NUTRIENT RETENTION WITH BHA AFTER STORAGE IN FOOD SYSTEMS HEAT-PROCESSED IN CANS AND POUCHES

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INTRODUCTION

For those who manufacture, transport, store, or use foods, the time or period during which processed foods will retain acceptable quality is important.

Due to a noticeable increase in consumer and governmental interest in the nutrient content of foods, processors have given special attention also. Among food scientists and food processors, this is producing a greater awareness of the extent which vitamins are lost during storage, the means by which they are lost, and the need to find ways to minimize these losses.

New and modified techniques used in the processing and preservation of foods are being developed in order to retain quality and extend the shelf life of the product from harvest to consumption.

Quality deterioration of a food during storage can be manifested in the form of nutritional degradation, changes in color and texture, development of off-flavor, and occasionally microbial development or insect infestation.

Factors which determine the storage life of processed foods may include the type of product, method of processing, packaging, temperature, humidity within and without the package, and exposure to light. There is a need for scientifically testing the close relationship between these different factors. Every food is different regarding its storage potential. Ideal storage conditions are not always possible.

Packaging materials, containers, and processing methods have been developed recently in order to obtain thermostabilized products of better quality. Foods thermally processed in plastic laminated pouches may permit higher retention of heat labile and water-soluble nutrients than foods processed in cans. Pouches are flat and rectangular in shape which favors rapid heat transfer, reducing heat penetration time to obtain commercial sterilization in the center of the pouch.

During processing, nutrients are reduced and/or destroyed due to sensitivity to pH, oxygen, light, heat, or a combination of these factors.

Some nutrients included as additives may have synergistic effects. But some components may have an antagonistic effect upon one another (e.g., effect of the anthocyanins on retention of vitamin C added to prune juice).

As far as temperature is concerned, there is some loss of bioavailability, if not in actual content of all nutrients during prolonged storage, and the rate of this loss is diminished with reduction of storage temperature.

LITERATURE REVIEW

The type of container used in processing and storing foods can make a difference in the overall chemical and physical attributes of the corresponding food.

Since pouches require reduced heat sterilization, it has been suggested that food processed in a pouch is equal in quality to frozen food: they are shelf stable and do not require refrigeration. However, vegetables, and probably other foods, regardless how they are processed and packaged, are not equal in quality to the frozen, with possible exception of "stew-type" foods (Kramer, 1979).

Thorne (1976) mentions some advantages of pouches over cans. He says that pouches are easier poured, adapt themselves to shape of food, the amount of liquid used is less in fruits and vegetables, and the processing time is reduced compared to cans of similar volume. In addition, the reduced time of heat processing is economical to the processor and less damaging to the food.

Cans of foods are generally sterilized in steam at about 120°C. Since flexible pouches are less rigid than metal cans they tend to burst when heated due to expansion of the food and the residual air within. To eliminate this disadvantage, pouches are heated in water at about 120°C with superimposed air pressure to prevent the water boiling and to keep the pouches flat in form. During processing, flexible containers must be separated to prevent close

contact between the numbers of pouches, otherwise, an effective large container can be produced with a danger of under-processing. For this reason, pouches are processed in racks to keep them apart. Another disadvantage is that can processing can be achieved very fast, and if pouches are to compete, great capital investment will have to be made (Thorne, 1976). Jimenez (1976) made a comparative study of the quality and nutritional adequacy of beef stew processed and stored in pouches and cans and stored for 6 and 12 months at 74°C. He found a better ascorbic acid retention in pouches than in cans in both 6 and 12 months storage. In addition, he reported a taste panel preference for the beef stew processed in pouches over the cans counterpart.

Abou-Fadel and Miller (1983) compared the color, texture, and retention of ascorbic acid after storage in pouched and canned green beans, as well as Royal Ann cherries. They found that after 4 months of storage in both at ambient temperature and at 38°C there was a higher retention of ascorbic acid in pouched green beans and pouched Royal Ann cherries than in cans.

Chen and George (1981) observed lower ascorbic acid values in pouched green beans than in canned, which they assume was due to over-processing of the pouched green beans. However, approximately 40% of the ascorbic acid in the canned product was leached into the brine while only 10% of the ascorbic acid in the pouched beans was found in the

brine. They also noticed that the loss was fast at the beginning of storage before reaching a steady state at which time only 20% of ascorbic acid was retained in pouches and 25% in cans. This agreed with Heidelbaugh and Karel's (1970) findings of a loss of 80% ascorbic acid in vegetables and bacon processed in metal cans and retort pouches after 5 weeks storage at 37^{0}C. But ascorbic acid retention was consistently higher in canned green beans than in pouches throughout 11 weeks of storage at room temperature.

Thorne (1976) found the ascorbic acid content for canned potato was 11mg, for canned liquor, 10 mg, and for flexible pouch potato, 19mg, per 100 gm wet weight basis.

All preserved foods tend to deteriorate with increasing time and temperature of storage including canned foods that have been heat sterilized (Kramer, 1979).

Cecil and Woodroof (1963) studied 35 different canned foods during storage of 4 to 7 years, at temperatures ranging from 37.8 to -28.9^{o}C. They found that there is a direct relationship of temperature to fading or browning of colors, softening or graining of texture, development of caramelized, stale or rancid flavor, loss of vitamins, and damage to containers.

Low temperature of storage of canned foods can be beneficial to nutrient retention in various ways. For instance, foods packed in less nutritive media such as water, sugar syrup, or starch gravies gradually suffer a leaching effect

of the nutrients from the solids into the liquid. This process can be retarded by low temperature storage (Gangal and Magar, 1967).

Guerrant et al., (1945) said that storage conditions used in many studies involve constant temperatures and, therefore is not exactly comparable to those found in a commercial warehouse storage where the temperature of storage usually varies with seasons and with night and day. They determined vitamin retention in canned foods stored under actual warehouse conditions. They found that both time and temperature of storage had an adverse effect on vitamin content and that the degree of effect depends on the particular food and on the specific vitamin.

Ascorbic acid and beta-carotene are two nutrients that are affected differently by time and temperature of storage. Both are unstable in air or oxygen, light and heat. Ascorbic acid has 100% maximum cooking losses, whereas beta-carotene has 30%. But both react differently to pH, at pH 7 ascorbic acid is unstable and beta-carotene is stable. At pHs less than 7 ascorbic acid is stable and beta-carotene is unstable; at pHs greater than 7 ascorbic acid is unstable and beta-carotene is stable (Lund, 1979).

Regarding the rate of deterioration of vitamins under all storage temperatures, there are two divided opinions. Salunkhe et al., (1979) say that vitamin losses occur relatively slowly during the first several weeks up to 6 months of

storage. Then losses continue at a faster rate during the storage period of 6 to 24 months, after which vitamin destruction continues at a slower rate. That is, further destruction of nutrients between the two and a half and five years from time of packing is continuous but usually almost negligible. These findings do not fit the prevailing notion that these changes are in accordance with a first order reaction.

According to Kramer (1979) foods deteriorate most rapidly immediately after harvest and/or processing. The deterioration rate slows with time; when this is true, the half-life for a product in storage can be calculated. For example, if beef stew in pouches stored at 70°F have a half-life of three months, its acceptable quality has been reduced to a point half way to where the product is no longer acceptable. Then, after three-months additional storage, the remaining shelf life is again reduced in half, and so on. Plotting storage time against residual shelf life results in a curve which drops sharply and then to goes down at a continuously slower rate. When the same plot is made on semi-log paper, the resultant line is linear, which would mean that the changes occurring are of a type called "first order reaction".

Salunkhe et al., (1979) measured the effects of long term storage of seven food items, including beef stew, on the quality of processed foods in pouches. They noted that the least stable of the seven pouch-packed products was beef stew, having a quality shelf life of 2, 6, and 12 months

when stored at 100, 70 and 40°F respectively. Practical storage life is 4, 24 and 60 months respectively at the same storage temperatures. Beef stew stored at 40°F for 54 months underwent a significant decrease in thiamin but not riboflavin or niacin. However, storage at 70°F significantly decreased all three vitamins. At all temperatures, percent loss of thiamin was higher than of riboflavin and niacin.

The amount of residual air in the container can be detrimental not only for cans but for pouches as well. Yamaguchi et al., (1977) found deterioration of the contents directly related to the amount of residual air within the pouch. However, 10-15 ml of air per pouch (170 x 130 mm) is desirable to prevent bursting of the pouch when it is sterilized at 120°C. The heating rate and lethality within the pouch also varies significantly depending on the amount of residual air.

Ascorbic acid may be very stable during storage in a sealed container, but rapid destruction may occur as the container is opened and contact is made with air (Bender, 1978).

Ascorbic acid is very stable in a strongly acid solution when oxygen is excluded. But, in a neutral or alkaline environment or in the presence of small traces of some metals, e.g. Cu and Fe, decomposition is greatly speeded up by oxygen (Heimann, 1980).

Cupric ions, even as low as 10^{-9} ppm, are effective prooxidants. Ascorbic acid oxidation depends on the conversion

of Cu^{2+} to Cu^{+} and reoxidation of the autoxidisable Cu^{+} by oxygen. The speed and amount of oxidation catalyzed by Cu increases with an increase in dissociation of ascorbic acid (Heimann, 1980).

Ascorbic acid is considered the most time and temperature sensitive nutrient in food (Kramer, 1979; Fennema, 1977; Guerrant et al., 1945; Salunkhe et al., 1979; and Moschette et al., 1975). Ascorbic acid loss in storage frequently parallels sensory quality loss, so ascorbic acid analysis is often used to indicate sensory quality (Kramer, 1977; Kramer, 1979).

The rate of ascorbic acid loss differs greatly with the food product. Slicing and increased surface exposure of the product is also very detrimental. Spinach, a rich source of ascorbic acid, loses 50% of its original content in about two days at 71°F (Fennema, 1977). Guerrant et al., (1945) found that ascorbic acid was affected more by temperature of storage than by time. Canned foods stored at 50°F showed better retention of vitamins than canned foods stored at higher temperatures. The adverse effect of each factor varied with different canned foods, eg., retention of ascorbic acid in lima beans decreased with time of storage but showed no appreciable difference attributable to the temperature.

Moschette et al., (1975) found that canned foods held at a constant temperature of 50°F for 12 months showed no significant losses of thiamin or carotene. They reported an

excellent retention of ascorbic acid in all canned fruits and fruit juice. These findings agree with those of Guerrant et al., (1945) for canned tomato juice, lima beans, and whole kernel corn. At 80°F significant losses of ascorbic acid in grapefruit juice after 4 months and in peaches after 8 months were found. In several foods held at 80°F, the average ascorbic acid decreased with time, and the rate of loss became disproportionally higher at higher temperature.

The absolute amount of water in the food and the physicochemical state in which water exists are important variables which regulate quality of food in storage. The relationship between the loss of food quality and the moisture content is best represented by the term "water activity". Lee and Labuza (1975) found that the loss rate of ascorbic acid increases as water activity increase. Activation for the destruction of ascorbic acid also increases as moisture content and water activity increases.

Beta-carotene in food systems has been broadly researched. Vegetables do not contain vitamin A as such but provitamin pigments called carotenes. These pigments are converted to vitamin A in the wall of the small intestine during absorption, therefore many vegetables have considerable vitamin A activity. There are several carotenes, but the most important is beta-carotene, also called pro-vitamin A. Beta-carotene is a red solid and was first isolated from carrots.

The chemical structure of beta-carotene is made up of a series of double bonds which make it highly suseptible to oxidation. The purified and synthetic compounds are very unstable but in foods they dissolve in fats with natural antioxidants and are more stable (Bender, 1978).

Substantial evidence has been gathered indicating that a loss of color in foods with high carotene content can be inhibited by using food-grade antioxidants. A type of discoloration frequently found in carbohydrate-containing foods is the loss of color due to oxidation of natural pigments such as carotenoids. These pigments suffer oxidation by free-radical type reactions (Stuckey, 1968).

Guerrant et al., (1945) found that carotene is not seriously affected by storage conditions; and that carotene retentions were considerably higher than ascorbic acid. The adverse effect of storage temperature on retention of this vitamin was less marked than the time.

Moschette et al., (1975) in a food storage experiment found that carotene was completely stable at 50 and 60°F for 1 year in tomato, tomato juices and peaches. The temperature effect was definitely less striking than with ascorbic acid. Carotene in tomatoes showed no statistically significant loss even at 80°F; Kramer (1977) reported some beta-carotene increase during storage.

Food containers are labeled stating a vitamin content which should be retained at the end of a storage period at

normal temperatures. Apparently beta-carotene is not only well retained in such products as carrots and sweet potatoes, but seems to increase sometimes during storage. In other products, such as leafy greens, it is rapidly lost unless stored at low temperatures (Kramer, 1977).

The oxidation of beta-carotene depends on the rate of oxidation of the fat since it is attacked by peroxides and free radicals formed from fats. Its destruction, then, depends on temperature, exposure to air, and is enhanced by light, traces of iron and especially traces of copper. Antioxidants protecting fats will also protect carotene (Bender, 1978).

It has been assumed that oxygen attacks the carotenoid molecule at the double bond, consequently producing beta-ionone. However, Eskin et al., (1971) determined that this compound has not been detected even by gas chromatography in stored dried carrots.

Upon oxidation, beta-carotene changes in color from a fairly deep-reddish-orange of carotene to the oxidized product which is a light, yellowish gray. Carotenes being the main fat pigments, oxidation becomes important in keeping quality (Stuckey, 1968).

Kanner and Budowski, (1978) investigated the effects of oxidizing factors on carotene using cellulose powder containing lipid extract from paprika. Carotenoid stability was compared with that of paprika powder. As the water activity

increased, the stability of carotenoids increased slightly. The effect of moisture becomes considerable when the system includes copper sulfate and ascorbic acid. They found that at high water-activity, the prooxidant effect of a water soluble protein is overcome by the marked antioxidant action of the ascorbic acid-copper system. However, in a dry product the decisive factor in carotenoid bleaching is the proteinous fraction which has peroxide activity.

The influence of initial carotene concentration and the effect of unsaturated fats and some antioxidants and synergists on the autoxidation of carotene in paraffin solution was studied by Budowski and Bondi (1960). They found that dilute carotene solutions are more stable than more concentrated ones. This causes longer induction periods and lower rates of autoxidation. Addition of unsaturated fat results in shorter induction periods and higher autoxidation rates. The prooxidant effect of fat increases with increasing degree of unsaturation. Antioxidants added cause a lengthening of the induction periods, and decrease the subsequent rate of autoxidation.

Prooxidant and antioxidant properties of ascorbic acid and beta-carotene has been reported. Burton and Ingold (1984) in a series of experiments in vitro showed that beta-carotene belongs to a class of biological antioxidants. Beta-carotene exhibits good radical-trapping antioxidant behavior only at partial pressures of oxygen significantly

less than 150 torr. Such oxygen partial pressures exist in most tissues under physiological conditions. At higher oxygen pressures, beta-carotene loses its antioxidant activity and shows an autocatalytic, prooxidant effect, particularly at relatively high concentrations.

The prooxidant effects of ascorbic acid in fats and particularly in aqueous fat systems was proved by Scarborough and Watts, (1949); Watts and Wong, (1951); and Kelly and Watts, (1957).

Olsen and Brown (1942) reported that ascorbic acid promoted lipid oxidation in milk products.

The situation became even more complicated when it was found that ascorbic acid can act as a prooxidant at low concentrations, but as an antioxidant at high concentrations in milk cream (Bauernfeind and Pinkert, 1970), or meat (Sato and Hegarty, 1971).

Metal ions seem to be involved in the prooxidative activity of ascorbic acid, as shown by oxidative inhibition by metal-chelating compounds such as ethylene-diaminetetraacetic acid (EDTA) or polyphosphates (Kelly and Watts, 1957; Allan and Wood, 1970; and Kanner et al., 1976).

In fact, iron and copper were reported to accelerate the prooxidant activity of ascorbic acid toward lipids (Allan and Woods, 1970).

The interacting effects of ascorbic acid and metal ions on carotene oxidation in an aqueous carotene-linoleate

solution at pH 7 was studied by Kanner et al., (1977a). Ascorbic acid at concentrations up to 0.01 M was a prooxidant. Iron, and to a lesser extent cobalt, acted synergistically with ascorbic acid. The prooxidant effect of ascorbic acid increased with metal concentration. A prooxidant system was formed between copper and ascorbic acid at low concentration. As the copper concentration increased, reversion of activity occurred and the copper-ascorbic acid system exerted a stabilizing action on carotene. By adding linoleate hydroperoxides, prooxidant effects were enhanced and antioxidant effects weakened. Oxygen depletion, brought about by the rapid oxidation of ascorbic acid, may be partially responsible for the carotene stabilizing effect of the copper-ascorbic acid couple. It is believed that additional stabilization is due to the radical-scavenging properties of copper or of a copper chelate formed by ascorbic acid and/or dehydroascorbic acid.

The chemical mechanisms for the prooxidant and antioxidant effect of ascorbic acid-copper ions has been discussed by Kanner et al., (1977a). They imply formation of a chelate between copper and ascorbic acid which apparently exerts a double action as free radical scavenger and peroxide-decomposing agent. It seems that ascorbic acid at high concentrations could be used in most foods to inhibit lipid oxidation. Therefore, weight ratios of above 10^{-1} and $5x10^{-5}$ for ascorbic acid/linoleic acid, and Cu/linoleic acid would cause good

stability, whereas values of 10^{-3} and $5x10^{-5}$ for the above ratios would be prooxidants. They conclude that, for ascorbic acid to be effective as a chelating agent, it will depend on the quantity and quality of the lipids present in the food.

In another study, two active carotene-bleaching fractions were separated from aqueous pepper extract, one a protein and the other was identified as ascorbic acid accompanied by dehydroascorbic acid. This latter low-molecular fraction also fitted ascorbic acid and dehydroascorbic acid with regard to the effect on carotene oxidation and the changes in bleaching activity caused by the addition of EDTA, changes in pH and in the concentration of the fraction (Kanner et al., 1976).

The carotene-bleaching properties of ascorbic acid can be explained if the following facts are considered: a) In the above assay, carotene-bleaching is coupled to the peroxidation of linoleate. Hence, any factor influencing linoleate oxidation will also be expected to affect the rate of bleaching; b) Ascorbic acid tends to exhibit prooxidant properties toward linoleate when cupric or ferric ions are present, and catalyze the breakdown of lipid peroxides.

It is clear from the results reported above that the bleaching activity of ascorbic acid will depend on several factors, such as the concentrations of ascorbic acid, metal ions,lipid peroxide,the presence of water, etc.

ANTIOXIDANTS: B H A

None of the known antioxidants entirely prevent oxidation, but they delay it. Antioxidants are only one way of delaying oxidation. The oxygen needed to initiate and maintain the oxidative process must be considered and how hard and expensive to remove the last traces of air from the food product. Antioxidants can extend the shelf life of a food, reduce nutritional losses, and widen the range of fats which can be used (Coppen, 1983).

A series of acidic compounds, such as citric acid, phosphoric acid, ascorbic acid and EDTA, help increase the shelf life of a fat when used in conjunction with an antioxidant.

The above acidic compounds complex small amounts of metals which would otherwise accelerate oxidation. These acidic compounds are commonly known as sequestrants. They are not real antioxidants and do not exhibit any antioxidant activity in a fat from which all traces of metal have been removed (Coppen, 1983).

Heimann (1980) discovered that antioxidants must be added above a certain level, otherwise a prooxidative effect or inversion effect can occur.

Antioxidants react with the fat hydroperoxides formed and will prevent the radical chain from progressing further. Antioxidants are used up during the autoxidation process and as a consequence only provide protection for a limited

time. A fat which has been oxidized cannot be restored to its original fresh state by the addition of antioxidants.

The effect of many phenolic antioxidants is increased by so-called synergists, such as ascorbic acid. They act partially as complex formers for the binding of trace metals such as copper and iron, which are known to have a prooxidant effect. Furthermore they also partially take over the regeneration of the phenolic antioxidants used in the course of autoxidation.

Stuckey (1968) found that metal catalysts in oxidation reactions increase the rate of chain generation to a level where normal concentrations of antioxidants are not longer effective in stabilizing the free radical. One of the main problems related to using antioxidants under commercial conditions has been the failure to accomplish complete dispersion especially to food products which contain comparatively small amounts of lipids.

The effect of BHA is much less marked in a vegetable oil which has sufficient natural tocopherol content (Coppen 1983). One disadvantage of BHA is its steam volatility. Not all combinations of antioxidants show synergism. BHA exerts a synergistic action with both BHT and gallate esters.

The mode of action of an antioxidant such as BHA is to donate a proton to the fatty acid free radical which then reverts back to the original molecule so that it cannot form a hydroperoxide (Eskin et al., 1971).

The effectiveness of an antioxidant increases as Aw increases for the adsorption systems since it is expected that more chelator is dissolved as moisture increases (Chou and Labuza, 1974).

The antioxidative effect of BHA can only be estimated within certain limits of BHA concentrations, according to Lingnert et al., (1979). They found that BHA inhibits the Cu^{++} action as characterized by the existence of a critical Aw in the intermediate region surpassing the antioxidative action of water.

A combination of additives may be very effective in preserving both pigments and lipids. Greene et al., (1971) investigated the effects of antioxidants and ascorbate alone and in combination toward prolonging the shelf-life of refrigerated raw ground beef. When combinations of additives thiobarbituric acid + ascorbic acid or BHA + ascorbic acid were used, pigment oxidation was remarkably retarded throughout the 8 days of storage. In general, the effects of antioxidant and ascorbic acid together were greater than those of either additive alone.

To determine if phenolic antioxidants were protecting ascorbic acid from destruction by free radicals from lipid oxidation, sample combinations of pyrogallic acid + ascorbic acid,or BHA + ascorbic acid alone were assayed for ascorbic acid loss over a period of 10 days by Loeffler and Ponting (1942). They found that all samples lost 60-80% of the

ascorbic acid within 6 days. Therefore phenolic antioxidants were not protecting ascorbic acid. They conclude that the effectiveness of the combination cannot be readily explained except as synergism.

In another study, Greene et al., (1971) measured the effect of adding BHA with fat or propylene glycol on the storage life of drum-dried carrot powder. Results showed that adding 0.5% fat (fresh weight basis) containing 0.025% BHA to a carrot slurry, reduced beta-carotene loss and enhanced shelf life as determined by sensory evaluation.

This study was conducted to determine if the antioxidant, BHA, could be used as an indicator of deterioration of ascorbic acid and/or beta-carotene. A model system composed of starch, soy protein isolate, salt and water was chosen as well as beef stew with beef, potatoes, carrots and several lesser ingredients. This study also sought to detect any differences in deterioration due to possible oxygen permeability of the pouch versus the standard tinned can.

MATERIALS AND PROCEDURES

Beef stew and a model system food were prepared and ascorbic acid was added to both. Beta-carotene was added only to the model system. Carrots in the beef stew provided beta-carotene.

Both the beef stew and the model system were divided separately in two parts: one part was thermally processed in pouches and the other part in tin cans. The total number of pouches and cans in both systems was divided in two equal groups. To one group, BHA was added, the other group had no added BHA.

All containers were heat processed, properly labeled and divided into four groups: Initial time (analysed immediately after processing), and four-month storage at three temperatures: 40F, 70F, and 100F.

The formulations used for the model system and beef stew were as follows:

Table 1 **Components in Model System per Package**

Component	**%**	**Can(g)**	**Pouch(g)**
Soy protein isolate	7.40	33.40	21.31
Starch	2.50	11.40	7.20
NaCl	1.00	4.50	2.88
Water	89.10	400.70	256.61
Total	100.00	450.00	288.00

Table 2 **Formulation of Beef Stew per Package**

ITEM	**%**	**Can (g)**	**Pouch(g)**
Beef	24.80	111.60	71.40
Potato	14.80	66.60	42.60
Carrots	12.00	54.00	34.00
Onions	3.62	16.30	10.40
Starch	1.40	6.30	4.00
Seasoning Stange # 76799	0.90	4.00	2.60
NaCl	0.30	1.40	0.90
Caramel color	0.13	0.60	0.40
Water	42.05	189.20	121.70
Total	100.00	450.00	288.00

The ascorbic acid was added at the rate of 70mg/450 g of beef stew product, and 20 mg/100g of model system product. Beta-carotene was added at the rate of 6 mg/100 g wet basis and added vegetable oil represented 4% of the total weight of the model system. BHA was added at the rate of 0.02% of total fat content in both food products. With beef stew, it was assumed that 15% of the beef weight was fat (USDA Handbook #8).

Containers used were # 303x406 C enamel tin cans and 4" x 61/2" thermal pouches consisting of an outer layer of polyester for strength, a middle layer of aluminum foil to keep water, vapor and light from reaching the product, and an inner layer of polyethylene as a heat seal and food contact material. Cans and pouches were steam exhausted, sealed and processed according to heat-penetration time-temperature data obtained previously with a computer using the BSPORD data file (Table 3).

Table 3

HEAT PROCESSING DATA FOR THE BEEF STEW AND THE MODEL SYSTEM IN CANS AND POUCHES

Food	Container	IT(^{O}F)*	RT(^{O}F)**	MIN
Beef Stew	Can	130F	250	80.0
Beef Stew	Pouch	130F	250	26.0
Model System	Can	160F	250	59.0
Model System	Pouch	160F	250	24.0

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* Initial temperature.

** Retort temperature.

Three containers each of pouches and of cans of beef stew without BHA were processed and set aside for taste paneling after 4 month-storage at 40^0F, 70^0F, and 100^0F.

Twenty people participated in the above evaluation on separate days for pouches and cans. They scored the samples following a hedonic scale. A sample score sheet is included in Appendix D.

SAMPLE PREPARATION FOR ANALYSIS

For ascorbic acid and beta-carotene analysis, the container was emptied into a Waring blender. The container was rinsed with water or as necessary with hexane for fatty material. The mixture was then blended and poured into a labeled plastic bag.

For BHA analysis, part of the blended product was freeze-dried overnight to obtain a powder which was used in extracting BHA. Nitrogen was flushed into the freeze-drier during drying to prevent oxidation of BHA.

Prior to all analyses, a sample was taken from the above blended product and freeze-dried overnight, then transferred to the vacuum oven for 12 hours at 80^0C for moisture determination.

Samples were analyzed for ascorbic acid, beta-carotene and BHA. Ascorbic acid was analyzed by the method of Roe and Kuether (1943) with some modifications as is indicated in Appendix A. Beta-carotene was analyzed by the standard

procedure of the AOAC (1980) as is indicated in Appendix B. BHA was analyzed by the standard procedure of the AOAC (1980) with some modifications as is indicated in Appendix C.

STATISTICAL ANALYSIS

Analysis of variance tests were run separately on ascorbic acid, beta-carotene, BHA, and the taste panel data. Then the differences between the means were compared in ascorbic acid, beta-carotene, and BHA under a 95% confidence interval through an LSD test.

R E S U L T S

It was found that the type of container made a difference in the percent retention of ascorbic acid. Pouches retained significantly higher levels of ascorbic acid than cans. The container did not make a difference in the per cent retention of beta-carotene (Table 4).

Time and temperature affected beta-carotene and ascorbic acid differently in beef stew (Table 5). Time of storage affected beta-carotene more than temperature. There was a significant difference in percent retention of beta-carotene between the initial time and all three temperatures after 4 months storage. However, no significant difference in beta-carotene retention was obtained between the three temperatures after 4 months storage.

There was a gradual decline in percent retention of ascorbic acid with time and as temperature of storage increased. It should be observed that the initial sample was at 0 time.

Analysis of the BHA effect on ascorbic acid and beta-carotene retention showed that ascorbic acid and beta-carotene were not affected by BHA.

The effects of container and time-temperature of storage on the retention of ascorbic acid in beef stew were as shown in Table 6. After 4 months of storage, at 40F, both pouches and cans showed no significant difference in the retention of ascorbic acid. However, at 70F and 100F differences were significant between cans and pouches. Ascorbic acid retention

TABLE 4

THE EFFECT OF CONTAINER ON ASCORBIC ACID AND BETA-CAROTENE RETENTION IN BEEF STEW

Container/Nutrient	Mean Percent
Pouch, Ascorbic Acid	90.45
Pouch, Beta-Carotene	86.80
Can, Ascorbic Acid	84.22
Can, Beta-Carotene	86.97

Comparison	Mean Difference
Pouch, As.Ac. - Pouch, BetaC.	3.65
Pouch, As.Ac. - Can, As.Ac.	6.24*
Pouch, BetaC. - Can, Beta C.	-0.17
Can, As.Ac. - Can, Beta C.	-2.75

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*Significantly Different at 0.05 level

TABLE 5

INTERACTION OF TIME AND TEMPERATURE ON ASCORBIC ACID AND BETA-CAROTENE RETENTION IN BEEF STEW

Storage Conditions/Nutrient	Mean Percent
Initial, Ascorbic Acid	100.00
Initial, Beta-Carotene	100.00
4 mo. 40°F, Ascorbic Acid	93.71
4 mo. 40°F, Beta-Carotene	81.38
4 mo. 70°F, Ascorbic Acid	86.05
4 mo. 70°F, Beta-Carotene	83.57
4 mo. 100°F, Ascorbic Acid	69.59
4 mo. 100°F, Beta-Carotene	82.58

Comparison	Mean Difference
Initial,As.Ac. - 40F, As.Ac.	6.29*
Initial,As.Ac. - 70F, As.Ac.	13.96*
Initial,As.Ac. - 100F, As.Ac.	30.41*
Initial,BetaC. - 40F, Beta C.	18.62*
Initial,BetaC. - 70F, Beta C.	16.43*
Initial,BetaC. - 100F,Beta C.	17.42*
40 F, Asc.Ac. - 40 F,Beta C.	12.33*
40 F, Asc.Ac. - 70 F., As.Ac.	7.66*
40 F, Asc.Ac. - 100F, Asc.Ac.	24.12*
40 F, Beta C. - 70 F, Beta C.	-2.19

continued on next page

Table 5 continued

40 F, Beta C.	- 100F, Beta C.	-1.19
70 F ,Asc.Ac.	- 70 F, Beta C.	2.47
70 F, Asc.Ac.	- 100F, Asc.Ac.	16.45*
70 F, Beta C.	- 100F, Beta C.	0.10
100F, Asc.Ac.	- 100F, Beta C.	-12.90*

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*Significantly Different at 0.05 level

TABLE 6

INTERACTIONS OF CONTAINER, TIME AND TEMPERATURE ON ASCORBIC ACID RETENTION IN BEEF STEW

	Ascorbic Acid
Container/Storage Conditions	**Mean Percent**
Pouch, Initial	100.00
Pouch, 4 mo. 40°F	94.31
Pouch, 4 mo. 70°F	97.67
Pouch, 4 mo. 100°F	69.84
Can, Initial	100.00
Can, 4 mo. 40°F	93.11
Can, 4 mo. 70°F	74.43
Can, 4 mo. 100°F	69.34

Comparisons	**Mean Difference**
Pouch, Initial -Pouch, 40F	5.69
Pouch, Initial -Pouch, 70F	2.34
Pouch, Initial -Pouch, 100F	30.16*
Pouch, 40F - Pouch, 70F	-3.36
Pouch, 40F - Pouch, 100F	24.46*
Pouch, 40F - Can, 40F	1.20
Pouch, 70F - Pouch, 100F	27.82*
Pouch, 70F - Can, 70F	23.24*
Pouch, 100F - Can, 100F	0.51
Can, Initial - Can, 40F	6.89

continued on next page

Table 6 continued

Can, Initial - Can, 70F	25.57*
Can, Initial - Can, 100F	30.66*
Can, 40F - Can, 70F	18.68*
Can, 40F - Can, 100F	23.77*
Can, 70F - Can, 100F	5.09

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*Significantly Different at 0.05 level

in cans was severely decreased at 70F and 100F. Whereas in pouches it seemed to be protected at 70F but drastically decreased at 100F.

Beta-carotene was severely affected by time of storage in both cans and pouches (Table 7). At 40F both cans and pouches did not show any difference in retention of beta-carotene. At 70F and 100F both cans and pouches responded differently. Pouches retained more at 70F but showed a drastic drop in beta-carotene at 100F. Cans, however, dropped slightly in beta-carotene from 40F to 70F but showed some significant increase in beta-carotene at 100F.

Ascorbic acid was significantly better retained in pouches than in cans (Table 8). However, in either cans or pouches when comparing with and without BHA treatments, there were no significant differences.

The effects of container and BHA on the retention of beta-carotene in beef stew was measured. There was no significant differences in either pouches or cans with or without BHA in the retention of beta-carotene.

After 4 months of storage at 40F, ascorbic acid did not significantly decrease from the initial without BHA, however, with BHA the decrease was significant (Table 9). At 70F, with BHA beef stew retained ascorbic acid as well as at 40F, but the treatments without BHA suffered a significant drop in ascorbic acid. Hence, at 70F ascorbic acid was significantly better retained in treatments with BHA. The

TABLE 7

INTERACTION OF CONTAINER, TIME AND TEMPERATURE ON BETA-CAROTENE RETENTION IN BEEF STEW

Container/Storage Conditions	Beta-Carotene Mean Percent
Pouch, Initial	100.00
Pouch, 4 mo. 40°F	81.39
Pouch, 4 mo. 70°F	87.34
Pouch, 4 mo. 100°F	78.48
Can, Initial	100.00
Can, 4 mo. 40°F	81.38
Can, 4 mo. 70°F	79.81
Can, 4 mo. 100°F	86.67

Comparison	Mean Difference
Pouch, Initial - Pouch, 40F	18.61*
Pouch, Initial - Pouch, 70 F	12.66*
Pouch, Initial - Pouch 100F	21.52*
Pouch, 40F - Pouch, 70F	-5.95
Pouch, 40F - Pouch,100F	2.91
Pouch, 40F - Can, 40F	0.01
Pouch, 70F - Pouch, 100F	8.86*
Pouch, 70F - Can, 70F	7.53
Pouch, 100F- Can, 100F	-8.20*

continued on next page

Table 7 continued

Can, Initial	- Can, 40F	18.62*
Can, Initial	- Can, 70F	20.19*
Can, Initial	- Can, 100F	13.33*
Can, 40F	- Can, 70F	1.57
Can, 40F	- Can, 100F	-5.30
Can, 70F	- Can, 100F	-6.86

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*Significantly Different at 0.05 level

TABLE 8

INTERACTIONS OF CONTAINERS AND BHA ON ASCORBIC ACID RETENTION IN BEEF STEW

	Ascorbic Acid
Container/BHA	**Mean Percent**
Pouch, With BHA	89.23
Pouch, Without BHA	91.67
Can, With BHA	82.92
Can, Without BHA	85.52

Comparison	**Mean Difference**
Pouch, With BHA-Pouch, Without BHA	-2.44
Pouch, With BHA - Can, With BHA	6.32*
Pouch, Without BHA-Can, Without BHA	6.15*
Can, With BHA - Can, Without BHA	-2.61

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*Significantly Different at 0.05 level

TABLE 9

INTERACTIONS OF TIME-TEMPERATURE AND BHA ON ASCORBIC ACID RETENTION IN BEEF STEW

	Ascorbic Acid
Storage Conditions/BHA	Mean Percent
Initial, With BHA	100.00
Initial, Without BHA	100.00
4 mo. 40°F, With BHA	89.24
4 mo. 40°F, Without BHA	98.17
4 mo. 70°F, With BHA	91.03
4 mo. 70°F, Without BHA	81.06
4 mo. 100°F, With BHA	64.03
4 mo. 100°F, Without BHA	75.16

Comparisons	Mean Difference
Initial, With BHA - 40F, With BHA	10.76*
Initial, With BHA - 70F, With BHA	8.97*
Initial, With BHA - 100F, With BHA	35.97*
Init., Without BHA-40F, Without BHA	1.83
Init., Without BHA-70F, Without BHA	18.94*
Init., Without BHA-100F, W/out BHA	24.84*
40F, With BHA - 40F, Without BHA	-8.93*
40 F, With BHA - 70F, With BHA	-1.79
40 F, With BHA - 100F, With BHA	25.21*
40 F, Without BHA- 70F, Without BHA	17.11*

continued on next page

Table 9 continued

40F, Without BHA - 100F, Without BHA	23.02*
70 F, With BHA - 70 F, Without BHA	9.97*
70 F, With BHA - 100F, With BHA	27.00*
70 F, Without BHA- 100F, Without BHA	5.90
100F, With BHA -100F, Without BHA	-11.10*

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*Significantly Different at 0.05 level

decrease in ascorbic acid was significantly greater at 100F than at 40F or 70F. At 100F, significantly better protection was given to ascorbic acid without than with BHA.

The effects of time-temperature of storage and BHA on the retention of beta-carotene in beef stew was determined (Table 10). Comparison of treatments with and without BHA were not significantly different at all temperatures. All were sharply affected by time of storage but not by temperatures during storage.

The percent retention of BHA in beef stew was compared with that of ascorbic acid and beta-carotene (Table 11). It was noted that beta-carotene and ascorbic acid were retained at about 80%. BHA was the lowest retained with significant differences from both ascorbic acid and beta-carotene.

Comparing the retention of BHA in pouches and cans at initial (0 months) and 4 months with temperatures of storage in beef stew it was found that BHA was significantly lower after time at all temperatures of storage in both pouches and cans (Table 12). Pouches and cans were parallel in BHA retention during time and at the temperatures of storage. BHA was retained significantly better at 70F than either at 40F or 100F. No significant difference in retention was observed between 40F and 100F.

The retention of BHA over 0 and 4 months at three temperatures of storage in beef stew demonstrated that BHA retention was significantly more affected by time than by

TABLE 10

INTERACTIONS OF TIME, TEMPERATURE AND BHA ON BETA-CAROTENE RETENTION IN BEEF STEW

	Beta-Carotene
Storage Conditions/BHA	Mean Percent
Initial, With BHA	100.00
Initial, Without BHA	100.00
4 mo. 40°F, With BHA	80.81
4 mo. 40°F, Without BHA	81.96
4 mo. 70°F, With BHA	85.07
4 mo. 70°F, Without BHA	82.08
4 mo. 100°F, With BHA	84.82
4 mo. 100°F, Without BHA	80.33

Comparison	**Mean Difference**
Initial, With BHA- 40F, With BHA	19.19*
Initial, With BHA - 70F, With BHA	14.93*
Initial, With BHA -100F, With BHA	15.18*
Init., Without BHA-40F, Without BHA	18.04*
Init., Without BHA-70F, Without BHA	17.92*
Init., Without BHA-100F, Without BHA	19.67*
40 F, With BHA - 40F, Without BHA	-1.15
40 F, With BHA - 70, With BHA	-4.27
40 F, With BHA -100F, With BHA	-4.02

continued on next page

Table 10 continued

40 F, Without BHA-70F, Without BHA	-0.12
40 F, Without BHA-100F, Without BHA	1.63
70 F, With BHA- 70F, Without BHA	2.99
70 F, With BHA- 100F, With BHA	0.25
70 F, Without BHA-100F, Without BHA	1.75
100F, With BHA - 100F, Without BHA	4.49

*Significantly Different at 0.05 level

TABLE 11

COMPARISON OF ASCORBIC ACID, BETA-CAROTENE, AND BHA RETENTIONS IN BEEF STEW

Compound	Mean Percent
Ascorbic Acid	79.00
Beta-Carotene	81.34
BHA	43.22

Comparison	Mean Difference
Asc.Acid - Beta Carotene	-2.34
Asc.Acid - BHA	35.78*
Beta Carotene- BHA	38.12*

*Significantly Different at 0.05 level

TABLE 12

INTERACTIONS OF BHA, CONTAINERS, TIME AND TEMPERATURE IN BEEF STEW

	BHA
Container/Storage Conditions	**Mean Percent**
Pouch, Initial	100.00
Pouch, 4 mo. 40°F	59.22
Pouch, 4 mo. 70°F	69.41
Pouch, 4 mo. 100°F	59.46
Can, Initial	100.00
Can, 4 mo. 40°F	44.41
Can, 4 mo. 70°F	60.21
Can, 4 mo. 100°F	50.10

Comparison	**Mean Difference**
Pouch, initial-Pouch, 40F	40.78*
Pouch, initial-Pouch, 70F	30.59*
Pouch, Initial-Pouch, 100F	40.54*
Pouch, 40F - Pouch, 70F	-10.18*
Pouch, 40F - Pouch, 100F	-0.24
Pouch, 40F - Can, 40F	14.81*
Pouch, 70F - Pouch, 100F	9.95*
Pouch, 70F - Can, 70F	9.19*
Pouch, 100F - Can, 100F	9.36*
Can, Initial- Can, 40F	55.59*

continued on next page

Table 12 continued

Can, Initial- Can, 70F	39.79*
Can, Initial- Can, 100F	49.90*
Can, 40F - Can, 70F	-15.80*
Can, 40F - Can, 100F	-5.69
Can, 70F - Can, 100F	10.11*

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*Significantly Different at 0.05 level

temperatures of storage (Table 13). It was also significantly better retained at 70F than at 40F or 100F.

The retentions of ascorbic acid and beta-carotene each with BHA added and that of BHA were compared at 0 and 4 months at three temperatures of storage in beef stew (Table 14). BHA had the lowest retention during time and at the temperatures of storage than ascorbic acid and beta-carotene. At 40F and 100F beta-carotene was significantly better retained than ascorbic acid, but at 100F ascorbic acid retention was significantly higher.

Ascorbic acid and beta-carotene retention and that of BHA were compared in cans and pouches in beef stew (Table 15). BHA and ascorbic acid were retained at a significantly higher percentage in pouches than in cans.

Comparing ascorbic acid and beta-carotene retention in beef stew it was found that there was no significant mean difference in percent retention of beta-carotene and ascorbic acid in beef stew. However, as indicated above there were differences depending on the conditions.

In the model system, it was found that the container type made a difference in retention of ascorbic acid (Table 16). Pouches retained significantly higher ascorbic acid than cans, but container type gave no significant difference in beta-carotene retention. Comparing ascorbic acid and beta-carotene retention in pouches, ascorbic acid was retained significantly higher than beta-carotene. However, in cans

TABLE 13

RETENTION OF BHA WITH TIME AND TEMPERATURE IN BEEF STEW

Storage Condition	BHA
	Mean Percent
Initial	100.00
4 months 40°F	51.82
4 months 70°F	64.81
4 months 100°F	54.78

Comparison	Mean Difference
Initial - 40F	48.18*
Initial - 70F	35.19*
Initial - 100F	45.22*
40 F - 70 F	-12.99*
40 F - 100F	-2.96
70 F - 100F	10.03*

*Significantly Different at 0.05 level

TABLE 14

INTERACTION OF BHA, ASCORBIC ACID AND BETA-CAROTENE WITH TIME AND TEMPERATURE IN BEEF STEW

Storage Condition/Compound	Mean Percent
Initial, Ascorbic Acid	100.00
Initial, Beta-Carotene	100.00
Initial, BHA	100.00
4 mo. 40°F, Ascorbic Acid	62.55
4 mo. 40°F, Beta-Carotene	72.72
4 mo. 40°F, BHA	20.18
4 mo. 70°F, Ascorbic Acid	67.15
4 mo. 70°F, Beta-Carotene	81.98
4 mo. 70°F, BHA	45.30
4 mo. 100°F, Ascorbic Acid	86.30
4 mo. 100°F, Beta-Carotene	70.65
4 mo. 100°F, BHA	7.40

Comparison	Mean Difference
Initial,As.Ac. - 40F, As.Ac.	37.45*
Initial,As.Ac. - 70F, As.Ac.	32.85*
Initial, As.Ac.- 100F, As.Ac.	13.70*
Initial,Beta C.- 40F, Beta C.	27.28*
Initial, Beta C.- 70F, Beta C.	18.02*
Initial, Beta C.-100F, Beta C.	29.35*

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Table 14 continued

Initial, BHA - 40 F, BHA	79.82*
Initial, BHA - 70F, BHA	54.70*
Initial, BHA - 100F, BHA	92.60*
40F, As.Ac. - 40F, Beta C.	-10.18*
40F, As.Ac. - 40F, BHA	42.37*
40F, As.Ac. - 70F, As.Ac.	-4.61
40F, As.Ac. - 100F, As.Ac.	-23.75*
40F, Beta C.- 40F, BHA	52.54*
40F, Beta C.- 70F, Beta C.	-9.26*
40F, Beta C.-100F, Beta C.	2.08
40F, BHA - 70 F, BHA	-25.12*
40F, BHA -100F, BHA	12.79*
70F, As.Ac.- 70F,Beta C.	-14.82*
70F, As.Ac.- 70F, BHA	21.85*
70F, As.Ac.- 100F,As.Ac.	-19.15*
70F, Beta C.- 70F, BHA	36.68*
70F, Beta C.- 100F,Beta C.	11.33*
70F, BHA - 100F, BHA	37.90*
100F, As.Ac. - 100F, Beta C.	15.66*
100F, As.Ac. - 100F, BHA	78.91*
100F, Beta C.-100F, BHA	63.25*

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*Significantly Different at 0.05 level

TABLE 15

COMPARISON OF RETENTION OF BHA, ASCORBIC ACID, AND BETA-CAROTENE IN CANS AND POUCHES IN BEEF STEW

Container/Compound	Mean Percent
Pouch, Ascorbic Acid	85.77
Pouch, Beta-Carotene	81.44
Pouch, BHA	48.86
Can, Ascorbic Acid	72.23
Can, Beta-Carotene	81.23
Can, BHA	37.58

Comparison	Mean Difference
Pouch, Asc.Ac. - Pouch, Beta C.	4.33
Pouch, Asc.Ac. - Pouch, BHA	36.91*
Pouch, Asc.Ac. - Can, Asc. Ac.	13.54*
Pouch, Beta C. - Pouch, BHA	32.58*
Pouch, Beta C. - Can, Beta C.	0.21
Pouch, BHA - Can, BHA	11.27*
Can, Asc.Ac. - Can,Beta C.	-9.00*
Can, Asc.Ac. - Can, BHA	34.65*
Can, Beta C. - Can, BHA	43.65*

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*Significantly Different at 0.05 level

TABLE 16

THE EFFECT OF CONTAINER ON ASCORBIC ACID AND BETA-CAROTENE RETENTION IN THE MODEL SYSTEM

Container/Nutrient	Mean Percent
Pouch, Ascorbic Acid	89.44
Pouch, Beta Carotene	82.21
Can, Ascorbic Acid	73.18
Can, Beta-Carotene	83.14

Comparison	Mean Difference
Pouch, As.Ac. -Pouch, Beta C.	7.23*
Pouch, As.Ac. -Can, As.Acid	16.26*
Pouch, Beta C. -Can, Beta C.	-0.94
Can, As.Ac. - Can, Beta C.	-9.97*

*Significantly Different at 0.05 level

beta-carotene was retained significantly higher than ascorbic acid.

The effect of time and temperature of storage on ascorbic acid and beta-carotene retention in beef stew was determined (Table 17). Time and temperature affected beta-carotene and ascorbic acid differently. Time of storage significantly decreased both ascorbic acid and beta-carotene. There was a significant difference in beta-carotene between the initial time and all temperatures of the 4-month storage, but no significant difference was obtained between the three temperatures.

Ascorbic acid retention was affected by both time and temperature of storage, with significant differences between the three temperatures of storage. Ascorbic acid was retained significantly higher at 100F than at 70F or 40F.

Analysis of the BHA effect in the model system showed that ascorbic acid retention was significantly higher when BHA was present. Beta-carotene retention was not affected by BHA (Table 18).

The effects of container, time and temperature of storage on the retention of ascorbic acid in the model system is shown in Table 19. Ascorbic acid was affected by time and temperature of storage similarly in both pouches and cans. In both cases, ascorbic acid was significantly better retained at 100F than at 70F or 40F.

TABLE 17

INTERACTION OF TIME AND TEMPERATURE ON ASCORBIC ACID AND BETA CAROTENE RETENTION IN THE MODEL SYSTEM

Storage Conditions/Nutrient	Mean Percent
Initial, Ascorbic Acid	100.00
Initial, Beta-Carotene	100.00
4 mo. 40°F, Ascorbic Acid	67.81
4 mo. 40°F, Beta-Carotene	74.70
4 mo. 70°F, Ascorbic Acid	72.34
4 mo. 70°F, Beta-Carotene	79.35
4 mo. 100°F, Ascorbic Acid	85.07
4 mo. 100°F, Beta-Carotene	76.66

Comparison	Mean Difference
Initial, As.Ac.- 40F, As.Ac.	32.19*
Initial, As.Ac.- 70F, As.Ac.	27.66*
Initial, As.Ac.-100F, As.Ac.	14.93*
Initial,Beta C. - 40F, Beta C	25.30*
Initial ,Beta C - 70F, Beta C.	20.65*
Initial,Beta C.- 100F, Beta C.	23.34*
40 F, As.Ac. - 40 F, Beta C.	-6.89*
40 F, As.Ac. - 70 F, As.Ac.	-4.53
40 F, As.Ac. - 100F, As.Ac.	-17.3*
40 F, Beta C.- 70 F, Beta C.	-4.64

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Table 17 continued

40 F, Beta C.- 100F, Beta C.	-1.96
70F, As.Ac. - 70 F, Beta C.	-7.00*
70 F, As.Ac. - 100F, As.Ac.	-12.70*
70 F, Beta C.- 100F, Beta C.	2.68
100 F, As.Ac.- 100, Beta C.	8.41*

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*Significantly Different at 0.05 level

TABLE 18

THE EFFECT OF BHA ON ASCORBIC ACID AND BETA-CAROTENE RETENTION IN THE MODEL SYSTEM

BHA/Nutrient	Mean Percent
With BHA, Ascorbic Acid	79.00
With BHA, Beta-Carotene	81.34
Without BHA, Ascorbic	83.61
Without BHA, Beta-Carotene	84.02

Comparison	Mean Difference
With BHA, As.Ac. - With BHA, Beta C.	-2.34
With BHA, As.Ac. - Without BHA, As.Ac.	-4.61*
With BHA, Beta C. - Without BHA, BetaC.	-2.68
Without BHA, As.Ac. - Without BHA, Beta C.	-0.40

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*Significantly Different at 0.05 level

TABLE 19

INTERACTION OF CONTAINER, TIME AND TEMPERATURE ON ASCORBIC ACID RETENTION IN THE MODEL SYSTEM

	Ascorbic Acid
Container/Storage Conditions	**Mean Percent**
Pouch, Initial	100.00
Pouch, 4 mo. 40°F	76.76
Pouch, 4 mo. 70°F	85.04
Pouch, 4 mo. 100°F	95.95
Can, Initial	100.00
Can, 4 mo. 40°F	58.86
Can, 4 mo. 70°F	59.65
Can, 4 mo. 100°F	74.20

Comparison	**Mean Difference**
Pouch, Initial-Pouch, 40F	23.24*
Pouch, Initial-Pouch, 70F	14.96*
Pouch, Initial-Pouch, 100F	4.05
Pouch, 40F - Pouch, 70F	-8.30
Pouch, 40F - Pouch, 100F	-19.20*
Pouch, 40F - Can, 40F	17.90*
Pouch, 70F - Pouch, 100F	-10.90*
Pouch, 70F - Can, 70F	25.38*

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Table 19 continued

Pouch, 100F - Can,100F	21.75*
Can, Initial- Can, 40F	41.14*
Can, Initial- Can, 70F	40.35*
Can, 40F - Can, 100F	25.80*
Can, 40F - Can, 70F	-0.79
Can, 40F - Can, 100F	-15.30*
Can, 70F - Can, 100F	-14.50*

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*Significantly Different at 0.05 level

The effects of container, time and temperature of storage on the retention of beta-carotene in the model system is shown in Table 20. Beta-carotene was severely affected by time of storage in both cans and pouches. Pouches did not show any significant difference between the three temperatures of storage. However, in cans, 70 F provided a significantly better protection to beta-carotene than at 40F or at 100F.

The effects of container and BHA in the retention of ascorbic acid in the model system was measured (Table 21). Ascorbic acid was significantly better retained in pouches without BHA.

The effects of container and BHA in the retention of beta-carotene in the model system was measured (Table 22). Beta-carotene was significantly better retained in cans without BHA than cans with BHA. In pouches, the difference was not significant. No difference in retention was observed between cans and pouches.

The analysis of the effects of time and temperature of storage and BHA in the retention of ascorbic acid in the model system showed that treatments with and without BHA were similarly affected by time of storage. At 40F and 70F, there was a significantly better retention of beta-carotene in the treatments without than with BHA. At 100F it was not significant (Table 23).

TABLE 20

INTERACTIONS OF CONTAINER, TIME, AND TEMPERATURE ON BETA-CAROTENE RETENTION IN THE MODEL SYSTEM

Container/Storage Conditions	Beta-Carotene Mean Percent
Pouch, Initial	100.00
Pouch, 4 mo. 40°F	76.57
Pouch, 4 mo. 70°F	75.21
Pouch, 4 mo. 100°F	77.06
Can, Initial	100.00
Can, 4 mo. 40°F	72.84
Can, 4 mo. 70°F	83.48
Can, 4 mo. 100°F	76.27

Comparison	Mean Difference
Pouch, Initial - Pouch, 40F	23.43*
Pouch, Initial - Pouch, 70F	24.79*
Pouch, Initial - Pouch, 100F	22.94*
Pouch, 40F - Pouch, 70F	1.35
Pouch, 40F - Pouch,100F	-0.49
Pouch, 40F - Can, 40F	3.73
Pouch, 70F - Pouch, 100F	-1.84
Pouch, 70F - Can, 70F	-8.26*
Pouch, 100F - Can,100F	0.79

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Table 20 continued

Can, Initial - Can, 40F	27.16*
Can, Initial - Can, 70F	16.52*
Can, Initial -Can, 100F	23.74*
Can, 40F - Can, 70F	-10.60*
Can, 40F - Can, 100F	-3.43
Can, 70F - Can, 100F	7.21*

*Significantly Different at 0.05 level

TABLE 21

INTERACTIONS OF CONTAINERS AND BHA ON ASCORBIC ACID RETENTION IN THE MODEL SYSTEM

	Ascorbic Acid
Container/BHA	**Mean Percent**
Pouch, With BHA	85.77
Pouch, Without BHA	93.10
Can, With BHA	72.23
Can, Without BHA	74.12

Comparison	**Mean Difference**
Pouch, With BHA- Pouch, Without BHA	-7.33*
Pouch, With BHA- Can, With BHA	13.54*
Pouch, Without BHA- Can, Without BHA	18.98*
Can, With BHA- Can, Without BHA	-1.89

*Significantly Different at 0.05 level

TABLE 22

INTERACTIONS OF CONTAINER AND BHA ON BETA-CAROTENE RETENTION IN THE MODEL SYSTEM

	Beta-Carotene
Container/BHA	Mean Percent
Pouch, With BHA	81.44
Pouch, Without BHA	82.98
Can, With BHA	81.23
Can, Without BHA	85.06
Comparison	**Mean Difference**
Pouch, With BHA- Pouch, Without BHA	-1.54
Pouch, With BHA- Can, With BHA	0.21
Pouch, Without BHA- Can, Without BHA	-2.08
Can, With BHA - Can, Without BHA	-3.82*

*Significantly Different at 0.05 level

TABLE 23

INTERACTIONS OF TIME, TEMPERATURE AND BHA ON ASCORBIC ACID RETENTION IN THE MODEL SYSTEM

Storage Conditions/BHA	Ascorbic Acid Mean Percent
Initial, With BHA	100.00
Initial, Without BHA	100.00
4 mo. 40°F, With BHA	62.55
4 mo. 40°F, Without BHA	73.08
4 mo. 70°F, With BHA	67.15
4 mo. 70°F, Without BHA	77.53
4 mo. 100°F, With BHA	86.30
4 mo. 100°F, Without BHA	83.84

Comparison	Mean Difference
Initial, With BHA- 40F, With BHA	37.45*
Initial, With BHA- 70F, With BHA	32.85*
Initial, With BHA- 100F, With BHA	13.70*
Initial, Without BHA - 40F, Without BHA	26.92*
Initial, Without BHA - 70F, Without BHA	22.47*
Initial, Without BHA - 100F, Without BHA	16.16*
40F, With BHA - 40F, Without BHA	-10.50*
40F, With BHA - 70F, With BHA	-4.61
40F, With BHA - 100F, With BHA	-23.80*

continued on next page

Table 23 continued

40F, Without BHA - 70F, Without BHA	-4.46
40F, Without BHA - 100F,Without BHA	-10.80*
70F, With BHA - 70F, Without BHA	-10.40*
70F, With BHA - 100F, With BHA	-19.10*
70F, Without BHA - 100F, Without BHA	-6.31
100F, With BHA - 100F, Without BHA	2.46

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*Significantly Different at 0.05 level

The effects of time, temperature and BHA on beta-carotene retention in the model system was determined (Table 24). After 4 months of storage, beta-carotene was severely and significantly affected with and without BHA.

At 70F, BHA protected ascorbic acid significantly better than at 40F or 100F. At 100F the retention dropped significantly with BHA.

The percent retention of BHA in the model system was also measured and compared to the retentions of ascorbic acid and beta-carotene each with BHA added (Table 25). There was no significant difference in retention between ascorbic acid and beta-carotene. BHA was significantly lower in retention from both ascorbic acid and beta-carotene.

Comparing the retention of BHA in pouches and cans at initial time and at 4-months temperatures of storage in the model system, BHA was significantly lower after time at all temperatures of storage in both pouches and cans (Table 26). Both pouches and cans followed similar trends of BHA retention with time and at the three temperatures of storage. BHA was lowest at 100F in both cans and pouches.

BHA retention was significantly affected by both time and temperature of storage, and was lowest at 100F (Table 27).

The retentions of ascorbic acid and beta-carotene each with BHA added only, and that of BHA were compared in the model system at initial time and at 4-month temperatures of storage (Table 28). BHA showed the lowest rates of retention

TABLE 24

INTERACTIONS OF TIME, TEMPERATURE AND BHA ON BETA-CAROTENE RETENTION IN THE MODEL SYSTEM

	Beta-Carotene
Storage Conditions/BHA	Mean Percent
Initial, With BHA	100.00
Initial, Without BHA	100.00
4 mo. 40°F, With BHA	72.72
4 mo. 40°F, Without BHA	76.68
4 mo. 70°F, With BHA	81.98
4 mo. 70°F, Without BHA	76.71
4 mo. 100°F, With BHA	70.65
4 mo. 100°F, Without BHA	82.68

Comparison	Mean Difference
Initial, With BHA- 40F, With BHA	27.28*
Initial, With BHA- 70F, With BHA	18.02*
Initial, With BHA- 100F, With BHA	29.35*
Initial,Without BHA - 40F, Without BHA	23.32*
Initial,Without BHA - 70F, Without BHa	23.29*
Initial,Without BHa - 100F,Without BHA	17.32*
40F, With BHA - 40F, Without BHA	-3.96
40F, With BHA- 70F, With BHA	-9.25*
40F, With BHA- 100F, With BHA	2.08

continued on next page

Table 24 continued

40F, Without BHA - 70F, Without BHA	-0.03
40F, Without BHA - 100F, Without BHA	-5.99*
70F, With BHA - 70F, Without BHA	5.27*
70F, With BHA- 100F, With BHA	11.33*
70F, Without BHA - 100F, Without BHA	-5.96*
100F, With BHA- 100F, Without BHA	-12.00*

*Significantly Different at 0.05 level

TABLE 25

COMPARISON OF RETENTION OF ASCORBIC ACID, BETA-CAROTENE, AND BHA IN THE MODEL SYSTEM

Compound	Mean Percent
Ascorbic Acid	86.07
Beta-Carotene	87.68
BHA	67.20

Comparison	Mean Difference
Ascorbic Acid - Beta Carotene	-1.60
Ascorbic Acid - BHA	18.08*
Beta Carotene - BHA	19.68*

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*Significantly Different at 0.05 level

TABLE 26

INTERACTIONS OF BHA, CONTAINERS, TIME AND TEMPERATURE IN THE MODEL SYSTEM

Container/Storage Conditions	BHA Mean Percent
Pouch, Initial	100.00
Pouch, 4 mo. 40°F	78.73
Pouch, 4 mo. 70°F	88.63
Pouch, 4 mo. 100°F	66.16
Can, Initial	100.00
Can, 4 mo. 40°F	74.91
Can, 4 mo. 70°F	79.26
Can, 4 mo. 100°F	56.97

Comparison	Mean Difference
Pouch, Initial - Pouch, 40F	21.27*
Pouch, Initial - Pouch, 70F	11.37*
Pouch, Initial - Pouch, 100F	33.84*
Pouch, 40F - Pouch, 70F	-9.90*
Pouch, 40F - Pouch, 100F	12.57*
Pouch, 40F - Can, 40F	3.83
Pouch, 70F - Pouch, 100F	22.47*
Pouch, 70F - Can, 70F	9.37*
Pouch, 100F - Can, 100F	9.19*
Can, Initial - Can, 40F	25.09*

continued on next page

Table 26 continued

Can, Initial	- Can, 70F	20.74*
Can, Initial	- Can,100F	43.03*
Can, 40F	- Can, 70F	-4.35
Can, 40F	- Can 100F	17.93*
Can, 70F	- Can, 100F	22.29*

*Significantly Different at 0.05 level

TABLE 27

RETENTION OF BHA WITH TIME AND TEMPERATURE IN THE MODEL SYSTEM

BHA	
Storage Conditions	Mean Percent
Initial	100.00
4 mo. 40°F	76.82
4 mo. 70°F	83.95
4 mo. 100°F	61.57

Comparison	Mean Difference
Initial - 40F	23.18*
Initial - 70F	16.05*
Initial - 100F	38.43*
40 F - 70 F	-7.13
40 F - 100 F	15.25*
70 F - 100 F	22.38*

*Significantly Different at 0.05 level

TABLE 28

INTERACTION OF RETENTION OF BHA, ASCORBIC ACID AND BETA-CAROTENE WITH TIME AND TEMPERATURE IN THE MODEL SYSTEM

Storage Condition/Compound	Mean Percent
Initial, Ascorbic Acid	100.00
Initial, Beta-Carotene	100.00
Initial, BHA	100.00
4 mo. 40°F, Ascorbic Acid	89.24
4 mo. 40°F, Beta-Carotene	80.81
4 mo. 40°F, BHA	60.41
4 mo. 70°F, Ascorbic Acid	91.03
4 mo. 70°F, Beta-Carotene	85.07
4 mo. 70°F, BHA	75.73
4 mo. 100°F, Ascorbic Acid	64.03
4 mo. 100°F, Beta-Carotene	84.82
4 mo. 100°F, BHA	35.85

Comparison	Mean Difference
Init., As.Ac. - 40F, As.Ac.	10.86*
Init., As.Ac. - 70F, As.Ac.	8.98*
Init., As.Ac. - 100F, As.Ac.	35.97*
Init., Beta C.- 40F, Beta C.	19.19*
Init., Beta C.- 70F, Beta C.	14.93*

continued on next page

Table 28 continued

Init., Beta C.	- 100F, Beta C.	15.18*
Init., BHA	- 40F, BHA	39.59*
Init., BHA	- 70F, BHA	24.27*
Init., BHA	- 100F, BHA	64.15*
40 F, As.Ac.	- 40F, Beta C.	8.44*
40F, As.Ac.	- 40F, BHA	28.83*
40F, As.Ac.	- 70F, As.Ac.	-1.79
40F, As.Ac.	- 100F, As.Ac.	25.21*
40F, Beta C.	- 40F, BHA	20.40*
40F, Beta C.	- 70F, Beta C.	-4.27
40F, Beta C.	- 100F, Beta C.	-4.02
40F, BHA	- 70F, BHA	-15.30*
40F, BHA	- 100F, BHA	24.56*
70F, As.Ac.	- 70F, Beta C.	5.96
70F, As.Ac.	- 70F, BHA	15.30*
70F, As.Ac.	- 100F, As.Ac.	27.00*
70F, Beta C.	- 70F, BHA	9.34*
70F, Beta C.	- 100F, Beta C.	0.25
70F, BHA	- 100F, BHA	39.88*
100F, As.Ac.	- 100F, Beta C.	-20.80*
100F, As.Ac.	- 100F, BHA	28.18*
100F, Beta C.	- 100F, BHA	48.97*

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*Significantly Different at 0.05 level

after time and at temperatures of storage than ascorbic acid and beta-carotene.

The retention of ascorbic acid and beta-carotene each with BHA added only, and that of BHA were compared in cans and pouches in the model system (Table 29). BHA was significantly lower in cans than in pouches. BHA was significantly destroyed more than either beta-carotene or ascorbic acid.

Comparing the retention of the two vitamins in the model system, it was observed that beta-carotene was not better retained than ascorbic acid.

TASTE PANEL RESULTS

Making comparisons of beef stew from cans and from pouches in the taste panel, no significant differences were observed at 40F or at 70F in the four attributes: texture, flavor, color, and overall.

At 100F pouched beef stew scored significantly higher in texture than canned, but no significant differences were observed in the other three attributes.

A temperature comparison of pouched and canned beef stew separately showed no significant differences between the three temperatures of storage, except that canned beef stew scored significantly higher in texture at 70F than at 100F.

TABLE 29

COMPARISON OF RETENTION OF BHA, ASCORBIC ACID AND BETA-CAROTENE IN CANS AND POUCHES IN THE MODEL SYSTEM

Container/Compound	Mean Percent
Pouch, Ascorbic Acid	89.23
Pouch, Beta-Carotene	87.48
Pouch, BHA	73.43
Can, Ascorbic Acid	82.92
Can, Beta-Carotene	87.87
Can, BHA	62.56

Comparison	Mean Difference
Pouch, As.Ac.- Pouch, Beta C.	1.76
Pouch, As.Ac.- Pouch, BHA	15.80*
Pouch, As.Ac.- Can, As.Ac.	6.32*
Pouch, Beta C.- Pouch, BHA	14.04*
Pouch, Beta C.- Can, Beta C.	-0.40
Pouch, BHA - Can, BHA	10.87*
Can, As.Ac.- Can, Beta C.	-4.96
Can, As.Ac.- Can, BHA	20.35*
Can, Beta C.- Can, BHA	25.31*

*Significantly Different at 0.05 level

DISCUSSION

Significantly higher retention of ascorbic acid in both beef stew and the model system in this study was in agreement with the studies reported by Jimenez, 1976; and Abou-Fadel and Miller, 1983. The difference in ascorbic acid retention in thermal pouches versus cans may be due to the longer heating required to process the canned products. These results disagree with Chen and George (1981) who observed that green beans processed in a thermal pouch contained less ascorbic acid than canned ones. However, they reported the retort pouched green beans were overprocessed due to additional time in the pressure cooker after sterilization. With a retort modified specifically for the pouch, better nutrient retention was obtained in beef stew (Jimenez, 1976).

Differences reported in vitamin retention are affected by such variables as size of container, amount of liquid used, time and temperature of processing.

According to Kramer, (1979); Fennema, (1977); and Bender, (1978), ascorbic acid is the most labile nutrient. Guerrant et al., (1945) also found that carotene retention was higher than ascorbic acid. The results of this study show that the degree of retention of ascorbic acid and beta-carotene were influenced differently by the variables involved. Some variables favored ascorbic acid retention and others beta-carotene.

The type of container did not make a difference in the percent retention of beta-carotene in both the model system and beef stew. These results are similar to those reported by Guerrant et al., (1945). They concluded that beta-carotene is highly stable to type of container and storage conditions.

Fox and Cameron (1977) mention that beta-carotene losses due to oxidation during normal cooking processes are small. Consequently, longer heating process of canned than the pouched products did not have a large effect on the destruction of beta-carotene as it did on ascorbic acid.

Guerrant et al., (1945) found that ascorbic acid is affected more by temperature of storage than by time. In this study, comparing time and temperature of storage, it was found that ascorbic acid was affected both by time and temperature of storage. Ascorbic acid in beef stew decreased gradually as time and temperature of storage increased. This was expected, for it is known that oxidation increases as the temperature increases. The speed of free-radical formation is increased. In addition, oxidases, if not yet destroyed will promote oxidation at higher temperatures.

Lee (1983) showed that intercellular air spaces in food, such as in potatoes and carrots, contain available oxygen. The beef stew formulation had chunks of potatoes and carrots whereas the model system did not.

Ascorbic acid in the model system seemed to be protected as the temperature of storage increased. This may have

been due to the fact that samples were analyzed according to the temperature of storage on a given day; therefore, analytical error could account for some of these differences. Another reason could be that ingredients in the model system did not contain trapped intercellular oxygen for oxidation as beef stew did. The difference in protein content and type of protein in each formulation should not be overlooked either. Fox and Cameron (1977) said that protein solutions help stabilization in a purely physical fashion by reducing the rate of oxygen diffusion as well as by the formation of hydrogen bridge between the ascorbic acid carbonyl groups and the sterically favored OH-, NH- , CO and COOH groups of the protein.

Beta-carotene in both beef stew and the model system was affected more by time than by temperature of storage. Beta-carotene has been reported Guerrant et al., (1945) to be highly stable to time and temperature of storage.

Ascorbic acid and beta-carotene retention were not affected by BHA. Heimann (1980) reported that antioxidants must be added above a certain level to protect nutrients otherwise a prooxidative or inversion effect can occur. In this particular study, an explanation could be that some of the initial amount of BHA was lost during processing, consequently, the amount of BHA at initial time of storage was probably less than the minimum amount required to properly protect ascorbic acid and beta-carotene.

Ascorbic acid retention in beef stew decreased gradually in cans as time and temperature increased. In pouches, the retention was higher at 70F, being significant than 100F, but not than 40F. The same type of protection at 70F was observed with beta-carotene in pouched beef stew. The reason why ascorbic acid in pouched beef stew experienced a better protection at 70F than at either 40F or 100F is unknown. It could possibly be that some activation reactions for oxidation were favored at 40F and even more at 100F but not at 70F. No explanation is apparent why this would happen in pouches and not in cans. Samples were analyzed by the type of container and the temperature of storage on a given day; therefore, analytical error could account for some of these differences also.

As expected, BHA in both the model system and beef stew was significantly less retained than ascorbic acid or beta-carotene. BHA is an antioxidant and is more sensitive to oxidation than beta-carotene and ascorbic acid. The chemical explanation for this theory is given by Eskin et al., (1971). The mode of action of an antioxidant such as BHA is to donate a proton to the free-radical which then reverts back to the original molecule so that it cannot form a hydroperoxide. BHA, due to its chemical configuration, can do this more readily than either ascorbic acid or beta-carotene.

The container type also made a difference in the destruction of BHA in the model system and beef stew. It was better retained

in pouches than cans similar to ascorbic acid. Cans having been processed longer might have affected BHA as they did ascorbic acid. Another reason could be that BHA as a phenolic compound can form complexes with iron, tin and other metals. This in the presence of oxidizing food components, an equilibrium may have been rapidly achieved in the presence of the metal walls of the container.

Storage time and temperature effects on BHA retention were almost parallel in beef stew and the model system. It decreased as time and temperature of storage increased except at 70F which in both cases was somehow better protected from destruction. The reason for the anomalous behaviour of BHA at 70F is not clear. Perhaps conditions were favorable for its destruction at both 40F and 100F but not at 70F.

In the taste panel results comparing canned and pouched beef stew, no significant differences were observed at 40F or at 70F in the four attributes: texture, flavor, color, and overall.

At 100F pouched beef stew scored significantly higher in texture than canned, but no significant differences were observed in the other three attributes. Here again the longer heat processing of cans probably had an adverse effect on texture.

A temperature comparison of pouched and canned beef stew separately showed no significant differences between the three temperatures of storage, except that canned beef stew

scored significantly higher in texture at 70F than at 100F. This indicated also that the higher the temperature of storage, the more adverse effect on the texture of the product.

CONCLUSIONS

Significantly higher ascorbic acid and BHA in both beef stew and the model system were retained by pouches than cans. The advantages of pouches over cans start at processing. Pouches require a reduced heat sterilization giving less thermal damage to the food and its nutrients. Therefore, better retention in pouches was probably due to the significantly shorter processing time.

The type of container did not make a difference in the percent retention of beta-carotene in both the model system and beef stew. This is not unusual, beta-carotene has been reported to be highly stable to processing and temperature of storage.

The temperature effect was definitely less striking to beta-carotene than to ascorbic acid in both the model system and beef stew.

Ascorbic acid and beta-carotene retention were not affected by BHA.

Ascorbic acid retention in beef stew showed significant differences between cans and pouches at 70F and 100F. Ascorbic acid in cans was severely decreased at 70F and 100F. Whereas in pouches it seemed to be protected at 70F but drastically decreased at 100F. In the model system pouches retained significantly higher ascorbic acid at all temperatures than cans.

In the model system and beef stew, beta-carotene was severely affected by time but not by temperature of storage.

Ascorbic acid retention in beef stew was reduced by both time and temperature of storage. Ascorbic acid in the model system was reduced significantly at 4 month storage at 40F than at 70F or 100F regardless of the presence of BHA.

BHA in both the model system and beef stew was significantly lower than both ascorbic acid and beta-carotene. This means that BHA acted as an antioxidant and it was oxidized before ascorbic acid or beta-carotene.

In both beef stew and the model system, BHA was more affected by time than by temperature of storage. It appeared to be better retained at 70F than at 40F or 100F.

BHA in both beef stew and the model system was significantly better retained in pouches than in cans.

Thermal pouches for low acid foods offer some advantages over tin cans when the process time is significantly reduced. Nutrient levels can be better retained in pouches.

The deterioration of BHA does not closely parallel the deterioration of either ascorbic acid or beta-carotene. However it does deteriorate at a much faster rate than either nutrient and could serve as a comparison for indicating deteriorative conditions. However, more data should be collected to establish this.

A P P E N D I C E S

APPENDIX A

ASCORBIC ACID DETERMINATION

A spectrophotometric method was used to determine the concentration of ascorbic acid in the samples. The method basically involved the extraction of the ascorbic acid and its subsequent mild oxidation as to convert all the ascorbic acid into dehydroascorbic acid. This was then reacted with 2, 4 dinitrophenylhydrazine producing an insoluble orange compound called 2, 4, dinitrophenylhydrazone. The hydrazone when dissolved in sulfuric acid gives a red solution which was measured by spectrophotometer and compared to a standard.

Triplicate samples were analyzed for ascorbic acid. Fifteen to twenty grams of sample was weighed. Samples were placed in a 250 ml beakers and 90 ml of 5% methaphosphoric acid solution and 10 ml of glacial acetic acid were added. It was stirred and allowed to stand for five to ten minutes, then blended on "high" for two minutes using a Waring blendor with a microblender cup. The mixture was then filtered, first through Whatman # 4 and then through Whatman # 2.

Since the samples contained pigments, charcoal (acid washed) was used as an oxidizing and decolorizing agent in the amount of about one tablespoon for every 100 ml of sample. The mixture was then filtered through Whatman # 42. A clear and colorless filtrate was obtained in most of the cases. When the sample contained starches, a cloudy filtrate was obtained. This was mixed with distilled isopropanol

in a 1:1 ratio, shaken and centrifuged for 2 minutes. A clear and colorless supernatant was thus obtained which was treated as a regular sample.

Two ml of the above colorless filtrate was pipetted into a matched colorimetric tube. The following then were added: two ml of 2% thiourea, 1 ml of 2, 4-DNPH (2, 4-dinitrophenylhydrazine). To a "blank" empty tube the following was added: 2 ml of 2% thiourea and 2 ml of 5% MPA (meta-phosphoric acid). Each tube was covered with Parafilm and mixed. They were incubated in a water bath at 37+- 0.5^{0}C for exactly 3 hours. After incubation the tubes were placed in an ice bath for five minutes. While in the ice bath, 4 ml of 85% sulfuric acid was dropped into each tube during one minutes time. After one more minute in the ice bath to cool, it was removed and allowed to stand at room temperature to complete 30 minutes from the time the first drop of sulfuric acid was added.

At the end of the color development period, 1 ml of 2% 2, 4 DNPH was added to the blank tube which was used to set the spectrophotometer at zero absorbance at 540 nm and the samples were then read as absorbance.

A standard curve was plotted. Absorbance represented the dependent variable and ascorbic acid the independent variable. With the aid of a computer a line of best fit through the points on the standard curve was determined using linear regression. The slope (m) and the y intercept

(b) of the line was recorded. The concentration of ascorbic acid in the sample expressed as mg/100g dry sample weight was determined as follows:

$$C = 0.1 \frac{Y}{W} \quad \text{or} \quad C = \frac{0.1\,(m * x) + b}{\frac{sw * \% dw}{100 + (sw * \% mc)}}$$

Where:

Y= ug ascorbic acid/ml in the final extract read in spectrophotometer.

W= g of dry sample/ml of final extract.

0.1= conversion factor from ug/g to mg/100g

m= slope = 0.0153588178

b= y intercept = 0.0156665151

x= absorbance of the final extract.

sw= sample weight.

% dw = % dry weight of sample.

100= ml of 90ml of 5%MPA plus 10 ml of glacial acetic acid.

%mc= % moisture content of the initial sample.

APPENDIX B

BETA-CAROTENE DETERMINATION

Analysis consisted of extracting the beta-carotene from the sample and reading the concentration by means of a spectrophotometer (AOAC Methods, 1980). It was extracted using acetone: hexane solution, and carotenes separated on a chromatographic column prepared from a 1:1 volume ratio of MgO and filter aid (aluminum oxide). The purified eluted beta-carotene solution was compared to a reagent blank using a spectrophotometer by reading the absorbance at 436 nm.

Triplicate samples were analyzed. Two and a half to three and a half grams of blended, dried food was weighed. The samples were placed in 250 ml beakers. Then 100 ml of water and 100 ml methanol were added along with one teaspoon of filter aid and stirred. A filtration suction apparatous with a safety trap was set up. The sample was then filtered using a filter paper Whatman # 4.

A slurry of filter aid was placed on the filter paper prior to filtration. The sample layer and part of the filter aid bed were scraped into a 250 ml beaker; 100 ml of 1:1 acetone-hexane solution was added and stirred. This solution was filtered through a Whatman # 42 repeating again the above procedure. This time the filtrate was collected as it had the carotene pigments dissolved in the 1:1 acetone-hexane solution. The filtrate was transferred to a 500 ml separatory funnel. Due to the possibility of still having

some carotene pigments on the filter aid bed, it was again scraped and the procedure repeated as above. The filter flask was then rinsed with hexane and added to the separatory funnel. About 50 ml of distilled water was added to the funnel and swirled gently. The lower (water) phase from the separatory funnel was carefully drained. Then 100 ml of distilled water was added, swirled, vented, swirled, vented, and allowed to settle. The water phase was drained off and the process repeated two more times.

Thirty five ml of methanolic potassium hydroxide was added to the funnel, agitated vigorously for 2 minutes, allowed to settle, and the bottom layer discarded. The solution was then washed 3 times with 100 ml portions of distilled water as above. The washed hexane solution containing the carotene pigments was transferred to a 250 ml beaker. Two tsp of sodium sulphate was added to remove excess water. The funnel was rinsed with 10 ml of hexane and added to the beaker.

Chromatographic columns were prepared and the solution was gradually poured onto the column. Once all the solution was poured in, the carotene pigments were eluted with 1:19 acetone-hexane solution.

The beta-carotene fraction was collected, brought to volume with 1:19 acetone-hexane solution and read in a spectrophotometer at 450 nm. A blank tube containing 1:19 acetone-hexane solution was used to calibrate the spectro-

photometer at 0 absorbance. The reading was then compared to a standard curve.

The concentration of beta-carotene was calculated by comparison to a standard curve using the following formula:

$Y = e/50ml \; ((m * x) + b) * \; 100/(sw * \% \; dw)$

Where:

Y = mg bC/100 g dry weight sample.

m = slope = 0.18011

b = y intercept = -0.00022

x = absorbance of sample.

sw = sample weight used.

% dw = % dry weight of the sample.

e = ml to which the final eluate was broght up to before reading in the spectrophotometer.

50 and 100 = constants.

APPENDIX C

BHA DETERMINATION

The concentration of BHA in the sample was determined by gas chromatography (AOAC Methods, 1980) modified due to the high concentration of fat in the sample. Five grams of the powdered food sample was weighed and poured into a 25 x 200 mm glass chromatographic tube which had a small drip tip (6mm od x 50mm long) with a medium porosity fritted disk. The chromatographic tube was plugged at the bottom with fine glass wool. With the sample in the column, another small glass wool plug was placed on top, and with a glass rod, the sample was tamped down to obtain better extraction.

About 50 ml of CH_2Cl_2 was gradually added in order to wash the fat and the BHA out of the sample. The 50 ml eluate was evaporated to about 10 ml in a rotary evaporator. Since the eluate had a high amount of fat, ion exclusion chromatography was used to separate BHA from fat. Bio-beads resin soaked in methylene chloride was used as column material and the 10 ml sample passed through the Bio-beads column with methylene chloride.

A standard solution was previously prepared with a known amount of BHA and fat and mixed in 4 ml of methylene chloride. This standard solution was used to make a calibration curve to determine the elution points of fat and BHA through the Bio-beads column. The eluate corresponding to the BHA fraction was evaporated to less than 5 ml and transferred

to a 5 ml vial. The solution containing the BHA in the vial was evaporated to dryness under a nitrogen stream.

The vial was then diluted to a known volume with methylene chloride. Then a standard, naphthalene, of known concentration was added to the vial. The vial was then mixed thoroughly and a sample was injected into the GC.

A solution of BHA was previously ran on the GC to know its elution time. The elution time of naphthalene was previously known.

The concentration of BHA in the vial was determined by peak area comparison of the naphthalene peak with the BHA peak through the following formula:

$$\text{mg BHA in vial} = \frac{(\text{BHA P.A.})\ (\text{ng naph.})\ (\text{Total volume})}{(\text{Naph.P.A.})\ (\text{ul injec.})\ (\ 1000000\ )}$$

Where:

BHA P.A. = peak area of BHA.

Naph.P.A.= peak area of naphthalene.

ng naph. = concentration of naphthalene in vial in ng.

ul injec.= amount of sample injected in ul.

Total volume = total volume of mixed solution in vial prior to injection.

1000000 = a conversion factor from ng to mg.

The concentration of BHA in the sample was calculated as follows:

$$\text{BHA mg/ 100g dry wt sample} = \frac{(100)}{(w)} (\text{mg BHA in vial})$$

Where: w= (powdered sample weight used) (% dry weight of sample)

100= a conversion factor to mg/100 g dry wt sample.

SENSORY EVALUATION SCORE SHEET

Product: ______________________________ Number: ________________

Please mark on the scales below your own personal preference after you examine this food. Any comments you may have relative to good or undesireable qualities of this food will be appreciated.

Code No. ______

Flavor: --------------------------------- / ---------------------------------
Very Poor Very Good

Color: --------------------------------- / ---------------------------------
Very Poor Very Good

Texture: --------------------------------- / ---------------------------------
Very Poor Very Good

Overall: --------------------------------- / ---------------------------------
Very Poor Very Good

Comments, if any: ____

Code No. ______

Flavor: --------------------------------- / ---------------------------------
Very Poor Very Good

Color: --------------------------------- / ---------------------------------
Very Poor Very Good

Texture: --------------------------------- / ---------------------------------
Very Poor Very Good

Overall: --------------------------------- / ---------------------------------
Very Poor Very Good

Comments, if any: ____

Code No. ______

Flavor: --------------------------------- / ---------------------------------
Very Poor Very Good

Color: --------------------------------- / ---------------------------------
Very Poor Very Good

Texture: --------------------------------- / ---------------------------------
Very Poor Very Good

Overall: --------------------------------- / ---------------------------------
Very Poor Very Good

Comments, if any: ____

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NUTRIENT RETENTION WITH BHA AFTER STORAGE IN FOOD SYSTEMS HEAT-PROCESSED IN CANS OR POUCHES

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ABSTRACT

Thermal pouches and tinned cans, were compared in the retention of ascorbic acid, and beta-carotene in a model system and in beef stew after storage for 4 months at 40, 70, and 100°F. The effect of BHA was measured against controls with no BHA present.

Significantly higher ascorbic acid and BHA in both beef stew and the model system were retained by pouches than cans, probably due to the shorter processing. The type of container did not make a difference in the retention of beta-carotene in both the model system and beef stew.

In the model system and beef stew, beta-carotene decreased more in time than by temperature of storage.

Ascorbic acid retention in beef stew was reduced by both time and temperature of storage.

Percent BHA in both the model system and beef stew was significantly less than ascorbic acid and beta-carotene. BHA was significantly higher in pouches than cans.

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