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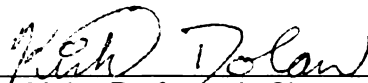
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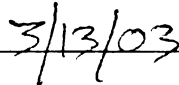
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Major Professor's Signature


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THERMAL AND MECHANICAL EFFECTS ON RETENTION OF FOOD-GRADE
 β -CAROTENE DURING EXTRUSION PROCESSING

By

Monali Manoj Yajnik

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ABSTRACT

THERMAL AND MECHANICAL EFFECTS ON RETENTION OF FOOD-GRADE β -CAROTENE DURING EXTRUSION PROCESSING

By

Monali Manoj Yajnik

To determine the separate thermal effects of extrusion, isothermal and nonisothermal experiments were conducted by heating wheat flour with 0.4616% (w/w) food-grade β -carotene in a shearless environment at 78, 138, and 149°C, at 28% (w/w) moisture content. To determine the total (thermal plus mechanical) effects of extrusion on retention of *trans*- β -carotene, the same mixture was extruded at 30/50/70/90/110°C and 50/70/90/110/130°C at screw speeds of 200, 250, 300, and 400 rpm on two separate days with a dough moisture content ranging from 30 – 36% (w/w). In the isothermal experiments at 78°C, retention did not change significantly. However, at 138°C for up to 60 minutes, the calculated first-order reaction rate constant was $6.83 \times 10^{-5} \text{ s}^{-1}$. The rate constant, $3.56 \times 10^{-4} \text{ s}^{-1}$, and activation energy, 18.82 kJ/g-mol, obtained from the nonisothermal experiments were used to calculate *trans*- β -carotene retention due to thermal effects during extrusion. For both temperature profiles, total *trans*- β -carotene retention ranged from 58 - 97%. Thermal effects accounted for less than 5% of the loss, showing that mechanical effects were the predominant cause of *trans*- β -carotene degradation. A linear statistical model worked well for separate days of extrusion; however, an exponential model was more effective for combined days of extrusion. Overall, food-grade β -carotene was more stable than other sources of β -carotene.

DEDICATION

To my parents, Manoj and Medha Yajnik, and my brother Manan, for their unconditional love and encouragement, and to Jeff Moore, for being a constant source of technical and emotional support.

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CHAPTER 1: INTRODUCTION

Food extrusion is a versatile and efficient cooking, mixing and forming method (Ilo and Berghofer, 1999). During this process, a material such as wheat flour is continuously mixed, heated, sheared, and conveyed by rotating screws inside a barrel at temperatures up to 200°C. The dough is formed into various shapes by being fed through a die at the end of the barrel. When extruding at temperatures greater than the normal boiling point of water, the product can expand upon exiting the die as superheated water flashes due to the sudden drop in pressure between the inside of the die and the atmosphere (Harper, 1978). Due to the intense thermal and mechanical energy inputs during extrusion, many physicochemical reactions can occur, such as starch gelatinization, protein denaturation, and degradation of vitamins and pigments (Padmanabhan and Bhattacharya, 1993). The quality of an extruded product can be assessed by the extent of chemical and structural transformations that occur. Examples of extruded products include ready-to-eat cereals, expanded snacks, breading substitutes, soup and gravy bases, full-fat soy flour, and texturized vegetable protein (Horn and Bronikowski, 1979).

Various thermosensitive indicators, such as thiamin (Guzman-Tello and Cheftel, 1987) and β -carotene, can be used to evaluate the overall intensity of extrusion cooking. β -carotene is commonly used in extruded food products as a source of vitamin A and as a coloring agent; therefore, monitoring its retention and color change during extrusion is important. When using β -carotene as an indicator, the color change after extrusion is typically measured by reflectance or absorbance colorimetry or high performance liquid chromatography (Guzman-Tello and Cheftel, 1990).

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β -carotene, which belongs to the chemical structure class of carotenoids, is recognized in the food industry as a valuable and natural colorant. In recent years, consumers have shown increased interest in the use of natural products for health benefits (Wisgott and Bortlik, 1996). Despite the use of stable synthetic additives and colors during processing, consumers are increasingly interested in the use of “natural” ingredients, such as β -carotene, that are perceived as safer and healthier (Berset, 1989).

β -carotene is one of the most important of more than 40 carotenoids that are converted to vitamin A. Vitamin A can play a role in normal vision, gene expression, reproduction, embryonic development, growth, and immune function (Food and Nutrition Board, 2001). Rich sources of β -carotene include carrots, squash, spinach, tomatoes, and broccoli. Observational epidemiological studies suggest that higher blood concentrations of β -carotene and other carotenoids obtained from foods are connected with a lower risk of several chronic diseases (Food and Nutrition Board, 2001). Within the class of carotenoids, β -carotene yields the highest amounts of vitamin A value, and is currently under intense research because of this potential to decrease chronic disease risk and alleviate vitamin A deficiency. Therefore, it is necessary to have a better understanding of its reactivity under different conditions (Parker, 1996).

The absorption of carotenoids is affected by food processing (Van het Hoff et al., 1998). Rock et al. (1998) found that the rise in serum β -carotene concentration was significantly greater in subjects consuming cooked carrots compared to those consuming equal amounts of raw carrots; therefore, to maximize absorption, it is important to minimize loss of β -carotene during processing. Carotenoid pigments are highly unsaturated, which makes them considerably reactive with light, heat, oxygen and acid.

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These sensitivities can cause problems during processing (Rodriguez-Amaya, 1999). While β -carotene occurs naturally as *trans* β -carotene, a significant amount of it is transformed into its *cis* form and other derivatives, when exposed to heat, shear, or oxygen. This chemical conversion relates to degradative changes in color and vitamin A bioavailability (Tsukida et al., 1982). During extrusion, several mechanisms have been suggested for the degradation of β -carotene, including *cis-trans* isomerization and oxidation into various derivatives (Berset and Marty, 1992; Marty and Berset, 1988). Kone and Berset (1982) reported that commercial, food-grade β -carotene powder underwent structural change during extrusion.

To obtain optimal β -carotene retention or desired color during extrusion, it would be useful to predict color and concentration changes throughout the extrusion process. Some studies have investigated β -carotene degradation during extrusion (Marty and Berset, 1990; Lee et al. 1978). However, these studies typically used either a pure form of *trans*- β -carotene or a mixture of isomers that were not in a protective, water soluble matrix. Food-grade β -carotene, which is used by food processors, is often encapsulated in gelatin and other ingredients that make it water soluble and possibly provide protection from degradation. This characteristic may make it more stable than all-*trans*- β -carotene. However, no studies were found, with the exception of Kone and Berset (1982), that investigated the degradation of food-grade β -carotene during extrusion.

During extrusion, the prediction of color and concentration changes is difficult due to the lack of knowledge of specific inputs, such as thermal and mechanical components (Shukla, 1991). No research was found that analyzed the separate contribution of thermal and mechanical effects, or that determined whether there is a

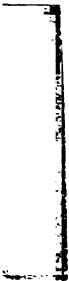
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synergistic effect of the two variables. Although one study (Guzman-Tello & Cheftel, 1990) modeled the overall effect of extrusion on degradation of β -carotene, the investigators did not account for the product temperature profile along the barrel. The assumption was that the product maintained the temperature at the die throughout the entire process. In the present study, the product temperature profile and varying residence time in each section of the extruder were taken into account.

Therefore, the objectives of this study were:

1. To determine the extent of thermal effects on concentration of food-grade *trans*- β -carotene in a shearless environment;
2. To measure the retention of food-grade *trans*- β -carotene in extruded products at high shear conditions and two different temperature profiles;
3. To quantify the separate thermal and mechanical effects on degradation of food-grade *trans*- β -carotene;
4. To validate a proposed model for predicting separate thermal and mechanical effects of extrusion on retention of food-grade *trans*- β -carotene.



CHAPTER 2: LITERATURE REVIEW

2.1. Extrusion Processing

2.1.1. Principles of Extrusion

Extrusion processing is commonly used for production of human and pet foods (Marty & Berset, 1986). Extrusion has great potential for production of many types of foods due to its high throughput, low operating cost and high-temperature, short-time treatment (Lee et al., 1978). Extrusion cooking is commonly used to produce breakfast foods, snack products and other convenience foods. During extrusion, moistened, expansile, starchy, and/or proteinaceous foods are plasticized and processed in a tube-shaped barrel using a combination of pressure, moisture, temperature, and mechanical shear (Hauck and Huber, 1989). The raw materials undergo many chemical and structural transformations, including starch gelatinization, protein denaturation, complex formation between amylose and lipids, and degradation of vitamins and pigments. The severity of shear forces in extrusion distinguishes it from conventional processing methods (Ilo & Berghofer, 1999). Batch or semi-continuous extrusion, which has been used for more than a century, utilizes a piston inside a tube to force a material through an opening to produce a specific shape. This process can be made continuous by using a helical screw instead of a piston to transport the material forward (Hauck and Huber, 1989).

The total development of an extrusion cooking process includes an evaluation of the function and operation of the extruder coupled with a characterization of the structural, physical, and rheological property changes within the raw material (Fletcher et al., 1985). Important parameters of extruders include barrel diameter and length, screw

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configuration, and die geometry, while operating characteristics include screw speed, feed rate, water injection, die temperature, power, and degree of fill. Product properties include moisture content, composition, residence time, temperature, and shear rate in the extruder (Clark, 1978b).

2.1.2.. Characteristics of Single-Screw vs. Twin Screw Extrusion

Extruders may be categorized as single-screw (SSE) or twin-screw (TSE). In an SSE, the material is fed into a hopper and then into the screw channel. While the food is conveyed along the channel, it is also simultaneously mixed, heated and sheared. As it approaches the die, it is transformed into a thermoplastic, viscoelastic material (Rossen and Miller, 1973). Mixing in a SSE poses some problems, because the small clearance between the screw and the barrel minimizes longitudinal material transfer. Another disadvantage is that material that fills the screw channels may stick to the screws and slip on the barrel's inner surface, thus only allowing for rotation with the screws and thereby hindering forward product movement (Martelli, 1971). The only force that prevents the material from rotating with the screw and pushes it to advance is its drag or friction against the barrel surface; therefore, if there were more friction, there would be less rotation of the material with the screw and more forward motion. This reliance on friction is often considered a limitation of the SSE process (Martelli, 1983).

In comparison, a TSE has numerous advantages for processing a variety of materials. TSE's can be used as mixers and formers and are categorized as co-rotating and counter-rotating types, depending on the directions that the screws rotate. These can then be subdivided into fully intermeshing, partially intermeshing, and non-intermeshing screws. The geometrical configuration of co-rotating, fully intermeshing TSE's is such

that the material is enclosed in compartments and allows material exchange lengthwise (Fichtali and van de Voort, 1989). One advantage of the TSE is its superior process stability, due to the reduction in fluctuating die pressure. When compared to the SSE, the TSE reduces the total dependence on frictional forces for transport, thereby allowing the process to operate with uniform flow and a more uniform die pressure as the viscosity of the material changes (Hauck and Huber, 1989).

2.1.3. Food Extrusion

Food extrusion is not a new development in the food industry, and has been used to make pasta for more than 60 years (Akdogan, 1999). Known as the pasta press, this initial application of extrusion was used to mix semolina flour, water, and other ingredients to form uniform dough and then force the mixture through specially designed dies without adding heat to the barrel. Ready-to-eat cereals are also manufactured on extruders; in this case, the extruder functions as a mixer, cooker, and former, and puffing of the product is accomplished simultaneously (Harper, 1978). In general, there is more mechanical energy input required to cook food products than is necessary to melt and homogenize plastics; as a result, food extruders usually have more kneading paddles, reverse pitch sections and other forms of high-shear screw elements. Food products are rheologically more complex, and the physical state of the material changes with the cooking process; therefore, there is a strong shear- and temperature history- dependent viscosity (Roberts and Guy, 1987). The costs of extrusion include the extruder and the energy required to cook the material at the required moisture and temperature. In an industrial setting, lower moisture contents and higher temperatures (up to 350°C) are used, requiring less steam but more electrical power for cooking; therefore, extruder

operating costs tend to rise exponentially as moisture content is lowered below 27% (Horn and Bronikowski, 1979).

Because of the complex network of most food systems, food extrusion is not a simple process. The conditions of food extrusion can include high temperature, pressure and shear, as well as low to intermediate water contents; therefore, the nutritional value of many extruded products is modified (Asp, 1987). Typically, the basic structures of extruded products are created due to the alteration and manipulation of natural biopolymers such as starches (wheat, maize, rice, and potato derivatives, and less frequently rye, barley, oats, sorghum, cassava, tapioca, buckwheat, and pea flours), as well as proteins (soy, soy flour, field bean, fava beans, and separated cereal proteins, such as wheat gluten) (Guy and Frame, 1994). Precooked cereal-based products are often fortified with vitamins, such as thiamin and β -carotene. Vitamin fortification is often done before the extrusion process to reduce microbial contamination that can occur if vitamins are sprayed on after a cooking process; therefore, the total vitamin content after extrusion needs to be investigated due to the potential loss during the thermally and mechanically intense process (Bjorck and Asp, 1983).

A thorough understanding of the flow pattern in an extruder is necessary in understanding all the other phenomena that occur, such as chemical and physical changes, heat transfer, and mixing (Clark, 1978a). The mixing conditions, flow patterns, and residence time distribution (RTD) in an extruder affect product quality, more so when the material is sensitive to heat or shear, or when greater mixing is required. The RTD is useful in determining optimal processing conditions for mixing, cooking, and shearing, and can be used to determine rates of chemical reactions during the process.



An RTD function allows estimation of the extent of mixing, the time a particle spends in the extruder, and the average total forces that are exerted on the material during the process, thus allowing for a direct comparison to a chemical reactor (Fichtali and van de Voort, 1989). An RTD is usually explained and analyzed in terms of a normalized concentration ($E(t)$ function) (Levenspiel, 1999), measured by injecting a tracer into a system. The output concentration of the tracer and the amount of time required for the tracer to exit from the extruder is then determined (Altomare and Ghossi, 1986). Most researchers evaluate residual color by using a colorimeter, which is easier, more straightforward and less rigorous than actually extracting the color, or dye, from the extrudate. Peng et al. (1994) studied the RTD in a TSE by injecting a red color during extrusion of rice flour. The mean residence times were calculated according to color values and then compared to mean residence times calculated according to concentration, based on a calibration curve of red dye concentration to color. At high concentrations, color values are not linear with concentration due to a saturation effect. The concentration approach is more accurate and results in shorter mean residence times and “sharper” $E(t)$ curves than those for the color approach.

2.1.4. Mechanical Effects of Extrusion

During extrusion, foods can undergo significant structural and chemical changes. Evaluation of the exact deformation that occurs in the barrel of an extruder is difficult to accomplish (Padmanabhan & Bhattacharya, 1993). Although high shear is usually necessary to achieve good mixing and reasonable rate of fluid transport in the extruder, it can also be responsible for decomposing networks or structures. A further explanation of

this may be that there is mechanical disruption of the structure, or perhaps degradative heat that is generated by shear-induced friction in the material (Clark, 1978a).

Mechanical effects can be described by specific mechanical energy, or functions of shear stress or shear rate. Specific mechanical energy (SME) is a function of the measured screw torque and can be defined as: $SME = (\text{torque} \times \text{screw speed}) / (\text{throughput})$ or the net rate of mechanical energy input divided by the mass flow rate. SME can be changed by varying screw speed, feed rate and temperature variables of the extrusion process (Frame, 1994; Harper, 1989).

Knowledge of the rheological properties of melted dough is important in modeling food extrusion systems because the properties affect extrudate expansion, texture, and appearance, as well as the thermal and mechanical energy inputs (Colonna et al, 1989). Shear rate affects viscous dissipation and can be related to the values of rotational speed and mechanical energy input of the system (Levine, 1989). In twin-screw extruders, complex mixing makes it very difficult to measure or predict a velocity profile or local shear rates in the dough. As an alternative, the average shear rate can be calculated by using the matching viscosity technique (Steffe, 1996). The matching viscosity method originated from a need to estimate power required to mix non-Newtonian fluids. Many mixing situations have ill-defined velocity profiles, thereby suggesting use of an average, or “overall” shear rate. By developing a relationship between the Power number and Reynolds number, Metzner and Otto (1957) established a relationship between impeller speed and the average shear rate of the non-Newtonian fluids that were being evaluated. Mohammed et al. (1990) applied this method to estimating the average shear rate in a co-rotating TSE. The average shear rate for three



screw configurations of a TSE was estimated by using Newtonian and non-Newtonian fluids. The average shear rate showed a power-law relationship to the screw speed and was also affected by the screw type.

2.2. β -carotene

2.2.1. Role of β -carotene as a Food Colorant

Colors, whether natural or synthetic, have been added to foods for centuries. However, over the last twenty years, there has been a greater shift towards natural additives in food products. Carotenoids such as β -carotene represent a class of naturally occurring food colorants in the plant and animal kingdom that have been used to color various foods (Wisgott and Bortlik, 1996). Products that contain or utilize colorants that also provide health benefits, such as β -carotene, are generally classified as functional foods. Functional foods are foods that, by nature or design, can give health benefits beyond simple sustenance. They bridge the gap between food and drugs by giving consumers the ability, to some extent, to make a contribution to their own health care (Howe, 2000).

Although β -carotene is extremely sensitive to certain food processing conditions, marketable forms of the color have been produced. β -carotene powders are an excellent replacement for FD&C Yellow No. 5, which has been under scrutiny for its potential harmful effects to humans when used as a food colorant (Emodi et al., 1976). Synthetic β -carotene, which was first promoted in 1954, captures about 40% of the colorant market in Europe and 17% of the global market. β -carotene is primarily used in yellow fats, such as margarines, and in low-fat spreads, soft drinks, confectionary, and bakery products (Downham and Collins, 2000). Because of its structure, β -carotene is susceptible to

degradation by oxidation, light, and heat, and is typically insoluble in water. Because β -carotene is difficult to dissolve in many food formulations, several approaches have been explored to create a more usable product. Various approaches to enhance water-dispersibility include formation of colloidal suspensions, emulsification in oily solutions, and dispersion in suitable colloids. These ingredients can be combined with protein, carbohydrate, and lipids and then stabilized with antioxidants. The final product is usually spray-dried to give a water-dispersible powder (Francis, 2000).

Hoffman la Roche has developed a 7% (w/w) food-grade, cold-water-soluble (CWS), beadlet formulation that appears yellow to orange and is almost translucent. The formulation, which is comprised of β -carotene crystals in a matrix of corn oil, fish gelatin, maltodextrin, sucrose, and silicon dioxide, along with the antioxidants ascorbyl palmitate and dl- α -tocopherol, is used in soft drinks, confectionary, and dairy products (Ruijter, 1998). Benefits of this formulation include increased light stability and extension to application in products such as sauces, dressings, and soft drinks (Hansen, 1999). Kearsley and Rodriguez (1981) studied the thermal stability of a similar food-grade, water-soluble β -carotene powder (Hoffman LaRoche). A known concentration of β -carotene was prepared in distilled water and introduced into a sample tube. Samples were heated for 30, 60, 120, 180, and 240 minutes at 20, 50, 74, and 100°C, after which the absorbance was measured and compared to the control at time zero. After four hours at 100°C, the total β -carotene content had decreased to less than half of the original amount, thus proving its thermal stability in distilled water for the given time-temperature conditions.

2.2.2. Chemistry of β -carotene and Vitamin A

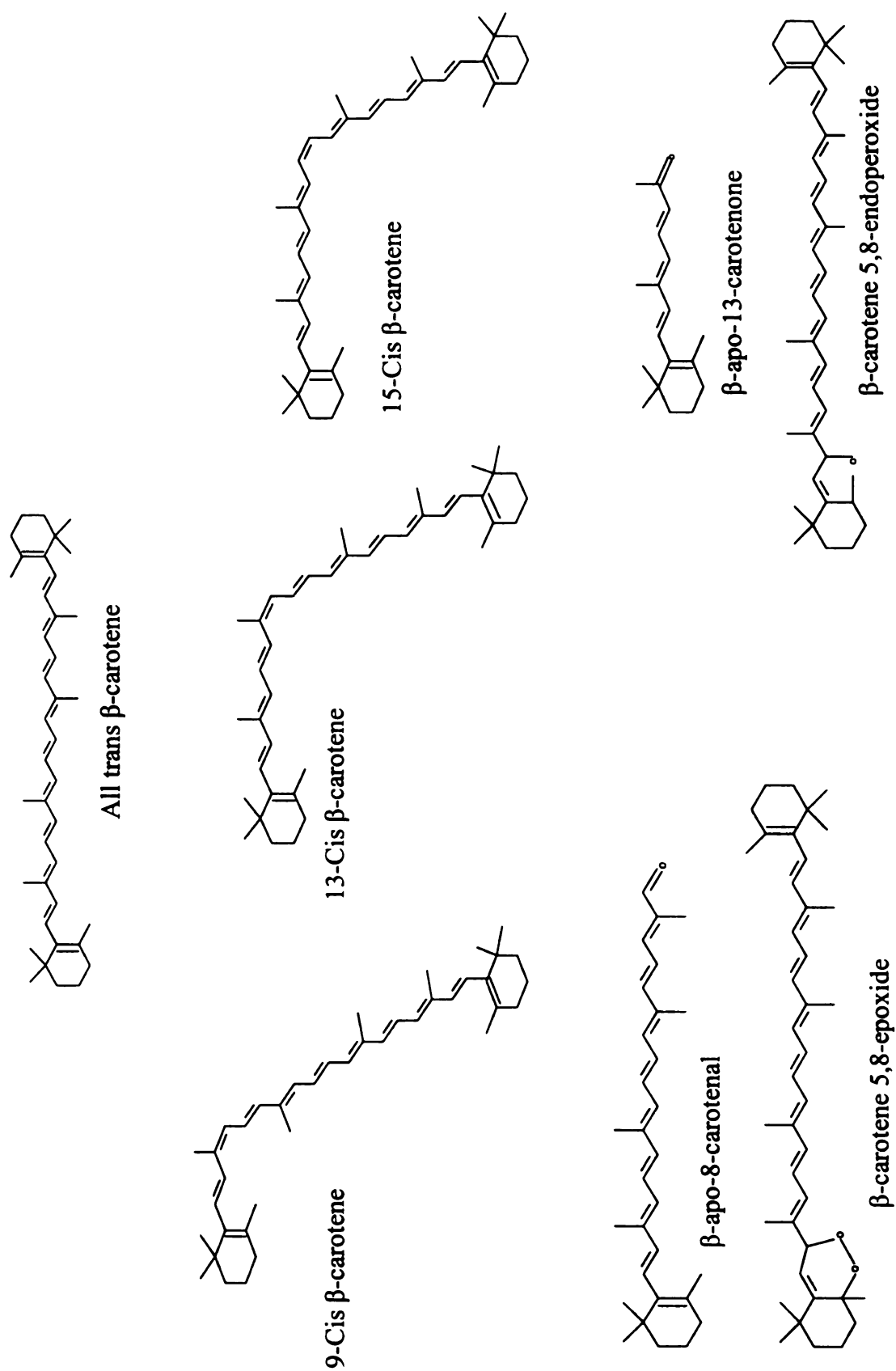
Carotenoids, a widely distributed family of tetraterpenes, can be used as natural colorants and antioxidants. Carotenoids are generally composed of isoprene units linked to form a conjugated double-bond system. Typically, they have eight isoprenoid units bonded so that the units form a mirror image of each other, thus making most carotenoids symmetrical. The two central methyl groups are set in a 1,6 position relative to each other with the remaining nonterminal methyl groups forming a 1,5 positional relationship (O'Neil and Schwartz, 1992). Davies (1976) stated that the linked isoprene units in the acyclic structure could be modified by hydrogenation, dehydrogenation, cyclization or oxidation. The oxygenated derivatives are called xanthophylls, while the hydrocarbon carotenoids are termed carotenes. They have a high degree of unsaturation that increases the probability of electron delocalization; therefore, the energetic proximity of excited states suggests that the molecule is able to absorb energy in the visible region of the spectrum and behave as a chromophore (Davies, 1976). The spectrophotometric features of carotenoids are due to this conjugated double bond system (Oliver and Palou, 2000). β -carotene, one of the carotenoids, is a conjugated polyene with 9 double bonds and two cyclic end groups known as β -ionone structures (Francis, 2000).

A unique characteristic of some carotenoids, such as β -carotene, is the ability to shift between *trans* and *cis* geometric conformations. Although the *trans* form is thermodynamically stable, it can be interconverted by light, thermal energy, or chemical reactions to form *cis* isomers and other compounds, such as apo-carotenals, apo-carotenones, endoperoxides and epoxides (Stahl and Sies, 1993).

The mechanism for this chemical conversion suggests that extra energy can break the conjugation sequence, possibly by pushing the electrons to anti-bonding orbitals, and consequently causing the loss of its chromophore properties, or color. The small amount of energy required to remove electrons makes β -carotene an effective antioxidant; unfortunately, this will result in a loss of color and other beneficial health effects (Sweeney and Marsh, 1970).

Carotenoid nomenclature is based on the carotene backbone. Due to the symmetrical structure, one half of the molecule is numbered 1-15 from the terminal ring to the center and additional methyl groups numbered 16-20. The other half is numbered similarly 1'-15' and 16'-20' (Goodwin, 1980). Double bonds allow for conformational changes in carotenoids. Using the International Union of Pure and Applied Chemists nomenclature rules for carotenoids, the stem name (β) implies *trans*, therefore *cis* configurations are named by citing the specific *cis* bond number and the term *cis* (IUPAC-IUB, 1975). Chemical structures of β -carotene and other degradation products are shown in Figure 2.1.

Figure 2.1. Chemical Structures of *Trans* β -carotene and Commonly Identified β -carotene Degradation Products



Carotenoids such as β -carotene, α -carotene, and β -cryptoxanthin among others, are also known for their vitamin A biopotency due to the presence of a polyene chain and at least one unsubstituted beta-ionone ring in their structure. Of this group, β -carotene has the highest provitamin A value or vitamin A biopotency, which refers to the amount of vitamin A that can be obtained from a compound (Goodwin, 1976). In general, *trans* forms of these carotenoids supply higher provitamin A value, or biopotency, than do their *cis* counterparts, because of the structural differences resulting from isomerization (Rock, 1997).

The mechanism of conversion from β -carotene to vitamin A is an important biosynthetic process. Two pathways have been suggested for the enzymatic cleavage of β -carotene to vitamin A in mammals as it passes through the gut mucosa (Parker, 1996). The primary pathway involves central cleavage at the 15,15' double bond of the molecule which results in two molecules of retinal that can either be reduced to retinol (vitamin A) or further oxidized to retinoic acid (Krinsky et al., 1990). During central cleavage, the enzyme should be able to convert one molecule of β -carotene to two molecules of vitamin A; however, the conversion is not always efficient. An alternative pathway is non-central (excentric) cleavage at the excentric double bonds, such as C-13', 14', C-11', 12', C-9', 10', and C-7', 8' to yield one molecule of vitamin A, and β -apocarotenals of different chain lengths (Blomhoff, 1994, Wang et al., 1992).

2.2.3. Health Effects of β -carotene and Vitamin A

For North Americans, recommended daily allowances (RDA) of vitamin A for men and women over the age of 19 are 900 and 700 μg , respectively. For children ages 1 to 18 years, RDA values range from 300 – 900 μg for boys and 300 – 700 μg for girls

(Food and Nutrition Board, 2001). Preformed vitamin A is typically present only in animal products such as liver, eggs, and milk products; therefore, in countries where consumption of animal products is low, carotenoids such as β -carotene are the primary source of vitamin A (Wouterson et al, 1999).

Common sources of β -carotene include carrots, spinach, mango, papaya, pumpkin, and many other plant products. Due to seasonal factors in some areas, however, the intake of β -carotene may vary because of the degradative effect of light and heat on the carotenoid content of foods (Olmedilla et al., 1994). Although there is a clear relationship between β -carotene and a lower risk of several chronic diseases, no evidence has pointed to the need for a certain percentage of dietary vitamin A to come from provitamin A carotenoids such as β -carotene. There are recommendations for increased consumptions of carotenoid-rich fruits and vegetables. As noted in *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids* (IOM, 2000), consumption of five servings of fruits and vegetables per day could provide 5.2 to 6 mg/day of provitamin A carotenoids (Lachance, 1997), which would contribute approximately 50 to 65 percent of the adult RDA for vitamin A (Food and Nutrition Board, 2001).

Vitamin A deficiency is estimated to affect approximately 124 million children and is one of the most common nutritional deficiencies worldwide (Brubacher and Weiser, 1984). Lack of vitamin A can result in blindness, which is estimated to affect 50,000 to 250,000 children annually (Sommer and West, 1996). The most specific clinical effect of vitamin A deficiency is xerophthalmia, a condition in which the glands that supply water to produce tears function inadequately. The effects of xerophthalmia include night blindness, conjunctival xerosis, corneal ulceration, keratomalacia and

corneal scarring. The problem of xerophthalmia is primarily confined to developing countries, where childhood malnutrition and mortality rates are high (Blomhoff, 1994).

β -carotene and vitamin A have been investigated for their potential anti-cancer properties. Numerous studies (Russell, 2002; Heinonen et al., 1998) have shown that retinoids such as retinol, retinyl esters and ethers, retinoic acid, retinoic acid esters, amides, and aromatic ring analogues, can be used as defenses against carcinogenesis in experimental animals and cell culture systems. Vitamin A interrupts chemical carcinogenesis *in vivo* as well as *in vitro*, decreases the mutagenicity of some carcinogens, and suppresses the induction of numerous promoters of carcinogenesis (Tomita, 1983).

β -carotene is also an interceptor of free radicals, which can be produced from oxygen and are unstable, highly reactive, energized molecules with unpaired electrons (Burton and Ingold, 1984). Examples of free radicals include super oxide (O_2^-), hydroxyl ($OH^\cdot$), hydroperoxyl ($HOO^\cdot$), peroxy ($ROO^\cdot$), and alkoxy ($RO^\cdot$) radicals. These free radicals have high reactivity with other compounds due to the necessity of capturing electrons for stability, which in turn produces a chain reaction of free radicals (Kaur and Kapoor, 2001). Beutner et al. (2001) stated that in order to stop this chain reaction, the body's natural defenses use antioxidants. The process that antioxidants, such as β -carotene, use to terminate the cycle of electron-stealing is to donate one of their own electrons. In other words, this process quenches singlet oxygen directly or intercepts harmful triplet states, to prevent the formation of singlet oxygen. However, the antioxidant does not become a free radical, due to its ability to remain stable in both forms (Beutner et al., 2001). This antioxidant mechanism in β -carotene may be

responsible for preventing other health conditions, such as cardiovascular disease, macular degeneration and cataracts (Burton and Ingold, 1984).

Although β -carotene has numerous beneficial effects, there have been a few studies published stating that β -carotene supplementation has detrimental effects on long-term smokers. In the Alpha Tocopherol and Beta Carotene Study (ATBC), which was a large, randomized, controlled study in Finland supported by the National Cancer Institute, β -carotene was given to 30,000 long-term smokers for six years. β -carotene was found to be ineffective in reducing the risk of lung cancer, and actually promoted lung cancer risk in smokers who took β -carotene (Albanes et al., 1996). In the Carotenoid and Retinol Efficiency Trial (CARET) in the United States, a large group of smokers and asbestos workers were given β -carotene. As in the ATBC study, results showed an increase, instead of an expected decrease, in the risk of lung cancer (Omenn et al., 1996). However, the validity of both of these studies has been disputed because of several factors. The subjects were smokers and asbestos workers, for whom it may have already been too late to prevent cancer. Also, the ATBC study was conducted immediately after the Chernobyl disaster (1986), in which Finland was one of the first areas to be heavily impacted. In addition, the Finnish men took 20 mg per day of synthetic β -carotene colored with quiniline yellow, which is a colorant known to have carcinogenic properties (Liede et al., 1998). The authors themselves indicated that there is no mechanism for toxic effect and no other studies have shown harmful effects of β -carotene.

2.2.4. Analysis of Beta-Carotene

More than 600 naturally occurring carotenoids have been isolated and identified. (Patrick, 2000). Because these carotenoids can form different geometrical isomers, each

isomer should be analyzed separately (O'Neil and Schwartz, 1992). The methods used for separation and quantification of carotene isomers are often complicated, time-consuming, and difficult to replicate due to the highly sensitive nature of carotenoid compounds. The traditional approach to measuring carotenoids is a crude measurement using spectrophotometric measurement at 450 nm using the extinction coefficient for β -carotene; however, it has limitations because it cannot distinguish effectively between different isomers (Nierenberg et al., 1994). In the past, pressurized liquid chromatography was used to separate and quantify carotene stereoisomers on a column support of calcium and magnesium hydroxides or nitrile bonded packing materials. A high performance liquid chromatography method has been developed to adequately analyze these compounds using a support of aluminum oxide with controlled water content in the mobile phase (Chandler and Schwartz, 1987). Until the mid 1990's, one of the more highly preferred analytical methods for determination of carotenoids was high performance liquid chromatography using a C_{18} polymeric stationary phase and ultraviolet detector. Khachik and Beecher (1985) utilized a monomeric C_{18} column coupled with a quaternary system of methanol- acetonitrile- methylene chloride- hexane (22:55:11.5:11.5) to separate α -carotene, β -carotene, and 15-cis- β -carotene; however, 15-cis- β -carotene was not completely resolved. In the early 1990's, a polymeric C_{30} column was developed and tailored specifically for the analysis of carotenoids. The C_{30} column results in adequate retention of polar carotenoids, as well as selectivity towards structural and geometrical isomers of polar and non-polar carotenoids (Emenheiser et al., 1996). Sander et al. (1994) obtained effective separation of all-*trans* carotenoids from mixtures of standards and extracts using a C_{30} column.

Carotenoids are thermally labile and should not be exposed to heat during extraction and storage. Procedures such as saponification, the cleavage of fatty acid ester linkages using a strong base and heat, should be done with extreme caution (Augustin et al., 1985). In addition to thermal degradation, carotenoids are also degraded by light and oxygen. If possible, carotenoid extraction and analysis should be performed under yellow or red light to minimize photodegradation and isomerization. Samples can also be protected using amber glass or opaque materials such as aluminum foil. Sunlight should be excluded from the laboratory (Schmitz et al., 1991). Oxidative degradation can be decreased by limiting the time exposed to air, and by using inert gases such as nitrogen or argon during evaporation and storage. Samples should not be allowed to remain at room temperature for long time periods. Antioxidants are also helpful in minimizing oxidation (Krinsky, 1989). Since β -carotene is a lipophilic compound, extraction solvents are typically hydrophobic organic solvents such as hexanes and ethers. Tetrahydrofuran is quickly oxidized in the presence of oxygen and yields highly reactive peroxides, thus promoting significant loss of β -carotene during analysis. Prolonged exposure of carotenoids to chlorinated solvents such as dichloromethane and chloroform can also lead to degradation (Khachik et al., 1988). During HPLC analysis, *trans* isomers of β -carotene generally absorb light between 400 – 500 nm (Figure 2). *Cis* isomers tend to absorb stronger at lower wavelengths (<400 nm), however standards are extremely unstable and difficult to obtain commercially.

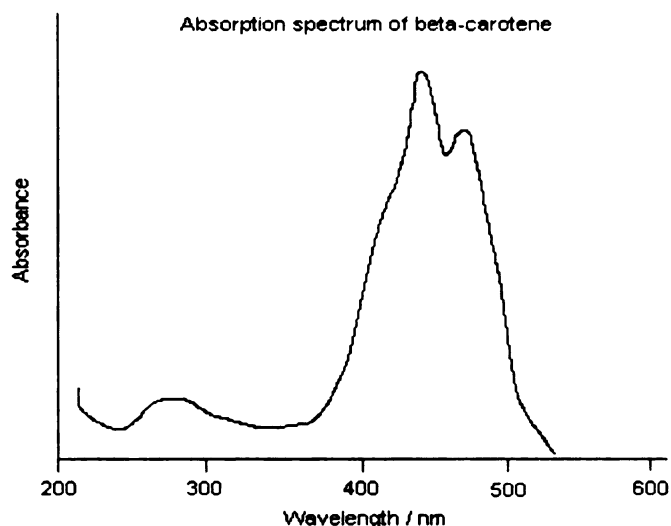


Figure 2.2. Absorption Spectrum of β -carotene

http://www.chm.bris.ac.uk/motm/carotene/beta-carotene_colourings.html

2.3. Degradation of β -carotene

2.3.1. Thermal Effects

Thermal processing of foods is used for cooking, flavor development, and inactivation of pathogens and other microorganisms, among others. However, these thermal processing treatments can simultaneously degrade nutrients, and affect other organoleptic qualities, such as color, taste, and texture (Barreiro et al., 1997). Thermal effects on rates of chemical reactions were first studied by van't Hoff in 1884, Hood in 1885, and Arrhenius in 1889. Process engineers need methods to predict and optimize nutritional content of processed and stored foods. The food industry has used kinetic models to predict quality changes during food processing (Thompson, 1982). Most of the quality factors, including color, can be described by degradation kinetics of zero or first order, with the effect of temperature in the rate constant usually accurately described by the Arrhenius equation (Karmas, 1988):

$$k = k_0 e^{-E_A / RT}$$

where: k = reaction rate constant, k_0 = pre-exponential constant, E_A = activation energy, R = gas constant, and T = temperature in °K. The activation energy can be estimated from the slope of a regression line of $\ln(k)$ versus $1/T$ (Labuza and Riboh, 1982).

β -carotene degradation has been studied in numerous model food systems. Chou and Breene (1972) (Table 2.1) studied β -carotene decoloration kinetics in simplified low-moisture model systems of microcrystalline cellulose with 0.5% β -carotene. The samples were held at constant water activities and constant temperatures for extended periods of time. Decoloration of β -carotene in microcrystalline cellulose was found to be an autoxidative reaction that appears to be first-order or pseudo-first order when oxygen is not a limiting factor. Ramakrishnan and Francis (1979) (Table 2.1) investigated the stability of β -carotene in model systems of cellulose and starch equilibrated under different relative humidities. Overall, increased water content was found to have an increased protective effect over β -carotene. The protective effect could be attributed to the formation of hydrogen bonds between water and hydroperoxide molecules. Degradation kinetics followed first-order. Minguez-Mosquera and Jaren-Galen (1994) (Table 2.1) determined the effects of moisture on the decoloration of β -carotene pigments by comparing an anhydrous medium versus an aqueous medium. The reactions that occurred in the anhydrous medium followed zero-order kinetics (Table 2.1) while those in an aqueous medium followed first-order kinetics (rate constants not listed in Table 2.1). Light and temperature were also investigated and were shown to accelerate the degradation reaction. Arya et al. (1979) also studied the effects of water activity on the

stability of β -carotene in isolated model systems of microcrystalline cellulose. The rate of β -carotene degradation decreased with the increase in water activity.

Arya et al. (1979) further investigated effect of water activity on β -carotene stability by adding several antioxidants, butylated hydroxy anisole (BHA) and propyl gallate (PG). Both BHA and PG stabilized β -carotene at all water activity levels. Papadoupoulou and Ames (1994) (Table 2.1) studied the effect of heating with and without phenylalanine, an antioxidant, on degradation of all *trans*- β -carotene. All-*trans*- β -carotene was heated in paraffin at 210°C for 15 minutes in the presence or absence of phenylalanine, to simulate deep-fat frying of foods. The sample containing only all-*trans*- β -carotene in paraffin showed a loss of 98% after 6 minutes at 210 °C, while the sample with phenylalanine showed an approximate 85% loss. An initial rapid depletion of all-*trans*- β -carotene was found within the first minute, with the rate decreasing over time. First-order kinetics was reported for both the presence and absence of phenylalanine. The rates of degradation were very close for samples heated with and without phenylalanine (Table 2.1).

Stefanovich and Karel (1982) (Table 2.1) examined the kinetics of β -carotene degradation during air-drying by also using a model system of microcrystalline cellulose and β -carotene. A first-order model and a simplified free radical reaction model were used for kinetic analyses and were shown to fit the experimental data. However, the degradation showed initial concentration dependence, indicating a first-order model was not ideal. A food system was also used in this study, but results could not be related to those of the model system because of the less complicated nature of the model system and the presence of pro- and anti-oxidant components present in the food system.

Henry et al (1998) (Table 2.1) studied the thermal and oxidative degradation kinetics of all-*trans* β -carotene in an oil model system to determine the stability of β -carotene and formation of isomers. All-*trans* β -carotene was heated in safflower seed oil at 75, 85, and 95°C for 24, 12, and 5 hours respectively. The major isomers that were formed were 13-*cis*, 9-*cis*, and an unidentified *cis* isomer. Degradation kinetics followed first-order.

Henry et al. (2000) (Table 2.1) investigated the effects of ozone and oxygen on degradation of all-*trans* β -carotene in an aqueous model. A mixture of carotenoids was adsorbed onto a C-18 solid phase and exposed to a continuous flow of water saturated with oxygen or ozone at 30°C. Almost 90% of all-*trans* β -carotene was lost after exposure to ozone. The samples that were exposed to oxygen degraded at a much slower rate and followed zero-order reaction kinetics.

The photodegradation of 10% (w/w) β -carotene beadlets and 1% (w/w) CWS β -carotene powder in model dispersions was investigated by Pesek and Warthersen (1988) (Table 2.1). Stock solutions of each type of β -carotene were made in distilled water, stoppered, and layed horizontally in a light chamber at a constant light intensity of 250 ft-c (2690 lux) at 28, 6, or -15°C. Degradation of β -carotene due to heat, oxidation, or light followed first-order or pseudo-first-order behavior. For the 10% β -carotene beadlet dispersion, degradation rates decreased with decreased temperature and approximately 50% was retained; however, the 1% CWS beadlet dispersion showed greater stability in comparison with 80-90% β -carotene retained after 5 days of light exposure at all temperatures. These differences can be attributed to the differences in the sample matrices of the 10% β -carotene beadlets, which has a gelatin/sugar matrix, and the 1%

CWS β -carotene powder, which has a gum acacia/sugar matrix (Pesek and Warthesen, 1988).

Table 2.1. Rate Constants of β -carotene Degradation in Various Model and Food Systems

Medium	Conditions	Reaction Rate Constants	Activation Energy	Reaction Order	Reference
β -carotene in microcrystalline cellulose	5°C	$k_{5^\circ\text{C}} = 2.87 \times 10^{-2} \text{ day}^{-1}$	40.59 kJ/g·mol	First-order or Pseudo-first order	Chou and Breene (1972)
	10°C	$k_{10^\circ\text{C}} = 10.5 \times 10^{-2} \text{ day}^{-1}$			
	35°C	$k_{35^\circ\text{C}} = 21.0 \times 10^{-2} \text{ day}^{-1}$			
β -carotene in microcrystalline cellulose	25°C ^a RH ₁ = 0% RH ₂ = 11% RH ₃ = 23% RH ₄ = 52% RH ₅ = 75%	$k_1 = 9.50 \times 10^{-2} \text{ day}^{-1}$	41.86 kJ/g·mol	First-order	Ramakrishnan and Francis (1979)
		$k_2 = 8.10 \times 10^{-2} \text{ day}^{-1}$			
		$k_3 = 6.50 \times 10^{-2} \text{ day}^{-1}$			
		$k_4 = 4.60 \times 10^{-2} \text{ day}^{-1}$			
		$k_5 = 4.00 \times 10^{-2} \text{ day}^{-1}$			
β -carotene in cyclohexane	15°C	^b $k_{15^\circ\text{C}} = 0.011[\text{conc}] \text{ hour}^{-1}$	107.5 kJ/g·mol	Zero-order	Minguez-Mosquera and Jaren-Galen (1994)
	25°C	^b $k_{25^\circ\text{C}} = 0.596[\text{conc}] \text{ hour}^{-1}$			
	35°C	^b $k_{35^\circ\text{C}} = 0.798[\text{conc}] \text{ hour}^{-1}$			
	45°C	^b $k_{45^\circ\text{C}} = 1.045[\text{conc}] \text{ hour}^{-1}$			
	darkness				
β -carotene in water	15°C	$k_{15^\circ\text{C}} = .002 \text{ hour}^{-1}$	48.81 kJ/g·mol	First-order	Minguez-Mosquera and Jaren-Galen (1994)
	25°C	$k_{25^\circ\text{C}} = .003 \text{ hour}^{-1}$			
	35°C	$k_{35^\circ\text{C}} = .005 \text{ hour}^{-1}$			
	45°C	$k_{45^\circ\text{C}} = .015 \text{ hour}^{-1}$			
	darkness				

^aRH = relative humidity ^bConcentration units not reported

Table 2.1. (continued). Rate Constants of β -carotene Degradation in Various Model and Food Systems

Medium	Conditions	Reaction Rate Constants	Activation Energy	Reaction Order	Reference
<i>Trans</i> - β -carotene in liquid paraffin	210°C	$k = 0.981 \text{ min}^{-1}$	-----	First-order	Papadoupoulou and Ames (1994)
<i>Trans</i> - β -carotene and phenylalanine in liquid paraffin	210°C	$k = 0.998 \text{ min}^{-1}$	-----	First-order	Papadoupoulou and Ames (1994)
<i>Trans</i> - β -carotene in safflower seed oil	75°C 85°C 95°C	$k_{75^\circ\text{C}} = 4.2 \times 10^{-2} \text{ hour}^{-1}$ $k_{85^\circ\text{C}} = 1.19 \times 10^{-1} \text{ hour}^{-1}$ $k_{95^\circ\text{C}} = 3.26 \times 10^{-1} \text{ hour}^{-1}$	109.67 kJ/g·mol	First-order	Henry et al. (1998)
β -carotene in microcrystalline cellulose	60°C 70°C 80°C	$k_{60^\circ\text{C}} = 3.7 \times 10^{-3} \text{ min}^{-1}$ $k_{70^\circ\text{C}} = 11.9 \times 10^{-3} \text{ min}^{-1}$ $k_{80^\circ\text{C}} = 23.9 \times 10^{-3} \text{ min}^{-1}$	90.84 kJ/g·mol	First-order	Stafanovich and Karel (1982)
<i>Trans</i> - β -carotene On C ₁₈ solid phase	oxygen ozone	$^b k_{\text{oxygen}} = .0013[\text{conc}] \text{ hour}^{-1}$ $^b k_{\text{ozone}} = .0039[\text{conc}] \text{ hour}^{-1}$	-----	Zero-order Zero-order	Henry et al. (2000)
10% β -carotene beadlets	28°C 6°C -15°C	$k_{28^\circ\text{C}} = 7.12 \times 10^1 \text{ day}^{-1}$ $k_{6^\circ\text{C}} = 5.23 \times 10^1 \text{ min}^{-1}$ $k_{-15^\circ\text{C}} = 2.08 \times 10^1 \text{ min}^{-1}$	-----	First-order	Pesek and Warthesen (1988)
1% CWS β -carotene	28°C 6°C -15°C	$k_{28^\circ\text{C}} = 4.20 \times 10^{-2} \text{ day}^{-1}$ $k_{6^\circ\text{C}} = 2.50 \times 10^{-2} \text{ min}^{-1}$ $k_{-15^\circ\text{C}} = 4.80 \times 10^{-2} \text{ min}^{-1}$	-----	First-order	Pesek and Warthesen (1988)

2.3.2. Effects of Extrusion

Vitamins and pigments, including β -carotene, can be particularly susceptible to degradation during extrusion due to the intense thermal and mechanical conditions to which a food product is exposed. The stability of β -carotene can vary with the composition of the food product, form of β -carotene, and the type/temperature of process. Lee et al. (1978) (Table 2.2) were first to show that a mixture of β -carotene and white corn flour retained only 30% β -carotene after extrusion. The vitamin A biopotency of β -carotene was lost due to the conversion of all-*trans* β -carotene to stereoisomers with lower vitamin A biopotency.

In India, Bhavani and Kamini (1998) (Table 2.2) studied the development and acceptability of β -carotene-rich maize-based extruded products as a possible solution to vitamin A deficiency (Kotareddy and Devi, 1998). β -carotene-rich sources like curry leaf, carrot, and red palm oil were mixed with maize and extruded. The loss of β -carotene in curry leaf and carrots after extrusion and storage was between 6-20%.

Marty and Berset (1990) (Table 2.2) assessed the impact of extrusion on the thermal degradation of all-*trans* β -carotene in corn starch. Using high and medium pressure liquid chromatography as analytical tools, they isolated and identified approximately 25 breakdown products of all-*trans* β -carotene. These products were grouped into 6 categories: mono- or polycis isomers of β -carotene, mono- or diepoxy derivatives, apocarotenals, polyene ketones, one dihydroxyl derivative and monohydroxyl diepoxy derivatives.

Although most extrusion studies investigating β -carotene have used all-*trans* standards, Kone and Berset (1982) (Table 2.2) investigated the stability of a food-grade

β -carotene commercial preparation (Hoffman-LaRoche, 0.5% (w/w) CWS) during extrusion cooking and storage. During extrusion, *cis-trans* isomerization occurred, and resulted in the formation of 15-*cis* β -carotene. The loss of color during storage at ambient temperature and in darkness appeared to follow a two-step first-order kinetic reaction, with a greater loss during light exposure.

Guzman-Tello and Cheftel (1990) (Table 2.2) investigated the color loss and formation of 9-*cis* and 13-*cis* isomers during extrusion as a function of heating and oxidation. During extrusion, the thermal and/or oxidative color loss from a wheat flour/all-*trans* β -carotene mix was modeled as a first-order kinetic reaction. Rate constants at 128, 152, and 160°C were $3.3 \times 10^{-3} \text{ s}^{-1}$, $6.0 \times 10^{-3} \text{ s}^{-1}$, and $8.0 \times 10^{-3} \text{ s}^{-1}$, respectively. Although this extrusion study modeled degradation kinetics, no studies found have accounted for the product temperature profile along the barrel. The researchers assumed the product was at the die temperature during the entire residence time in the extruder, leading to underestimated rate constants (as much as three orders of magnitude) and overestimated activation energies (up to 30%) (Dolan, 2003).

The effect of an antioxidant, butylated hydroxy toluene (BHT), on the overall rate constant of color loss during extrusion was also investigated by Guzman-Tello and Cheftel (1990). In general, the presence of 0.1% BHT decreased the reaction rate constant only at certain water contents. The presence of 0.1% BHT did not affect the rate constant at higher water contents or when the barrel temperature was low ($\leq 100^\circ\text{C}$).

Berset and Marty (1992) (Table 2.2) further investigated the effect of added antioxidants on the extrusion of corn starch with *trans* β -carotene and during storage of the extrudates. Five different antioxidants, including 100 ppm butylated hydroxy anisole

(BHA), 250 ppm extract of rosemary, 500 ppm extract of rosemary, 599 ppm dl- α -tocopherol, and 50 ppm butylated hydroxy toluene (BHT), were added to individual batches of corn starch and β -carotene and extruded at constant conditions. Results showed that all of the antioxidants had a similar effect except for BHA, which showed a significantly lower efficacy. It was concluded that in the absence of an effective antioxidant, formation of low molecular weight colorless compounds is accelerated, while the presence of an antioxidant impedes the process of oxidation. Storage studies showed that BHT provided the most long-term protection of β -carotene.

Berset et al. (1989) (Table 2.2) compared the effect of rosemary oleoresin, a natural antioxidant, to BHT, a synthetic antioxidant often used in extrusion cooking. Results showed that the efficacy of rosemary oleoresin was close to that of BHT, with approximate equivalency of 1000 ppm rosemary oleoresin to 500 ppm BHT. The study also showed that α -tocopherol was effective but a much higher dosage than that of rosemary is required.

Table 2.2. Conditions of β -carotene Extrusion Studies and Degradation Products

Raw Material	Screw Speed	Moisture Content (%)	Die Temperature	Mass Feed Rate	Degradation Products	References
β -carotene in white corn meal	700-100 rpm	-----	-----	-----	Unidentified stereoisomers	Lee et al. (1978)
Food-grade β -carotene in corn starch	-----	-----	150°C	-----	15- <i>cis</i> β -carotene	Kone and Berset (1982)
<i>Trans</i> - β -carotene in corn starch	150 rpm	-----	180°C	25 kg/hr	9-, 13- <i>cis</i> - β -carotene, epoxide derivatives	Marty and Berset (1986, 1988, 1990)
β -carotene in corn starch	150 rpm	-----	170°C	-----	9-, 13- <i>cis</i> - β -carotene, epoxide derivatives	Berset et al. (1989)
<i>Trans</i> - β -carotene in wheat flour	125-150 rpm	14-24 %	200°C	30 kg/hr	9-, 13- <i>cis</i> - β -carotene	Guzman-Tello and Chefel (1990)
<i>Trans</i> - β -carotene in corn starch	150 rpm	-----	170-185°C	25 kg/hr	<i>cis</i> , di- <i>cis</i> , tri- <i>cis</i> isomers, epoxide	Berset and Marty (1992)

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CHAPTER 3: THEORY

3.1. Overall Model

The model used in our research was based on a model proposed by Guzman-Tello and Cheftel (1987). The model in this research describes retention of β -carotene during extrusion, or total retention (R_T), as following exponential decay with time and as a product of two functions

$$f_1(\text{Temperature, Moisture Content}) \times f_2(\text{Screw Speed, Residence Time});$$

In our study, $f(\text{temperature})$ was expressed as retention due to thermal effects, or R_β . The time-temperature parameters of R_β (section 3.1.2.1) were estimated through separate experiments (section 3.1.1) in a shearless environment. The effect of screw speed, shear history, and SME were expressed as retention due to mechanical effects, or R_ϕ . The values of R_ϕ were calculated by mathematically removing the time-temperature effect from the total measured retention (section 3.1.2.2). The functional form of R_ϕ was empirically determined.

3.1.1. Thermal

3.1.1.1. Isothermal Kinetic Parameters

An equation commonly used to describe the reaction rate is

$$-\frac{dC}{dt} = kC^n \quad (1)$$

where C is concentration of *trans*- β -carotene (relative units) in flour (dry basis), k is the reaction rate constant $[\text{conc}]^{1-n} (\text{min}^{-1})$, and n is the reaction order (Levenspiel, 1999). We refer to equation (1) as the primary model. In the case of a first-order reaction ($n = 1$), the equation is

$$-\frac{dC}{dt} = kC \quad (2)$$

Integrating equation (2) from C_o to C over time 0 to t ,

$$\int_{C_o}^C \frac{dC}{C} = -k \int_0^t dt \quad (3)$$

$$\ln \frac{C}{C_o} = -kt \quad (4)$$

Therefore, retention of *trans*- β -carotene at constant temperature can be expressed as

$$\frac{C}{C_o} = e^{-kt} \quad (5)$$

3.1.1.2. Nonisothermal Kinetic Parameters

The reaction rate k is commonly assumed to follow an Arrhenius relationship with temperature:

$$k = k_r \exp \left[\frac{-\Delta E}{R} \left(\frac{1}{T(t)} - \frac{1}{T_r} \right) \right] \quad (6)$$

where k_r is the rate constant at a reference temperature T_r (sec^{-1}), ΔE is activation energy of *trans*- β -carotene degradation in flour ($\text{kJ/g}\cdot\text{mol}$), R is the ideal gas constant, and T is temperature of sample (K) (Thompson et al., 1976). We refer to expressions of k as “secondary models”.

When temperature is not held constant, k must remain within the time integral in equation (3)

$$\int_{C_o}^C \frac{dC}{C} = - \int_0^t k dt \quad (7)$$

Substituting equation (6) into equation (7) and integrating yields

$$C = C_o \exp(-k_r \beta) \quad (8)$$

where time-temperature history (Dolan, 2003) is

$$\beta = \int_0^t \exp \left[\frac{-\Delta E}{R} \left(\frac{1}{T(t)} - \frac{1}{T_r} \right) \right] dt \quad (9)$$

3.1.2. Effects of Extrusion

3.1.2.1. Thermal Effect

When calculating the thermal effect of extrusion, the same reasoning can be applied as shown in section 4.2.4.2. Equation (8) states:

$$\left(\frac{C}{C_o} \right)_{element} = \exp(-k_r \beta) \quad (10)$$

However, in the case of a continuous reactor such as an extruder, the time-temperature history of an element with exit age t is defined by equation (9)

$$\beta = \int_0^t \exp \left[\frac{-\Delta E}{R} \left(\frac{1}{T(t)} - \frac{1}{T_r} \right) \right] dt \quad (11)$$

where $T(t)$ is temperature of an element inside the extruder barrel, and dt is residence time of an element at temperature $T(t)$.

The element residence time [dt in Eq. (11)] in barrel section i was approximated as follows: mean residence time of dough in section i is $m_i / \dot{m}$ (Cai and Diosady, 1993);

$$\text{element residence time in section } i \text{ is estimated as } \Delta t_i \approx \frac{m_i}{\dot{m}} \left(\frac{t}{\bar{t}} \right) \quad (12)$$

Where m_i is the weight of the product in screw section i , kg, $\dot{m}$ is the mass feed rate in kg/sec, t is the exit time in seconds, and $\bar{t}$ is the mean residence time in seconds.

The sum of the sectional mean residence times must be equal to the mean residence time over the entire barrel,

$$\sum_i \frac{m_i}{\dot{m}} = \bar{t} \quad (13)$$

Because $\sum_i \frac{m_i}{\dot{m}}$ (m_i weighed after a dead stop) was different from $\bar{t}$ (calculated from injected red dye) due to loss of moisture or weighing inaccuracies, we defined a correction factor

$$CF = \frac{\bar{t}}{\left(\sum m_i / \dot{m}\right)} \quad (14)$$

to obtain a more accurate approximation of element residence time. Rewriting equation (12)

$$\Delta t_i \approx \frac{m_i}{\dot{m}} (CF) \left(\frac{t}{\bar{t}} \right) = \frac{m_i}{\dot{m}} \frac{\bar{t}}{\left(\sum \frac{m_i}{\dot{m}}\right)} \frac{t}{\bar{t}} = \frac{m_i}{\sum m_i} t \quad (15)$$

Therefore, substituting Eq. (15) into Eq. (9) for dt , the time-temperature history of an element was numerically calculated using the trapezoidal rule on equation (9) and the following equation:

$$\beta(t) = \sum_i \exp \left[\frac{-\Delta E}{R} \left(\frac{1}{T(x_i)} - \frac{1}{T_r} \right) \right] \left(\frac{m_i}{m} \right) t \quad (16)$$

where $T(x_i)$ was the measured product temperature profile of the dough in barrel section i , m_i was the measured mass of the product in section i , and $m = \sum_j m_j$ was the total measured mass of product in the extruder at dead-stop.

Extrusion has a distribution of residence times; therefore, only the mean concentration of *trans*- β -carotene can be measured. The mean retention in an extrudate, $\bar{C}/C_o$, due to thermal effects only was calculated by numerically integrating equation (8) over the residence time distribution (Levenspiel, 1999), using the trapezoidal rule

$$\left(\frac{\bar{C}}{C_o}\right)_{at\ exit} = \sum_i \exp[-k_r \beta(t_i)] E(t_i) \Delta t_i \quad (17)$$

3.1.2.2. Mechanical Effect

A general model for retention in an extruded product was proposed (Cha et al., 2001)

$$R_T = R_\beta R_\phi \quad (18)$$

where R_T is the measured total mean retention: $R_T = \left(\frac{\bar{C}}{C_o}\right)_{at\ exit}$ (19);

R_β is the calculated retention due to thermal effects: $R_\beta = \int_0^\infty \exp(-k_r \beta(t)) E(t) dt$ (20),

which is the integral form of equation (17);

and R_ϕ the retention due to mechanical effects is an unknown, and was expected to be a function of some measure of shear, such as screw speed, shear history, or specific mechanical energy. R_ϕ was solved for at each extrusion condition as:

$$R_\phi = R_T / R_\beta \quad (21).$$

3.2. Moisture Effect

The effect of moisture content on retention of *trans*- β -carotene was investigated by extruding at a constant screw speed of 300 rpm and 3 moisture contents, 30, 33, and

36%. A secondary model for k is commonly assumed to have an exponential relationship with moisture for various compounds (Guzman-Tello and Cheftel, 1987; Ilo and Berghofer, 1990). Therefore, equation (6) can be expanded by

$$k = k_r \exp \left[\frac{-\Delta E}{R} \left(\frac{1}{T} - \frac{1}{T_r} \right) + b(MC - MC_r) \right] \quad (22)$$

For a nonisothermal process at constant moisture, substitution of equation (22) into equation (7) will yield, after integration,

$$\frac{C}{C_o} = e^{-k_r \beta e^{b(MC - MC_r)}} \quad (23)$$

where MC is moisture content of flour and MC_r is reference moisture content. This equation assumes β and $E(t)$ are nearly constant for all 3 constant moisture content conditions.

Taking the log of both sides, equation (23) becomes

$$\ln(C / C_o) = -k_r \beta e^{b(MC - MC_r)} \quad (24)$$

Multiplying by -1 and taking log of both sides again, equation (24) becomes

$$\ln[-\ln(C / C_o)] = \ln(k_r \beta) + b(MC - MC_r) \quad (25)$$

There, the moisture parameter b can be estimated as the slope of the double-log of retention versus $MC - MC_r$.

CHAPTER 4: MATERIALS AND METHODS

4.1. Raw Material Preparation

4.1.1. Mixing

Food grade β -carotene, 7% Cold Water Soluble (CWS) was donated by Hoffman-LaRoche, Nutley, NJ. 7% CWS β -carotene is a fine, orange powder, with individual particles containing a finely dispersed oily solution of β -carotene in a matrix of gelatin, sucrose, maltodextrin, and corn oil. Ascorbyl palmitate and dl- α -tocopherol are added as antioxidants and silicon dioxide as a processing aid. Soft white wheat pastry flour was donated by Star of the West Milling Company (Frankenmuth, MI) and Mennel Milling Co. (Fostoria, IL). Average moisture content of flour was approximately 14% (wet basis).

Mixing of flour was done in 1-kg batches. One kg of flour and 4.616 g of 7% CWS β -carotene were split into three equal portions and mixed together for 5 minutes each in a Kitchen Aid Mixer (Hobart Manufacturing Company, Troy, OH) using a wire mixing attachment. Each portion was then placed in a twin-shell dry mixer (Patterson Kelly Co. Inc., East Stroudsburg, PA), which was comprised of a rotating v-shaped plastic chamber and a counter-rotating bar extending across the chamber. The bar consisted of 15 spokes 3.81 cm in length protruding from equal distances along the length of the bar, which was approximately 33 cm long. The twin-shell dry mixer was set to mix for 40-minute time periods.

The initial concentration of β -carotene used was based on values commonly found in fruits or vegetables containing β -carotene (0.1 – 1.0 g β -carotene/kg flour):

$$\% \beta\text{-carotene} = \frac{4.616 \text{ mg } \beta\text{-carotene}}{\text{g flour}} \times 0.07 = \frac{323 \text{ mg } \beta\text{-carotene}}{\text{kg flour}} \quad (26)$$

4.2. Thermal Experiments

During a complex process like extrusion, it is very difficult to quantify the separate energy inputs, such as thermal and mechanical forces, acting simultaneously on the product. In this research, separate thermal experiments were conducted to determine retention of *trans*- β -carotene found in commercial food grade β -carotene in a shearless environment. In this manner, all mechanical effects were eliminated and temperatures comparable to those found in extrusion were used to treat all samples. Isothermal experiments were conducted at 78 and 138°C. A nonisothermal experiment was conducted at an oil bath temperature of 149°C.

4.2.1. Equipment and Sample Configuration

A Fisher Scientific Isotemp 1013P heating bath with Fisher High Temperature Bath Oil (#02-20) (Hanover Park, IL) was used. Temperatures were recorded using a 12-channel thermocouple scanner data acquisition unit (Cole-Parmer Instrument Co., manufactured by Barnant Co., Barrington, IL) and T-type thermocouples.

Steel cells [102 mm diameter, 4.5 mm inner height, and 1 mm wall thickness] were used to hold flour mixtures. Each steel cell was covered by a small steel cap, which sealed tightly around the open portion of the steel cell. Each cap included a small hole to allow for thermocouple placement. A wire basket consisting of 10 sections, with dimensions approximately the same as the opening of the bath unit, was used to place samples vertically into the oil bath such that the oil level was above the flour level.

Thermal conductivity of flour was estimated using the following equation obtained from Rao and Rizvi (1986):

$$k = 0.58X_w + 0.155X_p + 0.25X_c + 0.16X_f + 0.135X_a \quad (27)$$

where: k is thermal conductivity, in Watts/m $\cdot$ °C, X_w (28%) is percent moisture, X_p (10%) is percent protein, X_c (59%) is percent carbohydrate, X_f (2%) is percent fat, and X_a (1%) is percent ash. Values for percent moisture, protein, carbohydrate, fat, and ash of β -carotene/flour mixture were estimated based on Pyler (1988) and specifications from Roche.

Preparation of flour samples for thermal experiments was done in the same manner as outlined in section 4.1; however, moisture was added to raw material to increase moisture content from 14% to 28%. This addition was done by spraying water onto the raw material mixture, mixing for 30 minutes in a Hobart mixer, and allowing settling overnight in a sealed container at 4°C.

Approximately 12 g of the flour mixture was transferred into steel cells. A thermocouple was drawn through the steel cap and a small piece of paper was folded around the tip of the thermocouple to ensure its placement inside the center of the mixture and to prevent the thermocouple tip from touching the edges of the steel cell. The thermocouple was then placed in the center of the flour mixture, halfway between the steel walls, to monitor approximate center temperature of the mixture. A small paper towel was placed inside the cap to absorb any escaping moisture and to prevent moisture from condensing on the wall and flowing back into the sample to form a clump.

4.2.2. Isothermal and Near-Isothermal Experiments

The high temperature bath was pre-heated to the desired temperature. Duplicate samples at initial moisture content of $28\% \pm 2\%$ were placed in the sample basket. The sample basket was placed in the oil bath and temperature readings were started and collected every 4 seconds. A 12 ± 2 minute period, the lag time, was observed for the

internal temperature of the samples to come up to the target temperature, $\pm 5^{\circ}\text{C}$. After each cell was heated for a specific time period at a certain temperature, cells were removed individually and immediately placed in an ice bath to stop further degradation. The isothermal experiment was done at 78°C , during which samples were removed at 10, 30, 60, and 100 minutes. One near-isothermal experiment was conducted at 138°C , during which samples were removed at 10, 30, and 50 minutes.

4.2.3. Nonisothermal Experiments

Nonisothermal experiments were conducted at an oil bath temperature of 149°C and samples were removed at 2, 3, 6, and 8 minutes at varying temperatures. After cooling, the flour samples were placed in opaque bags, sealed, and placed into a dark freezer (-4°C) until further analysis.

4.2.4. Data Analysis

Kinetic parameters were calculated for both isothermal and nonisothermal experiments. Isothermal kinetic parameters that were obtained included reaction rate constants, which define the rate at which *trans*- β -carotene degrades at a particular temperature. Rate constants for isothermal experiments were estimated by linear regression using equation (4) using Excel®. C_o was the concentration at the end of lag time. The isothermal temperature reported was the mass average sample temperature, calculated per equation (28) in the following section.

Nonisothermal kinetic parameters included activation energy (ΔE), k_r (rate constant at reference temperature) and an initial concentration value (C_o) for *trans*- β -carotene.

The parameters k_r , ΔE , and C_o were estimated by minimizing the sum of squares of residuals using the nonlinear regression package “Solver” in Excel®. The temperature $T(t)$ in equation (9) was calculated as the sample mass average temperature, T_{ma}

$$T_{ma}^i = \frac{T_c^i + T_w^i}{2} \quad (28)$$

where T_c^i was measured center temperature (horizontally halfway between steel cell walls) at time index i and T_w^i was the wall temperature at time i , approximated using a heat flux balance

$$\frac{k(T_w^i - T_c^i)}{\Delta x / 2} = h(T_{oil} - T_w^i) \quad (29)$$

where k is the thermal conductivity of raw material in W/m·°C, Δx is the inner height of steel cell in meters, h is the heat transfer coefficient in W/m²·°C, T_{oil} is the temperature of the oil, and i is a superscript index for each time (representing 4 or 5 seconds) (Holman, 1997).

The heat-transfer coefficient values, h , were taken from Lai (2003) using lumped capacity analysis. A brass plate (5 mm thickness, 0.0127 m² area, and 236.9 g mass) was immersed in the oil bath at constant temperatures 80, 105, or 145°C and temperature data were recorded every 4 seconds using a thermocouple sealed within a hole drilled in the plate. The h value was calculated based on:

$$\ln \left[\frac{T - T_\infty}{T_0 - T_\infty} \right] = \left(\frac{-hA}{\rho c_p v} \right) t \quad (30)$$

where T was measured temperature of the brass plate, in °C, T_∞ was temperature of the oil bath, in °C, T_0 was initial temperature, in °C, A was surface area, in m², c_p was

specific heat in Joule/kg·°C (Holman, 1997). The mass of the brass plate (kg) and volume v was used as the value for density, ρ .

Confidence intervals and correlation coefficients (ρ) for parameters for nonisothermal estimation were obtained using methods of Van Boekel (1996) and Dolan (2003).

4.2.4.1. Isothermal and Near-Isothermal Kinetic Parameters

Typically, isothermal experiments are simple to conduct and mathematically less complicated than nonisothermal experiments. A near-isothermal experiment, which was conducted at close to isothermal conditions, was done because isothermal conditions could not be reached throughout the entire heating period.

The original intent of conducting these experiments was to obtain rate constants for several temperatures as well as an activation energy (ΔE) and reaction rate constant (k_r) at a reference temperature that could be used for estimation of thermal parameters in extrusion. Retention of *trans*- β -carotene using isothermal and near-isothermal conditions was expressed using equation (5).

However, there were several limiting factors with isothermal and near-isothermal experiments. First, the time of heating at a certain high temperature necessary to cause degradation of *trans*- β -carotene was much longer than typical extrusion residence times; therefore; it was difficult to compare to the extrusion process. Also, using high temperatures and long times caused a loss in moisture content; as a result, moisture content could not be held constant at typical moistures used in extrusion. Finally, because the product that was heated was a solid, there were large temperature gradients across the sample at short heating times.

4.2.4.2. Nonisothermal Kinetic Parameters

Because of the limitations with isothermal and near-isothermal experiments, nonisothermal experiments were conducted. Although the mathematical analysis was more complicated, nonisothermal experiments were more representative of heating that occurs during extrusion. The times of heating and temperatures used were closer to that used during extrusion, and moisture changes were also comparable. Retention of *trans*- β -carotene for nonisothermal heating was expressed using equation (8).

4.2.5. Moisture

Changes in moisture throughout the heating process were monitored. Moisture content of all samples was measured by using a Sartorius MA 30 moisture analyzer (Sartorius, Bohemia, NY). Approximately 2.5 g of sample was placed in the moisture analyzer and moisture content was determined at 130°C for 10 minutes.

4.3. Extrusion Experiments

Extrusion experiments were conducted to obtain the effect of extrusion at different conditions on degradation of *trans*- β -carotene.

4.3.1. Extrusion Setup and Conditions

A lab scale APV Baker MP19TC-25 co-rotating, intermeshing twin-screw cooking extruder was used to extrude all samples. The die was composed of one 3 mm-diameter circular hole. Each barrel diameter was 19 mm with a barrel length-to-diameter ratio of 25:1.

A high-shear screw configuration (Table 4.1) was used for all extrusion runs to obtain the maximum shear effect. The configuration consisted of either twin-lead or single-lead feed screws and kneading paddles. Feed screws were necessary to convey the

materials through paddle sections and the barrel. Kneading paddles themselves have little conveying ability but were necessary for agitation, melting and distribution of ingredients. Kneading paddles can either be situated in 30, 60, or 90° orientations and placed in a forward (clockwise) or reverse (counter-clockwise) direction.

Table 4.1. Screw Configuration for Extrusion Experiments
High-Shear Screw Configuration,
from Feed Inlet to Die

8 D TL
7 x 30° FKP
4 D TL
4 x 60° FKP
4 x 30° RKP
2 D TL
6 x 60° FKP
4 x 30° RKP
1 D SL
7 x 90° KP
2 D SL

Where D = 19 mm; TL = twin lead screw; F/RKP = forward/reverse kneading paddle; SL = single lead screw; KP length = 0.25D.

Temperatures were programmed along the barrel, which consisted of 5 heating zones. Heating was accomplished using electrical heating rods placed along the barrel. Barrel temperatures were set on a control panel, which was also used to monitor product temperature and other processing parameters. Barrel and product temperatures in the five zones were measured by thermocouples located along the distance of the barrel. Two temperature profiles were used: 50, 70, 90, 110, 130°C (High) and 30, 50, 70, 90, 110°C (Low), where barrel temperatures are listed from the feed inlet towards the die. Temperature profiles were determined specifically for this product after conducting

preliminary experiments at higher temperatures. The nature of the food-grade β -carotene required that temperatures be maintained low enough to avoid excessive burning of the product at the die yet high enough to melt the product sufficiently for extrusion.

Base torque was collected at each screw speed by allowing lubricated screws to run empty at each screw speed for approximately 1 minute. The raw material was metered into the extruder using a double-screw volumetric feeder (K-Tron Corp., Pitman, NJ) at feed rates selected to approximate constant fill (section 4.3.3) at all screw speeds. Water pump stroke was controlled by an EZ Metripump positive displacement metering pump (Bran and Luebbe, Northampton, UK).

All extrusion samples were collected after constant torque was maintained and process variables were recorded for each condition. Variables included screw speed (rpm), torque (%), die pressure (psi), feed rate (rpm), % water pump stroke, barrel temperature profile ($^{\circ}\text{C}$), and product temperature profile ($^{\circ}\text{C}$). Die pressure was monitored using a Dynisco pressure transducer (Model # EPR3-3M-6 Dynisco, Morgantown, PA). Temperature of product inside the die ($^{\circ}\text{C}$) was measured by inserting a needle thermocouple into the die for approximately 5 seconds.

4.3.2. Calibration of Feeder and Water Pump Stroke

Calibration of the double-screw raw feeder was accomplished by setting a range of feed rates (rpm) and weighing the feed raw material over 1 minute. Each collection was done in duplicate. Two calibration equations were obtained for extrusion. For all preliminary work (sections 4.3.3 and 4.3.5), the calibration equation (Feed Calibration A) was: $\text{g flour/minute} = 0.0336 (\text{feed rpm}) + 12.1$, $R^2 = 0.9825$. Another calibration was done for samples because there was a change in the level of raw material in the double-

screw feeder. The calibration equation (Feed Calibration B) used for all samples (section 4.3.6) was: $\text{g flour/minute} = 0.0777 (\text{feed rpm}) + 2.68$, $R^2 = .9998$.

Calibration of the water pump was done identically to calibration of the feeder. Each collection was done in triplicate. The calibration equation for all work was $\text{g water/minute} = 1.29 (\% \text{pump stroke}) - 2.23$, $R^2 = .9948$.

4.3.3. Analysis of Percent Fill as a Function of Screw Speed and Feed Rate

A change in screw speed at a constant feed rate will also change the degree of fill (Altomare and Ghossi, 1986), which will subsequently affect average shear rate (Suparno et al., 2002). The average shear rate may influence *trans*- β -carotene degradation. To minimize this effect, we attempted to maintain a constant degree of fill at all screw speeds. To predict the feed rates at each screw speed to maintain constant fill, we first conducted factorial experiments with 3 feed rate ranges at 3 screw speeds (Table 4.2) using the high temperature profile.

Table 4.2. Extrusion Conditions for Analysis of Percent Fill (at temperature profile 50/70/90/110/130°C, feed inlet to die)

Screw Speed (rpm)	Flour Feed Rate (g flour/ minute)	Water Feed Rate (g water/ minute)
200	48.2	9.45
200	51.5	10.10
200	54.8	10.74
300	51.5	10.10
300	54.8	10.74
300	58.1	11.39
400	54.8	10.74
400	58.1	11.39
400	61.4	12.03

Residence time samples were collected and mean residence times were calculated (section 4.3.4.2) for all 9 conditions. % Fill was calculated for each condition and results

were used to approximate a constant % fill at varying screw speeds. % Fill was calculated as follows:

$$\%Fill = \left(\frac{\dot{m} \times \bar{t}}{v \times \rho} \right) \times 100 \quad (31)$$

where $\dot{m}$ = mass flow rate of dough = $\dot{m}_{flour} + \dot{m}_{water}$ (kg/second),

$\bar{t}$ = mean residence time, sec ,

v = void volume of extruder, m^3 and ρ = dough density, kg/m^3 .

The method for estimating dough density is found in section 4.3.7.4.

4.3.4. Residence Time Distribution (RTD) Analysis Methods

The RTD is a useful tool for scaleup and optimization of process conditions (Harper, 1978). RTD's are especially valuable in the case of twin-screw extruders, due to the complex mixing and geometry. The RTD was useful in this research because it allowed for estimation of average time that one particle spent in the extruder at each condition, and calculation of % fill.

4.3.4.1. Preparation of Dyed Flour to Measure RTD

Twenty grams of flour was mixed with 8 ml of water-based blue dye #1080-0500 (Craft Store, Cordova, TN) by weight. The dye was poured onto the flour and mixed until the dye was completely absorbed. The mixture was then placed in a drying oven at 90°C until sufficient moisture had evaporated for the sample to be milled. The samples were pulverized in a mortar and pestle. The mixture was brought to a uniform particle size by milling in a Udy Cyclone mill (Udy Corporation, Fort Collins, CO) with 0.5 mm steel screen size.

4.3.4.2. Residence Time Sample Collection Methods

At each condition, once steady state torque was attained and all system parameters were documented, 0.2g of the residence time dye was added instantaneously to the feeder inlet. Time was measured from when the blue flour was added until color in the extrudate leaving the die could no longer be observed. Samples were collected every 10 seconds from the time color appeared in the extrudates until the color disappeared from the extrudates. The samples were placed on a pan in sequence and covered with aluminum foil to allow for drying.

After drying for several days, each sample was split into half or thirds, due to some obvious difference in color uniformity in certain strands. The total length for a ten-second strand was measured and used to calculate the time represented by each extrudate sample length.

Each strand was ground in a coffee grinder for approximately 15 seconds, or until the sample was a fine powder, and tested for color per section 4.4.3. Normalized residence time $E(t)$ and mean residence time $\bar{t}$ were calculated using (Levenspiel, 1999):

$$E(t) = \frac{C(t_i)}{\sum C(t_i) \Delta t_i} \quad (32)$$

$$\bar{t} = \frac{\sum t_i C(t_i) \Delta t_i}{\sum C(t_i) \Delta t_i} = \sum t_i E(t_i) \Delta t_i \quad (33)$$

Where $C(t_i)$ is the concentration of dye in an extrudate with exit age t_i ; $\bar{t}$ is the mean residence time. $C(t_i)$ is calculated from the measured color ($H - H_{\text{control}}$) and the color-concentration calibration curve, described in section 4.4.3.

4.3.5. Color-Concentration Calibration Curve

The concentration approach for RTD's, developed by Peng et al. (1994), was applied to all residence time samples collected in this research. Color concentration for eight batches of flour was calculated as g dye/1 kg flour. Flour was mixed with blue dye (by weight) in a metal pan by spraying the dye on uniformly using a spray bottle to atomize the dye. The flour mixtures were then mixed by hand and placed in a drying oven at 90°C for drying. After all samples reached a state where they could be milled, the samples were then milled to a small, uniform particle size using the Udy flour mill. Food-grade β -carotene was mixed into dyed flour as outlined in section 4.1.1.

Table 4.3. Concentration of Dye and Screw Speeds Used for Color-Concentration Calibration Curve

Concentration of Dye (ml dye/kg flour)	Screw Speed (rpm)
0.1	300
0.2	250
0.2	300
0.2	400
0.25	250
0.25	300
0.25	400
0.3	300
0.4	300
0.5	250
0.5	300
0.5	400
0.6	300
1.2	300
3	300
6	300
10	300

Table 4.3 shows the concentrations of each batch of flour and screw speed used in extrusion. The batches with concentration 0.2, 0.25, and 0.4 g dye/kg flour were also

extruded at 3 different screw speeds to investigate the effect of screw speed on color. Each batch of prepared dyed flour was placed into the feeder of the extruder and extruded using the high-temperature profile and 28% moisture content. After steady state torque was attained, samples at each concentration were collected for approximately 1 minute. Sample were placed in plastic bags, covered with aluminum foil, and allowed to dry at room temperature. Samples were then ground in a coffee grinder for approximately 15 seconds and 1.5 g of the sample was used to take color readings in triplicate. Color readings were plotted versus concentration to establish a calibration curve. This relationship was applied to all residence time data that was collected, and was used to estimate mean residence time.

4.3.6. Experimental Design

Flour mixtures were prepared as outlined in section 4.1. Moisture content of raw material was measured prior to extrusion. Conditions for all extrusion runs are listed in Table 4.4. A replicate for each condition was run on a different day.

Table 4.4. Experimental Design for Extrusion Experiments

Sample #	Barrel Temperature (°C)	Moisture Content %	Screw Speed (rpm)	Flour Feed Rate (g flour/minute)	Water Feed Rate (g water/minute)
1	50/70/90/110/130	30	200	93.6	22.3
2	50/70/90/110/130	30	250	83.1	19.7
3	50/70/90/110/130	30	300	93.6	22.3
4	50/70/90/110/130	30	400	107.5	23.6
5	50/70/90/110/130	33	300	93.6	26.9
6	50/70/90/110/130	36	300	93.6	31.4
7	30/50/70/90/110	30	200	57.1	14.6
8	30/50/70/90/110	30	250	64.8	15.9
9	30/50/70/90/110	30	300	72.6	17.2
10	30/50/70/90/110	30	400	80.4	18.5

After steady-state torque was attained, samples were collected for 1 minute, sealed in plastic bags, covered with aluminum foil, and stored in a dark freezer (-4°C) until extrudates were dry enough to grind for extraction. Residence time samples were collected and analyzed for each condition.

A dead-stop shutdown was used to measure % fill and observe color within the extruder. To accomplish this, steady state torque was attained, screws and feed were stopped instantly and simultaneously, and the extruder barrel was opened rapidly. Each section of screws and mixing paddles was weighed with material that was left after extrusion. To obtain an estimate of the fill level, the weight of all screw sections were subtracted from the total weight to obtain weight of material in each section. Table 4.5 lists the weight of each screw section and the length of each screw section.

Table 4.5. Weights and Lengths of Screw Sections

Screw Section	Weight (g)	Length (cm)
8 D TL	193.5	15.2
7 x 30 FKP	41.9	3.3
4 D TL	97.3	7.6
4 x 60 FKP + 4 x 30 RKP	48.3	3.8
2 D TL	48.4	3.8
6 x 60 FKP + 4 x 30 RKP	60.8	4.7
1 D SL	27.2	1.9
7 x 90 KP	42.4	3.3
2 D SL	48	3.8

In preparation for β -carotene extraction, extrudates were ground in a small coffee grinder and milled in Udy cyclone mill to obtain a small particle size for extraction. Samples were then placed in opaque plastic bags, sealed, and placed in a dark freezer (-4°C) until extraction.

4.3.7. Data Analysis

4.3.7.1. Thermal Effect on Extruded Product

Nonisothermal kinetic parameters obtained at 149°C (Section 4.2.4.2) were used to calculate thermal effects on retention of *trans*-β-carotene. Mean retention due to thermal effects on extrudates can be expressed using equation (17).

4.3.7.2. Mechanical Effect

A general model for retention was proposed (Cha et al., 2001) and is expressed in equation (18). Therefore, retention due to mechanical effects was calculated using equation (21).

4.3.7.3. Shear History

Shear history in an extruder may be a good indicator of the extent of mechanical effects that the product is exposed to. Shear history is a function of shear rate and mean residence time. Shear history, which is dimensionless, can be calculated as follows:

$$\Phi = \dot{\gamma}_a \bar{t} \quad (34)$$

where average shear rate, $\dot{\gamma}_a = k'(\text{screw speed})^\alpha$ in s^{-1} , where screw speed was in rps. k' and α were adapted from Suparno et al., 2002. Two equations were used to estimate k'

$$k' = 0.849(\% \text{ fill})^{0.7845}, \text{ for flow behavior index} = 0.7 \quad (35)$$

$$k' = 0.128(\% \text{ fill})^{1.3055}, \text{ for flow behavior index} = 0.3 \quad (36)$$

Because flow behavior index for the dough in this study was unknown and was changing along the barrel, the average k' from these two equations was used .

Values for α were calculated according to Suparno (2002)

$$\alpha = (-0.0022 * \% \text{ fill}) + 1.5748 \quad (37)$$

4.3.7.4. Specific Mechanical Energy

Another method for evaluating the mechanical effect of extrusion is by calculating specific mechanical energy. Specific mechanical energy (SME) is affected primarily by the torque, screw speed and by the feed rate to the screw. It can be defined as the net mechanical energy input divided by the mass flow rate (Levine, 1989). Therefore,

$$SME \text{ (kJ/kg)} = \frac{E_v}{\dot{m}} \quad (38)$$

where $E_v = \frac{(P_w - \Delta P Q)}{1000}$, in kJ/sec and

$\dot{m}$ = mass flow rate of dough = $\dot{m}_{flour} + \dot{m}_{water}$ (kg/second)

with $P_w = (2.64)(\%torque - \%base\ torque)(N)$, in J/sec (from the manufacturer),

N = screw speed, rps, and ΔP = die pressure, Pa

Q = volumetric flow rate of dough = $\frac{\dot{m}}{\rho}$, m³/sec,

with ρ = dough density, kg/m³

Dough density was estimated as 1,150 kg/m³ based on Schmid et al. (2001) and Rao and Rizvi (1986).

4.3.7.5. Statistical Analysis

Statistical evaluation of the effect of screw speed, shear history (ϕ), and SME on retention due to mechanical effects (R_ϕ) was performed using SAS software. Each day of extrusion was analyzed separately. A full statistical model was used to determine if there was an interaction between temperature and mechanical effects (screw speed, ϕ , or SME).

An individual statistical model was also used to analyze effect of screw speed, ϕ , and SME at each temperature profile. Both days of extrusion were combined and analyzed using the full and individual models, to determine if there was a difference between days.

For both high and low temperature profiles on each day, we tested whether there was a statistically significant ($\alpha = 0.10$) linear trend of R_ϕ with mechanical effect = screw speed, shear history, or SME. Using the following equation,

$$R_\phi = A(\text{Mechanical Effect}) + B \quad (39)$$

if the p-value was greater than 0.10 (90% confidence), it was assumed that the slope was equal to zero and mean retention due to mechanical effects was constant over all screw speeds. In this case, mean $R_\phi \pm$ standard deviation was reported. In the cases where the effect was significant ($p \leq 0.10$), the slope and intercept were reported. Theoretically, at a screw speed of zero, retention due to mechanical effects was 1.0; however, it was not included in the statistical analysis. Therefore, equation (39) was not applicable to screw speed = zero. Values of retention due to mechanical effect at 50 rpm were not investigated due to equipment and product limitations.

We further tested combined days of extrusion to determine if retention due to mechanical effects, R_ϕ , followed an exponential trend with screw speed, shear history, or SME. In the following equation,

$$R_\phi = A \left(e^{-d(\text{Mechanical Effect})} \right) \quad (40)$$

if the p-value was greater than 0.10 (90% confidence), retention due to mechanical effects did not follow an exponential trend with screw speed, shear history, or SME. In this case, a screw speed of zero was used in the analysis.

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4.4. β -carotene Analysis and Color Evaluation

4.4.1. Extraction

All-*trans*- β -carotene 95% synthetic powder was obtained from Sigma Chemical Co., St. Louis, MO. All extraction solvents except ethanol were Baker analyzed solvents (Mallinckrodt Baker, Inc, Phillipsburg, NJ) and included methanol, diethyl ether (anhydrous), petroleum ether, and methyl-tert-butyl-ether (MTBE). Protex protease (Genencor International, Palo Alto, CA), a food-grade enzyme, was used to break apart the β -carotene food-grade matrix.

A wooden nitrogen dispensing apparatus [wood, 14 in. height, 10 in. width, and 17.5 in. length, 8 compartments (4 by 4 in.)], with metal pipes protruding from an adjustable top to dispense nitrogen at various distances from flasks, was used to dry all samples at room temperature. 99% pure compressed nitrogen was obtained from BOC Gases (Murray Hill, NJ) and AGA (Cleveland, OH). A Precision Scientific 360 Orbital Shaker Bath (Winchester, VA) was used to mechanically agitate all samples at room temperature.

The following extraction method was adapted from Roche Technical Marketing Analytics. All samples were evaluated for moisture content prior to extraction using the same method as outlined in section 4.2.5. Six-gram flour or extrudate samples were weighed in aluminum weighing boats and transferred into 500 (ml) bottles. 100 ml distilled water were added. Approximately 10 ml of the water was poured into the aluminum weighing dishes to dissolve residual sample left in the weighing boats, and then poured into the bottle. Three ml of Protex were pipetted into each bottle. Each bottle was placed in a 65°C water bath for 10 minutes, and then allowed to cool for 15

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minutes. 200 ml of petroleum ether and 100 ml of diethyl ether were added to each bottle. Each bottle was capped tightly and shaken by hand for one minute. The caps were unscrewed and 75 ml of 3A alcohol were added.. Each sample was wrapped in aluminum foil and placed on a shaker for 60 minutes. After shaking, samples were placed on a flat surface and allowed to settle for 5 minutes. 50 ml of the upper ether layer were pipetted into a 125 ml Erlenmeyer flask and placed in a nitrogen dispensing apparatus. Each sample was dried under nitrogen at room temperature until a thin, dry film was observed in each flask. During the drying process, the top of nitrogen dispensing apparatus was brought lower to dry extract at a closer range. After each sample was completely dry, 15 ml of methyl-tert-butyl-ether (MTBE) were immediately added to the flask to reconstitute the dried extract. The solution was transferred to a 50 ml volumetric flask and brought to volume with 100% MTBE. A glass stopper was placed in each flask, and it was wrapped in parafilm and aluminum foil, and placed in a dark freezer (-4°C) until preparation for high performance liquid chromatography (HPLC).

To prepare for HPLC, each flask was unwrapped and the solution was poured into a beaker. Approximately 1 ml was drawn up using a plastic syringe and then filtered through a 0.45 μ m syringe-driven filter unit (Millipore Corp., Bedford, MA) into an HPLC conical vial. Each vial was then placed into a spring, put inside a small HPLC bottle, and sealed with a cap containing a septum. Each sample was then placed inside the HPLC injection tray and prepared for auto-injection. The HPLC method and conditions are outlined in the following section. All steps were conducted under yellow light.

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A standard curve for *trans*- β -carotene (Sigma-Aldrich) 95% synthetic powder was prepared by dissolving 425 mg of *trans*- β -carotene in 250 ml of MTBE for preparation of a stock standard solution (stock 1). One ml of the first stock was transferred to another 250 ml volumetric flask and diluted to 250 ml (stock 2). Six solutions were prepared by taking aliquots (0, 1, 6, 11, 15, and 20 ml) of stock 2 and diluting to 25 ml using volumetric flasks. Each solution was then filtered with a 0.45 μ m filter. 20 μ l of each solution was injected into the Waters HPLC system. A standard curve was not used in calculating retention, but was performed to ensure linearity within the region for all samples.

4.4.2. HPLC Method

A Waters HPLC system (Milford, MA) consisting of a Waters 1500 Series HPLC Pump, Waters 2487 Dual Wavelength Absorbance Detector with deuterium lamp, Waters 717 Autosampler and Waters Breeze Software was used. Sample equipment consisted of conical vials, a spring, small sample bottles with caps, and septums purchased from Waters. HPLC solvents were Baker analyzed HPLC grade methanol and HPLC grade MTBE.

A Devilosil Reverse Phase Aqueous C₃₀ polymeric column (Nomura Chemical Co., Anado-cho Seto, Japan) was used to perform separation of isomers. The C₃₀ column is specifically tailored to separate *cis-trans* isomers of β -carotene. Sander et al. (1994) obtained effective separation of all-*trans* carotenoids from mixtures of standards and extracts using a C₃₀ column. Results from analytical evaluation of the column, performed by Nomura Chemical Co., are shown in table 4.6.

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Table 4.6. Analytical Results for Devilosil Reverse Phase Aqueous C₃₀ Column

Characteristic	Specification	Result
Median Particle Size (µm)	5.4-6.0	5.6
Surface Area (m ² /g)	285-315	299
Pore Volume (ml/g)	1.1-1.2	1.17
Median Pore Diameter (nm)	13-15	14
Total Carbon (%)	17.5-19	18.2

The HPLC method used in this research was adapted from a method described by Lessin et al. (1997) and Emenheiser et al. (1996). Our method consisted of an isocratic separation using a mobile phase of 89%:11% (v/v), methanol:MTBE. Solvents were degassed for 30 minutes and the column was conditioned for one hour each time new solvent was added. The column temperature was ambient laboratory temperature (22°C) and column effluent was monitored using a UV-vis detector set at 410 nm. Flow rate of solvent was 1.25 ml/min with an injection volume of 20 µl for samples that were dissolved in 100% MTBE.

Integration of peak areas was conducted from 40 to 120 minutes, which was the time period in which all major peaks appeared. This was done to ensure baseline noise was not being integrated as a peak. Minimum peak area and peak height values were set at zero for all chromatograms. All chromatograms were integrated in the same manner. Roche food-grade β-carotene is a mixture of *cis-trans* isomers of β-carotene; therefore, it was assumed that all peaks integrated on the chromatograms would represent total β-carotene, or all isomers present. The peak of interest in this research was the *trans* peak.

$$\text{Concentration of } trans - \beta - \text{carotene (relative units)} = \frac{\text{Peak Area of } trans \text{ isomer}}{\text{Total Area of All Peaks}} \quad (41)$$

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Sample size was not considered because *trans*- β -carotene was calculated based on a proportion, therefore, if the sample size were to increase, the proportion would remain the same because all peak area values would increase in the same manner.

It was also assumed that all peaks on the chromatograms were either *cis* or *trans* isomers and react in the same manner to thermal and mechanical effects.

4.4.3. Color

In the food industry, color is a very important characteristic that often indicates how appealing a food product will be to a consumer. The color of products containing β -carotene during extrusion have been investigated (Guzman-Tello and Cheftel, 1990), however, little research has been conducted on commercial food-grade β -carotene products. Typically, color changes in β -carotene products are reasonable indicators of a loss in concentration of β -carotene.

Color changes throughout the heating process were investigated to determine if there is a relationship between color of heated samples and concentration of *trans*- β -carotene. 1.5 grams of the sample was poured into a black sample cup (1.75 in. diameter). Color was evaluated using a Hunter Colorimeter (Hunter Associates Lab Inc., Reston, VA). The black sample cup was placed in the colorimeter and values that were recorded included a^* and b^* parameters. A color value was established using:

$$H = \sqrt{(a^*)^2 + (b^*)^2} \quad (42)$$

Where H is color, a^* is a-parameter, and b^* is b-parameter. Several control strands (color strand without dye added) were measured and H was then adjusted by subtracting the color value for the control strand.

5.1. HPLC

5.1.1. Chromatography

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CHAPTER 5: RESULTS AND DISCUSSION

5.1. HPLC

5.1.1. Chromatograms

Results from HPLC are found in Figures 5.1 and 5.2. Figure 5.1 shows an example of a chromatogram for a mixture of food-grade β -carotene and flour. Five major peaks eluted during the 120 minute sample run time, however, as is shown in Figure 5.2, the *trans*- β -carotene standard eluted at approximately the same time as the largest peak in Figure 5.1. Therefore, it was determined that the *trans*- β -carotene peak eluted at approximately 94 minutes.

The other peaks were assumed to be *cis* isomers of β -carotene, however, no further work was done to elucidate their identities. No other compounds were formed during the heating or extrusion process, therefore all peaks on the chromatogram were assumed to be either *cis* or *trans* isomers.

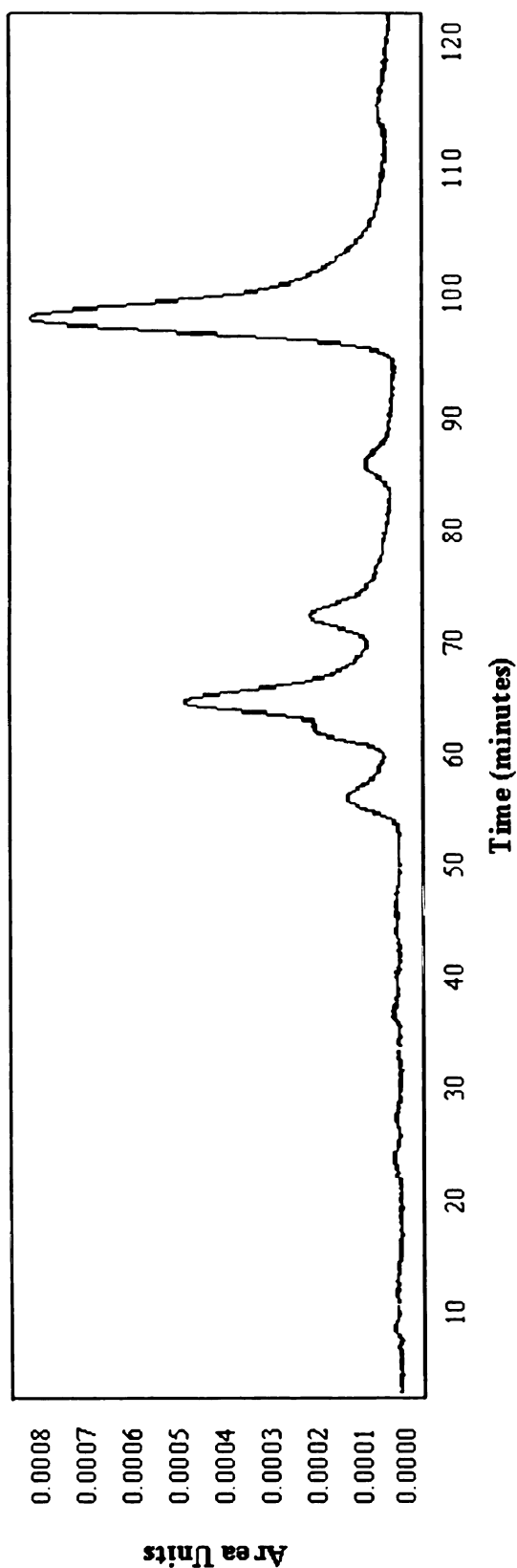


Figure 5.1. Representative HPLC Chromatogram for Food-Grade β -carotene/Flour Mixture.

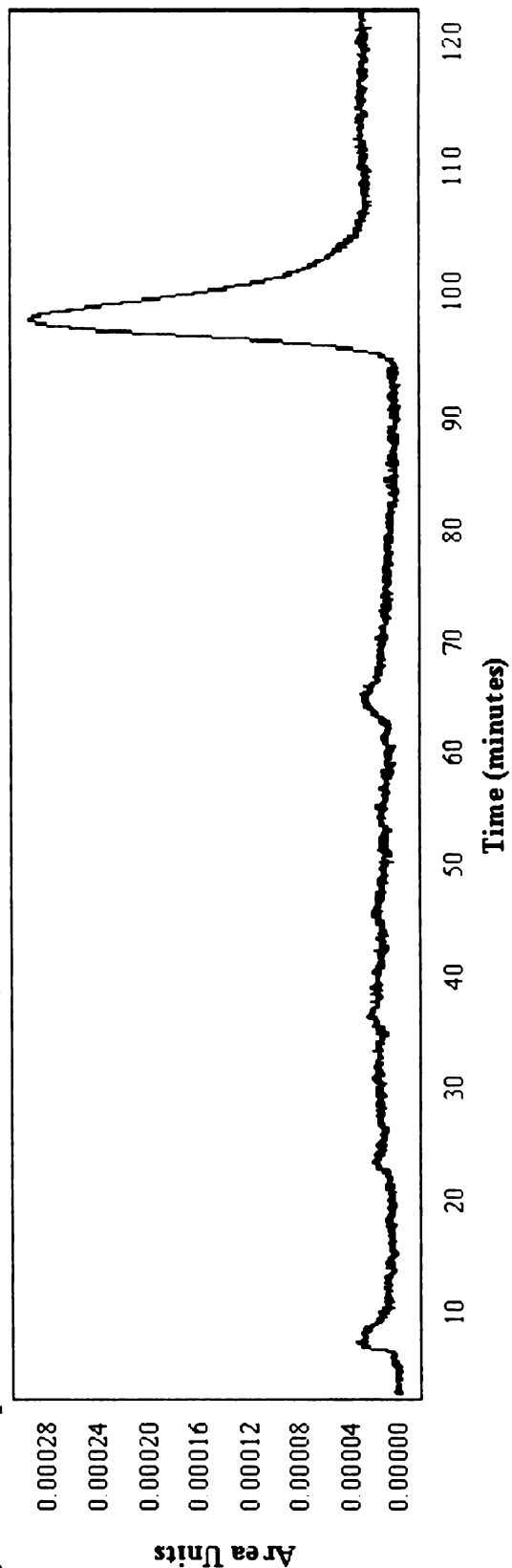


Figure 5.2. Representative HPLC Chromatogram for 95% All-*Trans*- β -carotene Standard.

5.2. Thermal Effects

Results were calculated as concentration of *trans*- β -carotene (relative units) and will be referred to as “*trans*- β -carotene”.

5.2.1. Isothermal Kinetic Parameters for *Trans*- β -carotene

At 80°C oil bath temperature, an approximate 10 minute time period was observed for the internal temperature of the samples to come up to a mass average temperature, $T_{ma} = 78^{\circ}\text{C} \pm 1^{\circ}\text{C}$ (Figure 5.3). Moisture was relatively stable, decreasing from 28% to ~24% throughout the 120 minute time period.

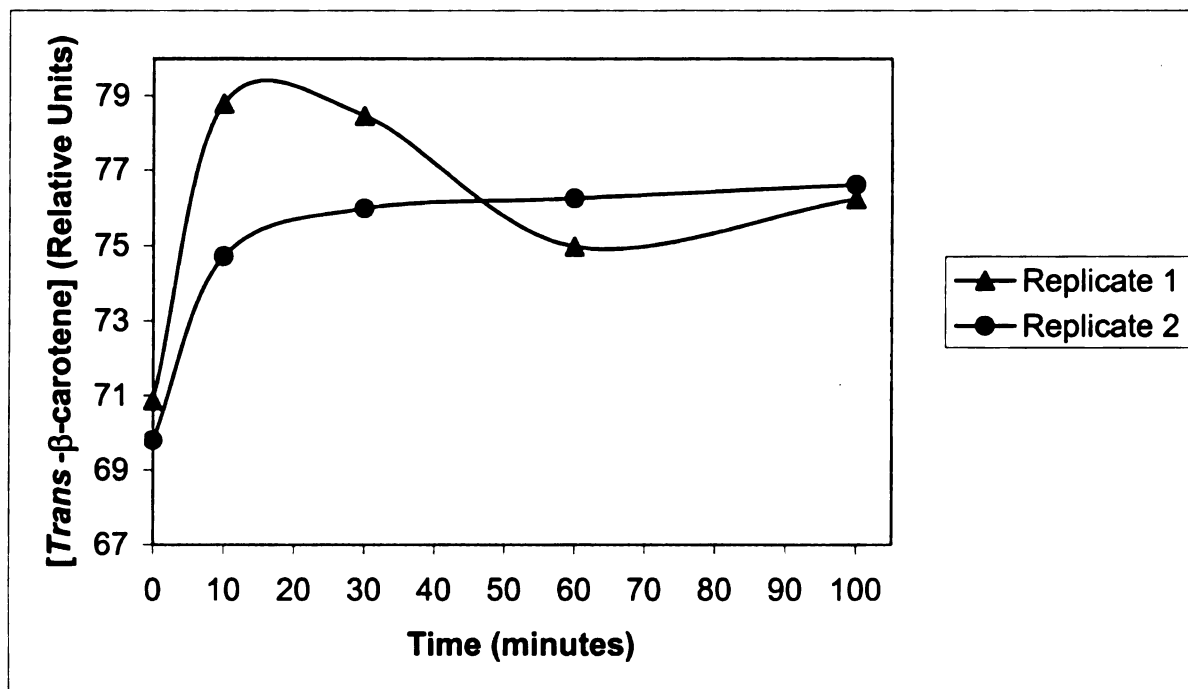


Figure 5.3. Concentration of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture vs. Time for Samples Heated Isothermally at 78°C.

There was a seeming increase within the first 10 minutes (Figure 5.3). This result may have been caused by a release of the β -carotene from its matrix during heating for the first 10 minutes. After 10 minutes of heating, the protective effect of the matrix may have decreased, and a slight decrease in concentration occurred.

Linear regression of $\ln (C/C_0)$ vs. time, where C_0 was concentration at 10 minutes, produced a very low R^2 . The effect of heating at 78°C on retention of *trans*- β -carotene was significant (p-value = 0.74) (Table 5.1). Therefore, *trans*- β -carotene did not show significant degradation throughout the entire 100-minute time period and proved to be stable at 78°C.

Table 5.1. Kinetic Parameters for Isothermal Experiments

Temperature (°C)	Moisture Content (%)	k (s ⁻¹)	r ²	P-value*
78	24-28	1.66 x 10 ⁻⁶	0.019	0.740
138	2.5-28	6.83 x 10 ⁻⁵	0.622	0.062

*90% Confidence ($\alpha = 0.10$)

During heating at 138°C (Figure 5.4), a true isothermal experiment was impractical because of the large sample size creating large temperature gradients, and moisture loss at temperatures greater than 100°C. Therefore, the experiment was performed at “near-isothermal” conditions. The lag time (time for sample to reach $T_{ma} = 138 \pm 1^\circ\text{C}$) was approximately 20 minutes, and samples taken out at 10 minutes had not quite reached isothermal temperatures ($T_{ma} = 119.35^\circ\text{C} \pm 1^\circ\text{C}$). From 0 to 60 minutes, moisture content decreased from 28 to 2.5% (Figure 5.4).

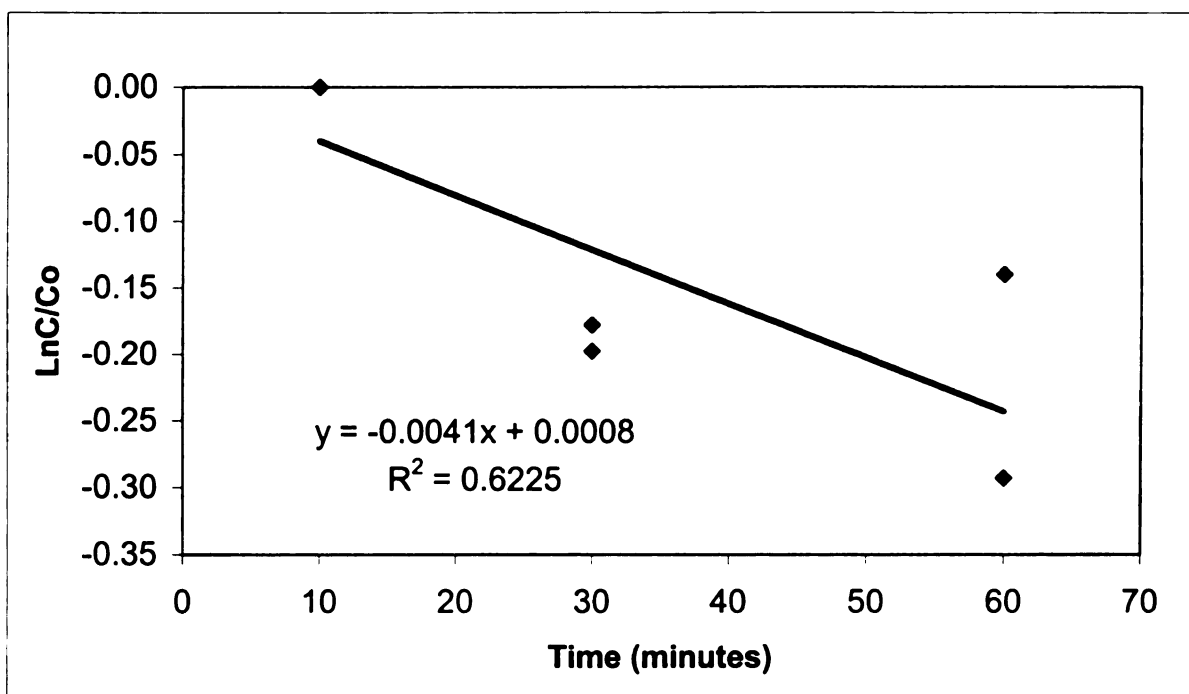


Figure 5.4. Log of Retention of *Trans*-β-carotene in Food-Grade β-carotene/Flour Mixture vs. Time for Samples Heated Near-Isothermally at 138°C. [Slope of line = -k (reaction rate constant)]

As listed in Table 5.1, the reaction rate constant at 138°C was $6.83 \times 10^{-5} \text{ s}^{-1}$, with a p-value = 0.062 for $\ln (C/C_o)$ versus time, where C_o was the value at the end of 10 minutes. Although the R^2 of 0.622 was low, the effect of heating near-isothermally at 138C on *trans*-β-carotene was significant at $\alpha = 0.10$.

The results at both 78°C and 138°C on food-grade *trans*-β-carotene in flour were different (larger amount retained) from other studies involving effects of heat on β-carotene in other environments. Papadoupoulou and Ames (1994) studied the effect of heating all-*trans*-β-carotene in paraffin at 210°C for 15 minutes. After only 6 minutes, approximately 98% of all-*trans*-β-carotene was degraded. Henry et al. (1998) studied the thermal degradation kinetics of all-*trans*-β-carotene in an oil model system at 75, 85, and 95°C for 24, 12, and 5 hours, respectively. After 19, 8, and 3 hours, there were only trace

amounts of the carotenoids remaining. The main difference between these studies and our study was the type of β -carotene used and the system it is contained in.

Kearsley and Rodriguez (1981) studied the thermal stability of a water-soluble β -carotene powder obtained from Hoffman LaRoche that was similar to the one used in our study. They found that after heating in water for nearly four hours at 100°C, the total β -carotene content had decreased to slightly less than half the original amount. Although this study investigated total β -carotene concentration as opposed to only looking at the *trans* isomer, it is a good indicator of the superior thermal stability of commercial β -carotene powders compared to pure *trans*- β -carotene.

The thermal stability of Roche food-grade β -carotene was probably due to numerous factors. The formulation contains two antioxidants, dl- α -tocopherol and ascorbyl palmitate. The presence of these compounds is principally to prevent any oxidative degradation and virtually eliminates auto-oxidation as a degrading factor. The other components in the formulation include gelatin, sucrose, maltodextrin, and corn oil. The primary purpose of these ingredients is to aid in hydrodispersibility of the inherently non-polar β -carotene; however, there is potential that these ingredients may provide a protective effect on *trans*- β -carotene. Therefore, the composition of an individual system containing β -carotene is evidently a very important factor on the extent of degradation of β -carotene.

There were two main difficulties with these isothermal studies. Primarily, the rate of degradation was so slow that the heating times were much longer than typical residence times in the extruder (2-4 minutes). Also, at lag times, the moisture loss was considerable, making the moisture content much lower than at extrusion. Therefore, the

nonisothermal results were more useful because the heating time was closer to the range of typical extrusion residence times, and the shorter times prevented such high moisture loss.

5.2.2. Nonisothermal Kinetic Parameters for *Trans*- β -carotene

Moisture decreased from 28 to 11.16% during heating at 149°C oil bath temperature. As with heating at 78°C (Figure 5.3), *trans*- β -carotene appeared to increase at the beginning of the nonisothermal heating period at 149°C (Figure 5.5). Because the rate can be calculated over any time period, the values at time = 0 were excluded from the nonlinear estimation procedure. The h values at each temperature from equation (30) in materials and methods were used to develop an empirical equation:

$$h = 1.8964(T) - 36.37$$

where T was in°C. The heat transfer coefficient, h , was calculated as 246 Watt/m²·°C at 149°C.

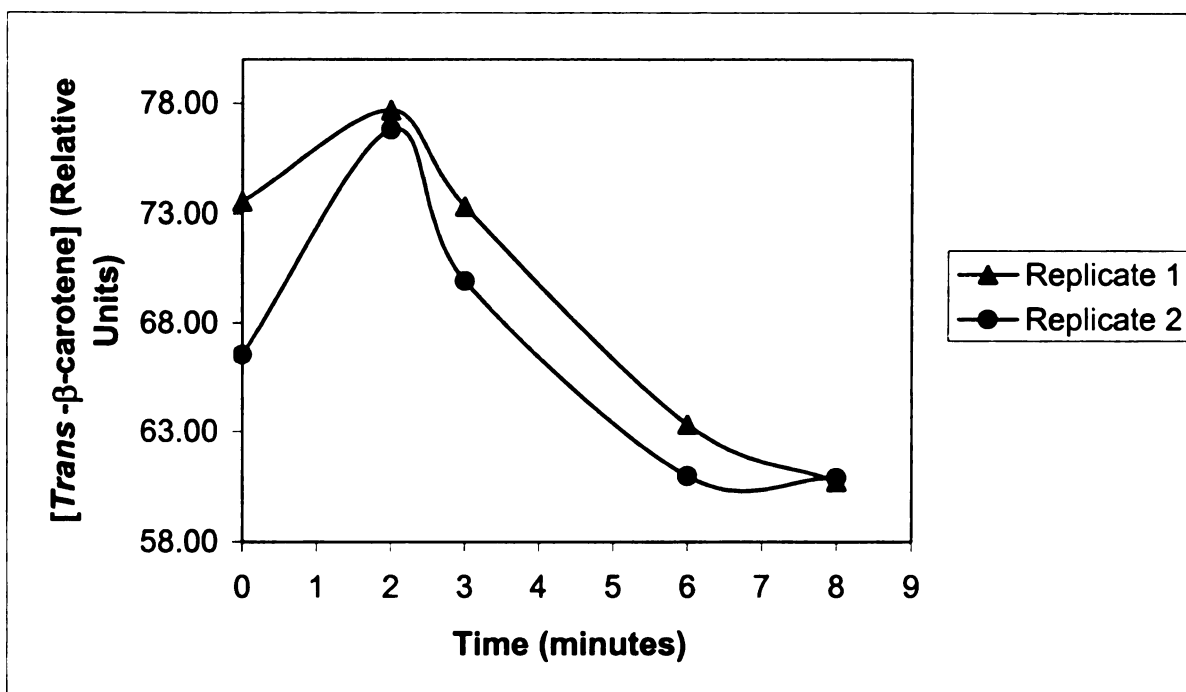


Figure 5.5. Concentration of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture vs. Time for Samples Heated Nonisothermally at Oil Bath Temperature = 149°C.

The time-temperature history (β) was calculated for each sample using its temperature profile (Figure 5.6). An activation energy (ΔE), reference reaction rate constant (k_r), and initial concentration value (C_0) were estimated (Table 5.2) at a reference temperature of 373.15°K (100°C).

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* $\alpha = 0.10$

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(Table 5.2). H

β -carotene in s

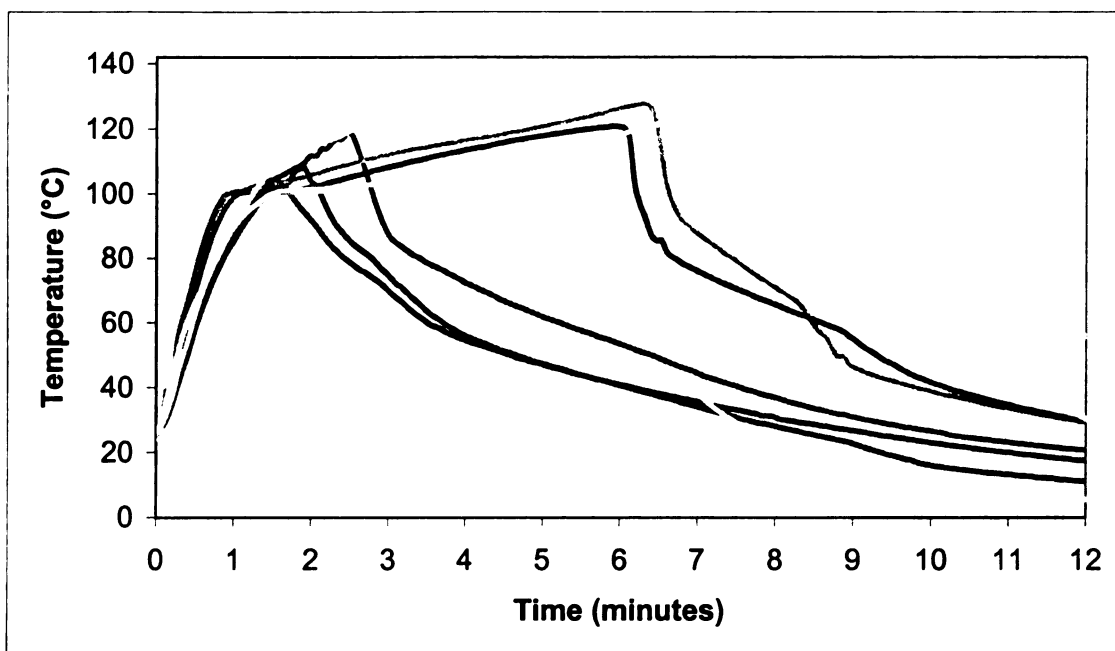


Figure 5.6. Temperature Profiles for Samples Heated Nonisothermally at Oil Bath Temperature = 149°C.

Table 5.2. Estimates of Reaction Rate Constant at Reference Temperature (k_r), Activation energy (ΔE), and Initial Concentration (C_0) for Nonisothermal Samples Containing 0.4616% Food-Grade β -carotene/Flour Mixture with Moisture Content Ranging from 28 to 11%

Parameter	Estimate	Standard Error	90% Confidence Interval*	R^2	Correlation Coefficient ρ_{ij}
$k_{100^\circ\text{C}}$	$3.56 \times 10^{-4} \text{ s}^{-1}$	2.85×10^{-4}	$\pm 5.75 \times 10^{-4}$	0.836	$\rho_{k_r \Delta E} = -0.968$
ΔE	18.820 kJ/g·mol	30.60	± 61.7	---	$\rho_{k_r C_0} = 0.993$
C_0	86.73 (relative units)	14.3	± 21.9	---	$\rho_{\Delta E C_0} = 0.953$

* $\alpha = 0.10$

The values for activation energy and reaction rate constant, 18.82 kJ/g·mol and $3.56 \times 10^{-4} \text{ s}^{-1}$, respectively, were much lower than those reported in previous studies (Table 5.2). Henry et al. (1998) found an activation energy of 109.67 kJ/g·mol for *trans*- β -carotene in safflower seed oil heated at 75, 85, and 95°C (Table 2.1). Minguez-

Mosquera and Jaren-Galen (1994) found an activation energy of 48.81 kJ/g·mol for β -carotene in water at 15, 25, 35, and 45°C (Table 2.1). Although the temperatures used in both of these studies were lower than what was used in our study, the activation energies were significantly higher than our value over the range 25 - 149°C. This confirms that Roche food-grade β -carotene has much increased thermal stability compared to other natural or pure β -carotene sources, such as the ones used by Henry et al. (1998) and Minguez-Mosquera and Jaren-Galen (1994).

Although the standard errors and confidence intervals are large, the data fit reasonably well ($R^2 = 0.836$) (Table 5.2). Predicted values for *trans*- β -carotene were calculated and compared to actual values. Figure 5.7 shows the best-fit line for the nonlinear estimation results.

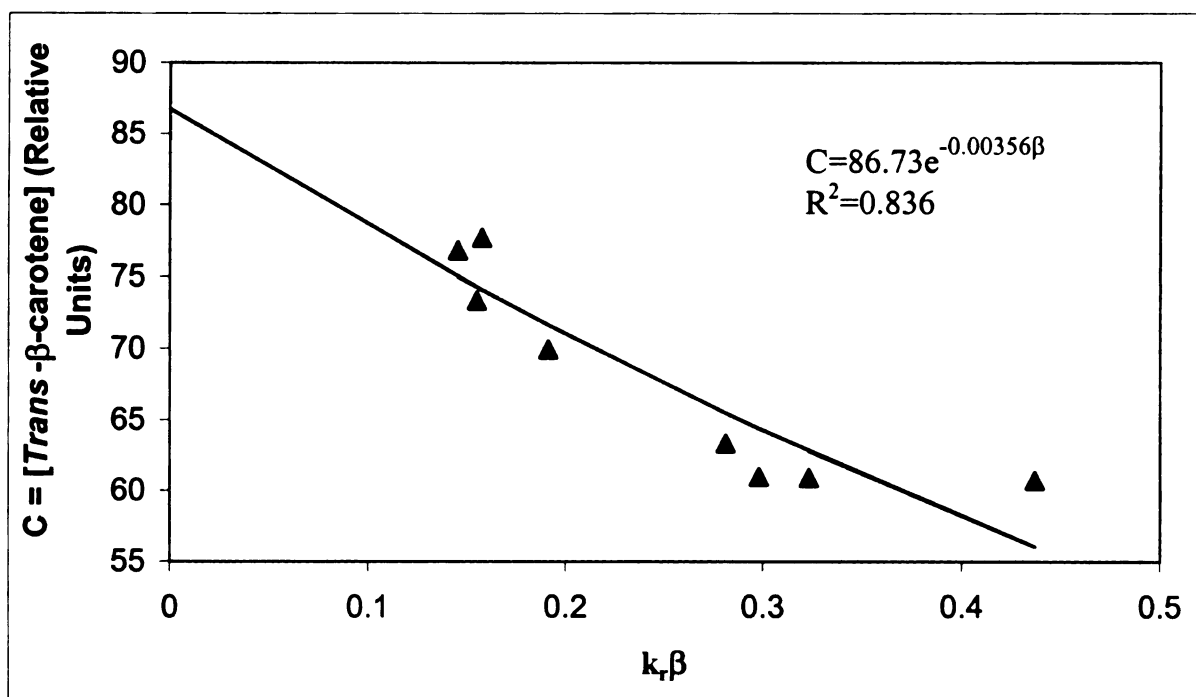


Figure 5.7. Measured Concentration of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture and Best-Fit Line (Estimated Activation Energy 18.82 kJ/g·mol).

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Correlation between parameters estimated in nonisothermal analysis was estimated by calculating correlation coefficients (ρ) from the confidence interval matrix (Table 5.2). Although the correlation coefficient for k_r and C_o was high (>0.99 is critical, Bates and Watts, 1988), the nonlinear regression converged rapidly to the same value for a wide range of starting values of parameters: $1 \times 10^{-6} < k_r < 1 \times 10^{-2}$, $100 < \Delta E < 600,000$, $0 < C_o < 275$.

The rate constant at 138°C was also calculated as $6.23 \times 10^{-4} \text{ s}^{-1}$, based on the nonisothermal parameter estimates, using the Arrhenius equation [equation (6)]. This value was almost ten times higher than the rate constant found at a lower moisture content (2.5%) at 138°C ($6.83 \times 10^{-5} \text{ s}^{-1}$) (Table 5.1). This indicates that at higher moisture contents, *trans*- β -carotene degrades faster than at lower moisture contents. This finding is opposite that found by Ramakrishnan and Francis (1979), who studied the stability of β -carotene in model systems of cellulose and starch equilibrated under different relative humidities. They found that overall, increased water content was found to have a protective effect over β -carotene, which can be attributed to the formation of hydrogen bonds between water and hydroperoxide molecules.

Several factors could be contributing to the results that we obtained; however the primary factor is the β -carotene matrix. Since the form of β -carotene is water soluble, more β -carotene may be liberated at higher moisture contents and therefore available for reaction. More extensive work on the effect of moisture content on degradation of *trans*- β -carotene should be conducted to determine trends for predictive models.

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5.3. Extrusion

5.3.1. Estimating % Fill

% Fill is a function of screw speed and feed rate; therefore, a different feed rate at each screw speed is required to maintain a constant fill. Figure 5.8 shows the mean residence times for nine combinations of screw speeds and feed rates. These data were used to calculate the % fill at each of the nine conditions (Figure 5.9). Although a constant fill could be maintained for 300 and 400 rpm ($\approx$ 50-53%), there was no % fill that overlapped for 200 rpm (Figure 5.9). Therefore, feed rates were chosen from Figure 5.8 to maintain a range of fills. The feed rates chosen are listed in Table 4.4 (in Materials and Methods). (Feed rates actually used were slightly higher than what was previously estimated due to a higher fill level in the double screw feeder, causing the flour to be fed at a faster rate. Feed calibration curve B was used in this case).

Mean Residence Time (seconds)

Figure 5.

Mass Feed Rate (g/min)

72
68
64
60
56
48

Figure 5.9. A
Figure 5.8.

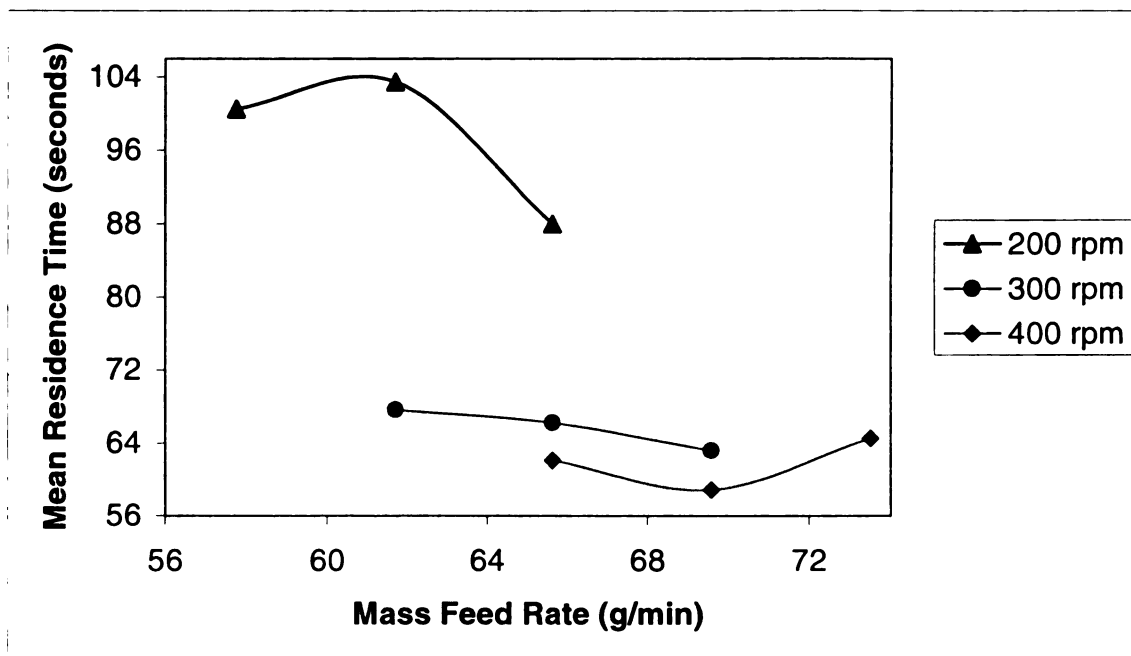


Figure 5.8. Mean Residence Times vs. Mass Feed Rates for Three Screw Speeds

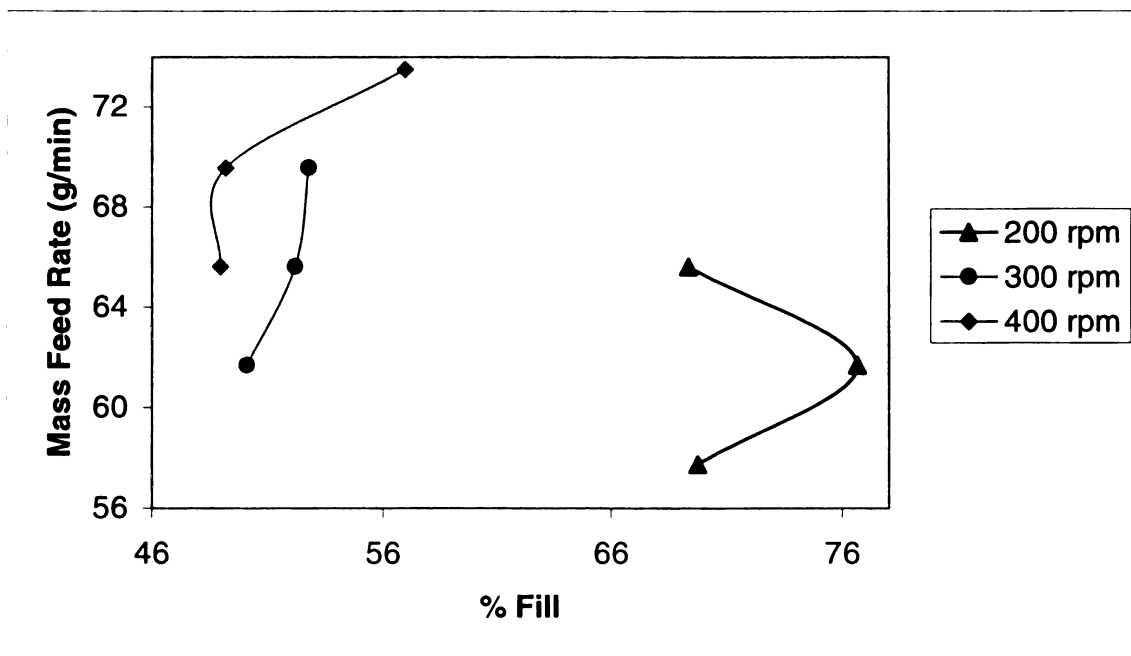


Figure 5.9. Mass Feed Rate vs. % Fill for Three Screw Speeds, Based on Data from Figure 5.8.

5.3.2. Residence Time Distribution Analysis

5.3.2.1. Color-Concentration Calibration Curve

A relationship between color and concentration was established (Figure 5.10). Two equations were fit to the data and used to calculate concentration for all residence time samples:

$$x \geq 3: y = -0.0132x^3 + 0.3913x^2 - 1.0435x + 0.734, \quad R^2 = 0.9479$$

$$x < 3: y = 0.0479x + 0.2906, \quad R^2 = 0.0485 \quad (\text{p-value} = 0.062)$$

Where x is the color in Hunter units, y is the concentration of blue dye in g dye/ kg flour.

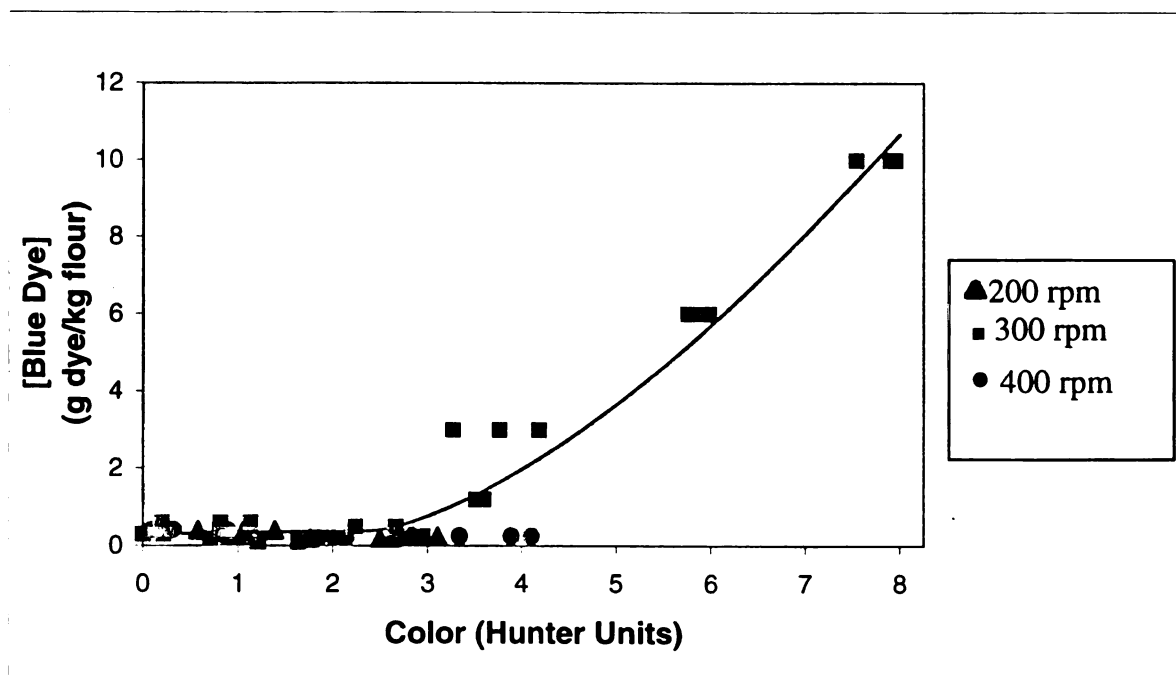


Figure 5.10. Color vs. Concentration of Blue Dye for Extruded Wheat Flour with 0.4616% Food-Grade β -carotene at Varying Screw Speeds.

5.3.2.2. Residence Time Distribution Curves and Mean Residence Times

Figure 5.11 shows an example of a residence time distribution (RTD) for 1 replicate at the high temperature profile for each screw speed. Increasing the screw speed shifts the RTD curve to the left, thus shortening the mean residence time (Altomare and Ghossi, 1986). Only the RTD's at 200 and 250 rpm did not follow this trend, but their curves were nearly identical (Figure 5.11).

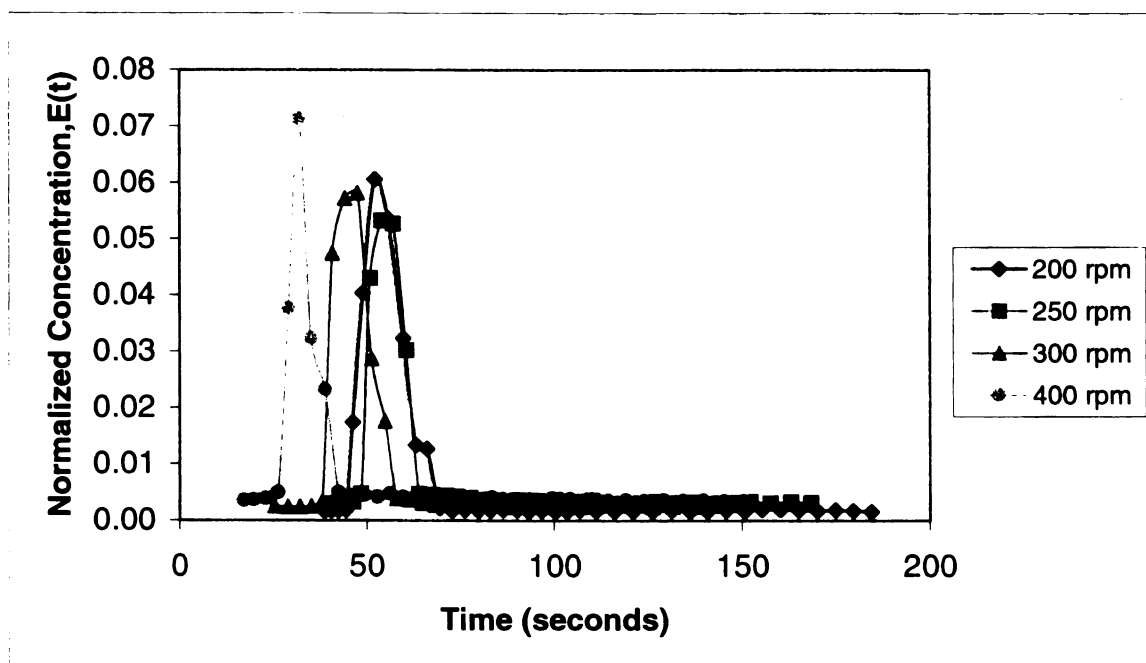


Figure 5.11. RTD Curves for High Temperature 1 Extrusion Runs at Screw Speeds = 200, 250, 300, and 400 rpm.

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Mean residence times for each of the 16 extrusion runs are shown in Figure 5.12. The mean residence times (MRT) decreased with an increase in screw speed as expected (Figure 5.12), and were reasonably repeatable from one day to the next, with the exception of low and high temperature runs at 200 rpm. Mean residence times indicated the average time the β -carotene was exposed to higher temperatures.

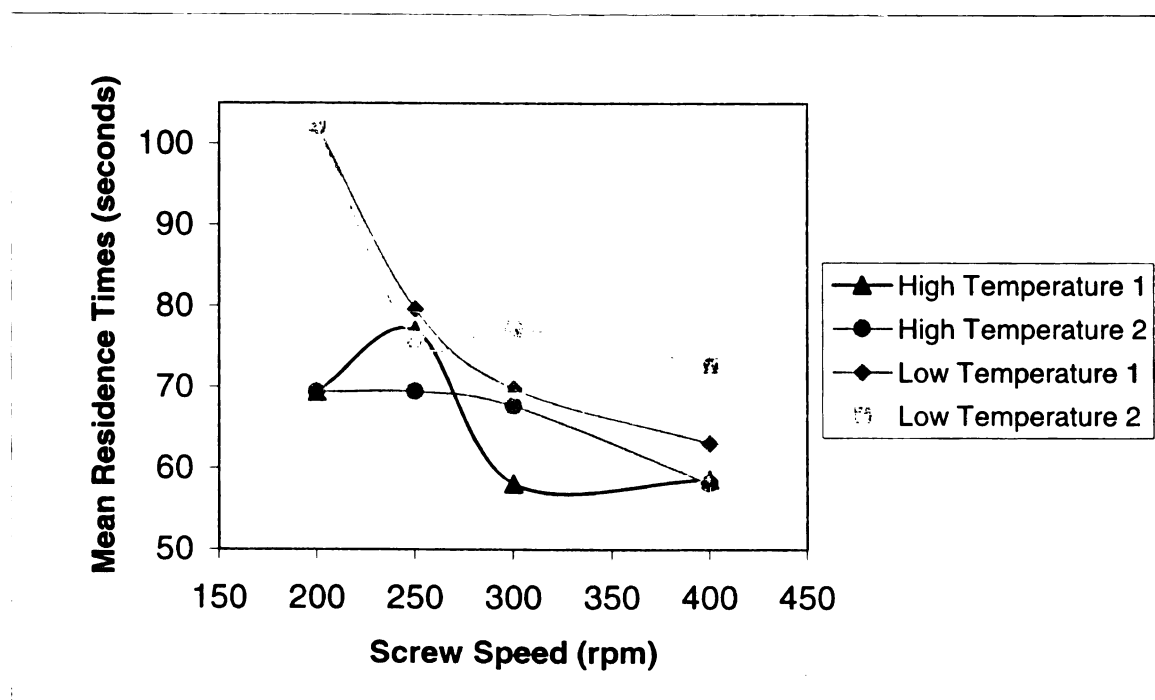


Figure 5.12. Mean Residence Times vs. Screw Speed for 16 Extrusion Runs at High and Low Temperature Profiles.

5.3.3. Thermal and Mechanical Effects of Extrusion on Retention of *Trans*- β -carotene – Separate Days

The following results are grouped according to the day of extrusion (day 1 or day 2) and include both temperature profiles (high and low). Because extrusion is a dynamic process and often does not give highly reproducible results from day-to-day, duplicate days were not grouped together. A simple linear model was used to analyze the results of separate days.

Any references to loss of *trans*- β -carotene will be defined as:

$$Loss = 1 - Retention$$

where retention is expressed as a decimal from 0.00 to 1.00. Retention was assumed to be a function of mechanical effects. Three different measures of mechanical effects were chosen: screw speed, shear history, and SME.

Theoretically, if a constant degree of fill is maintained, screw speed is essentially a measure of average shear rate only (Suparno et al., 2002). Shear history is a combination of both average shear rate and mean residence time; therefore, a high shear rate and short residence time may result in the same retention as a low shear rate and long residence time. Note that as screw speed increases, mean residence times are shorter, but average shear rate increases. Therefore, as screw speed is changed, thermal and mechanical effects move in reverse directions. SME, which measures the net energy input per unit mass, does not account for mean residence time, and it includes 3 variables: screw speed, shear rate, and mass flow rate of dough.

The following sections show which, if any, of these measures of mechanical effect (screw speed, shear history, or SME), has the most effect on retention. Although

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the three measures have some relationship to each other, they are not the same and were analyzed separately.

Each graph contains the average of two measurements for total retention (R_T); a value for calculated retention due thermal effects (R_β) (only one thermal history existed at each condition); and the average of two calculated values for retention due to mechanical effects (R_ϕ). All replicates for retention values (R_T , R_β , and R_ϕ) are listed in Table A.1.3. To avoid cluttering figures, error bars were not used. Instead, standard deviations for values of R_T during extrusion are listed in Table 5.3. Because R_ϕ is calculated from R_T , it had the same proportional standard deviations (standard deviation of R_T divided by R_β).

Table 5.3. Standard Deviations of Measured Total Retention of *Trans*- β -carotene at Each Extrusion Condition

Condition*	Standard Deviations			
	200 rpm	250 rpm	300 rpm	400 rpm
High, Day 1	---	0.034	0.049	0.014
Low, Day 1	0.003	0.093	0.032	0.126
High, Day 2	0.130	0.150	0.014	0.084
Low, Day 2	0.036	0.005	0.009	0.041

*Conditions listed as: Temperature Profile, Day

Values for statistical significance (p-value) for all variables (screw speed, shear history, SME) and their interaction with temperature are listed in Table 5.4 (2 data points at each screw speed = 8 data points total at each temperature profile on each day).

Table 5.4. P-values for Retention Due to Mechanical Effects (R_ϕ) for Screw Speed, Shear History, and SME

Condition	P-value		
	Screw Speed	Shear History	SME
High, Day 1	0.0847*	0.3041	0.1147
Low, Day 1	0.0350*	0.0538*	0.2585
Interaction of Temperature and Variable, Day 1	0.2100	0.0742*	0.4687
High, Day 2	0.2131	0.2538	0.7557
Low, Day 2	0.1666	0.4050	0.2359
Interaction of Temperature and Variable, Day 2	0.0847*	0.1439	0.4526

*Indicates significant effect at 90% Confidence ($\alpha = 0.10$)

5.3.3.1. Effect of Screw Speed

In theory, an increase in screw speed would result in a shorter mean residence time and subsequently shorter time of exposure to any high temperatures. If the compound were highly heat sensitive and thermal effects dominated, retention would be expected to increase with screw speed. More *trans*- β -carotene would be degraded at the lower screw speeds because the time of exposure to high temperatures is increased due to the product moving at a slower rate through the extruder barrel. An increase in screw speed, however, could also cause an increase in mechanical energy or shear effects on the product inside the extruder. If mechanical effects dominated, retention would typically decrease with screw speed.

If the temperature profile is constant at all screw speeds, and mean residence times decrease with screw speed, then retention due to thermal effects will increase with screw speed (less degradation). Conversely, if the product temperature increases with screw speed because cooling cannot remove sufficient heat, retention due to thermal effects will not increase with screw speed as rapidly, and may decrease or not change at all, depending on how much the temperature increase compensates for the shorter residence times.

The trend of retention due to mechanical effects with screw speed can be interpreted by examining mean residence times (Figure 5.12) and die temperature (Table 5.5). Die temperature for all screw speeds could not be maintained due to equipment limitations (Table 5.5). In general, there was an increase of die temperature with screw speed.

Table 5.5. Die Temperature for Extrusion Experiments at Each Condition

Condition	Die Temperature			
	200 rpm	250 rpm	300 rpm	400 rpm
High, Day 1	133	133	128	138
Low, Day 1	120	116	120	130
High, Day 2	128	137	142	145
Low, Day 2	119	123	128	134

High temperature extrusion performed on day 1, shown in Figure 5.13, shows that the majority of loss of *trans*- β -carotene during extrusion was due to mechanical effects.

Retention due to thermal effects remained fairly stable (> 0.95) as screw speed increased, because die temperature increased (Table 5.5). The effect of screw speed on retention due to mechanical effects (R_ϕ) was statistically significant ($\alpha = 0.10$) on this day at the high temperature profile, with p -value = 0.0847 (Table 5.4), slope = -3.6×10^{-4} , and intercept = 0.981, $R^2 = 0.4794$. This implies that screw speed did have a significant linear effect on R_ϕ .

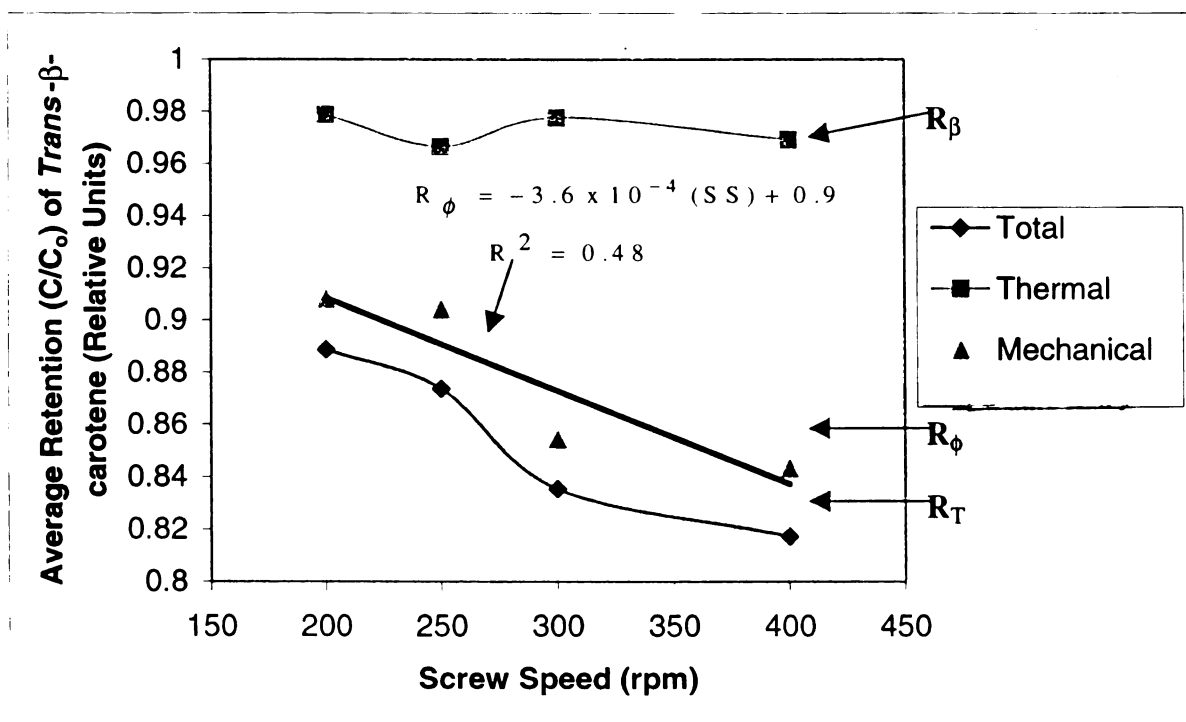


Figure 5.13. Average Retention of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture vs. Screw Speed at High Temperature Profile on Day 1 {“Total” is measured total retention [HPLC results] (R_T), “Thermal” is calculated retention due to thermal effects (R_β) [calculated from: $\int_0^\infty \exp(-k_r \beta) E(t) dt$], and “Mechanical” is calculated retention due to mechanical effects ($R_\phi = R_T / R_\beta$)}.

Low temperature extrusion performed on day 1 (Figure 5.14) showed a more significant trend. R_β consistently increased with screw speed (Figure 5.14), because mean residence times decreased with screw speeds (Figure 5.12) and die temperature remained fairly constant except at 400 rpm (Table 5.5). At this condition, R_ϕ was statistically significant ($\alpha = 0.10$) with p-value = 0.0350 (Table 5.4), slope = -8.8×10^{-4} , and intercept = 1.14, $R^2 = 0.5507$.

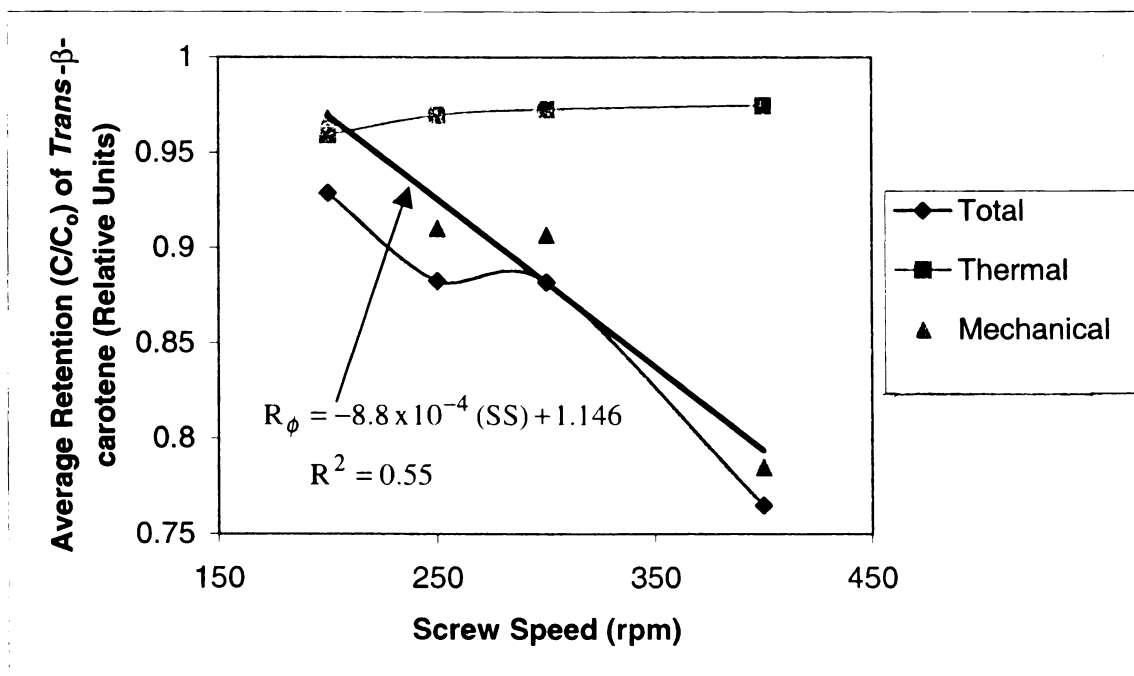


Figure 5.14. Average Retention of *Trans*-β-carotene in Food-Grade β-carotene/Flour Mixture vs. Screw Speed at Low Temperature Profile on Day 1 [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

Results from statistical analysis indicated that there was no significant effect of interaction between temperature and screw speed on day 1, with p-value = 0.2100 (Table

5.4) implying that mechanical effects on retention were the same for both temperature profiles and there was no synergistic effect of temperature and mechanical effects.

On the second day of extrusion, results varied somewhat compared to the first day. Figure 5.15 shows the retention of *trans*- β -carotene vs. screw speed at the high temperature profile. Although mechanical effects dominated, effect of screw speed on R_ϕ was not significant with p-value = 0.2131 (Table 5.4). In this case, mean R_ϕ was 0.90 ± 0.098 . R_β was nearly constant because temperature at the die (Table 5.5) was compensating for shorter mean residence times (Figure 5.12).

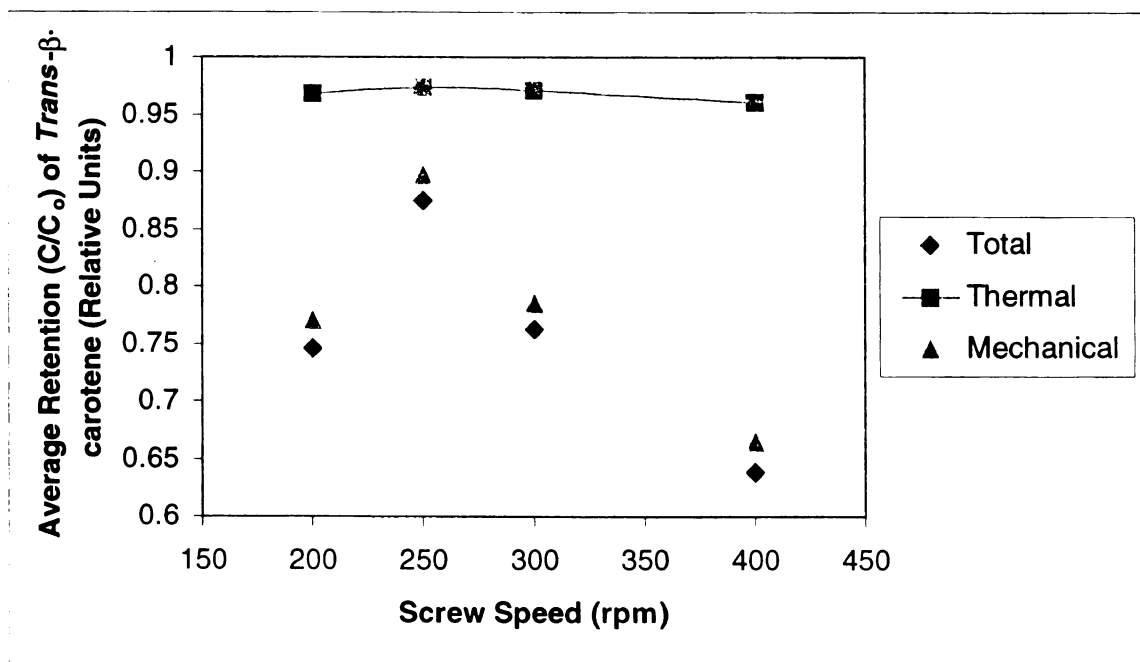


Figure 5.15. Average Retention of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture vs. Screw Speed at High Temperature Profile on Day 2 [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

On day 2 of low temperature extrusion (Figure 5.16), mechanical effects also dominated; however, there was no significant trend of R_ϕ with screw speed ($p = 0.1666$) (Table 5.4). Mean R_ϕ was 0.95 ± 0.06 at all screw speeds. R_β at increasing screw speeds did not follow a particular trend, due to an increase in die temperature (Table 5.5) and an unexpected increase in mean residence time (Figure 5.12).

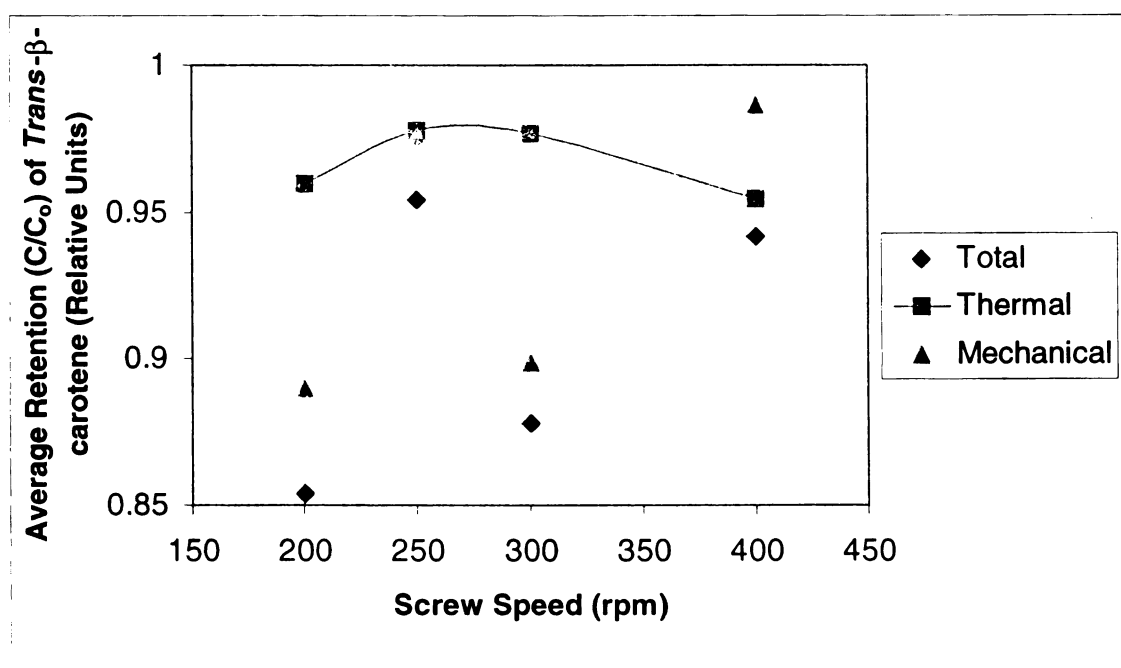


Figure 5.16. Average Retention of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture vs. Screw Speed at Low Temperature Profile on Day 2 [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

Statistical results showed that on day 2, an interactive effect between temperature and screw speed on R_ϕ was significant ($\alpha = 0.10$), with p -value = 0.0847 (Table 5.4). This implies that mechanical effects on retention were different at both temperature profiles and a synergistic effect of temperature and mechanical effects existed.

Overall, although the trends of retention could not be replicated on different days, the range of retention values was repeatable (Table 5.6). At all conditions, mechanical effects dominated and thermal effects were minimal.

Table 5.6. Retention Ranges for Total (R_T) and Mechanical (R_ϕ) Effects at Each Temperature Replicate

Condition	Retention Ranges	
	R_T	R_ϕ
High, Day 1	0.80 – 0.89	0.81 – 0.90
Low, Day 1	0.67 – 0.95	0.69 – 0.97
High, Day 2	0.58 – 0.97	0.60 – 1.00
Low, Day 2	0.82 – 0.96	0.86 – 1.00

The range of measured total (R_T) retention at both temperature profiles was between 0.58 – 0.97, giving an approximate range of 3 - 42% loss of *trans*- β -carotene. The total effects of extrusion on retention of *trans*- β -carotene were much larger than that of thermal effects, thus implying that either mechanical effects (Day 1 results, Table 5.4) or a synergistic effect (Day 2 results, Table 5.4) of temperature and mechanical inputs had the greatest impact on retention.

In comparison to other β -carotene/extrusion published results, the % *trans*- β -carotene retention in this research was much higher than what was found in other researchers work. Lee et al. (1978) found an approximate 70% degradation of β -carotene after extrusion of β -carotene and white corn flour. The vitamin A biopotency was lost due to a conversion of *trans*- β -carotene to stereoisomers with lower vitamin A biopotency. Guzman-Tello and Cheftel (1990) investigated the loss of *trans*- β -carotene

during extrusion of all-*trans*- β -carotene/wheat flour. They found that approximately 73% of *trans*- β -carotene was degraded at die temperature of 173°C.

The work by these researchers, however, cannot be directly compared to our research because of the particular source of β -carotene that was used during extrusion. Lee et al. (1978) used pure β -carotene and Guzman-Tello and Cheftel (1990) used pure all-*trans*- β -carotene, while the formulation used in this research was a food-grade preparation with numerous components that could have affected the retention of %*trans*- β -carotene.

The components in the formulation that potentially had the most significant protective effect on % *trans*- β -carotene were the antioxidants dl- α -tocopherol and ascorbyl palmitate. Guzman-Tello and Cheftel (1990) found that the effect of an antioxidant butylated hydroxy toluene (BHT) on the loss of all-*trans*- β -carotene contained in flour mixes during extrusion decreased the rate constant.

5.3.3.2. Effect of Shear History

Retention results discussed in the previous section were also investigated to determine the effect of shear history. Results are grouped in a similar manner.

Typically, because shear history is also a function of screw speed, increases in shear history would also mean an increase in shear effects on the product. If the compound is sensitive to both the shear rate level and the time of shearing, then there would be a decrease in retention with an increase in shear history.

On day 1 of extrusion at the high temperature profile, in Figure 5.17, an increase in shear history did not appear to have an effect on the extent of degradation. Shear histories at this condition ranged from 13,900 – 29,300 (dimensionless). Statistical

evaluation of the effect of shear history on retention of mechanical retention of *trans*- β -carotene gave a p-value of 0.3041 (Table 5.4), which indicates that there was no trend of retention with shear history.

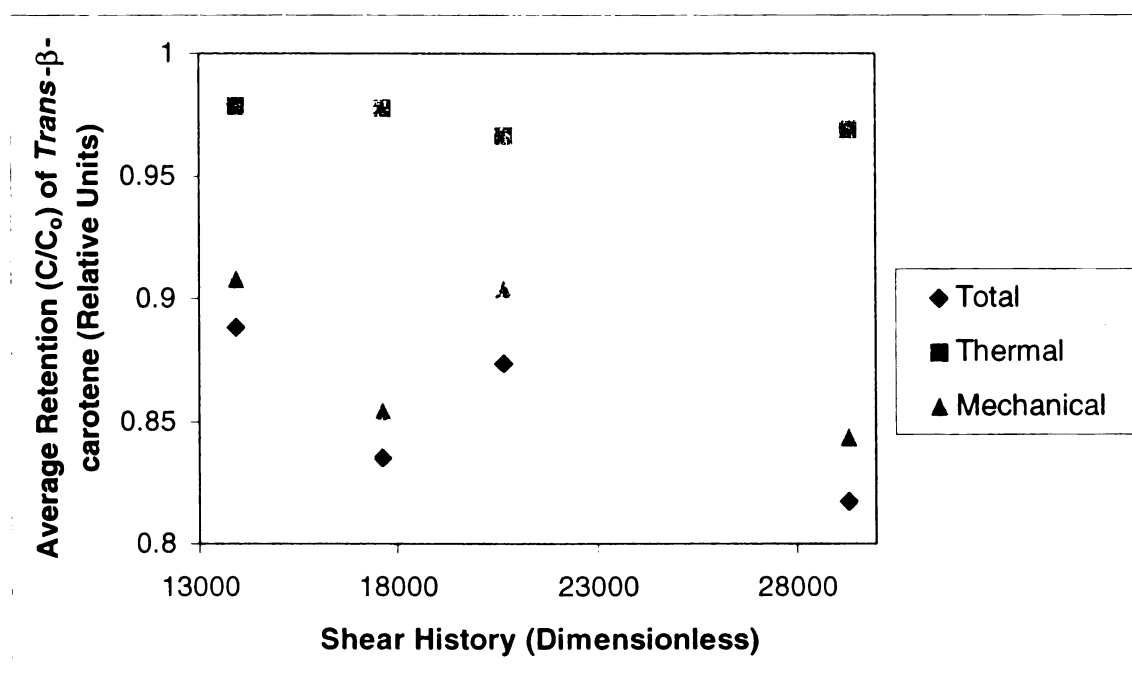


Figure 5.17. Average Retention of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture vs. Shear History at High Temperature Profile on Day 1 [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

At the low temperature profile on day 1 (Figure 5.18), shear histories ranged from 18,900 – 27,000 (dimensionless) and did have a significant effect ($\alpha = 0.10$) on mechanical retention of *trans*- β -carotene. Statistical evaluation of this relationship gave a p-value of 0.0538 (Table 5.4), slope = -1.7×10^{-5} , intercept = 1.25, and $R^2 = 0.4882$, indicating that there was more significance than at the higher temperature profile (Figure 5.17).

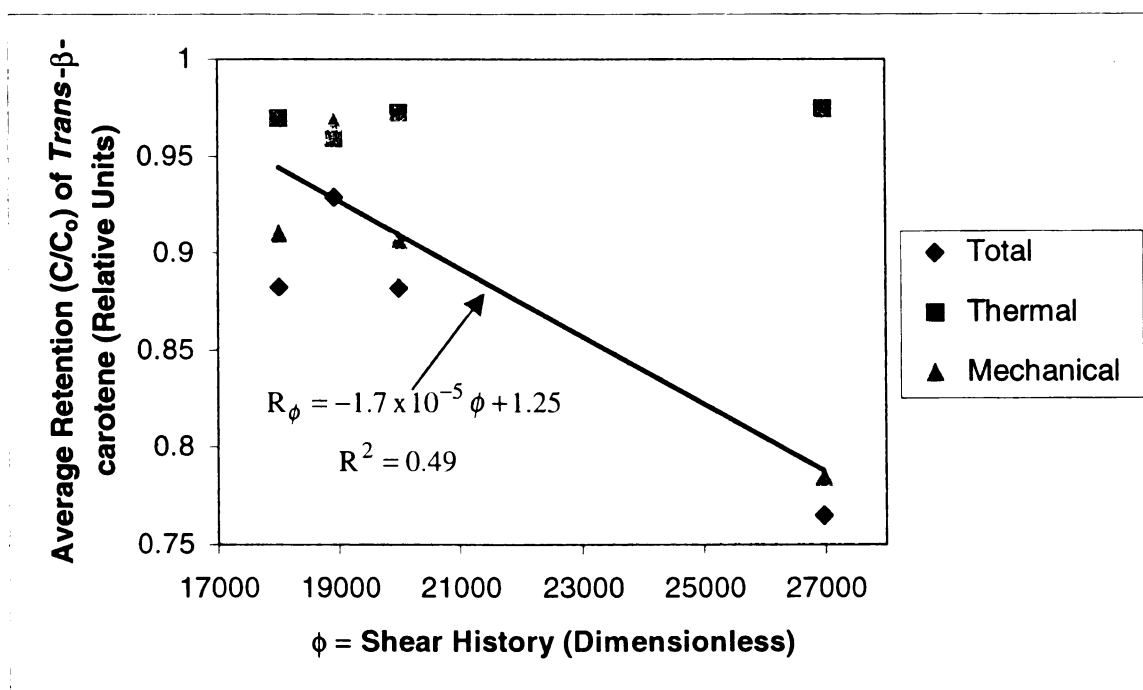


Figure 5.18. Average Retention of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture vs. Shear History at Low Temperature Profile on Day 1 [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

Statistical evaluation of the interaction between temperature and shear history on day 1 gave a p-value of 0.0742 (Table 5.4), indicating significance at $\alpha = 0.10$. The

interaction between temperature and shear history was more significant (p-value = 0.0742) than the interaction of temperature and screw speed on day 2 samples (p-value = 0.0847, Table 5.4). Therefore, some synergistic effect of temperature and shear history did exist on day 1.

High temperature extrusion on day 2, shown in Figure 5.19, gave similar results to day 1 samples. Retention of *trans*- β -carotene did not follow a trend with shear history, which ranged from 12,500 to 28,900 [p-value = 0.2538 (Table 5.4) at $\alpha = 0.10$].

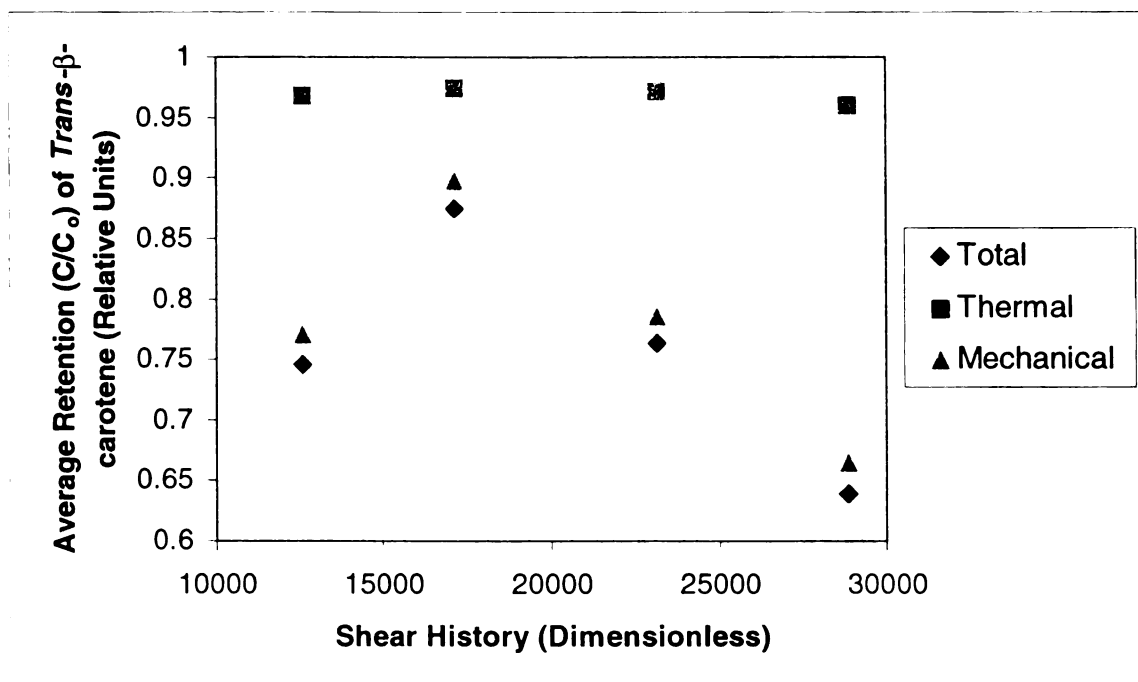


Figure 5.19. Average Retention of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture vs. Shear History at High Temperature Profile on Day 2 [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

Low temperature samples on day 2 (Figure 5.20) also gave similar results to previous samples, even though shear histories covered a larger range from 19,000 – 34,800 (dimensionless). The p-value for this condition was 0.4050 (Table 5.4) at $\alpha = 0.10$ and was much larger than that of low temperature extrusion on day 1 (Figure 5.18).

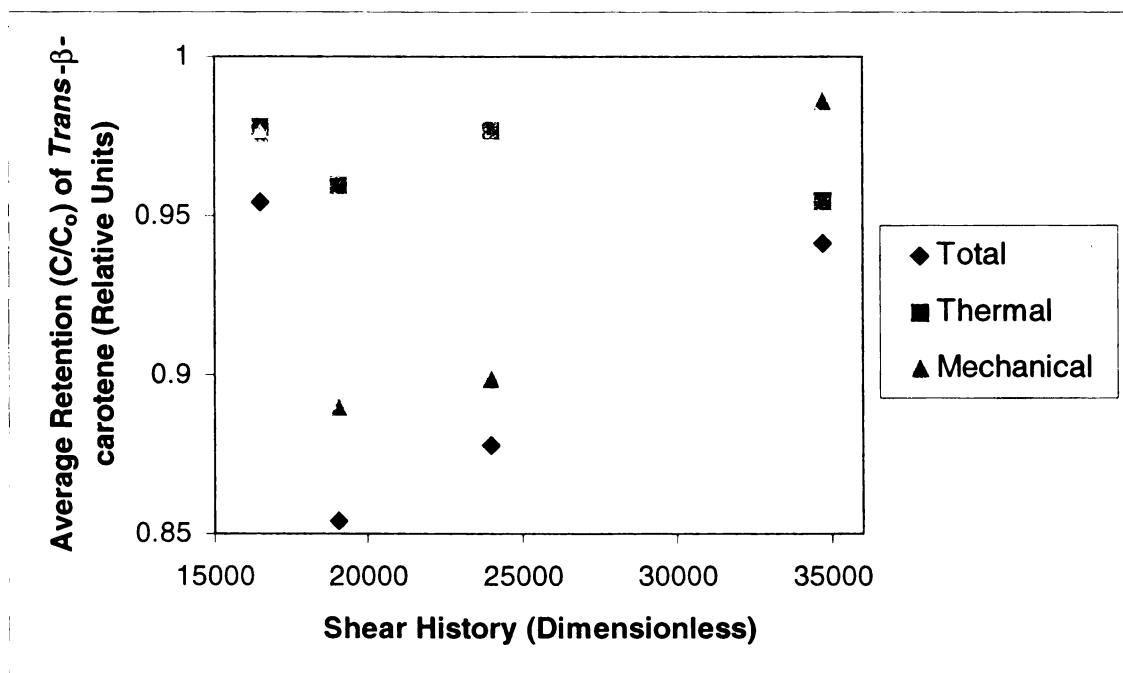


Figure 5.20. Average Retention of *Trans*-β-carotene in Food-Grade β-carotene/Flour Mixture vs. Shear History at Low Temperature Profile on Day 2 [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

An interactive effect of shear history and temperature on day 2 also was not statistically significant with p-value = 0.1439 (Table 5.4).

Overall, results from this comparison showed that there was a statistically significant trend of retention due to mechanical effects with of shear history only on day

1 at the low temperature profile. This was not the case with screw speed, which was significant at both temperature profiles on day 1. The fact that retention (R_ϕ) followed trends with screw speed more strongly than with shear history indicates that there may be a threshold value of screw speed (shear rate), above which the shearing residence time is not influential. That is, for the conditions tested, the level of shear rate, and not time of shearing, was the predominant factor causing *trans*- β -carotene degradation.

5.3.3.3. Effect of Specific Mechanical Energy

Similar to screw speed and shear history, the effect of specific mechanical energy on retention of *trans*- β -carotene was evaluated at both temperature profiles over both days. SME has three major influences: shear rate (screw speed), torque, and mass flow rate of dough. Since SME is not a function of mean residence time, its effect on R_ϕ is difficult to predict.

On day 1 of extrusion at the high temperature profile, in Figure 5.21, no apparent trend was found. SME values ranged from 250 – 375 kJ/kg and showed an overall increase with screw speed (mass flow rate). Statistical evaluation of the effect of SME on R_ϕ resulted in a p-value = 0.1147, which was not significant at $\alpha = 0.10$ (Table 5.4).

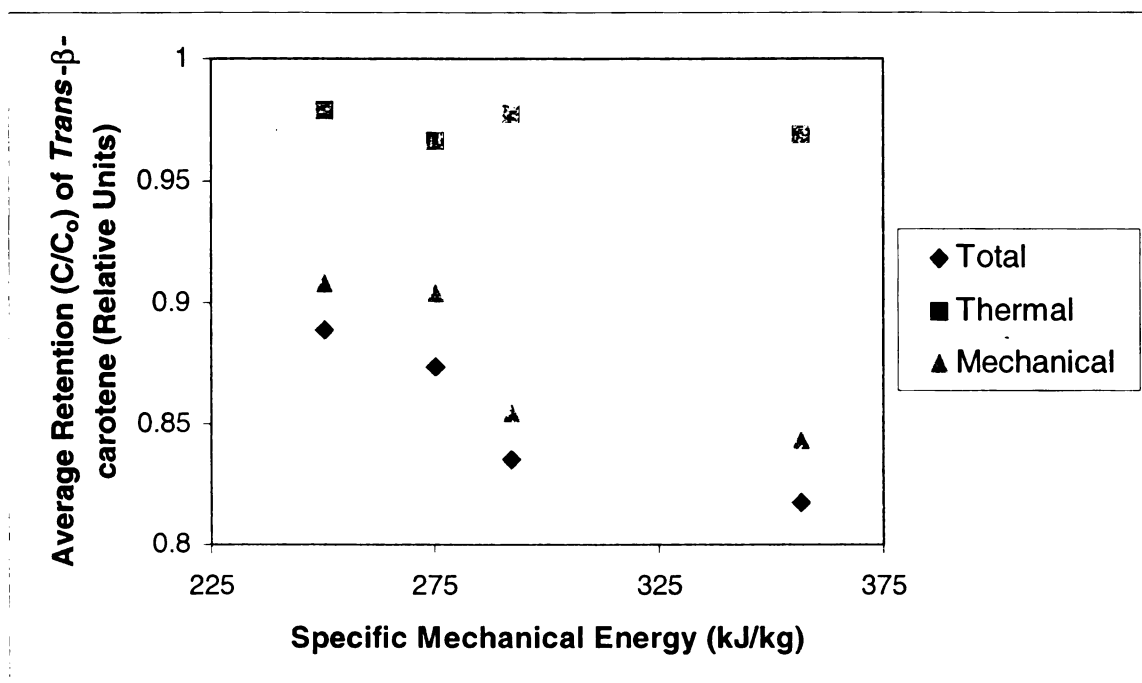


Figure 5.21. Average Retention of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture vs. Specific Mechanical Energy at High Temperature Profile on Day 1 [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

At the lower temperature profile on day 1 (Figure 5.22), the p-value for this interaction was 0.2585 (Table 5.4) at $\alpha = 0.10$, showing that R_ϕ did not follow a significant trend with SME.

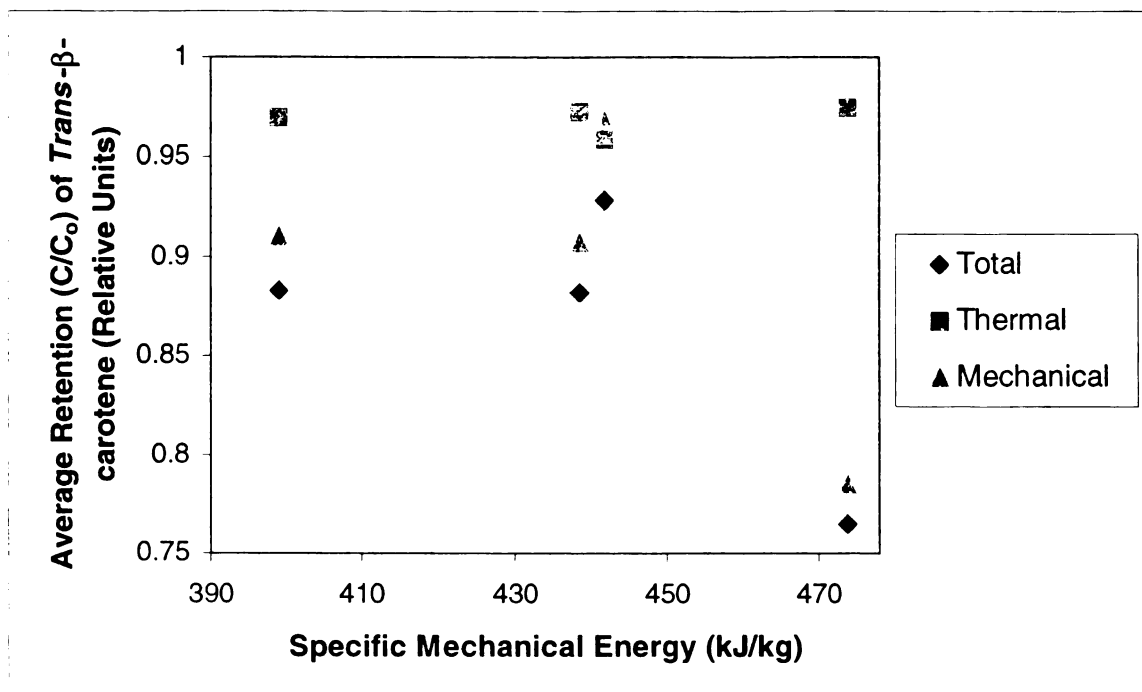


Figure 5.22. Average Retention of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture vs. Specific Mechanical Energy at Low Temperature Profile on Day 1 [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

Statistical evaluation of the effect of interaction between temperature and SME on R_ϕ on day 1 gave a p-value of 0.4687 (Table 5.4) at $\alpha = 0.10$, which shows there was no interaction at all. Therefore, there was no synergistic effect of temperature and SME on R_ϕ on day 1.

On day 2, high temperature extrusion (Figure 5.23) gave similar results to day 1. However, the p-value (Table 5.4) was much higher (p-value = 0.7557) at $\alpha = 0.10$, showing that there was no trend of R_ϕ with SME.

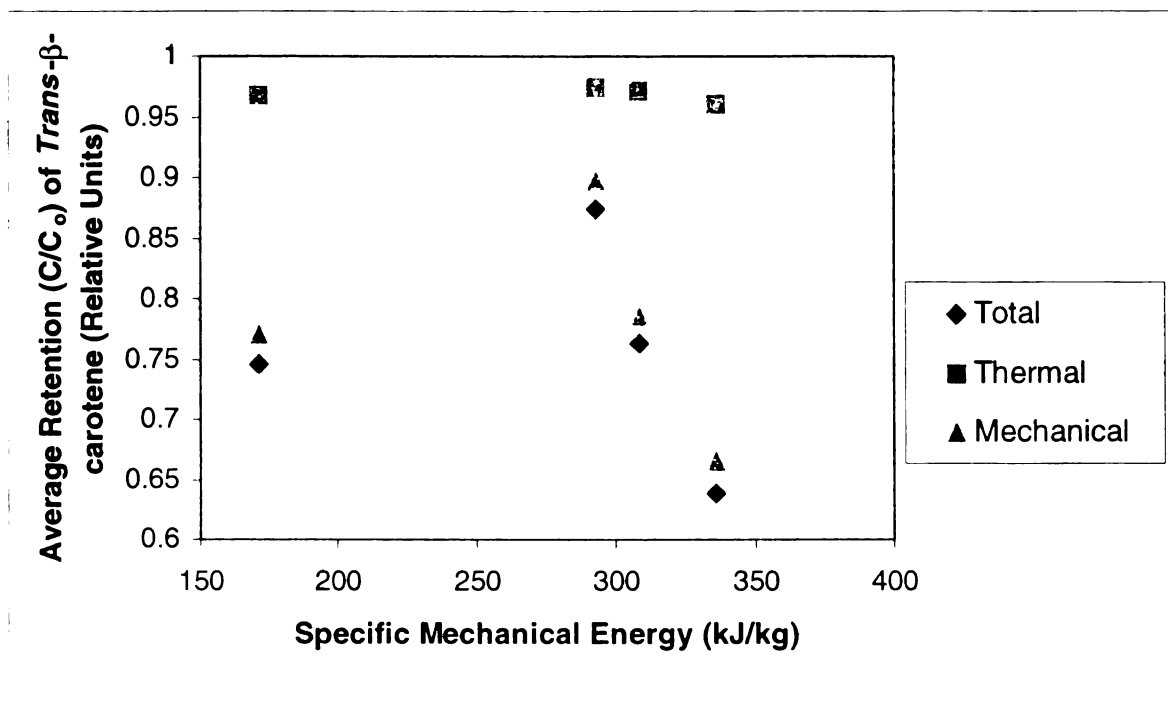


Figure 5.23. Average Retention of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture vs. Specific Mechanical Energy at High Temperature Profile 2 on Day 2 [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

Low temperature samples on day 2 (Figure 5.24) also gave similar results to previous samples. Similar to high temperature samples on that day, the effect of SME on R_ϕ was not significant with p-value = 0.2359 (Table 5.4) at $\alpha = 0.10$.

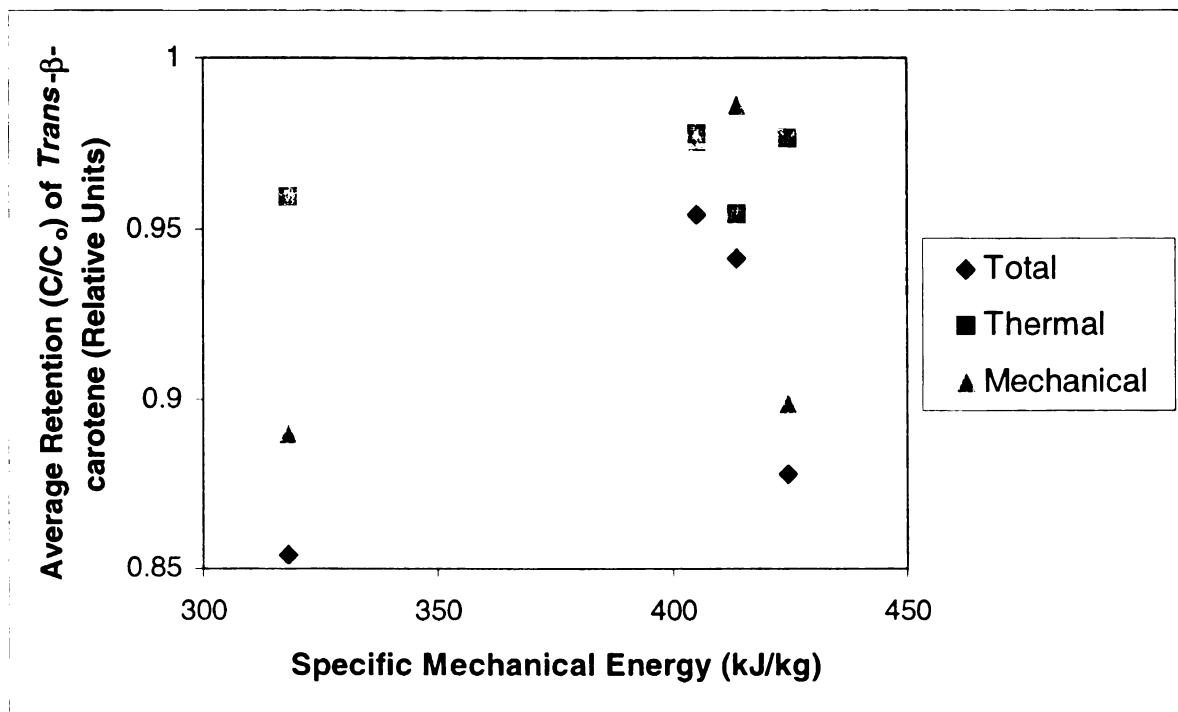


Figure 5.24. Average Retention of *Trans*-β-carotene in Food-Grade β-carotene/Flour Mixture vs. Specific Mechanical Energy at Low Temperature Profile on Day 2 [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

The effect of interaction between temperature and SME was also not significant on day 2, with a p-value of 0.4526 at $\alpha = 0.10$.

Overall, results from this comparison indicated that there was no statistically significant trend of retention due to mechanical effects (R_ϕ) with SME. These results indicate again, that the level of shear rate is more influential on *trans*-β-carotene degradation than total energy input.

5.3.4. Thermal and Mechanical Effects of Extrusion on Retention of *Trans*- β -carotene – Combined Days

The days of extrusion were separated in the previous sections; therefore, it was important to determine if combining days made a difference. In the following section, days were combined and analyzed statistically using the linear model to determine if there was a difference. Table 5.7 shows the p-values for high and low temperature samples for combined days using linear analysis. It is clear that all of the p-values are not significant. Therefore, combining days using the linear model did not give additional useful information.

Table 5.7. P-values for Retention Due to Mechanical Effects (R_ϕ) for Screw Speed, Shear History, and SME for Combined Days – Linear Model

Condition	P-value		
	Screw Speed	Shear History	SME
High	0.1549	0.2226	0.7916
Low	0.3105	0.8328	0.4851
Interaction of Temperature and Variable	0.5623	0.3902	0.7849

*Indicates significant effect at 90% Confidence ($\alpha = 0.10$)

Although the simple linear model did not work on the combined days of extrusion, using an exponential model did show significant trends with screw speed, shear history, and SME. Table 5.8 shows the p-values for high and low-temperature samples for combined days using an exponential model.

Table 5.8. P-values for Retention Due to Mechanical Effects (R_ϕ) for Screw Speed, Shear History, and SME for Combined Days – Exponential Model

Condition	P-value		
	Screw Speed	Shear History	SME
High	0.001	0.002	0.009
Low	0.025	0.077	0.041

*Indicates significant effect at 90% Confidence ($\alpha = 0.10$)

Each graph shows the average of two measurements and standard deviations for values of R_T are listed in Table 5.9.

Table 5.9. Standard Deviations of Measured Total Retention of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture At Each Extrusion Condition

Condition*	Standard Deviations			
	200 rpm	250 rpm	300 rpm	400 rpm
High	0.101	0.001	0.051	0.126
Low	0.053	0.050	0.003	0.125

*Conditions listed as: Temperature Profile (Combined Days)

5.3.4.1. Effect of Screw Speed

Combining both days of extrusion, at the high temperature profile (Figure 5.25), the effect of screw speed on retention due to mechanical effects using the exponential model was statistically significant ($\alpha = 0.10$) with p-value = 0.001 (Table 5.8), slope = 0.0007, and $R^2 = 0.4528$. Although the R^2 was low, the p-value showed a strong exponential effect.

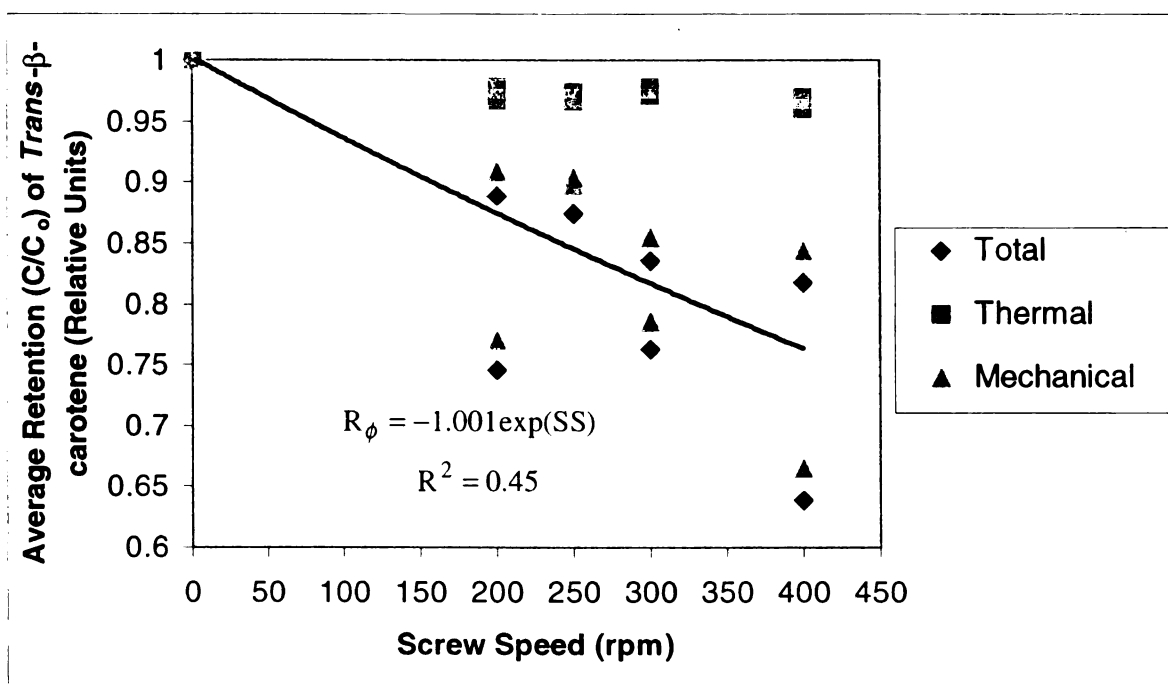


Figure 5.25. Average Retention of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture vs. Screw Speed at High Temperature Profile (Combined Days) [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

Combining both days of extrusion, at the low temperature profile (Figure 5.26), the effect of screw speed on retention due to mechanical effects using the exponential

model was statistically significant ($\alpha = 0.10$) with p-value = 0.025 (Table 5.8), slope = 0.0003, and $R^2 = 0.2493$. The data showed more scatter than the high temperature samples (Figure 5.25) and were not reproducible. Similar to the high temperature analysis, although the R^2 was low, the p-value showed that R_ϕ followed a strong exponential trend.

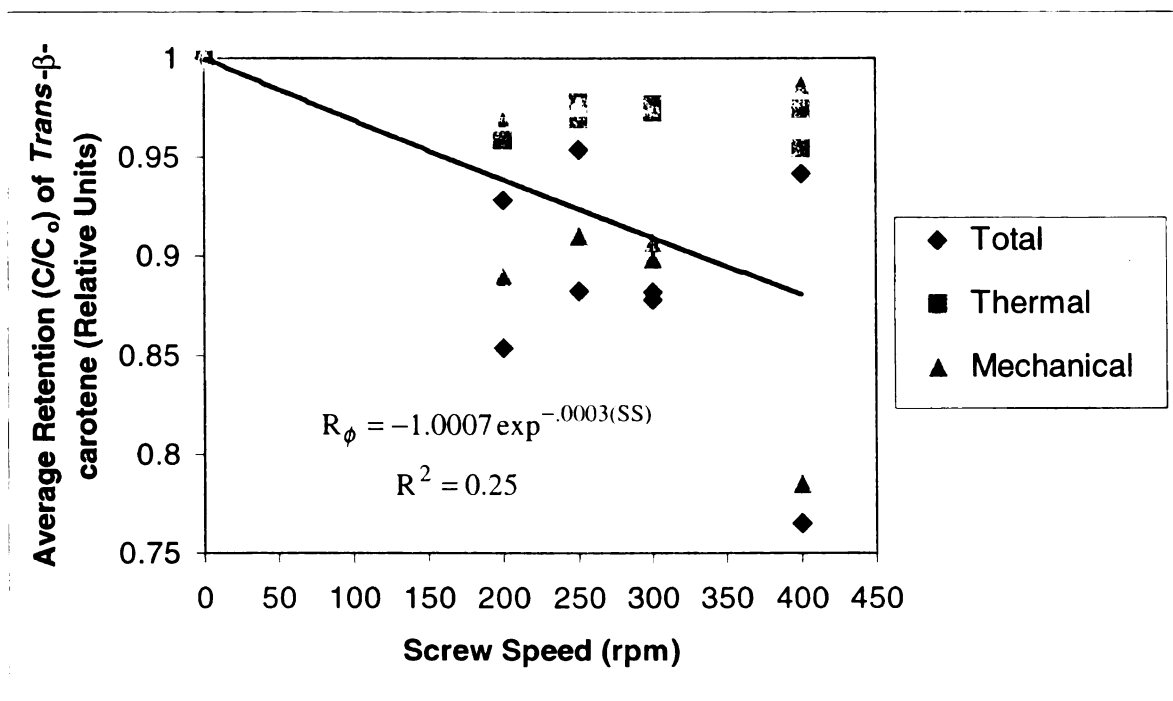


Figure 5.26. Average Retention of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture vs. Screw Speed at Low Temperature Profile (Combined Days) [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

Overall, both temperature profiles showed strong exponential trends with screw speeds, although the high temperature profile had a higher R^2 .

5.3.4.2. Effect of Shear History

Combining both days of extrusion, at the high temperature profile (Figure 5.27), the effect of shear history on retention due to mechanical effects using the exponential model was statistically significant ($\alpha = 0.10$) with p-value = 0.002 (Table 5.8), slope = 9×10^{-6} , and $R^2 = 0.4227$. Similar to screw speed at both temperature profiles, although the R^2 was low, the p-value showed that R_ϕ followed a strong exponential trend.

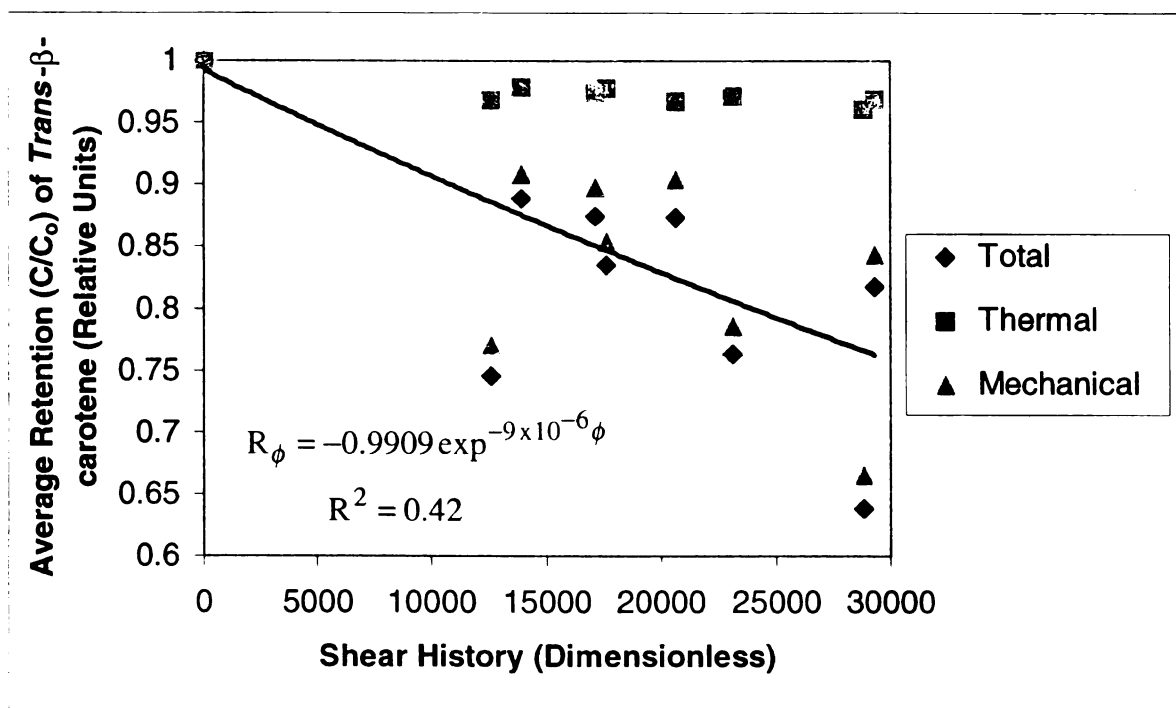


Figure 5.27. Average Retention of *Trans*-β-carotene in Food-Grade β-carotene/Flour Mixture vs. Shear History at High Temperature Profile (Combined Days) [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

At the low temperature profile (Figure 5.28), the effect of shear history on retention due to mechanical effects using the exponential model was statistically significant ($\alpha = 0.10$) with $p\text{-value} = 0.077$ (Table 5.8), $\text{slope} = 3 \times 10^{-6}$, and $R^2 = 0.1627$, when combining both days of extrusion. Again, the data were difficult to reproduce and showed high scatter. Similar to screw speed at both temperature profiles and shear history at the high temperature profile, although the R^2 was low, the $p\text{-value}$ showed that R_ϕ followed a strong exponential trend with shear history.

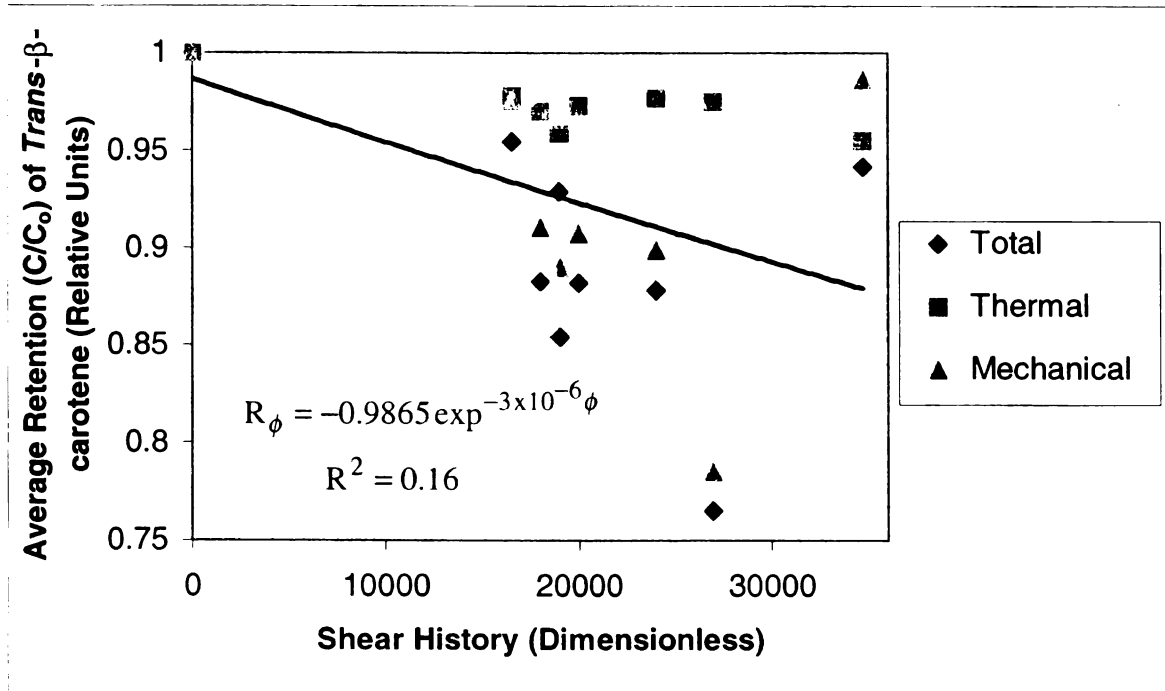


Figure 5.28. Average Retention of *Trans*-β-carotene in Food-Grade β-carotene/Flour Mixture vs. Shear History at Low Temperature Profile (Combined Days) [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

Similar to screw speed, when combining the two days of extrusion, R_ϕ followed a significant trend with shear history at both temperature profiles using the exponential model.

5.3.4.3. Effect of Specific Mechanical Energy

At the high temperature profile (Figure 5.29), retention due to mechanical effects followed a statistically significant exponential trend ($\alpha = 0.10$) with SME when combining both days of extrusion, with p-value = 0.009 (Table 5.8), slope = 0.0006, and $R^2 = 0.3223$. Similar to screw speed and shear history at both temperature profiles, at the high temperature profile, although the R^2 was low, the p-value showed that R_ϕ followed an exponential trend with SME.

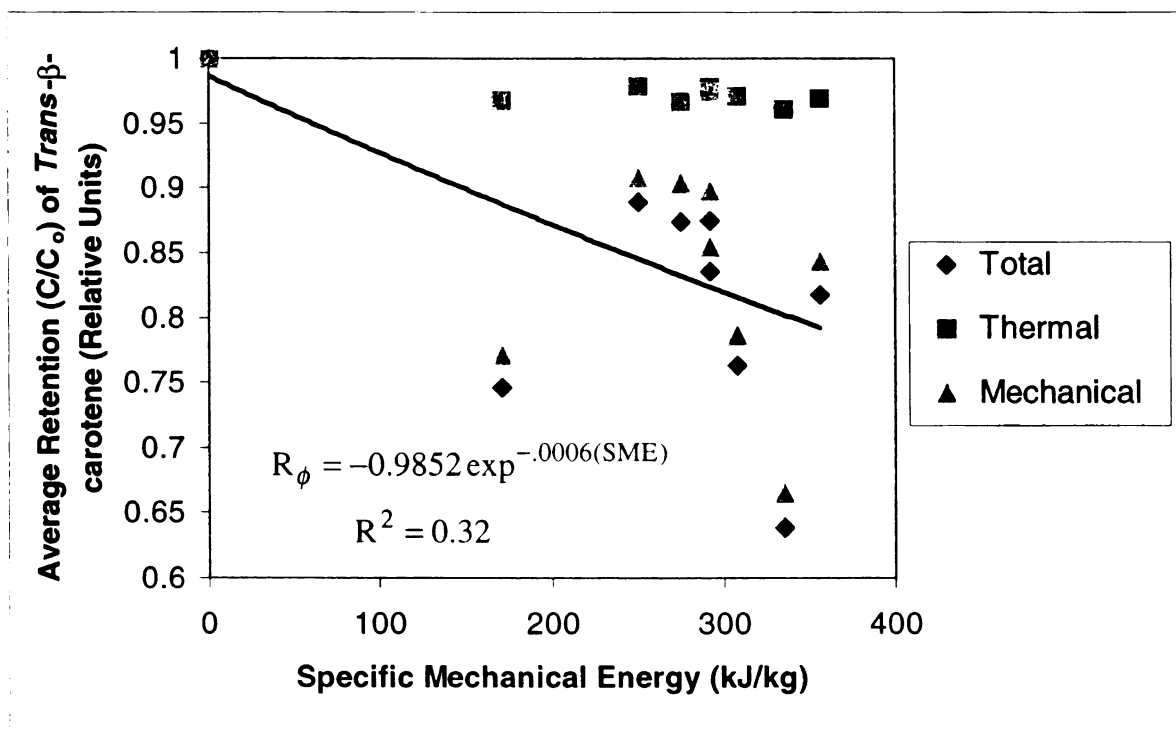


Figure 5.29. Average Retention of *Trans*-β-carotene in Food-Grade β-carotene/Flour Mixture vs. Specific Mechanical Energy at High Temperature Profile (Combined Days) [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

At the low temperature profile (Figure 5.30), retention due to mechanical effects followed a statistically significant exponential trend ($\alpha = 0.10$) with SME when combining both days of extrusion, with p-value = 0.041 (Table 5.8), slope = 0.0002, and $R^2 = 0.2121$. At the low temperature profile, although the R^2 was low, the p-value showed that R_ϕ followed a strong exponential trend with SME, which was what was found with screw speed and shear history at both temperature profiles and SME at the high temperature profile.

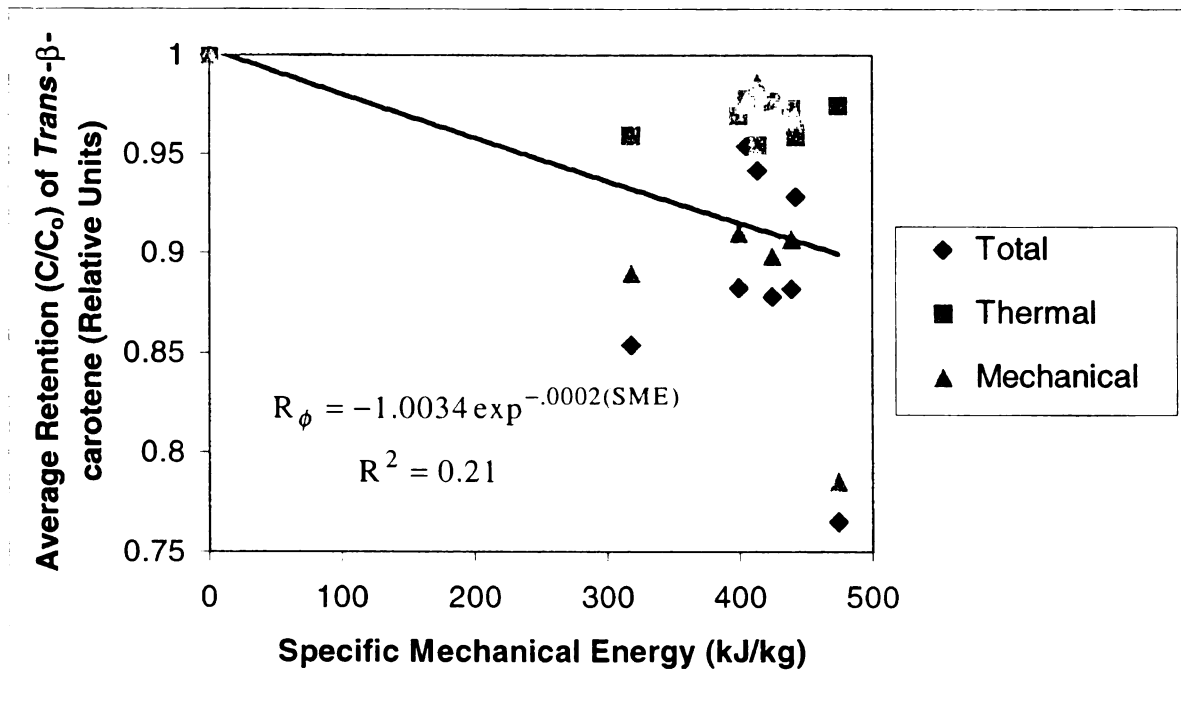


Figure 5.30. Average Retention of *Trans*-β-carotene in Food-Grade β-carotene/Flour Mixture vs. Specific Mechanical Energy at Low Temperature Profile (Combined Days) [“Total” is measured total retention (R_T), “Thermal” is calculated retention due to thermal effects (R_β), and “Mechanical” is calculated retention due to mechanical effects (R_ϕ)].

Overall, at both temperature profiles, when combining both days of extrusion, retention due to mechanical effects followed a significant trend with SME using the exponential model. Although a linear model did not work well when both days of extrusion were combined, the exponential model appeared to work better at the high temperature profile for screw speed and shear history and poorly at the low temperature profile for screw speed and shear history. The model worked poorly for the high and low temperature profiles for SME. Statistical evaluation showed that the R^2 values at the low temperature profile were somewhat lower than those at the high temperature profile.

5.3.5. Validity of Model

Although it was determined that a simple linear model worked well when separating different days of extrusion in this study, the linear model did not work when combining days. Therefore, an exponential model was applied and showed that R_ϕ followed a significant trend with screw speed, shear history, and SME at both temperature profiles. Our study, however, was limited because only 2 replicates were done at each condition.

Overall, the basic model of separating energy inputs worked reasonably well, although the thermal portion showed the most consistency. Further study should be done to determine the cause of inconsistencies, such as HPLC problems, storage issues, and raw material. The basic idea behind the model, however, can possibly be used by any food processor that is attempting to predict loss of a compound during the extrusion process.

5.3.6. Effect of Moisture on Retention of *Trans*- β -carotene

The effect of moisture content on the retention of *trans*- β -carotene is shown in Figure 5.31. There appeared to be no significant effect of moisture content on retention, with a p-value of 0.495 at $\alpha = 0.10$. These results are true in the moisture content range that was used in this research (30-36%).

Although there may be significant effect of moisture on retention, we were unable to extrude at a sufficiently large range of moisture contents to measure such an effect. Extrusion outside of this range on our extruder was very difficult for several reasons: If the moisture content were set lower than the reference of 30%, product tended to burn on at the die; and at moisture contents higher than 36%, the product was very moist and would not puff..

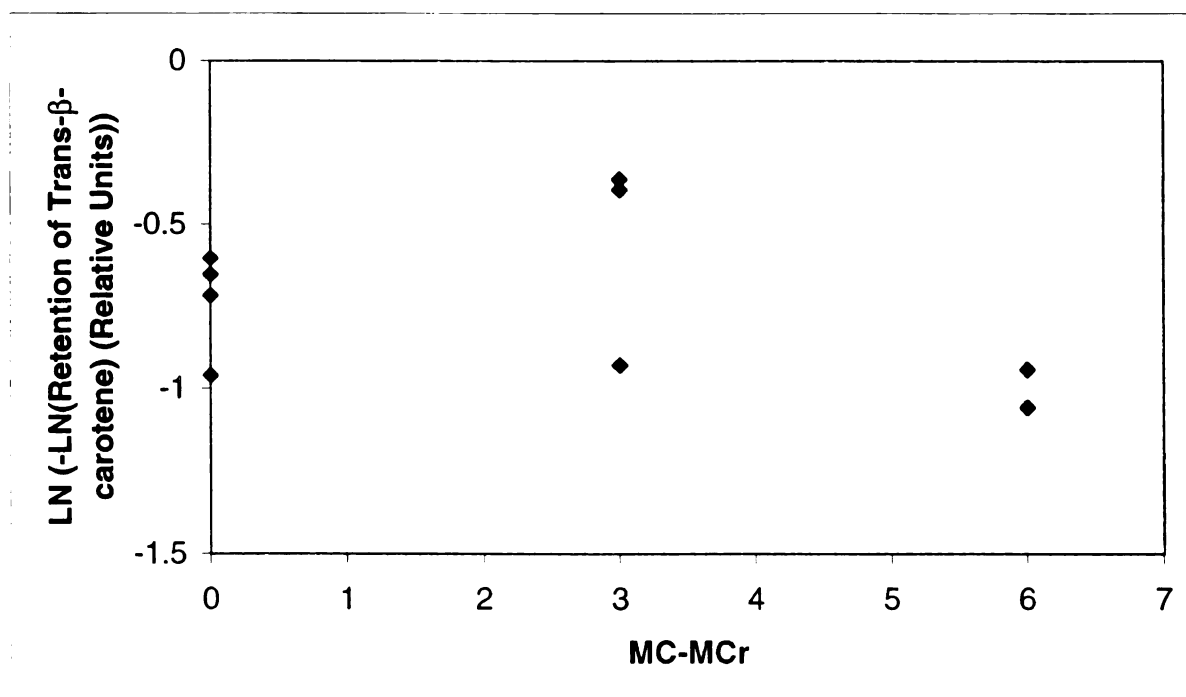


Figure 5.31. Double Log of Retention of *Trans*- β -carotene in Food-Grade β -carotene/Flour Mixture vs. Moisture Content (Reference moisture content was 30%; other moisture contents 33 and 36%)

5.4. Color

5.4.1. Effect of Heat and Moisture

Figure 5.32 shows the effect of moisture addition and heating on color, with the points at time = 0 as unheated β -carotene/flour mixture at 28%. Color values for raw β -carotene/flour mixtures at 14% moisture content were used as the control, and set equal to zero. There was an increase in color values (H) when moisture was added to raw flour and during heating. For both isothermal heating treatments at 78 and 138°C, color values increased slightly at 10 minutes, and remained fairly stable throughout the heating period. At 78°C, there was no significant effect of heating for 100 minutes (p-value = 0.88 at α = 0.10); however, at 138°C, a significant effect of heating for 60 minutes existed (p-value = 0.026 at α = 0.10). This indicates that a shorter time, higher temperature treatment (60 minutes at 138°C) had a greater influence on color values of food-grade β -carotene/flour mixes than did a longer time, low temperature treatment (120 minutes at 78°C).

For both heating treatments, color was influenced much more by moisture addition (moisture content increase from 14 to 28% caused a color change from 0 to 5.2,) than by heating (color ranged from 6.2 to 7.3 during heating, Figure 5.32).

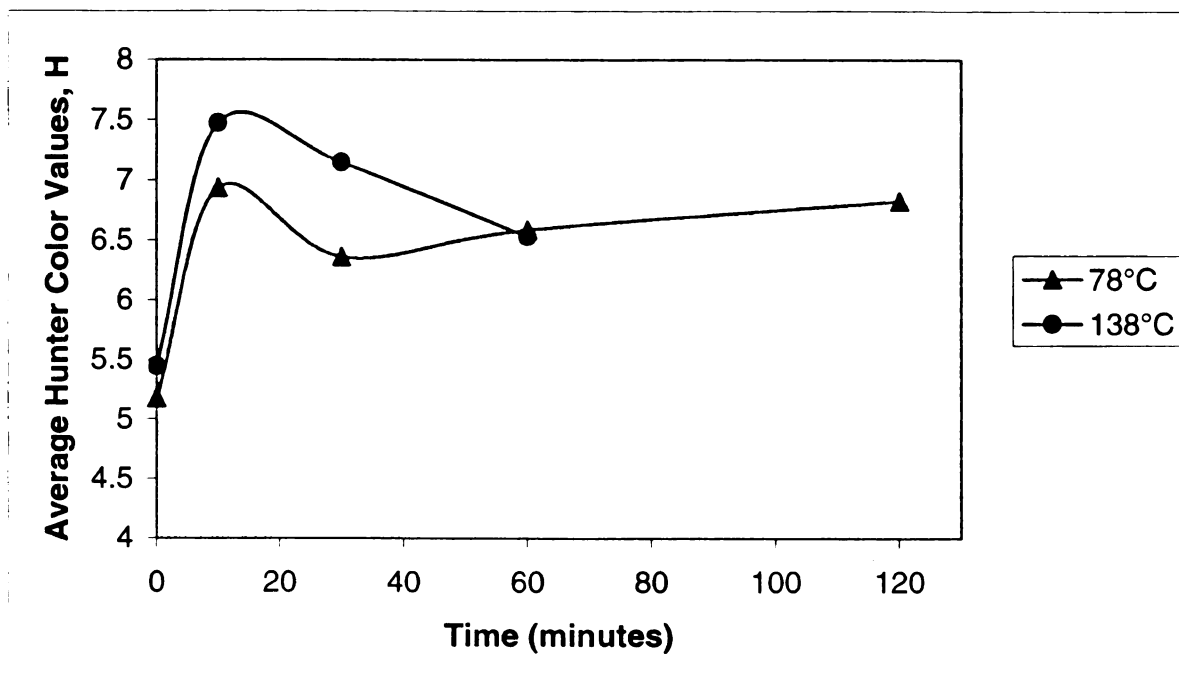


Figure 5.32. Average Color Values of Food-Grade β -carotene/Flour Mixture During Moisture Addition and Heating Where Color at 14% Moisture Content Was Set to Zero. (Moisture content decreased during heating from 28% at time 0 to 2.4% at 120 minutes at 145C. At 80C, moisture content decreased from 28% to 24%.)

Typically, it is thought that β -carotene products would lose color when heat-treated, due to a conversion from the *trans* isomer to certain *cis* isomers, or complete loss of β -carotene. When referring to color loss of β -carotene, many researchers evaluated color by determining the amount of β -carotene that was lost. This was usually done with spectrophotometry (Minguez-Mosquera and Jaren-Galen, 1994; Arya et al., 1979). This measurement was possible with natural or unprotected sources of β -carotene because degradation of β -carotene is directly related to color. The color of the carotenoid pigment is a function of the number of conjugated double bonds in the molecule; therefore, if the chain of double bonds is altered or cleaved, color is lost (Francis, 2000; Davies, 1976). The nature of our product, however, proved to be different. Although there was some color loss at 138°C after 60 minutes, it was not as large as the color

change found during moisture addition. This difference may have been due to the water dispersibility of the β -carotene formulation, which results in liberation of β -carotene from the matrix.

5.4.2. Effect of Extrusion on Color

Extrusion had a significant effect on color of β -carotene/flour mixtures. The extruder was dead-stopped at the high temperature profile and the barrel was opened. Figure 5.33 shows the visual color changes of the β -carotene/flour mixture as it proceeded through the extruder barrel. As the product entered the barrel, it maintained its pale pink color, however, as soon as water was injected into the product, the color changed significantly into a deep yellow color. As the mixture proceeded through the barrel, the yellow color appeared to deepen into a light orange color. As the product approached the die, the color maintained its light orange hue, however, as it exited the die, the color changed to a dark orange. Figure 5.34 shows a typical extrudate compared to a pre-extrusion β -carotene/flour mixture.

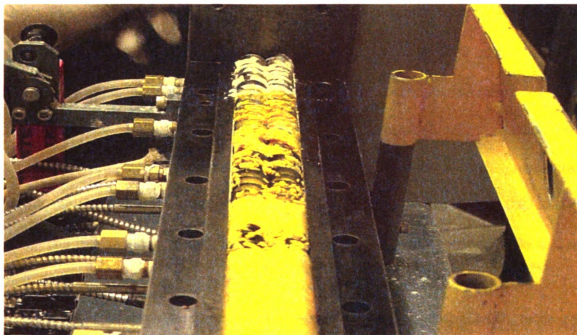


Figure 5.33. Open Extruder Barrel Displaying Color Changes of Food-Grade β -carotene/Flour Mixture Throughout the Extrusion Process



Figure 5.34. Comparison of Color for Pre-Extruded Food-Grade β -carotene/Flour Mixture vs. Extruded Product

Figure 5.35. shows results similar to what was found with β -carotene/flour mixtures that were heated. The first point shown is β -carotene/flour mixture at 28% moisture. β -carotene/ flour mixtures at 14% were used as the control and subtracted from subsequent color values. From the beginning of extrusion, color values increased and remained at similar values with increases in screw speed. At the high temperature profile, the effect of screw speed on color was not significant, with p -value = 0.23 at α = 0.10. The low temperature samples showed similar results, with p -value = 0.26 at α = 0.10. This was due to the short amount of time that was spent at high temperatures.

Overall, at both temperature profiles, color was influenced much more by moisture addition (moisture content from 14% to 28% in the barrel). Similar to the samples in Figure 5.32 that were heated, as moisture was increased, color values increased from 0 to 5.

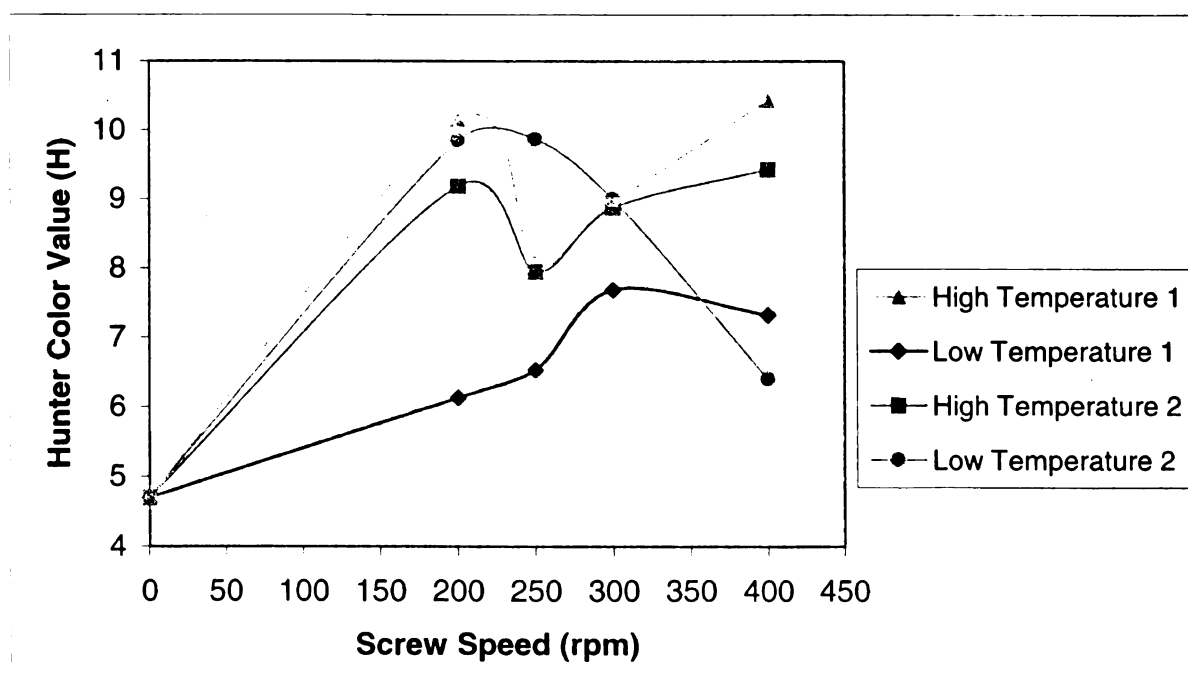


Figure 5.35. Average Color Values of Food-Grade β -carotene/Flour Mixture During Extrusion Where Color at 14% Moisture Content Was Set to Zero. (Moisture content decreased during heating from 28% at time 0 to 11% after extrusion)

Our results show an increase in color with extrusion, which is not a typical occurrence when β -carotene is processed. Similar to the case with thermal studies involving β -carotene, most extrusion studies that have investigated color of β -carotene during extrusion have approached it on the basis of β -carotene concentration changes because of the relationship between most sources of β -carotene and color. Guzman-Tello and Cheftel (1990) found there was an approximate 15 – 39% loss of β -carotene color in wheat flour mixes after extrusion. The main difference is, again, the form of β -carotene used during the process and the influence of moisture during the extrusion process.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1. Summary and Conclusions

During extrusion, thermal and mechanical effects are confounded at high-temperature, high-shear conditions. Without conducting separate experiments outside of extrusion, it is difficult, if not impossible, to quantify the proportions of nutrient degradation due to thermal and mechanical effects, respectively. Previous researchers investigated β -carotene retention during extrusion. However, most of the studies used pure all *trans*- β -carotene, which is too expensive and impractical to use, and is more heat-labile than food-grade β -carotene, which is used commercially. Also, these studies did not separate thermal and mechanical effects. Therefore, we conducted experiments to quantify the separate thermal and mechanical effects on food-grade *trans*- β -carotene during extrusion.

Thermal stability of *trans*- β -carotene was investigated in a shearless environment. Two sets of isothermal experiments were conducted at 78 and 138°C. Heating food-grade β -carotene/flour mixes at 78°C did not show significant degradation of *trans*- β -carotene for up to 120 minutes. During near-isothermal heating at 138°C up to 60 minutes, the calculated first-order reaction rate constant was $6.83 \times 10^{-5} \text{ s}^{-1}$, which was very low compared to previous research. These results indicated that the food-grade matrix was the reason for superior thermal stability. The isothermal experiments were difficult to compare to heating during extrusion because of the time scale, which was much longer than the typical residence time in the extruder (up to 4 minutes). Also, due to the extended heating time at 138°C, we were unable to maintain constant moisture

content. Therefore, to minimize these two difficulties, nonisothermal experiments were conducted at high temperatures and short times.

Samples were heated nonisothermally from 25°C to 145°C in a 149°C oil bath. At this condition, moisture content decreased from 28 to 11.6% over approximately 8 minutes. Nonlinear regression estimates of $k_{100^{\circ}\text{C}}$ (rate constant at 100°C), ΔE (activation energy), and C_0 (initial concentration) were $3.56 \times 10^{-4} \text{ s}^{-1}$, 18.820 kJ/g·mol, and 86.73 (relative units), respectively, $R^2 = 0.836$. The value for activation energy for *trans*- β -carotene (in food-grade β -carotene) was much lower (about one-fifth) than what was found in literature for pure *trans*- β -carotene and other natural sources. This was probably due to the protective nature of the matrix that surrounds the β -carotene molecule.

The rate constants and activation energy from the nonisothermal experiments were used to calculate the *trans*- β -carotene retention due to thermal effects during extrusion. Retention due to mechanical effects was calculated by mathematically removing thermal effects from the measured retentions. Two sets of extrusion experiments were conducted at 110°C and 130°C die temperature, with replicates on separate days. For extrusion at the high temperature profile (130°C die temperature), measured retention of *trans*- β -carotene (R_T) ranged from 58 - 97% (3 - 42% loss) as screw speed increased from 200 to 400 rpm. For extrusion at the low temperature profile (110°C die temperature), measured retention of *trans*- β -carotene ranged from 67 - 95% (23 - 5% loss). Retention due to thermal effects was greater than 0.95 on both days. **In the range of measured retention values for both temperature profiles, thermal**

effects accounted for less than 5% of the loss, showing that mechanical effects were the predominant cause of *trans*- β -carotene degradation.

Different days of extrusion were analyzed separately using a linear model. During extrusion, *trans*- β -carotene concentration followed a significant linear trend (p-value = 0.0847 for high temperature; p-value = 0.0350 for low temperature) with screw speed on day 1 at both temperature profiles; however, day 2 samples at both temperatures did not show a significant trend. Contrary to what was expected, *trans*- β -carotene followed a stronger trend with screw speed than with shear history or specific mechanical energy (SME). **This result indicates that the primary cause of *trans*- β -carotene degradation was the level of shear rate and not the time of shearing or the mechanical energy input.**

Due to the difficulty in obtaining highly reproducible results from day to day, both linear and exponential models were used to determine significance of combining replicated days of extrusion. The linear model did not work well when combining days, however, the exponential model showed significance at both temperature profiles for all three measures of mechanical effect. The exponential model worked better at the high temperature profile.

Overall, food-grade β -carotene had good thermal stability. Retention of *trans*- β -carotene in this study was much higher than previous published research, which was mostly due to the different source of β -carotene.

An interactive effect between temperature and mechanical effects gave mixed results over both days of extrusion. For both screw speed and shear history, there was a

significant interaction on day 1, which could indicate a synergistic effect of temperature and mechanical effects. There was no interaction on day 2.

Using the mathematical model to separate energy inputs worked reasonably well, although the thermal portion was the most consistent. This information could be useful to a food processor when predicting the retention of a compound during extrusion processing.

Color of heated and extruded samples was analyzed to determine if a correlation between color and concentration of *trans*- β -carotene existed. There was an increase in color during both heating and extrusion; however, the majority of the increase was during moisture addition. At 138°C, heating did have a significant effect (p -value = 0.026) on color, however heating at 78°C did not. Also, during extrusion, there was no significant effect of screw speed on color at either temperature profile. Color increases during moisture addition were probably due to a release of β -carotene from the water-soluble matrix. Overall, the color could not be directly correlated to concentration of *trans*- β -carotene.

6.2. Recommendations for Future Research

The following areas of research are recommended for future work:

1. Investigate the compounds that may be formed during heating/extrusion that may contribute to the increases in color values.

2. Conduct heating experiments and extrusion experiments using pure all *trans*- β -carotene at identical conditions to directly compare to food-grade β -carotene. Also, this will help in determining exactly what effect the food-grade matrix has on degradation of *trans*- β -carotene.
3. Investigate the retention of *trans*- β -carotene at various moisture contents during extrusion. The concentration of food-grade β -carotene may have to be decreased to prevent clogging of the extruder at lower moisture contents.
4. Plot color vs. moisture content for unheated food-grade β -carotene/flour mixes, using different concentrations of β -carotene to determine what the trend is, e.g., is there a threshold moisture above which color rapidly changes, or does color change gradually.
5. Investigate the effect of extrusion on different products (e.g., corn meal, other flours) containing β -carotene.

APPENDICES

Appendix 1

Appendix 1: Standard Curve for Concentration of *Trans*- β -carotene (Sigma Standard) ($\mu\text{g/ml}$) vs. Peak Area

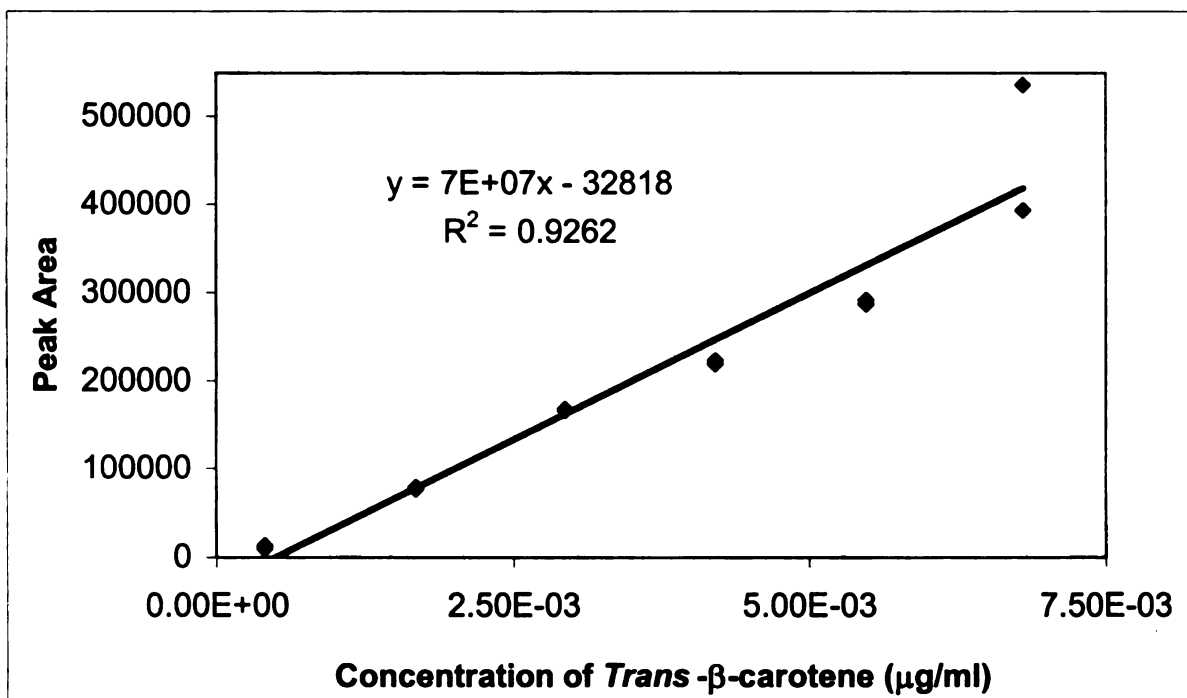


Figure A.1.1. Standard Curve for Concentration of *Trans*- β -carotene (Sigma Standard) ($\mu\text{g/ml}$) vs. Peak Area

Appendix 2

Appendix 2. Original (Unmodified) Extraction/HPLC Methods and Modifications

Original Roche Extraction Method

The original extraction method obtained from Roche Vitamins Inc. – Technical Marketing Analytics was created for spectrophotometric analysis, however, several modifications were made for HPLC analysis. The original Roche method consisted of the following:

Materials

3A alcohol (ethyl alcohol denatured with methanol)

Diethyl ether anhydrous, reagent grade

Petroleum ether (low boiling 35-60°C), reagent grade

Cyclohexane, certified ASC Spectroanalyzed

Protex, Bio-Cat, Inc. (alkaline protease R)

Iodine solution

Method

To prepare the iodine solution, dissolve 0.5 g of iodine crystals in 50 ml of 3A alcohol (solution 1). Pipet 1 ml of solution 1 into a 100 ml volumetric flask, dilute to volume with cyclohexane and mix well (solution 2). Pipet 5 ml of solution 2 into a 50 ml volumetric flask and dilute to volume with cyclohexane (iodine solution). This solution contains µg/ml iodine and is used in the isomerization step.

For extraction, weigh a 1.2 g sample and place in a 500 ml volumetric flask. Add 50 ml water and 60 drops of Protex the flask and place in a 65°C water bath for approximately 10 minutes, or until completely dispersed. Remove the solution from the water bath and allow to cool to room temperature. Add 150 ml of petroleum ether and 75

ml of diethyl ether to the solution and shake by hand. Add 75 ml of 3A alcohol and shake the flasks mechanically for 30 minutes. After shaking, remove the flasks and allow to settle. Pipet 10 ml of the upper layer into 50 ml volumetric flask and evaporate to dryness at room temperature under nitrogen. Immediately add 15 ml cyclohexane and 2 ml of the iodine solution. Place the flask in a 65°C water bath for 15 minutes, cool rapidly to room temperature, and dilute to volume with cyclohexane. Immediately measure the absorbance at 452 nm using a 1 cm cell against cyclohexane in the reference cell.

Modifications to Roche Method

Numerous changes were made to this method, although it was directly obtained from the company. Primarily, the method of analysis was changed from spectrophotometric analysis to HPLC. This was done by changing the reconstitution solvent from cyclohexane to 100% methyl-tert-butyl-ether.

The iodine addition step was completely eliminated from the extraction because its purpose was to isomerize any *cis* isomeris forms of β -carotene back to the *trans* form. This particular step was not necessary in our extraction because we were tracing the degradation of *trans*- β -carotene.

After running numerous extraction tests on raw material, it was discovered that there was a high degree of variability among samples; therefore, the sample size for extraction was increased from 1.2 to 6 g. After increasing the sample size, solvent volumes were also increased to compensate.

Original HPLC Method

The HPLC method used in this research was adapted from a method described by Lessin et al. (1997) and Emenheiser et al. (1996) with several modifications. The original method consisted of an isocratic separation using a mobile phase of 89% : 11% (v/v) methanol : MTBE. The column temperature was ambient laboratory temperature and column effluent was monitored using a UV-vis detector set at 410 nm, the isosbestic point for carotenoid isomers. (The isosbestic point is a wavelength or frequency at which the total absorbance of the sample does not change during a chemical reaction or physical change of the sample. Flow rate of solvent was 1 ml/min with an injection volume of 30 – 60 µl for samples that were dissolved in 50% : 50% (v/v) methanol : MTBE.

Modification of HPLC Method

Although the original method called for reconstitution of dried extract and standard preparations in 50:50 methanol:MTBE, we could not completely dissolve our samples/standard in the original solvent ratio. Therefore, the samples were dissolved in 100% MTBE, which is extremely non-polar.

The flow rate for the present work was also modified from 1 to 1.25 ml/min to allow for faster elution of the solute.

Extraction of Raw Flour to Determine Carotenoid Content

Wheat flour was extracted using the Roche extraction method to determine if there were any carotenoids present in the flour that may interfere with values of *trans*-β-carotene. The extract was injected into HPLC and the results obtained did not have any significant peaks that could change the values of *trans*-β-carotene.

Study to Determine Raw Material (Food-Grade β -carotene/Flour Mixture) Variability

The following study was conducted over a two-week period, using 3 separate flour mixtures to determine mixing and raw material variability

Table A.2.1. Values for Variability of Food-Grade β -carotene/Flour Mixture

Concentration of <i>Trans</i>-β-carotene (Relative Units) %	*Concentration of <i>Trans</i>-β-carotene (Relative Units) (Dry Basis)
47.92	55.9
47.87	55.8
50.40	58.8
49.78	58.1
58.16	67.8
52.73	67.5
55.79	65.1
55.53	64.8
56.24	65.6
49.05	57.2
44.44	51.8
44.84	52.3
48.21	56.2
49.12	57.3

*Average = 59.2

*Standard Deviation = 5.02

*CV = 8.5%

Appendix 3

Appendix 3: Data from Thermal Experiments

Table A.3.1. Values for Concentration of *Trans*- β -carotene (Relative Units), Moisture Content, and Color Values for Isothermal (78°C), Near-Isothermal (138°C) and Nonisothermal (149°C) Experiments of Food-Grade β -carotene/Flour Mixture

Temperature (°C)	Time (minutes)	Concentration of <i>Trans</i> - β -carotene (Relative Units) %	Moisture Content %	Concentration of <i>Trans</i> - β -carotene (Dry Basis)	Color a*	Color b*	Color H
78	0*	60.09	15.21	70.87	-1.8	4.4	0
78	0*	59.28	15.07	69.80	-0.9	3.7	0
78	10	58.18	26.16	78.79	1.6	11.0	6.36
78	10	54.88	26.55	74.72	2.2	11.1	7.51
78	30	57.98	26.10	78.46	2.0	10.5	5.93
78	30	56.79	25.26	75.98	2.0	10.4	6.75
78	60	57.26	23.63	74.98	1.9	10.7	6.11
78	60	57.04	25.20	76.26	1.9	10.7	7.06
78	100	57.89	24.07	76.24	1.7	10.7	6.28
78	100	56.36	26.44	76.62	2.0	11.0	7.37
138	0*	54.72	14.85	64.26	-1.2	4	0
138	0*	51.84	14.31	60.50	-0.6	3.8	0
138	10	52.06	11.29	58.69	2.7	11.2	7.34
138	10	54.77	10.37	61.11	2.8	11.1	7.60
138	30	46.50	3.46	48.17	2.6	10.7	6.83
138	30	49.30	3.62	51.15	2.6	11	7.45
138	60	42.62	2.63	43.77	2.9	10.1	6.33
138	60	51.52	3.03	53.13	2.8	10.2	6.73
149	0*	62.22	15.41	73.55	-0.3	4.1	0
149	0*	56.50	15.09	66.54	-1.3	3.6	0
149	2	56.98	26.67	77.70	2.8	10.1	6.36
149	2	57.25	25.48	76.83	1.6	10.3	6.59
149	3	55.35	24.52	73.33	2.5	9.9	6.09
149	3	53.82	23.04	69.93	2.7	10.8	7.30
149	6	52.97	16.36	63.33	2.8	9.9	6.17
149	6	52.79	13.43	60.98	2.0	11.2	7.54
149	8	53.95	11.16	60.73	2.4	10.3	6.46
149	8	53.25	12.56	60.90	2.1	10.9	7.27
Ambient**	—	—	—	—	1.7	9.4	4.80
Ambient**	—	—	—	—	2.2	9.1	5.55

*Zero-time sample were measured at 15% moisture content

**Values were not evaluated for *trans*- β -carotene content, only color at 28% moisture content

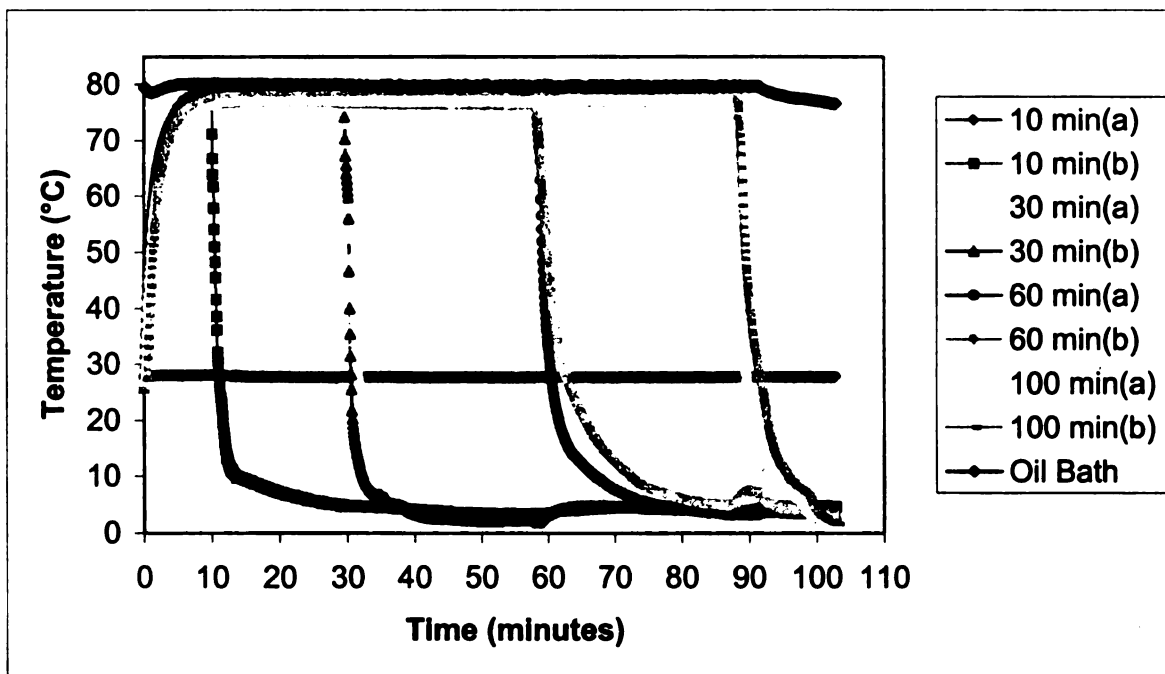


Figure A.3.1. Temperature Profiles for Samples Heated Isothermally at 78°C
(Samples correspond to those in Table A.3.1)

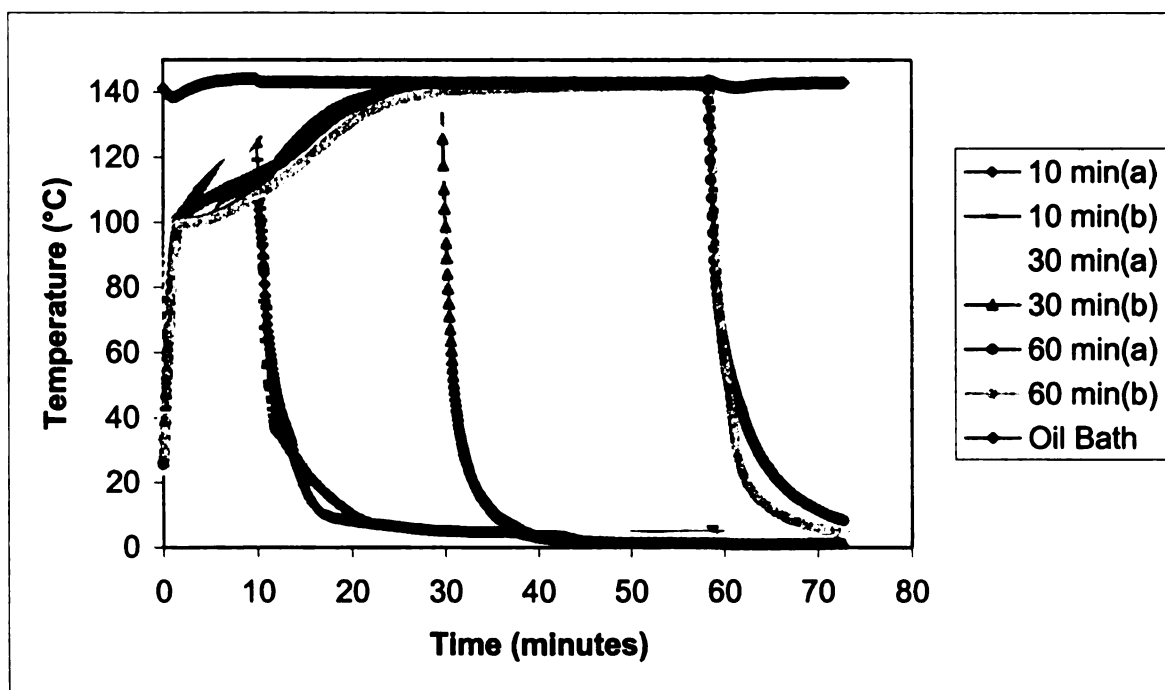


Figure A.3.2. Temperature Profiles for Samples Heated Near-Isothermally at 138°C
(Samples correspond to those in Table A.3.1)

Appendix 4

Appendix 4: Extrusion Data

Table A.4.1. Processing Conditions for High and Low Temperature Profile Extrusion on Replicate Days

% Moisture	Screw Speed (rpm)	Temperature Profile °C	Die Temperature °C	% Torque	Die Pressure
30	200	50/70/90/110/130	133	70	365
30	250	50/70/90/110/130	133	58	320
30	300	50/70/90/110/130	128	58	350
30	400	50/70/90/110/130	138	60	370
33	300	50/70/90/110/130	108	56	280
36	300	50/70/90/110/130	115	47	170
30	200	30/50/70/90/110	120	75	650
30	250	30/50/70/90/110	116	64	520
30	300	30/50/70/90/110	120	65	560
30	400	30/50/70/90/110	130	60	420
30*	200	50/70/90/110/130	128	53	290
30*	250	50/70/90/110/130	137	60	310
30*	300	50/70/90/110/130	142	60	410
30*	400	50/70/90/110/130	145	57	370
33*	300	50/70/90/110/130	131	38	140
36*	300	50/70/90/110/130	131	35	120
30*	200	30/50/70/90/110	119	57	370
30*	250	30/50/70/90/110	123	64	490
30*	300	30/50/70/90/110	128	63	520
30*	400	30/50/70/90/110	134	54	500

*Replicates

Table A.4.2. Specific Mechanical Energy, Shear History, % Fill, and Mean Residence Time Values for High and Low Temperature Profile Extrusion on Replicate Days

% Moisture	Screw Speed (rpm)	Temperature Profile °C	SME KJ/kg	Shear History	% Fill	Mean Residence Time (sec)
30	200	50/70/90/110/130	250.47	13909.48	96.60	69.37
30	250	50/70/90/110/130	275.17	20656.40	95.10	69.45
30	300	50/70/90/110/130	292.22	17615.40	80.89	67.68
30	400	50/70/90/110/130	356.59	29292.80	92.40	58.11
33	300	50/70/90/110/130	---	---	---	---
36	300	50/70/90/110/130	---	---	---	---
30	200	30/50/70/90/110	441.72	18914.25	87.95	102.46
30	250	30/50/70/90/110	399.01	17991.93	77.18	75.89
30	300	30/50/70/90/110	438.42	19973.82	75.28	77.23
30	400	30/50/70/90/110	473.78	26968.49	74.90	72.85
30*	200	50/70/90/110/130	171.25	12567.97	96.60	69.37
30*	250	50/70/90/110/130	292.55	17141.73	85.80	69.44
30*	300	50/70/90/110/130	308.25	23137.56	94.24	67.67
30*	400	50/70/90/110/130	335.67	28853.11	91.59	58.11
33*	300	50/70/90/110/130	---	---	---	---
36*	300	50/70/90/110/130	---	---	---	---
30*	200	30/50/70/90/110	318.18	19011.07	88.19	102.46
30*	250	30/50/70/90/110	404.91	16471.85	73.58	75.89
30*	300	30/50/70/90/110	424.55	23972.98	83.28	77.23
30*	400	30/50/70/90/110	413.49	34702.12	86.49	72.85

*Replicates

Figure A.4.3. Raw Data for Extrudates, Measured Total Retention, Calculated Retention Due to Thermal Effects, and Retention Due to Mechanical Effects for High and Low Temperature Profile Extrusion on Replicate Days

Temp. Profile °C	Screw Speed (rpm)	[<i>Trans</i> - β -carotene] (Relative Units)	Moisture Content %	[<i>Trans</i> - β -carotene] (Relative Units) (Dry Basis)	Measured Total Retention (R_T), C/C ₀	Calculated Retention Due to Thermal Effects (R_θ)	Retention Due to Mechanical Effects (R_μ)
HT, Day1	0	57.49	14.98	67.61	1	---	---
HT, Day1	0	57.49	14.98	67.61	1	---	---
HT, Day1	200	55.24	13.12	63.58	0.8886	0.9788	0.9078
HT, Day1	250	50.24	12.61	57.48	0.8501	0.9665	0.8795
HT, Day1	250	53.02	12.61	60.67	0.8972	0.9665	0.9282
HT, Day1	300	47.45	12.39	54.16	0.8009	0.9777	0.8191
HT, Day1	300	51.52	12.39	58.80	0.8696	0.9777	0.8894
HT, Day1	400	48.15	11.79	54.58	0.8072	0.9692	0.8328
HT, Day1	400	49.34	11.79	55.93	0.8271	0.9692	0.8534
LT, Day1	0	58.35	14.99	68.63	1	---	---
LT, Day1	0	57.50	14.99	67.63	1	---	---
LT, Day1	200	56.38	11.32	63.57	0.9262	0.9590	0.9657
LT, Day1	200	55.84	11.32	62.96	0.9369	0.9590	0.9706
LT, Day1	250	49.77	11.40	56.17	0.8183	0.9696	0.8439
LT, Day1	250	56.75	11.40	64.05	0.9469	0.9696	0.9765
LT, Day1	300	54.63	11.96	62.05	0.9040	0.9726	0.9294
LT, Day1	300	51.21	11.96	58.16	0.8599	0.9726	0.8841
LT, Day1	400	51.12	12.60	58.49	0.8521	0.9747	0.8742
LT, Day1	400	40.07	12.60	45.84	0.6778	0.9747	0.6953
HT, Day2	0	60.62	15.27	71.54	1	---	---
HT, Day2	0	59.81	15.27	70.58	1	---	---
HT, Day2	200	49.31	14.30	57.53	0.8347	0.9680	0.8623
HT, Day2	200	38.89	14.30	45.37	0.6564	0.9680	0.6781
HT, Day2	250	60.95	12.92	69.99	0.9783	0.9742	1.0004
HT, Day2	250	47.37	12.92	54.39	0.7706	0.9742	0.7911
HT, Day2	300	47.26	12.26	53.86	0.7528	0.9714	0.7751
HT, Day2	300	47.89	12.26	54.58	0.7732	0.9714	0.7959
HT, Day2	400	36.57	12.07	41.59	0.5813	0.9604	0.6052
HT, Day2	400	43.18	12.07	49.10	0.6956	0.9604	0.7242
LT, Day2	0	61.30	15.48	72.52	1	---	---
LT, Day2	0	55.13	14.94	64.81	1	---	---
LT, Day2	200	55.22	13.33	63.71	0.8784	0.9595	0.9154
LT, Day2	200	46.77	12.99	53.75	0.8293	0.9595	0.8642
LT, Day2	250	61.46	10.81	68.90	0.9501	0.9779	0.9715
LT, Day2	250	54.09	12.190	62.10	0.9582	0.9779	0.9798
LT, Day2	300	55.49	12.19	63.19	0.8713	0.9767	0.8920
LT, Day2	300	50.09	12.61	57.31	0.8843	0.9767	0.9053
LT, Day2	400	57.85	12.71	66.27	0.9137	0.9547	0.9571
LT, Day2	400	54.74	12.87	62.82	0.9693	0.9547	1.015

*HT = 50/70/90/110/130; LT = 30/50/70/90/110

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