unsaturation.

Arachidonic acid	>	Linolenic acid	>	Linoleic acid	>	Oleic acid
(40)		(20)		(10)		(1)

The reactivity of the various reactive substrates can be supported by observing the occurance of conjugated double bonds which stabilizes reaction intermediates (Carey and Sundberg, 1990).

During lipid oxidation, the primary products formed are hydroperoxides (ROOH). However, hydroperoxides are labile and decompose to form secondary compounds that give the characteristic off-odors associated with rancidity (Figure 3). These secondary products include aldehydes, ketones, peroxides, and epoxides. Carbonyl compounds appear to accumulate in a linear fashion over time (Boggs et al., 1964; Buttery and

Teranishi, 1963; Fritsch and Gale, 1976). However, after a certain time, known as the time of break point, these concentrations begin to deviate from linearity and begin to accumulate exponentially. This break point of rapid hexanal accumulation is of interest because it is relatively close to the time when consumers begin to detect rancidity (Figure 4).

Control of lipid oxidation in food systems: Oxidation of food lipids is inevitable if foods are subjected to long storage periods. There have been a number of advancements in the past two decades in off-setting the oxidation of lipid-containing products. The most prevalent approaches are the use of antioxidants and modified or controlled storage environments.

Antioxidants are often classified according to their chemical mode of action, which typically includes three different categories (Mielche and Bertelsen, 1994). The first class of antioxidants includes phenolic compounds such as butylated hydroxyanisole, butylated hydroxytoluene, tertiary-butylated hydroxyquinone, and propyl gallate. A proton donating compound can exhibit antioxidant properties if its conjugate base is thermodynamically more stable than the fatty-acid radical (i.e., the reactive substrate). If the protonation of the fatty-acid radical occurs then free-radical formation ceases.

The mechanism by which phenolic antioxidants function is by accepting and scavenging the free radicals that initiate and propagate oxidation.

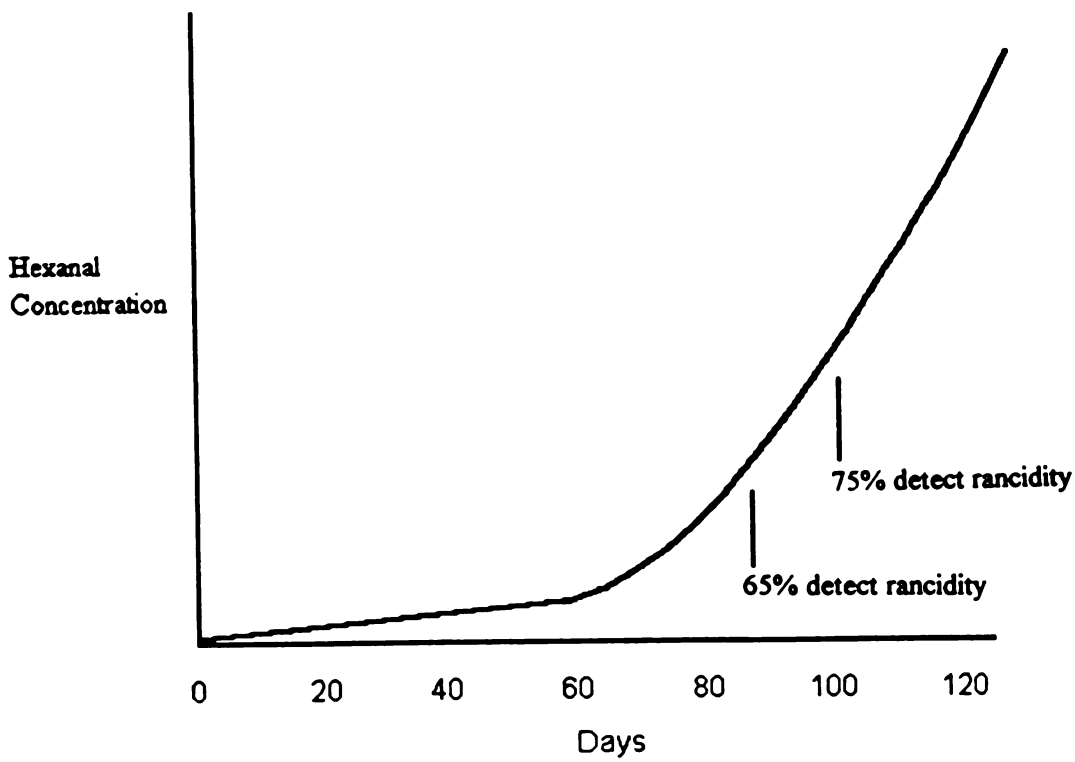

Figure 4. Hexanal concentration in the vapor above reconstituted potato granules (at 93°C) which had been air-packed and stored at 22°C (Boggs et al.,1964).

$$ROO^{\bullet} + A^{\bullet} \text{ ---> nonradical product}$$

$$ROO^{\bullet} + AH \text{ ---> } ROOH + A^{\bullet}$$

where A* is the antioxidant free radical, and AH is the phenolic antioxidant. The addition of naturally occurring antioxidants, such as the tocopherols, after processing of the food has also been investigated. However, Anderson et al. (1976) and Kumor (1986) reported that added tocopherols have no effect on the storage stability of cereals.

The second class of antioxidants includes free-radical production preventors which sequester the catalytic activity of reactive transition metals. Included in this class are ethylenediamine tetraacetic acid, citrate, and various phosphates. Depending on the mode of oxidant activity within the food, and the mole ratio of the oxidant to the catalyzing metal, these compounds can exhibit both pro-oxidant and antioxidant characteristics (Arora, 1997).

The third class of antioxidants are environmental components including compounds or processes that affect oxygen partial pressure, redox potentials, or the water activity of foods (Mielche and Bertelsen, 1994). This classification includes both controlled and modified atmospheric storage. The use of modified atmospheres involves flushing the product, at the time of packaging with a gaseous atmosphere that is virtually oxygen-free. Controlled-atmosphere packaging involves adjusting the package atmosphere and maintaining this modification throughout the life of the product.

Measurement of lipid oxidation: Measuring the loss of initial reactant compounds and the production of primary and secondary products of lipid oxidation are common approaches to assessing the extent of lipid oxidation in foods. Besides sensory analysis, St. Angelo (1996) discussed examples of assessing lipid oxidation including oxygen uptake, peroxide value, and the 2-thiobarbituric acid test (TBA test) that assesses the amount of malonaldehyde-like compounds present in the product. These methods are either not sensitive or selective enough, too labor intensive, or their execution produces artifacts that bias results for the intended use (Halliwell and Chirico, 1993; St. Angelo, 1996).

An alternative to the above techniques is to measure specific volatile secondary by-products of lipid peroxidation. For example, linoleate, a predominant polyunsaturated fatty acid in most foods, readily oxidizes and forms a number of compounds including hexanal. Quantification of hexanal can be used as a measure of the degree of oxidation (Boggs et al., 1964; Buttery, 1963; Fritsch and Gale, 1976; Kumor, 1986; Frankel et al., 1989; Koelsch et al., 1991).

Assessment of food stability

Many studies have been conducted on various products to assess the shelf-life of foods held under different conditions. Labuza and Ridoh (1982) predicted the extent of browning in whey stored under fluctuating temperature conditions. Labuza and Contreras (1981) studied water transmission rates of different films which could be used to predict moisture gain or loss from processed foods. Vojnovich and Pfeifer (1970)

assessed the times for a 50% reduction of vitamin C to occur in infant cereals at different water activities. Singh et al. (1976) used varying light intensities to determine the rate constants and the order of reaction for the degradation of ascorbic acid in a commercially available infant formula. Fritsch and Gale (1976) studied the shelf-life of two different cereals using the development of hexanal as a marker of rancidity. Kim and D'Appolonia (1977 a, b) studied the effects of different protein concentrations on rates of staling in bread.

The work of Fritsch and Gale (1976) is of particular interest to this current study as it dealt with ready-to-eat cereals. By following the rate of hexanal production as a marker of rancidity, these investigators were able to quantitatively assess the shelf-life of a corn cereal and a wheat cereal when stored at different temperatures. By making an Arrhenius plot using the natural logarithm of the rates at which the products became unacceptable (i.e., the rate of hexanal accumulation) as functions of temperatures [ln(k) vs. $1/T^{K}$], good predictions of the time required for the onset of rancidity to occur at different temperatures were possible.

The mathematical modeling of Fritsch and Gale dealt with the initial stages of oxidation (i.e., before the time of rapid hexanal accumulation). Koelsch et al. (1991) used kinetic models to illustrate the mechanisitic influence of oxygen on lipid oxidation in a model system before and after the time of breakpoint for hexanal accumulation. Their data show that the time at which the breakpoint occured is a logarithmic function of the oxygen concentration in the system.

Materials and Methods

Materials

Preparation of cereal product

Three batches of a corn-based ready-to-eat breakfast cereal were obtained from a commercial supplier. The three batches of cereal were produced at the same plant, but not from the same shift. All batches of cereal were prepared using the same formulation and utilized in the three experimental sets described later in this section. The cereal was placed in 176 cm^3 packages consisting of a high moisture and high flavor barrier metalized foil liner (known as "PC1" liners from Print Pack, Atlanta, GA).

Methods

Gas chromatography

Hexanal concentrations were monitored using a modified Photovac 10S Plus gas chromatograph (Photovac International Incorporated, West Deer Park, NY). This instrument was selected because it was furnished with a photoionization detector that has a high sensitivity for aldehydes (Langhorst, 1981). The gas chromatograph was equipped with a 25 m * 0.53 mm I.D., 1.5 μ film thickness, DB-1 column (J&W Scientific, Aston, PA).

The carrier gas was 99.99% pure helium (as specified by the manufacturers). The flow rate was 25 mL / minute at the exit port of the detector. In order to control the baseline sensitivity of the gas chromatographic detector, air at a rate of 25 mL / minute

was added to the carrier gas before entering the detector (Figure 5). This minimized the detector response to the ionizing differential caused by injected air, thereby allowing for the detection of eluting hexanal. The addition of air to the carrier gas also reduced the unwanted retention of gases within the detector. Details of the sampling procedure and validation of the response of the gas chromatograph to standardized hexanal concentrations are provided in Appendices A and B.

Generation of hexanal concentrations in the headspace

Known concentrations of hexanal in the headspace are required to assess the validity of the gas chromatographic method for quantitating hexanal concentrations in cereal packages. Hexanal headspace concentrations can be generated by instruments designed to deliver precise concentrations of gases for air pollution analyses. Dynacalibrators (Vici Metronics, Santa Clara, CA) can generate consistent concentrations of gases over an extended period of time. The dynacalibrator utilizes small inert capsules called permeation devices that contain the pure compound of interest (in this instance hexanal). When held at a constant temperature, the compound within the capsule exists in a two-phase equilibrium of gas and liquid. The gas permeates through the shell of the capsule and is swept away in a constant stream of nitrogen. Consequently, there is a relatively constant supply of hexanal in nitrogen emitted by the dynacalibrator over time for calibration. This is a desirable approach for generating constant concentrations of hexanal as the method of extraction closely resembles the method used to sample the headspace of packaged cereal in this study.

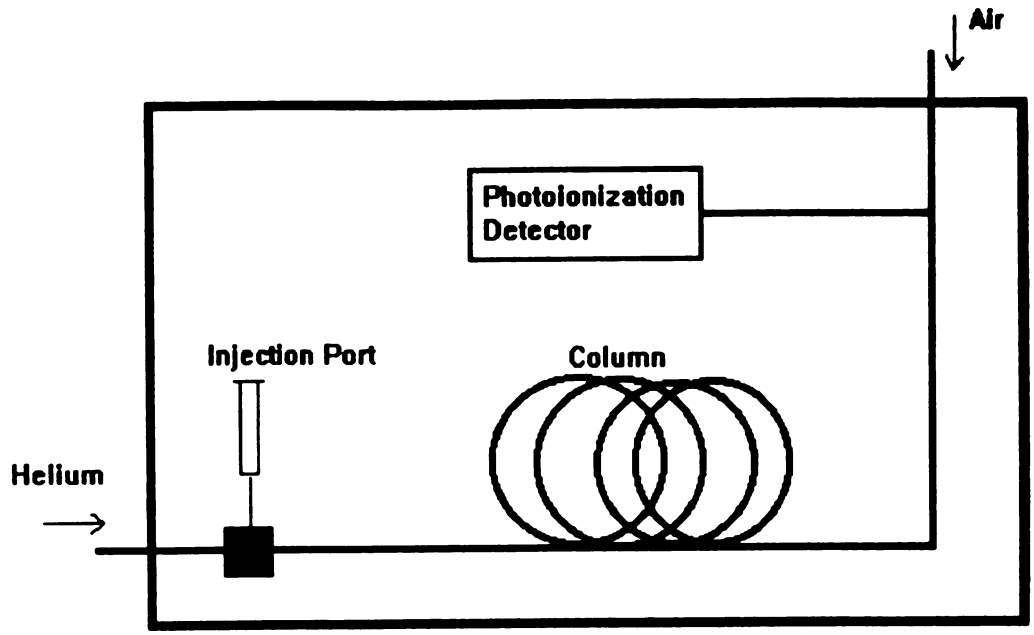

Figure 5. Major components of the Photovac gas chromatograph.

Standardization of hexanal headspace concentrations

A standard test method for the determination of formaldehyde and other carbonyl compounds (ASTM D5197) was adapted to verify the hexanal concentrations being emitted from the dynacalibrators. This method involves trapping carbonyls, including hexanal, onto a silica adsorbent cartridge coated with 2,4-dinitrophenylhydrazine (DNPH, Supelco Inc., Bellefonte, PA). Aldehydes react with DNPH to form stable derivatives. The resulting hydrazones are then eluted from the cartridges with 5 mL of high performance liquid chromatography (HPLC) -grade acetonitrile and then analyzed by HPLC.

Standard concentrations of carbonyl-DNPH derivatives were purchased from Supelco (1 mL vial of 1000 μg of hexanal-DNPH / mL in acetonitrile). Details of the procedure used to determine the concentrations of hexanal emitted by the dynacalibrator are provided in Appendix A.

Quantitation of hexanal in the headspace of packaged cereal

The Photovac gas chromatographic procedure was used to quantitate hexanal concentrations in the headspace of packaged cereal. An aliquot (0.5 mL) of headspace from the packaged cereal was removed via a Hamilton 1 mL gas-tight syringe (Reno, NV) and injected into the gas chromatograph. The area counts representing the hexanal concentrations in the packaged cereal were then compared to the area counts of injected standards drawn from the dynacalibrators (Appendix B). This is a desirable approach because it directly measures the volatile compounds equilibrated with packaged foods. In

addition, the chance of any artifacts forming using this procedure is minimal as neither harsh temperatures nor organic solvents are used (Chang et. al., 1995).

Experimental Design of the Storage Study

The cereal product was held at five different temperatures: 23, 29, 37, 43, and 48°C. This study was done in triplicate using three different batches of cereal. The higher temperatures (29 to 48°C) were used to predict the shelf-life of the cereal stored at 23°C. The studies performed at 23°C permitted an evaluation for the accuracy of the predictive value.

For each temperature, at least 100 packages containing 200 ± 5 g of product were prepared and stored in an environmental chamber, allowing for a minimum of 33 sampling points. At each sampling period, three packages of product were pulled, allowed to equilibrate for 24 hours at ambient temperature (subject to a temperature range of 19 to 24°C), and then analyzed for concentrations of hexanal in the headspace (Figure 6).

The frequency of sampling at each temperature permitted the determination of the linear range for hexanal accumulation prior to the time of breakpoint (t_{bp}) (i.e., the time at which hexanal begins to accumulate exponentially in the headspace of the packaged cereal), and the exponential accumulation of hexanal concentrations for all three sets of experiments. The time and the corresponding hexanal concentrations are in Appendix C.

The dynacalibrator was sampled periodically with the DNPH / HPLC method. This was done in order to accurately verify the concentrations of hexanal being emitted from the instrument over time. The response of the gas chromatograph to the rate of

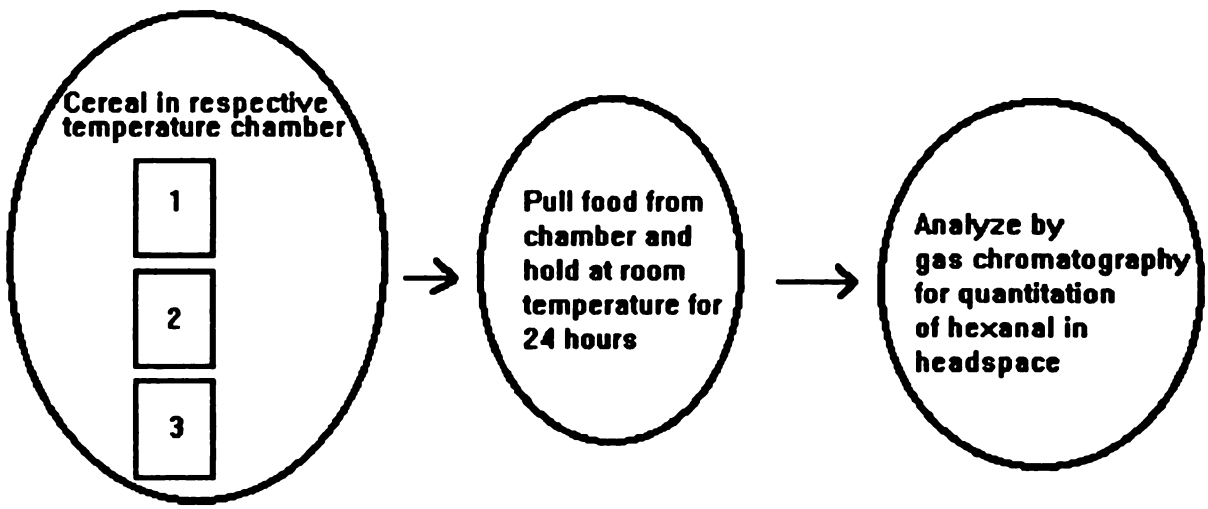

Figure 6. Overview of product sampling procedure[1].
[1] numbers refer to the experiment set.

hexanal transmission from the dynacalibrator was assessed on a daily basis. This information was used to establish a calibration factor by dividing the concentration being delivered from the dynacalibrator by that day's respective area counts from the gas chromatograph.

Multiplying sample average area counts by that day's particular calibration factor, gave the concentration of hexanal in parts per million (i.e.,- ppm or μmole hexanal / mole gas). The resulting hexanal concentrations were then plotted against their respective time of sampling.

Each experiment included other storage treatments in addition to the five temperatures reported above.

Experimental set 1: Additional packages were held at 16 and 41°C. The 41°C temperature was chosen because it was felt initially that a storage temperature of 48°C may be too high. The extra temperature storage was incorporated to assure adequate data for the Arrhenius plot. The 16°C condition was chosen to determine if a prediction could be made from the Arrhenius equation at the lower temperature.

Experimental set 2: Cereal was stored at additional temperatures of 54 and 60°C. These higher temperatures were chosen because the initial results from experimental set 1, indicated that the data generated at 48°C appeared to follow Arrhenius modeling. The higher temperatures were investigated to determine if a quicker prediction could be made.

Experimental set 3: One additional temperature of 22°C was investigated. Cereal was removed from storage and the headspace immediately sampled for hexanal concentration. Normalsampling procedures are subject to an approximate 5°C range (approximately 21 ±2.5 °C) during the 24 hour equilibration period. Better modeling may result by keeping cereal in an environmental chamber that has better temperature control.

Factors that may affect product stability

Several factors were measured prior to starting each of the three experimental sets. These data were used to determine if there were any trends with the relative stabilities among the three sets at room temperature. The factors measured were; moisture content (determined by method 14.004, AOAC, 1984), water activity (A_w as per the Instruction Manual for Hygroline Digital Humidity/ Temperature Indicators, Beckman Industrial Corporation, Cedar Grove, NJ.), and iron (in units of milligrams of iron/ 100 grams of sample by methods 965.09 and 968.08, AOAC 1990).

Results and Discussion

Validation of the hexanal assay

By developing a standard curve of the HPLC response to generated hexanal concentrations from the dynacalibrators, the linear range and the limit of quantification of hexanal were assessed. The DNPH / HPLC method produced a limit of quantification of 0.011 ppm for hexanal in the headspace gas (μmole hexanal / mole of headspace gas) with a 10% coefficient of variation, and a linear range of 0.011 to 1.572 ppm. The recovery of hexanal, as determined by the DNPH / HPLC method, was 91% or greater. This was achieved by spiking known amounts of hexanal into derivatizing SEP-PAK cartridges and analyzing the eluted hydrazone derivative by HPLC.

Several hexanal concentrations (standardized as described above) were used to determine the gas chromatographic response to hexanal. A standard curve was made of the GC response to 0.5 mL aliquots of varying hexanal concentrations in the headspace. The GC method was found to have a limit of quantification of 0.0317 ppm for hexanal in headspace gas with a 8% coefficient of variation, and a linear range of 0.0317 to 0.7379 ppm. This linear range of simulated headspace concentrations encompasses the anticipated concentrations for the ready-to-eat cereal at the time of rapid hexanal accumulation in the headspace.

The entire hexanal assay meets the requirements of remaining in statistical control (as per the parameters established above), and being both selective and sensitive enough to determine the linear accumulation of hexanal concentrations in packaged cereal prior to

the breakpoint (Dols and Armbretch, 1977). The described assay has a variation of 10% or less. Because of this low variation, it was anticipated that the hexanal assay could be used successfully for accelerated shelf-life testing. This hexanal assay was then applied to determine the shelf-life for packaged cereal.

Sensory analysis of packaged cereal

The low permeation properties of the PC-1 liner used for packaging in this study were necessary in order to properly execute the accelerated shelf-life test. However, off-odors from the packaging materials used in this study interfered with the ability of the panelists to detect rancidity. It was decided that the quantitative analysis of hexanal in headspace was more important than panelists being able to detect rancidity. Therefore, the PC-1 packaging material was used throughout this study. As a result, attempts to determine the concentrations at which panelists detect rancid odors were unsuccessful and subsequently aborted.

Storage stability of packaged cereal

Shelf-lives were determined by plotting hexanal concentrations as functions of time for each temperature (Fritsch and Gale, 1976). At each temperature, there was a gradual linear increase in hexanal concentration (i.e., a zero order reaction) until a transition point was reached where there was a rapid buildup of hexanal in the headspace (Figure 7). It is shortly after the time which hexanal rapidly begins to build up that consumers begin to detect rancidity. Boggs et al. (1963) observed this trend of hexanal development and

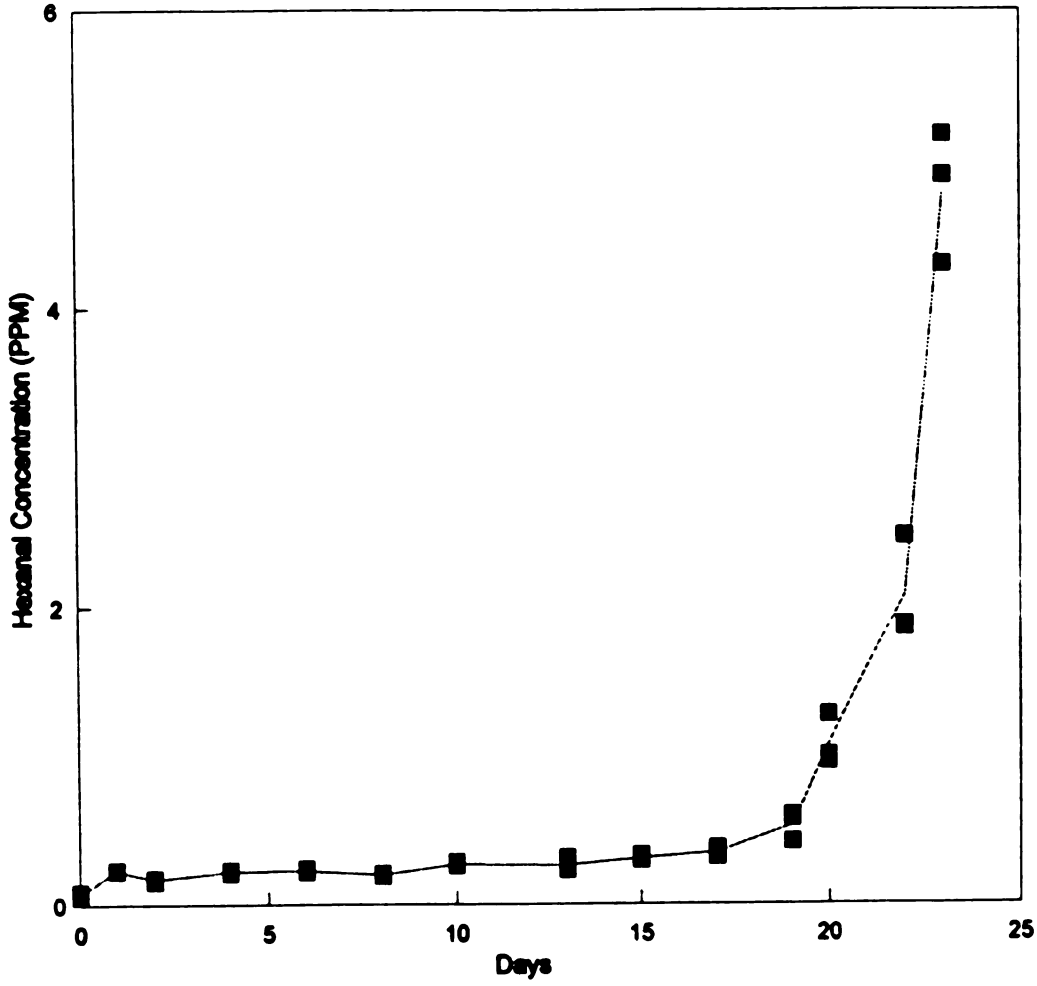

Figure 7. Oxidative stability of cereal stored at 48°C is 18 days, as determined by hexanal concentrations. Similar trends in hexanal development were observed for all temperatures of storage.

rancid odor detection while monitoring the development of hexanal in reconstituted potatoes.

The time at which the concentration of hexanal rapidly increased (called the breakpoint or t_{bp}) has been correlated to the time when customers and / or trained panelists begin to detect rancidity, thereby signifying the end of the shelf-life for the product (Boggs et al., 1963; Labuza, 1971; Fritsch and Gale, 1976).

It is generally assumed that the time at which the breakpoint of hexanal accumulation occurs is considered the end of the shelf-life of the product. Fritsch and Gale (1976) determined that panelists were able to detect rancidity at hexanal concentrations between 5 and 10 ppm (weight basis). Their work utilized a method that dealt with ground cereal and boiling water to force hexanal into headspace. The headspace was then sampled and the hexanal contained therein was quantitated by gas chromatography. Comparisons between the results of Fritsch and Gale (1976) and those of the current study should be made with caution because two different methodologies for hexanal extraction (isolation) and quantitation were used.

Development of a model to predict the shelf-life of ready-to-eat breakfast cereals

Arrhenius modeling

The times required to reach the breakpoint of hexanal development, and the rates of hexanal accumulation (i.e., the rate constants) at the various temperatures of storage are summarized in Table 1.

There were variations among the rate constants determined at similar temperatures

among the three experimental sets. These variations may be due to the fact that three different batches of cereal were utilized in the study. Thus, variables such as differences in fatty acid compositions and concentration profiles, surface area interactions, initial moisture contents, and the exposure of raw ingredients to different stresses would be introduced.

Table 1 -Rate constants and times to breakpoint (t_{bp}) for hexanal formation in packaged cereal stored at various temperatures.

Experimental set #	Temperature of storage (°C) 23	29	37	37	48
1	0.00166[1] (133)[2]	0.03172 (75)	0.00570 (34)	0.00911 (29)	0.01130 (17)
2	0.00623 (91)	0.01320 (50)	0.04016 (17)	0.05690 (13)	0.09397 (8)
3	0.00226 (111)	0.00367 (70)	0.00792 (32)	0.01790 (24)	0.01910 (13)

[1] Rate constants representing the linear accumulation of hexanal in headspace prior to t_{bp} are expressed in units of ppm day^{-1}.
[2] In parentheses, t_{bp} is expressed in units of day.

Arrhenius plots were developed by plotting the natural logarithm of the rate constants as functions of inverse absolute temperature, i.e., ln (slope) vs. $1/T^{(K)}$ (Figure 8). Linear regression analyses were performed on the Arrhenius data which provided

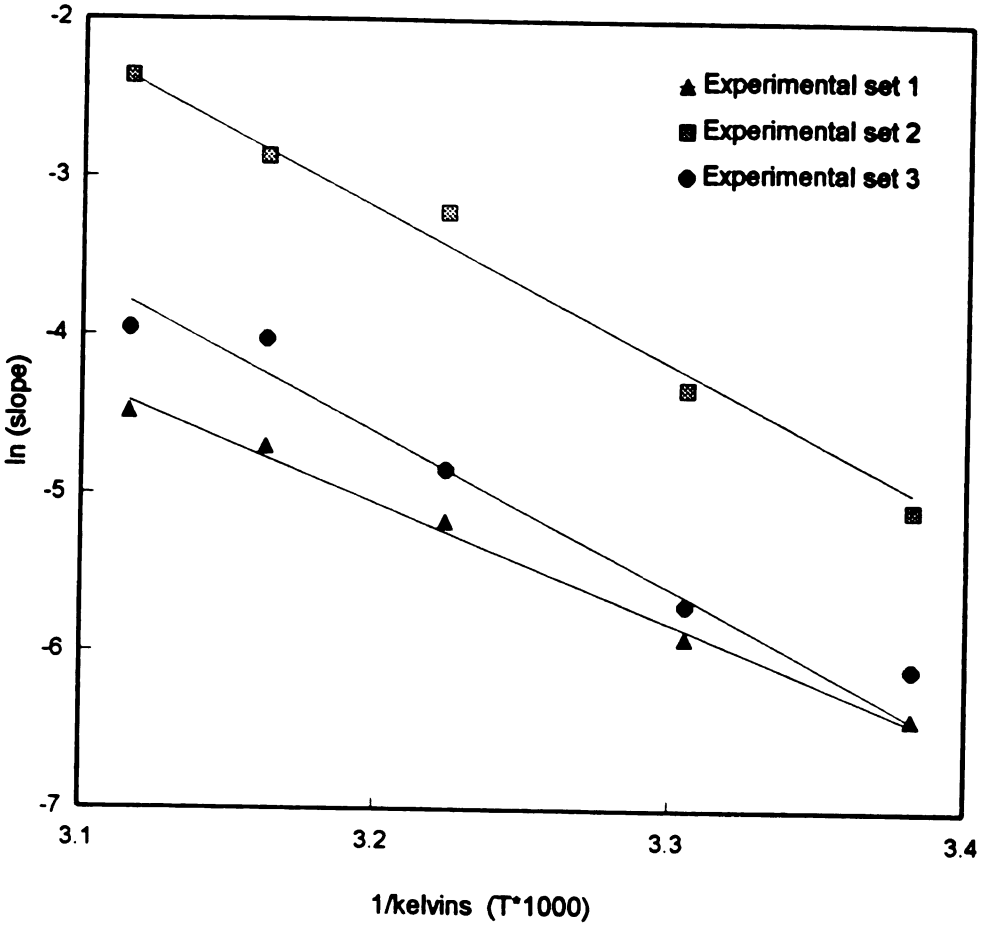

Figure 8. Arrhenius plots for the formation of hexanal in ready-to-eat cereals based on gas chromatographic headspace analysis.

predictions for the rate constants at room temperature and the apparent energies of formation for hexanal in the headspace of packaged Cereal (Table 2).

Table 2. - Predicted rate constants and energies of formation for hexanal in the headpsace of a corn base ready-to-eat cereal at 23°C.

Experimental set #	Predicted rate constant (ppm /day)	Energy of hexanal formation in the headspace of cereal (Kcal/mole)
1	0.00160	15.1
2	0.00689	19.5
3	0.00160	19.4

The calculated apparent energies of hexanal formation are close to those reported by Fritsch and Gale (1976) who reported an average of 14 Kcal / mole for stored cereal. Frankel (1993) reported an average energy of hexanal formation of 29 Kcal / mole in oxidizing fish and vegetable oils. The calculated energies of hexanal formation for ready-to-eat breakfast cereals, as determined by Fritsch and Gale (1976), are very similar to the calculated energies of hexanal formation in headspace in the current study. Keeping in mind that two different methods for quantitating hexanal formation were involved, the proximity of the data for the two investigations reinforces the results of both studies.

There are a couple of factors that may explain the differences in the calculated energies of hexanal formation between for the cereals and the fish and vegetable oils. The information reported by Fritsch and Gale (1976) indicates that the exposer to the different processing stresses and the types or amounts of catalysts that are present in cereals may

result in lower energies of hexanal formation when compared to those of fish and vegetable oils. In addition, the greater exposure of lipids in cereals to an oxidizing environment (i.e., greater surface area in contact with the air) may facilitate the oxidation process.

Averaged hexanal concentrations at t_{bp} for individual experimental sets were determined (Table 3). The average hexanal concentrations at which the breakpoints occur are similar for experimental sets 1 and 3. However, the hexanal concentration at which the breakpoint in experimental set 2 occurs is higher than those determined in the other two sets. A similar trend was found between rate constants of the three experimental sets (Table 2). Again, these differences can be explained on the basis that different batches of cereal were used. cereal packages at the time of breakpoint.

Table 3. - Hexanal concentrations[1] in the headspace of packaged cereal at the time of rapid hexanal accumulation (t_{bp}).

Experimental Set #	Temperature of storage (°C) 29	37	43	48	Avg.
1	0.332	0.316	0.380	0.347	0.344
2	0.764	0.718	0.805	0.747	0.759
3	0.356	0.301	0.345	0.311	0.328

[1] Hexanal concentrations (ppm) represent the average of the headspace concentrations of three cereal packages at the time of breakpoint.

The times to reach the breakpoint at room temperature were calculated by

applying the equation

$$x = y/m \qquad (10)$$

where y is the average concentration from each experimental set (Table 3), and m is the predicted rate of hexanal accumulation in the headspace of packaged cereal at room temperature (Table 2). The calculated times (x) at which t_{bp} would occur and the observed times at which t_{bp} occurred for each experimental set are listed in Table 4.

Table 4.- Measured and predicted times required to reach the breakpoint for hexanal formation in packaged cereal.

Experimental Set #	Actual time to t_{bp} (days)	Predicted time to t_{bp} (days)	Error relative to actual t_{bp}
1 (23°C)	133	210	+58%
2 (23°C)	91	108	+19%
3 (22°C)	133	204	+53%
1 (16°C)	234	786	+236%

The errors in the calculated shelf-life predictions for the three experimental sets are unacceptable as all of the shelf-life predictions resulted in overestimations. The results of the shelf-life predictions are not congruent with those of Fritsch and Gale (1976) who were able to calculate satisfactory shelf-life predictions. The differences between the two studies may be attributed to the different methodologies used. Fritsch and Gale (1976)

used boiling water to drive hexanal into the headspace of sample vials filled with ground cereal, whereas the methodology used in this study directly measured the equilibrated headspace of packaged cereals.

The observed rate constants for hexanal formation in cereal stored at 16°C in experimental set 1, and in cereal stored at 23°F in all three experimental sets, did not follow modeling trends set forth by tests performed at the higher temperatures (Figures 9, 10, and 11). As stated by Bromberg (1984), large deviations from exponential Arrhenius plots often mean that the observed rate constants are influenced by several competing reactions. Bromberg (1984) implied that the observed rate-limiting step for the formation of hexanal in headspace may be different for tests run at lower temperatures (i.e., $\leq$ 23°C).

The hexanal concentration at the time of breakpoint in the 16°C test of experimental set 1, was 0.174 ppm (Figure 12). This concentration at the time of breakpoint was vastly different than the averaged breakpoint concentration of 0.344 ppm from experimental set 1 (Table 3), as determined by the hexanal assay used in this study. It is quite possible that breakpoint concentrations of hexanal in the entire system at 16°C are similar to those encountered at higher temperatures. This change in the concentration at the time of breakpoint between 16 and 22°C would be consistent with a change in the reaction mechanism. Therefore, either the rate constant used by Fritsch and Gale (1976) and Kumor (1986) for determining the shelf-life may not be the proper rate constant, or Arrhenius modeling over this temperature range may not be permissible for the substrate used in this study, or there has been a change in the rate limiting step.

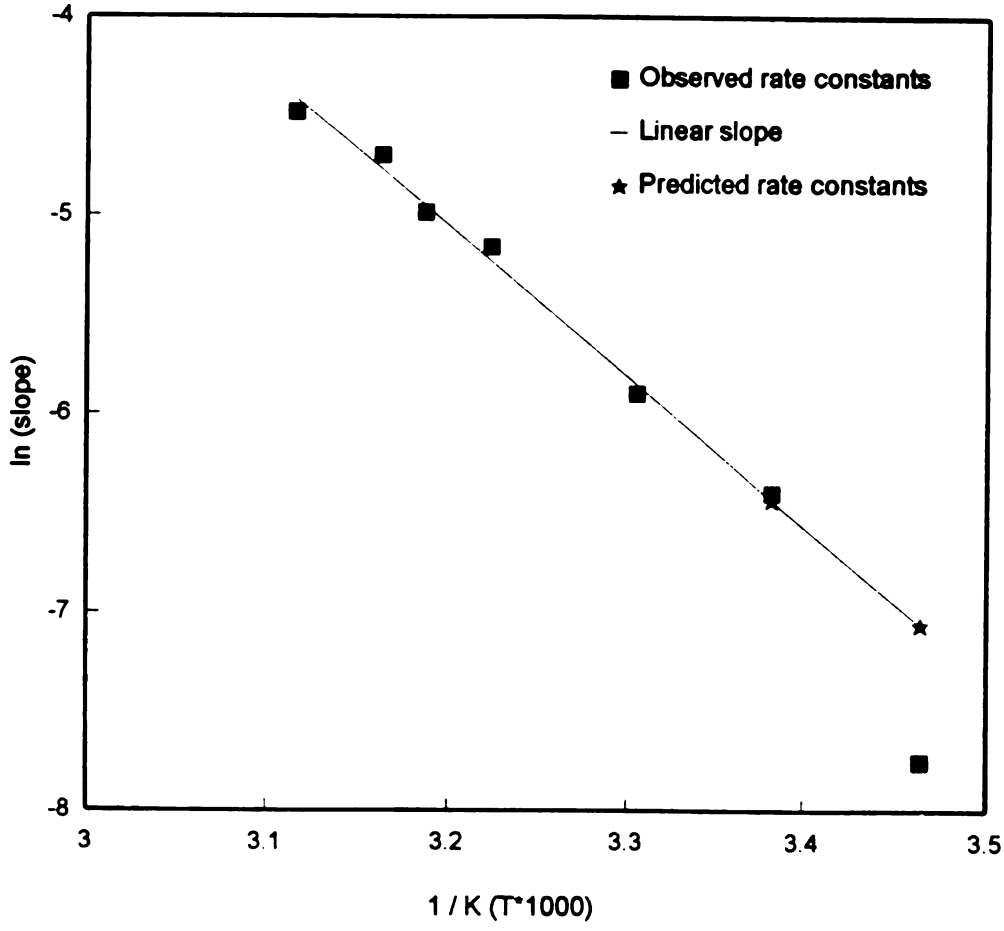

Figure 9. Arrhenius plot of the shelf-lives for a packaged cereal (experimental set 1) based upon the rate of formation of hexanal in the headspace as determined by gas chromatographic analyses.

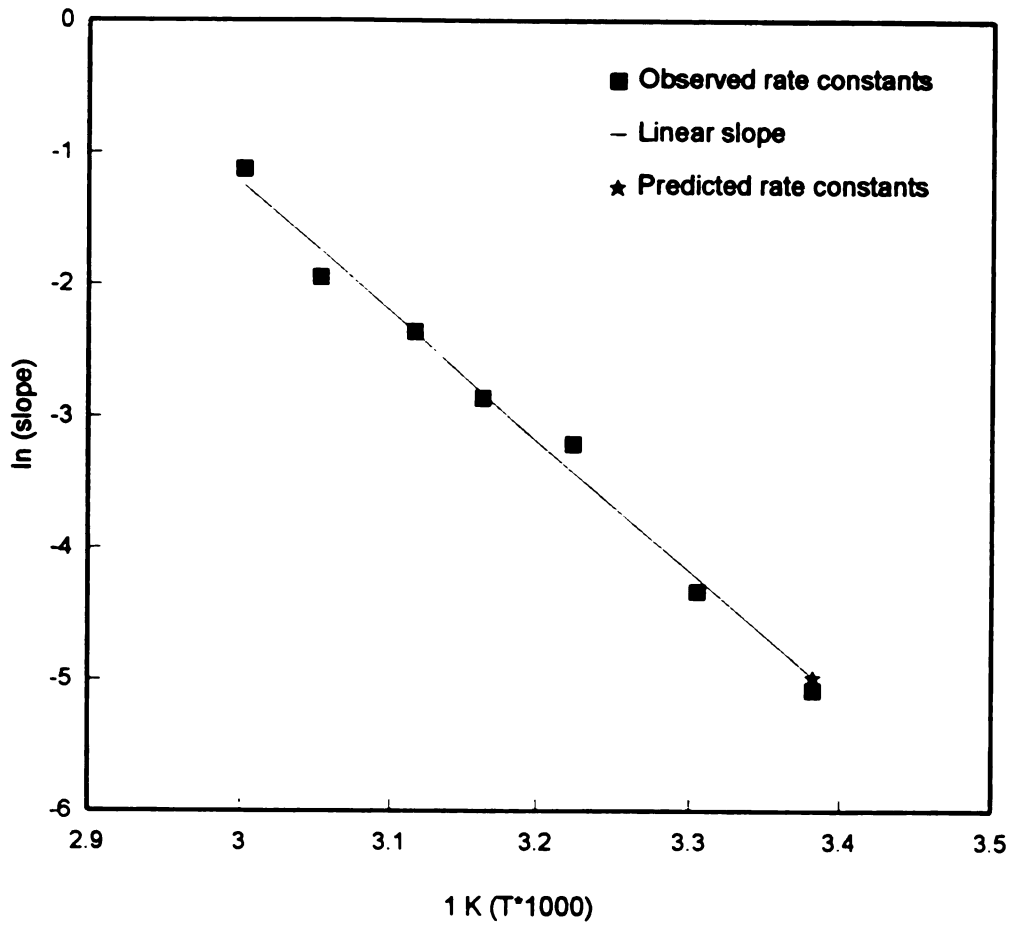

Figure 10. Arrhenius plot of the shelf-lives for a packaged cereal (experimental set 2) based upon the rate of formation of hexanal in the headspace as determined by gas chromatographic analyses.

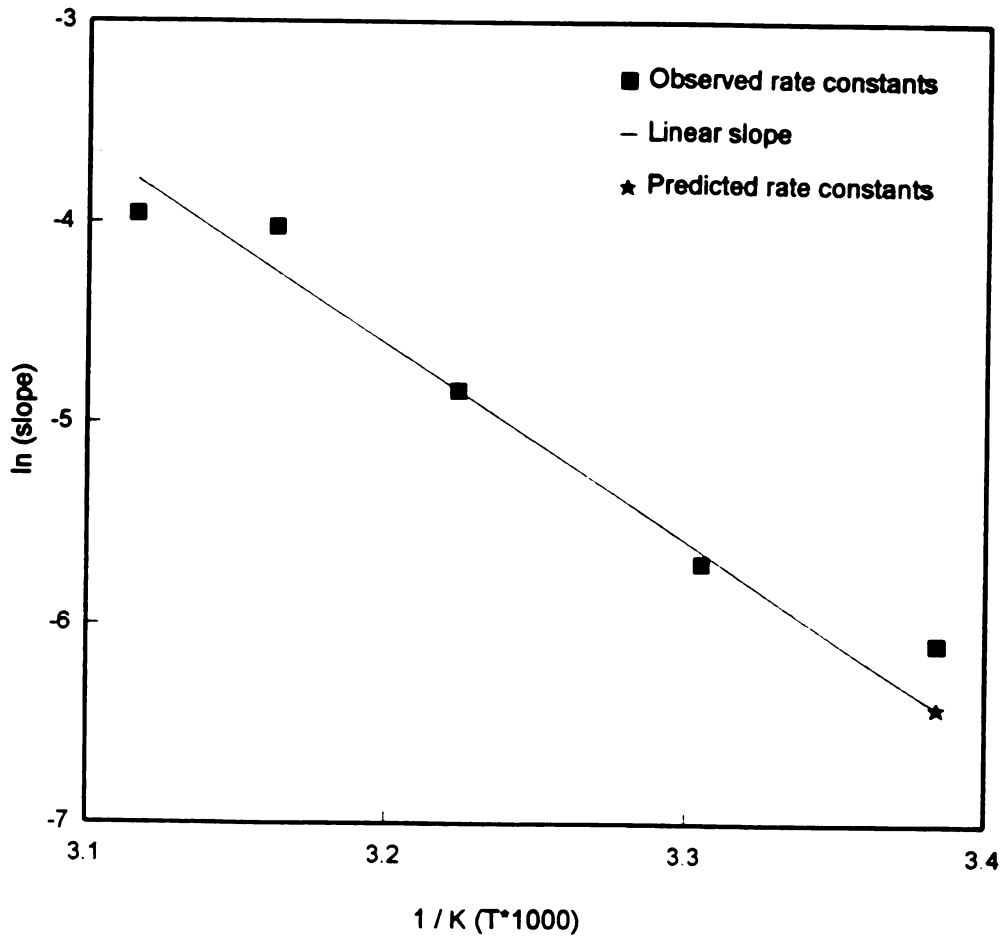

Figure 11. Arrhenius plot of the shelf-lives for a packaged cereal (experimental set 3) based upon the rate of formation of hexanal in the headspace as determined by gas chromatographic analyses.

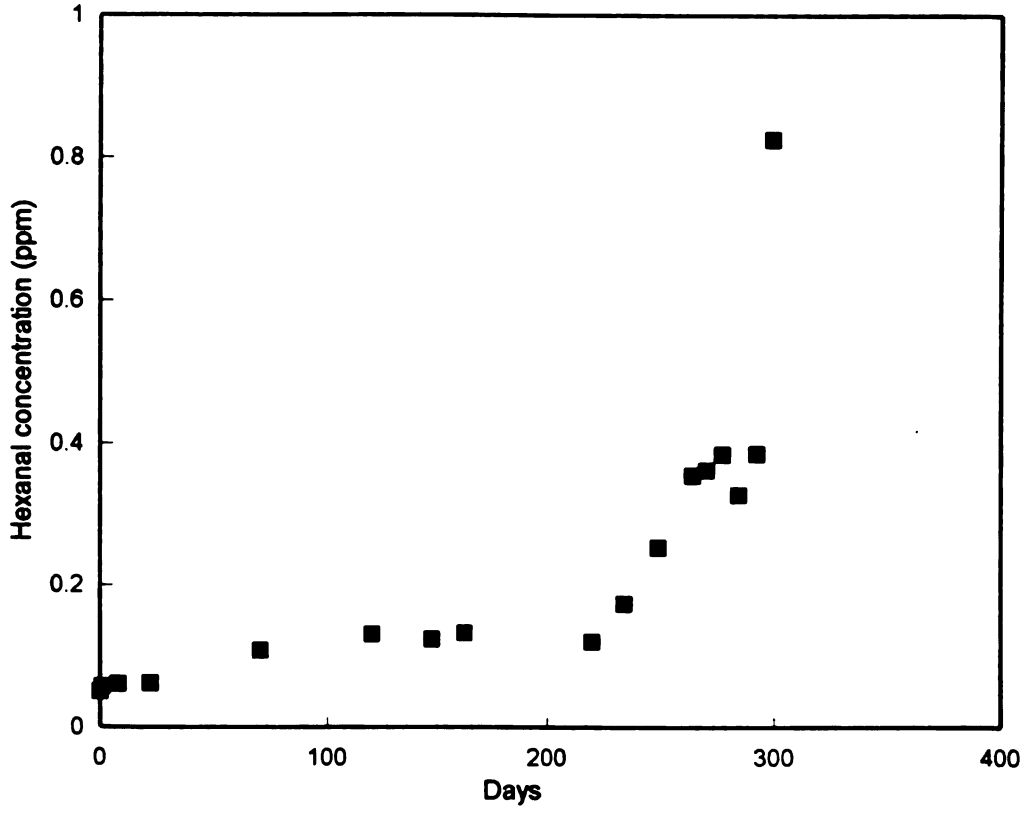

Figure 12. Oxidative stability of cereal stored at 16°C is 234 days, as determined by hexanal concentrations.

Assuming that Arrhenius modeling can be used to predict the oxidative stability of the ready-to-eat cereal used in this study, the deviation from the predicted rate constant at 16°C for the Arrhenius plot in experimental set 1 (Figure 9 and 12) indicates that a new limiting factor / reaction influencing the concentrations of hexanal in the headspace of packaged cereal at lower temperatures ($\leq$ 23°C) may be involved (Bromberg 1984). This limiting factor must have a higher energy of activation than the 15.1 Kcal / mole energy of formation for hexanal in the headspace of packaged cereal for experimental set 1 (Taoukis and Labuza, 1985). One may conceive that the reason for the limited amount of hexanal in headspace may be due to a diffusion mechanism. Taoukis and Labuza (1985) stated that energies of diffusion are typically around 10 Kcal / mole. Therefore, the limiting factor at 16°C probably is not likely to be the result of a diffusion mechanism unless there was a change in the structure of the product occurring between 16 and 23°C.

Modified Arrhenius modeling

The rate constants used in the previous Arrhenius modeling were functions of both hexanal concentration and time (Fritsch and Gale, 1976). The second objective of this study was to determine the time at which the product becomes oxidatively unstable. Therefore, a rate constant that is solely a function of the time at which there is a large change in the accumulation of hexanal in the headspace of packaged cereal was investigated.

A modified rate constant used by Koelsch et al., (1991) and Arrhenius modeling were used to more accurately predict the oxidative stability of cereal as determined by the

accumulation of hexanal in the headspace (k is ***1/days to break point***). Arrhenius modeling was again applied using the new rate constants to predict the stability of the cereal products (Table 5). The errors in the shelf-life predictions are within the variability of the hexanal assay. This approach permits the reliable prediction of the oxidative stability for cereals at room temperature.

Table 5.-Measured and predicted times required to reach the breakpoint for hexanal formation in packaged cereal.

Experimental set #	Actual time to t_{bp} (days)	Predicted time to t_{bp} (days)	Error relative to actual t_{bp}
1 (23°C)	133	127	- 5%
2 (23°C)	91	86	- 5%
3 (22°C)	133	139	+ 5%
1 (16°C)	234	232	- 1%

Results from additional storage treatments

Additional runs were performed in the three experimental sets in order to investigate the effects of various temperatures on the shelf-lives of the product.

Experiment 1: This experimental set included the storage of cereal at 16 and 41°C in addition to the five original storage temperatures. A linear regression analysis on the modeling data was used to calculate the variation (i.e., the R^2 value) for predicting the time at which hexanal would rapidly accumulate in headspace, assuming that the storage temperature is known. The R^2 value was determined to be 0.99; therefore the rate

constant for hexanal formation at 48°C was consistent with data acquired through Arrhenius modeling. This means that a storage temperature of 48°C can be used to predict the oxidative stability at room temperature. As a result, the 41°C storage temperature was not repeated in later experiments.

The rate constant for hexanal formation at 16°C was also consistent with data acquired through Arrhenius modeling as a result of the R^2 value of 0.99 reported earlier. Because the rate constant for the 16°C storage temperature followed trends from Arrhenius modeling, shelf-life predictions can be made at 16°C using storage temperatures of 23°C and higher.

Experiment 2: This experimental set included cereal stored at additional temperatures of 54 and 60°C. A linear regression analysis performed on the Arrhenius data from experimental set 2 produced an R^2 value of 0.99. Therefore, the rate constants from the 54 and 60°C storage tests followed trends from Arrhenius modeling. Future experimenters should use these higher temperatures to provide quick and reliable shelf-life predictions of cereal shelf-life quality.

Experiment 3: The equilibration period between pulling packaged cereal from the environmental chambers and analyzing the headspaces by gas chromatography is subjected to temperature fluctuations. A test was performed which involved holding cereal in a 22°C environmental chamber throughout storage and during the 24 hour equilibration period. The intent was to minimize any fluctuation in hexanal concentrations in the

headspace that may result in a erroneous shelf-life prediction. A linearity study on the accumulation of hexanal in the headspace prior to the breakpoint for the 22°C constant temperature test was determined (Figure 13). A regression analysis was performed on the hexanal data generated over a 140 day period. At this point, there was a rapid accumulation of hexanal in headspace. The data from days 125 and 133 were not included in the analysis because the environmental chamber temporarily lost its ability to control temperature. Therefore, the resulting storage temperature was subject to ambient temperatures (approximately 19°C). The data used to determine the rate of hexanal accumulation in the headspace with respect to time (i.e., <125 days) was determined to have an R^2 value of 0.98. These data have a higher R^2 value than other tests in this study that were conducted over 80 days. Therefore, using the temperature controlled storage environmental chamber permits for an better determination of the time at which hexanal begins to rapidly accumulate in the headspace of packaged cereal. It is recommended for future testing, that a controlled room temperature storage environment is used to hold cereal during the equilibration period. This will realize more accurate shelf-life predictions.

Factors affecting product stability

The parameters measured prior to the start of the three experiments are summarized in Table 6. Interactions of these data should be made with caution, as the experimental sets were not true replicates. The measured parameters (% moisture, water activity, and iron content) do not encompass all the factors that affect oxidative stability. Because

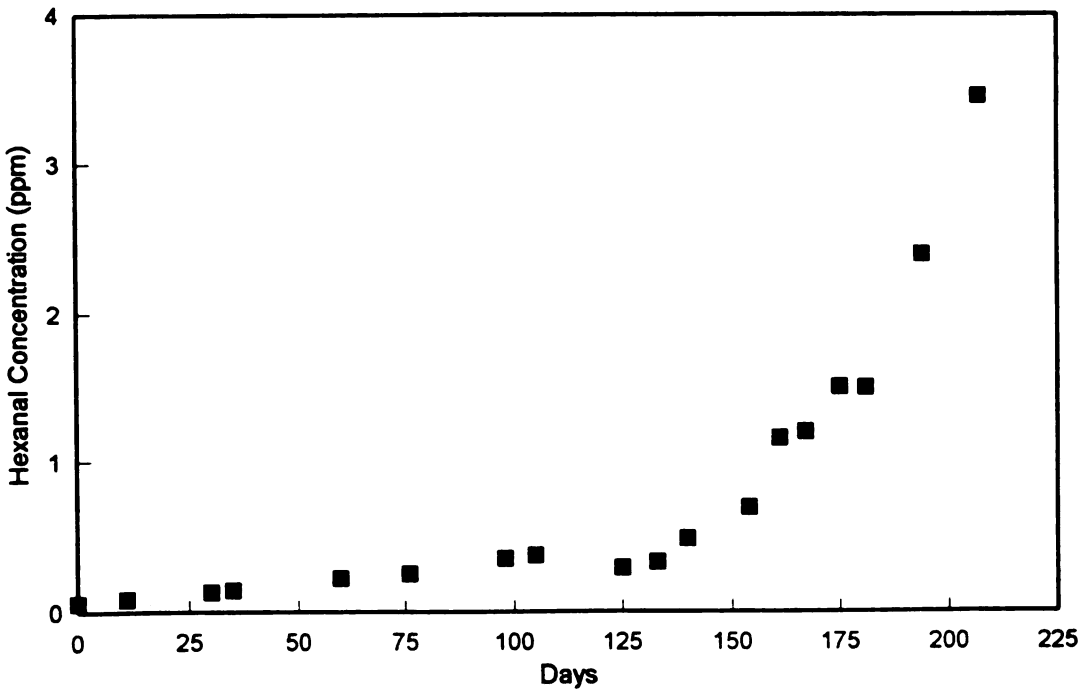

Figure 13. Hexanal formation in packaged cereal stored at 22°C.

there was limited characterization of the cereals, definitive conclusions or inferences drawn from information in Table 6 would be presumptuous.

Table 6.-Moisture levels, water activities, and Iron concentrations in packaged cereals.

Experimental set #	% Moisture	Water Activity (A_w)	Iron (mg/100g)
1	3.0	0.12	30.3
2	3.3	0.15	29.5
3	3.4	0.15	18.6

Times required to make shelf-life predictions

The shelf-lives observed at room temperature and the times required to predict the shelf-lives of cereals at room temperatures using data gathered from storage temperatures of 29, 37, 43, and 48°C are summarized in Figure 14. These data indicate that the time required to predict the room temperature (i.e., 23°C) shelf-life is approximately half of the time that it takes to determine the shelf-life of a product under storage at 23°C.

The time required to determine the shelf-life of a product under storage at 23°C can be reduced by approximately 80% using data gathered from storage temperatures of 37°C and higher. In this study, shelf-life predictions made over large temperature ranges (e.g.,

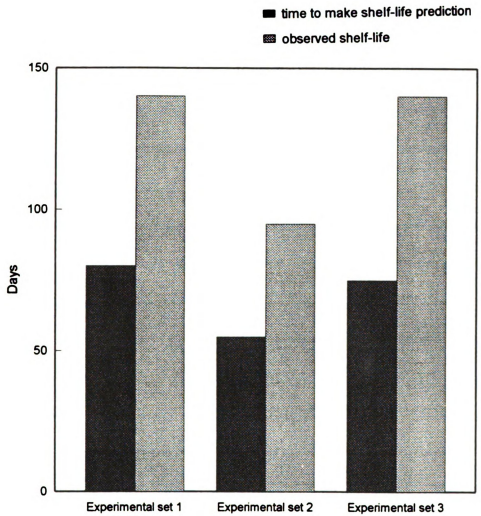

Figure 14. The times required to make room temperature shelf-life predictions using tests run at 29, 37, 43, and 48°C and the respective observed room temperature shelf-lives.

23 to 37°C) which used data from only three storage temperatures (e.g., 37, 43, and 48°C) were in error by an average of 17%. This error is greater than the 3% average relative error encountered when using data from four storage temperatures (e.g., 29, 37, 43, and 48°C). It is recommended that shelf-life predictions utilize four or more storage temperatures and that caution be used when dealing with shelf-life predictions made over large temperature ranges because of the increased shelf-life prediction errors encountered when using the three storage temperatures of 37, 43, and 48°C.

Summary and Conclusions

The validation of a hexanal assay that can be used to assess the degree of rancidity in ready-to-eat cereals was completed. This task involved two separate validations: one for determining the concentrations of hexanal being emitted by instruments that generate continuous concentrations of hexanal in the headspace (dynacalibrators), and the other for determining the GC response to different concentrations of hexanal in the headspace (see Appendices A and B). The combination of the two assays permitted the determination of the time at which hexanal begins to accumulate rapidly in the headspace of cereals.

Off-odors from the packaging material used in this study interfered with the ability of panelists to detect rancidity. As a result, a comparison between hexanal concentrations and panelists' perception of rancid odors in oxidizing cereal were not made.

An accelerated shelf-life test was performed on a corn-based ready-to-eat breakfast cereal using hexanal as a marker of lipid oxidation. This was repeated three times using three separate batches of product. Arrhenius plots were made by plotting rate constant, which were determined by observing the time at which hexanal concentrations began to accumulate exponentially in the headspace of the product, as functions of various temperatures. The data from this modeling had stability predictions errors of 5% or less at room temperature (23°C) and 16°C.

The work completed in this study has positive implications for companies wishing to introduce new products in the market place. The hexanal assay can be used as a tool to evaluate the oxidative stability of lipids in food products. The accelerated shelf-life

testing, utilizing Arrhenius modeling, and the hexanal assay can also be used to facilitate the introduction of new products to market by reducing the product development cycle.

Future Research

The results from this study sets the stage for a number of tests that could be used to improve the oxidative stability of ready-to-eat cereals. The evaluation of various raw ingredients and different processing conditions, and what impact they have on the oxidative stability of cereals should be explored. Such information would provide a basis for developing a more stable and desirable products.

It has long been known that temperature affects the rates of lipid oxidation. Both differential scanning calorimetery and thermal mechanical analysis have been used to determine the effects of temperature on physical characteristics of food. Based on the fact that temperature influences both the physical characteristics and the rates of reactions in foods, it may be possible to use instrumental analyses to characterize the oxidative stability of cereals. Fatty acid profiles (e.g., composition and amount) could also be performed on the products to see if there are any correlations with the oxidative stability of the food.

The packaging material used throughout this study contributed off-odors to the product. Although these odors did not interfere with the detection of hexanal in the headspace by gas chromatography, they did obstruct the sensory panel's ability to determine rancidity. Different packaging material should be investigated if the oxidative stability of cereal is to be determined by / or in conjunction with sensory panelists in future testing of cereal products.

Appendices

Appendix A
DNPH method validation

Scope

An outline of how to sample and quantitate a 500 mL hexanal headspace sample follows: This methodology can be used as an independent check to verify the concentration of hexanal being delivered from an instrument that generates precise concentrations of gases in headspace (i.e., a dynacalibrator).

Principle

This assay involves drawing "headspace" through a cartridge coated with a derivatizing agent. The reacted hexanal in the cartridge is eluted and then analyzed by HPLC. The resulting data are then used to quantitate hexanal concentrations for future headspace sampling. This approach is adapted from the American Standard Testing Method (ASTM D5197) for monitoring carbonyl emissions in the auto industry.

Apparatus

1. HPLC system including:

    a) Automated sampler (Waters 712 WISP).

    b) HPLC pump (Waters 510).

    c) Column- Zorbax ODS, 4.6*250 mm 5-Micron (part # 880952.702, serial # F48816. Rockland Technologies, Inc./ Hewlett-Packard Company, Newport DE).

    d) Ultraviolet Detector (Waters 490E).

    e) Hewlett-Packard Integrator model 3390A.

2. Solutions:

    a) The mobile phase for HPLC consisted of the following

    70% HPLC grade acetonitrile
    30% HPLC grade water

    b) Washing solution for eluting off the derivatizing agent in the cartridges

    >99.9 pure, HPLC grade acetonitrile

3. DNPH requirements:

-sample cartridges (Supelco # LPDNPH S1050, # 21014)
-standard solutions (Supelco # Hexanal-DNPH, 1000 µg/mL, #47178)

4. Personal protective equipment:

-Safety glasses with side shields must be worn at all times.
-Nitrile gloves must be worn when working with mobile phase, DNPH standards, and eluting cartridges.

Sampling Procedure

1. Attach a LP-DNPH cartridge to a 500 mL headspace sampling apparatus.

2. Place a sampling syringe, attached to the headspace sampler, "into" the emission stream (or headspace) of the dynacalibrator.

3. Sample 500 mL of headspace from the dynacalibrator. After sampling, remove the cartridge, properly seal the ends of the cartridge, and place into a refrigerator until further analysis. Repeat this sampling procedure 3 times.

Eluting Procedure

1. Remove the LP-DNPH cartridges from storage, and elute the reacted hexanal out of the cartridge. This is done by applying 5 mL HPLC-grade acetonitrile to the 3 mL reservoir on the cartridge. Collect the eluting material in a 5 mL volumetric flask. Once the material stops eluting from the cartridge, fill to 5 mL with acetonitrile if necessary.

2. Mix the contents in the 5 mL volumetric flask. Transfer the contents to a HPLC vial and seal with a cap. Place the vial in a refrigerator until HPLC analysis.

Preparation of Standards

Prepare a working standard solution of 0.005 μg hexanal-DNPH/mL acetonitrile standard by making serial dilutions from the 1000 µg of hexanal-DNPH/mL acetonitrile solution standard.

Analyzing samples

1. Prepare three "Blank" solutions. This is done by following the *Eluting Procedure* described above on three unadulterated cartridges (i.e.- cartridges that have not been deliberately exposed to any hexanal).

2. Analyze the standards, blank, and samples by HPLC.

Calculations

Determine the concentration of hexanal emission being delivered by a dynacalibrator from the following calculations:

1. Develop a standard curve from the area counts obtained from 1.00, 0.100, 0.010, and 0.005 μg/mL standards plotted against the respective concentrations.

2. Average the area counts from three blank cartridges.

3. Record the results from the analyzed samples.

4. Subtract the averaged area count of the blank cartridges from the averaged area counts of the samples.

5. Multiply the corrected area count by the concentration to area count ratio established from the linear regression (step 1).

6. Determine the amount of hexanal collected onto the cartridges.

7. Convert μg of hexanal to μmoles of hexanal.

8. Determine the moles of gas (i.e.- headspace) drawn through the cartridges assuming the headspace gas (i.e., hexanal in nitrogen) acts as an ideal gas at standard temperature and pressure.

9. Determine the concentration (ppm or μmole/mole) of headspace sample from the dynacalibrator by dividing the amount of hexanal by the amount of headspace gas.

Hexanal-DNPH assay validation

Several parameters of the hexanal-DNPH / HPLC assay were assessed. The parameters were: the range of linearity, the limit of reliable measurement, the accuracy, and the precision of the assay. Each parameter was determined by carrying out a separate study.

1. Linearity: A series of DNPH-hexanal standards were prepared from a hexanal-DNPH standard. The liquid standards were found to be linear from 0.03 to 0.7 μg hexanal-DNPH / mL acetonitrile. This is equivalent to headspace concentrations ranging from 0.067 to 1.57 μmoles hexanal/ mole at standard temperature and pressure, provided a 500 mL. headspace sample is used.

2. Accuracy: The accuracy (or rather recovery) of this method was determined by spiking three different concentrations of hexanal onto DNPH-cartridges in triplicate (e.g., 0.08, 0.82, and 8.20 μg). The three different concentrations of hexanal in acetonitrile were spiked into the reservoir of the cartridges. The cartridges were washed with 5 mL acetonitrile and analyzed. The recovery for the three different amount of hexanal was determined to be 88% or greater.

3. LQ or LRM: The limit of quantification (LQ) or the limit of reliable measurement (LRM) is considered to be the minimum concentration at which an analyte can be analyzed and give results that fall within a 95% confidence interval. Several LQs were determined for this method (Table 7).

Table 7.-The limit of reliable measurement for determining hexanal concentrations in headspace by HPLC.

Conc. in Soln.(ug/mL)	Conc. of Headspace (ppm) for a 500 mL sample	Coefficient of Variation
0.005	0.011	10
0.010	0.022	7
0.030	0.067	2

* data are the results of seven measurements.

4. Precision: A precision study was performed by sampling dynacalibrators. This was performed by sampling three different hexanal concentrations, in triplicate, over a 5 day period. The hexanal was generated by dynacalibrators and sampled onto Supelco's DNPH-coated cartridges. The three concentrations and the respective variations are in Table 8.

Table 8.-The between day and within day variation for determining hexanal concentrations in headspace by HPLC.

Concentration (ppm)	Between day variation	Within day variation
0.136	25%	75%
0.287	30%	70%
0.725	1%	99%

Appendix B
Photovac 10S Plus Validation

Scope

This write-up includes the theory, data, and evaluation of the data for the responce of a gas chromatograph to standardized hexanal concentrations.

Principle

This method involves sampling the headspace of a packaged food product. The headspace sample (0.5 mL) is injected into the gas chromatograph for separation and quantification, and then compared to the area counts of injected standards.

Apparatus

1. GC system including:

    a) Hamilton 1mL gas tight syringe (Hamilton Company, Reno NV).

    b) Photovac 10S Plus.

    c) Hewlett-Packard integrator model 3390A connected to Port 2 of the Photovac gas chromatograph via an analog cable.

Hexanal gas chromatograph assay validation

Several parameters of the hexanal gas chromatograph assay were assessed. The parameters were: the range of linearity, the limit of reliable measurement, the accuracy, and the precision of the assay. Each parameter was determined by carrying out a separate study.

Linearity: A series of 0.5 mL headspace aliquots with varying hexanal concentrations, were injected onto the Photovac gas chromatograph. The response of the gas chromatograph was found to be linear from 0 to0.738 μmoles hexanal/mole gas (ppm).

LQ or LRM: The limit of quantification (LQ) or the limit of reliable measurement (LRM) is considered the minimum level at which a hexanal concentration can be analyzed and give results that fall within a 95% confidence interval. The concentration, results, and Coefficient of Variation are in Table 9.

Table 9.-The limit of reliable measurement for measuring hexanal concentrations in packaged cereal by GC.

Conc. of Headspace (ppm) for a 500 mL sample	Coefficient of Variation
0.032	8
0.039	4

** data are the results of seven measurements.

Precision: A precision study was done by sampling 0.5 mL of headspace samples. This was done at three different levels, 5 times, over a 5 day period. The hexanal was derived from dynacalibrators and injected into the Photovac. The concentrations and the respective variations are in Table 10.

Table 10.-The between day and within day variation for measuring hexanal concentrations in packaged cereal by GC.

Concentration (ppm)	Between day variation	Within day variation
0.136	33%	67%
0.287	56%	44%
0.725	81%	19%

Appendix C

Column heading definitions:

Time pulled =The day at which samples were withdrawn from their environmental chamber.

Analyzed by G.C. = The day at which samples were analyzed by Gas Chromatography.

Temp. = Ambient temperature at the day of analysis.

Dyn. Conc. =Concentration in ppm being delivered by the Dynacalibrator.

Table 11-Data for Experimental set 1

Time pulled	Analyzed by G.C	Temp	16 C (ppm)	23 C (ppm)	29 C (ppm)	37 C (ppm)	41 C (ppm)	43 C (ppm)	48 C (ppm)
0	0		0.049615	0.049615	0.049615	0.049615	0.049615	0.049615	0.049615
1	2		0.056693	0.088117	0.118778	0.169549	0.187491	0.176192	0.229507
2	3			0.108697	0.114841	0.140835	0.127317	0.149634	0.172789
4	4			0.117575	0.1206	0.175233	0.180164	0.19417	0.224272
6	7	21.8				0.204681	0.197864	0.213142	0.227378
8	9	20	0.060556	0.126572	0.150666	0.18148	0.180266	0.171557	0.20217
10	11					0.219329	0.218413	0.21839	0.270954
13	14	19				0.194023	0.201468	0.203917	0.265524
15	16				0.143531		0.230292	0.212196	0.309389
17	18	19		0.169623		0.201883	0.247645	0.236248	0.347236
19	19	20							0.533859
20	21				0.161806	0.260005	0.244069	0.271945	1.075062
22	23		0.061607	0.156484	0.171418	0.245648	0.272829	0.3255	2.069265
23	24						0.315792	0.35645	
24	25					0.305497	0.344808	0.368579	4.70917
25	28					0.266513	0.233231	0.261116	
27	30						0.295699	0.447942	
29	32				0.202561	0.291399	0.36386	0.380251	
31	33	19		0.167465			0.320712	0.55177	
34	35	20.3				0.316308	0.428935	1.144079	
36	37	22		0.186231	0.211946		0.711873	2.301362	
38	39	19				0.506012	0.584337	2.683231	
41	42	19.5			0.244819	0.665948	1.479086	3.710222	
43	44	19			0.200134	0.559122	2.309746	6.569174	
45	46	19		0.182656	0.205686	0.576969	2.429849		
48	49	21.5			0.266583	1.396459	3.489131		
50	51	22.3		0.211265	0.202014	1.271688			
52	53	23.8			0.278157	2.490385			
56	57	22			0.216723				
57	58	22		0.239058	0.249844	4.164778			
59	60	22			0.272645				
62	63	23			0.27318				
64	65	21.5			0.283222				
66	67	23.3			0.306775				
67	68	22.3			0.2741				
68	69	22		0.193688	0.311683				
70	71	23.2	0.107594						
71	72				0.290517				
73	76	22.5			0.278104				
75	76	22.5			0.332819				
76	77			0.243341					
78	79				0.401744				
82	83	23.4			0.452115				

Table 11(continued) -Data for Experimental set 1

Time pulled	Analyzed by G.C.	Temp.	16 C (ppm)	23 C (ppm)	29 C (ppm)	37 C (ppm)	41 C (ppm)	43 C (ppm)	48 C (ppm)
84	85	19			0.38134				
86	87				0.36095				
90	92	20		0.21893	0.469818				
97	98	19.5			0.517611				
101	102	21		0.241141					
106	107				1.081478				
108	110				1.898733				
114	115				2.241914				
120	121	22.8	0.130328						
121	122	22.1			2.59013				
126	128	24.5		0.357761					
133	134	20.8		0.308559					
147	148	22.5	0.122962	0.441245					
149	153	21.5		0.392334					
156	157	21		0.602813					
160	161	23		0.504778					
162	163	21.5	0.131677	0.629556					
167	168	21		0.575739					
173	174	23		0.646728					
177	178	23		1.092926					
184	185	25.5		1.060556					
193	194	21		0.83837					
207	208	21		1.71161					
214	215	21		2.273419					
220	221	24	0.120255						
234	235	23	0.17387						
249	250	22	0.253367						
264	265		0.354804						
270	271	21	0.361739						
277	278	19	0.384736						
284	285	21	0.327571						
292	293	19	0.384933						
299	300		0.824						

Table 12-Data for Experimental set 2

Time pulled	Analyzed by G.C.	Temp.	23 C (ppm)	29 C (ppm)	37 C (ppm)	43 C (ppm)	48 C (ppm)	54 C (ppm)	60 C (ppm)
1	2		0.068097	0.068097	0.068097	0.068097	0.068097	0.068097	0.068097
2	3	21.5						0.210372	0.318026
3	4	22.5					0.238351	0.368912	0.714838
4	6	23.3						0.488806	1.802844
6	7	22.3			0.190939	0.234442	0.421804	1.491318	7.690348
7	8	22			0.228163	0.281805	0.641072	3.383777	
8	9	22.8	0.095976	0.127765	0.243344	0.313803	0.746663	5.576541	
9	10	23.2			0.25542	0.342219	1.088557		
10	11				0.291273	0.446996	2.473625		
13	14					0.805231	5.48663		
14	15	22.5	0.113677		0.483454	0.89111			
15	17	22.2		0.177049	0.453939	1.196151			
16	17				0.689767	1.841718			
17	18		0.117935	0.196668	0.718353	1.78633			
20	21	23	0.143558	0.209597	1.236508				
21	23	23.4	0.123735	0.178593	1.157125	5.159556			
22	23	19	0.128271	0.202307	1.573267				
23	25	19			1.779645	8.460402			
24	25		0.139083	0.215592	1.699197				
28	30	21.5	0.127828	0.203367	3.860442				
37	38	20	0.159705	0.327612	8.930546				
42	42	19.5	0.165893						
46	47	21	0.148051	0.599534					
47	48			0.606813					
50	53		0.192781	0.764603					
56	57			1.273686					
62	63	22		2.140124					
68	70	22.8	0.399588						
75	76	21	0.350921						
83	84	20.8	0.457948						
85	88	23	0.484792	7.647988					
89	91	21	0.617463						
91	95	22.5	0.794002						
96	97	21.5	0.83998						
98	99	21	0.984288						
103	104	22.5	1.211272						
105	106	23	1.244925						
110	111	21.5	1.406577						
116	117	21	1.540874						
120	121	23	2.32096						
126	127	23	2.404856						
134	135	21	2.80901						

Table 13-Data for Experimental set 3

Time pulled	Analyzed by G.C.	Temp.	23 C (ppm)	22 C (upstairs) (ppm)	29 C (ppm)	37 C (ppm)	43 C (ppm)	48 C (ppm)
0	3		0.06129	0.0612899259	0.06129	0.06129	0.06129	0.06129
3	5	21.5					0.093263	0.100255
6	7	22					0.194148	0.213133
7	10	21			0.087329	0.148961	0.157061	0.175991
11	12	20.3		0.0830758908		0.171063	0.231766	0.298589
12	13	22.8	0.107879			0.187678	0.248922	0.258639
13	14	22.1					0.248644	0.311872
15	18	21.8						0.799488
17	18	21.8					0.303018	1.714018
18	20	21				0.235328	0.352479	2.800799
19	20	21				0.286002	0.326096	
20	21	24.5			0.186253		0.420646	2.810971
24	25	21.3			0.17769	0.250765	0.345914	
25	26	20.8	0.111737			0.261232	0.67726	
26	27	19.5				0.245692	0.877994	
26	27	19.5					0.488014	
27	30	21.5			0.182691	0.22298		
30	30	21.5		0.1334174864				
30	32	25			0.183085	0.445566	3.426871	
32	33	23			0.254556	0.30113	4.343904	
35	38	21		0.1464020359	0.245137	0.504492	9.623612	
39	41	22.5	0.158506			0.623589		
41	45	21.5	0.170854			1.581498		
46	47	21			0.216596			
45	47	21				2.986541		
48	49	22.5			0.209598			
53	54	23			0.210667			
60	61	21		0.2241481137	0.277271			
66	67	23			0.313177			
70	71	23	0.268026		0.356202			
76	77	25.5		0.2541279585	0.507612			
84	85	21	0.226269		0.685083			
98	99	21		0.3543152242	1.653839			
105	106	21		0.3794109253	2.336219			
111	112	24	0.389901					
125	126	23	0.540753	0.2942148663				
133	134	22	0.784846	0.3306787142				
140	141	22	0.966747	0.4874793225				
154	155	20	1.816375	0.694338				
161	162	21	2.417315	1.164198				
167	168	19	1.226561	1.2047796591				
175	176	21	3.218512	1.5054203784				
181	182	19		1.5026034445				

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