STORAGE STABILITY OF DRUM DRIED NAVY BEAN POWDERS

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ABSTRACT

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Atmosphere and retort cooked drum dried navy bean powders were investigated to determine their storage stability and to determine the nature of some of the physical and chemical changes occuring during storage.

Sensory evaluations of the powders indicated a maximum shelf life of between 90 and 120 days without the use of shelf stabilizing additives. Storage at 0° C. only increased the shelf life about 30 days over those stored at 40° C.

Major changes were obtained in both the lipid and protein fractions and in the oxygen content of the headspace gas. The data indicated that headspace gas analysis might be the most promising test to use as a quality control method in the determining the rate of degradation during storage.

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INTRODUCTION

The use of legume seeds as a food source in the United States has been on the decline for the last decade, according to the U.S.D.A. 1965-1966, Report Number One, of Food Consumption of Households in the United States. According to Muneta, (1964), one of the contributing factors to this decline probably can be attributed to the long soak or cook time required to prepare these products.

One method of reducing preparation time and increasing the convenience of a legume seed is to produce an instant reconstituting powder by drum drying, as described by Bakker-Arkema, et al., (1968). In addition, when cooked prior to drum drying, the destruction of trypsin inhibitor and hemagglutinin and increase in nutritive value of these powders should help to make them a very desirable food source, (Liener, 1962).

Shelf life of these powders has not been clearly established, although Boggs, et al., (1964), reported drum dried Pinto bean powder had less than a 66 day period of flavor stability when stored at 32°C. in hermetically sealed tin cans with an atmosphere pack. Burr, et al., (1969), indicated that shelf stability of drum dried lima bean powders is less than 60 days when stored in an air pack without antioxidants. Neither of these two forementioned references determined the nature of the deterioration in these powders, but both have had success in the use of antioxidants and inert gas packs to markedly increase storage stability of powders.

Because drum dried legume powders have commercial possibilities, this study was conducted to determine the shelf life of atmosphere and retort cooked Navy bean powders in an atmosphere pack at different storage temperatures. An attempt was made to determine the nature of the flavor degradation of these powders by chemical analysis of the carbohydrate, lipid, and protein fractions, and by sensory flavor evaluation.

REVIEW OF LITERATURE

According to Ponting, et al., (1964). there are three broad classes of chemical deterioration that occur in dried vegetables, the first two of which will be considered here. They are: (1) lipid and essential oil reactions, including mainly oxidation, isomerization, and hydrolysis, and (2) non-enzymatic browning reactions involving carbonyl containing compounds, organic acids, and nitrogenous compounds.

Deterioration of lipids is known to cause rancidity, and by-products have been shown to cause off flavors by Buttery and Teranishi, (1963), Tarladgis, et al., (1960), and Lee and Wagenknecht, (1951).

Some of the lipid oxidation reactions do not require water and in fact are accelerated at very low moisture contents, according to Ponting, et al., (1964). Feustel, et al., (1964), state that: (1) the rate of oxidation is not affected by temperature of storage to any marked degree (the rate is increased only about 1.2-1.4 times for every 18^{o}F. temperature rise); (2) packing in nitrogen greatly retards oxidative change, but has

little effect on rate of browning; and (3) lowering the moisture content accelerates oxidative deterioration but retards non-enzymatic browning. In connection with the moisture effect. Brunnauer, et al., (1938) and Salwin, (1959, 1961), have shown evidence that moisture content for maximum resistance to oxidation is at the value corresponding to a monomolecular layer of water within the internal structure of the solid, and this value varies with different materials. Koch, (1962),gives this value as 5.21% water (as is basis) for Navy Beans.

Lundberg (1962), mentioned the effects of extraneous influences, such as trace metals, in acting as pro-oxidants on lipids. In addition, he summarized the initial stages of the lipid auto-oxidation reaction and the effect of unsaturation. Legume seeds are high in phospholipids (White, 1964), and Koch. (1962), cites evidence that shows phosphatides have an anti-oxidant effect, perhaps by enhancing the protective action of protein on other lipids.

The second class of deterioration of importance in powders is non-enzymatic browning. Hodge, (1953) has divided this into three groups: (1) carbonyl-amino reactions, including those of aldehydes, ketones, and reducing sugars with amines, amino acids, peptides, and proteins; (2) caramelization (which will not be considered here); and (3) oxidative reactions which for example, convert ascorbic acid and polyphenols into di- or poly-carbonyl compounds.

Ponting, et al., (1964), stated that browning of this

sort can be inhibited by removal or combination of carbonyl groups, and often by combination of amino groups, when these are major contributors to browning. They further stated that lowering the storage temperature is very effective in controling browning, giving a decrease of 5-7 fold for every 18°F. drop in temperature. This statement was qualified in that it is product moisture content dependent, the lower the moisture, the less the browning.

METHODS AND MATERIALS

Beans

Phaseolus vulgaris, var. Michelite, Navy beans of the 1968 crop, stored at 32°F. until time of drying, were used in this experiment.

Methods of Cooking

Two types of cooks were used:

(1) Atmosphere cook- beans were soaked and cooked in water at 210°F. for 90 minutes.

(2) Retort Cook- beans were soaked in water at 210°F. for 45 minutes, placed in a wire basket, and retorted in steam at 220°F. for 30 minutes.

Drum Drier

An American 12" x 19 1/8" double drum drier, manufactured by the Overton Machine Company of Dowagiac, Michigan, was used. This drier was set up to simulate a single drum operation by running one drum cold (Bakker-Arkema, et al. 1966). Drying conditions of 23 1/3 r.p.m., 4 layers, and 85 p.s.i.g. were

maintained for both cooks. Moisture content was 5.7% and 6.9% for the atmosphere and retort cook powders respectively. After drying, the sheets were pulverized with a Fitzpatric comminuting mill using a 0.125" screen, and were thoroughly mixed to obtain a homogenous product.

Containers and Storage

Three hundred grams of powder were filled into No. 2 (307 x 409) cans, and hermetically sealed with a semi-automatic double roll closing machine. The containers were then randomly distributed into three different storage cabinets with air circulation and maintained at $0^{\circ} \pm 1^{\circ}C.$, $20^{\circ} \pm 0.5^{\circ}C.$, and $40^{\circ} \pm 0.5^{\circ}C.$ Five random samples were removed from each of these cabinets at 0, 30, 60, 90, and 120 days for analysis and evaluation.

Physical and Chemical Evaluation

Moisture Content: Five 10 g. samples of each cook were taken from containers 12 hours after sealing, and the moisture content was determined using the AOAC, sixth ed. (1945), method for dried vegetables. Moisture was also determined by this method on two samples of each storage temperature lot at the end of the experiment.

Head Space Gas Analysis: A Beckman Model 778 Process Oxygen Analyzer, using a Beckman 76365 Polargraphic Oxygen Sensor in conjunction with a Beckman Head Space Sampler, was used to determine oxygen content of the cans.

Color: Color of the powders was determined using a Hunterlab D-25 L Color Difference Meter. A yellow tile,

number 2814, with values of L=83.0, a= - 3.5, and b= 26.5 was used as a standard.

Acid Value in Lipids: The method for grain of Baker, et. al., (1957) was used for the determination of fat acidity as an index of lipid deterioration. Forty grams of powder were extracted with 100 ml. of benzene in a Waring blender, filtered through Whatman No. 2 filter paper (funnels were covered with foil to prevent excessive evaporation), a 25 ml. sample pipetted into an Erlenmeyer flask, and titrated with 0.0356 N potassium hydroxide to the phenolphthalein endpoint described by Baker, et.al. (1957).

Separation of Glycerides: Thin layer chromatography (TLC) of glycerides was employed using the procedure recommended by Eastman Kodak Co., (1966), to show the development of free fatty acids to further confirm the acid value titration. A 2.00 g. sample was thoroughly mixed with 4.0 ml. of 2:1 (v/v) chloroform/methanol in a 40 ml. standard taper (S. T.) centrifuge tube using a Scientific Products Test Tube Mixer. These tubes were then centrifuged at 2500 r.p.m. in an I.E.C. Model U centrifuge using a number 279, 12 tube, 50 ml. head. The relative centrifugal force at tip was 1570 g.

A 0.05 ml. (50 ul) aliquot of the supernatant was applied to Eastman 5051 Silica Gel Sheets, and developed in 80:20:1 (v/v/v) hexane/diethyl ether/acetic acid solvent for thirty minutes. Knowns were also spotted, and the visualizing reagent was 0.2% 2',7'-Dichlorofluorescein in ethyl alcohol, used with long wave length ultraviolet light (3660 A).

Ultraviolet Absorption Spectra: Conjugated diene and triene lipid components (hydroperoxides and secondary decomposition products) were determined by recording the ultraviolet absorption spectra from 220 m u. to 320 mμ., (Privett, et al., 1953, 1954). A Beckman Model DB scanning spectrophotometer with a scanning speed of 40 mμ. per minute was used. A 2.00 g. sample was extracted with 10 ml. of 2:1 (v/v) chloroform/methanol in a 40 ml. S.T. centrifuge tube and centrifuged as previously outlined. A 3 ml. aliquot was placed in the cuvette, 3 ml. of chloroform/methanol used as a blank, and maximum absorption was determined to occur at 242 mμ.

Carbonyl Determination: 15 ml. of carbonyl free benzene, ca. 2.5 g. anhydrous sodium sulfate, and 5.00 g. of bean powder were thoroughly mixed into a 40 ml. S.T. centrifuge tube using the test tube mixer, and centrifuged as previously described under separation of glycerides. A 5.0 ml aliquot of supernatant was carefully withdrawn, and the method of Henick, et al. (1954), was followed.

Free Amino Groups in Lipid Fraction: The method of Siakotos, (1967), for the determination of free amino groups in lipids was used, with a 0.20 g. sample of powder added to 4.0 ml. of 2:1 (v/v) chloroform/methanol in a 40 ml. S.T. centrifuge tube. The samples were centrifuged as previously described, and a 1.0 ml. aliquot of supernatant analyzed.

Biuret Protein Determination: A 0.5 g. sample of powder was thoroughly mixed with 10 ml. of 1:1 (v/v) 1 N. sodium hydroxide/8 M. Urea using the test tube mixer, allowed

to stand for thirty minutes to insure protein extraction, and then centrifuged. A 0.5 ml aliquot of supernatant was pipetted into a B & L Spectronic 20 cuvette, and 5.0 ml of Biuret reagent (E & M Chemicals of Brinkmann Instruments, Westbury, New York) added. After mixing on a test tube shaker, color was allowed to develope for thirty minutes, and then read at 545 mu. A standard curve was made using protein serum supplied with the Biuret reagent.

Colorimetric Determination of Sugars: The method of DuBois, et al., (1956), was used. The sample was prepared as follows: A 1.00 g. sample of powder was blended with 50 ml. boiling water in a Waring blender for three minutes, and filtered through Whatman No. 5 filter paper into a 125 ml. Erlenmeyer flask (funnels were covered with foil during filtration to prevent excessive evaporation). A 1.0 ml. aliquot was removed from the filtered sample and diluted to 100 ml. In a volumetric flask with distilled water. After thoroughly mixing, a 1.0 ml. sample was transferred to a 1.6 mm cuvette and, 1.0 ml. of 5% redistilled phenol in water, and 5.0 ml. of concentrated sulfuric acid were added. A standard curve was made using D-glucose.

5-Hydroxymethylfurfural Determination: This method was not used until the 90 day storage analysis period, and at this and later dates (120 day storage period) no differences were noted between the three storage temperatures. A 1.00 g. powder sample was mixed into 25 ml. distilled water in a 40 ml. centrifuge tube and centrifuged as previously described.

5 ml. of supernatent were carefully removed, transferred to a 40 ml. S. T. centrifuge tube for a reaction vessel, and the method of Keeney, et al., (1959) followed for the determination of both free and total 5-Hydroxymethylfurfural (HMF).

Sensory Flavor Evaluation

Selection of Panel: Twenty four randomly selected persons from the Michigan State University Food Science Department were selected as an untrained flavor panel.

Preparation of Powders: 375 g. of powder (75 g. from each can), 11.25 g. of salt, and 1500 g. of boiling water were thoroughly mixed in a 2000 ml. beaker, covered with foil, and placed in a 60° C. constant temperature water bath. As individual panalists arrived to taste, the three beakers from the different storage temperatures were uncovered, one ounce samples withdrawn, and the beakers recovered and placed back in the water bath to insure uniform temperature and texture for each sample.

Method of Sample Presentation: A flavor difference procedure and a random method of presentation was divided into three parts to eliminate bias on the part of the judges: the first eight panelists to arrive received three randomly numbered samples, representing 0°, 20°C and 40°C stored powder and a lettered reference sample, ref, that was identical to the first sample; the second eight panelists to arrive received three numbered samples, and a lettered reference sample, ref, that was identical to the second sample; the last eight panelists to arrive received three numbered samples, and a lettered reference sample, ref, that was identical to the third sample. (In all

tests, the first sample was 0°C. Storage powder, the second sample was 20°C. storage powder, and the third sample was 40°C. storage powder). All judges were instructed to compare the flavor of the numbered samples with the reference sample. In each case the difference between the numbered sample and the reference was judged on a scale of five: much worse; worse; no difference; better; and much better. Comments about any or all samples were solicited on the ballots.

Statistical Analysis of Test Results

Analysis of variance using a factorial design (Little and Hills, 1966) including cooks, temperature, and time was used to determine significant differences in: (1) Head space gas; (2) Acid value in lipids; (3) Ultraviolet absorption spectra; (4) Carbonyl determination; (5) Free amino groups in lipids; (6) Biuret protein analysis; and (7) Water extracted sugars. A correlation coefficient (Little, 1966) was determined between all physical and chemical tests that showed differences at the 5% level or less.

Flavor panels were analyzed individually using the above mentioned procedure, but judges, references and temperatures were used as factors, following the procedure of Wiley, et al., (1957).

RESULTS AND DISCUSSION

Physical Evaluation

Moisture content was 6.9% and 5.7% for the retort and atmosphere cook respectively. There was no change in this value with storage. As the powders were both dried under the same drum conditions, some reasons for differences in moisture may be: (1) differences in moisture retention ability during drying between the two cooks; (2) differences in relative humidity on the two days of preparation; and (3) differences in solids content between the two cooks at the time of application to the drum.

Salwin, (1959), states that the moisture content for maximum resistance to oxidation is the value corresponding to a monomolecular layer of water within the internal structure of the solid (Brunnauer, et al., 1938). This value varies with different materials. Ponting, et al., (1964), gave optimum values of 6.5% for potatoes, 3.5% for protein foods like meat, and near zero for fruits and high sugar type foods. Since the monomolecular layer moisture content of these beans is known to be around 5.21% (Koch, 1962) and the retort cook powder was higher in moisture than the atmosphere cook powder (which was close to the optimum value), one might have expected it to have more browning than the atmosphere cook, when in fact they were about equal when comparing Hunter Color-difference values. Tests discussed later in this text may suggest reasons for this lack of difference.

The retort cooked bean powders had a slightly darker (lower L value) color than the atmosphere cooked powders, but less than one full unit on the Hunter Color Difference Meter. Readings on the "a" scale seemed to increase slightly in negative values, and "b" readings seemed to increase slightly in positive values in comparing 40°C. storage temperatures with 0°C. storage temperatures in the latter part of this experiment. However in trying to calculate this change either as hue (a/b) or chroma ($\sqrt{a^2 \pm b^2}$), no meaningful values could be found because of the neutral "a" scores giving large differences when in fact none existed, (Appendix A).

Headspace oxygen analysis was the single test in this experiment that showed the largest changes with both storage temperature and time (significant at the 1% level), (Figure I). There is a difference (5% level) between the two cooks, the retort cook showing an increase in oxygen absorption over the atmosphere cook.

Figure I. A logical assumption from this test would be that the use of antioxidants such as butylated hydroxyanisole (B.H.A.) and butylated hydroxytoluene (B.H.T.), as well as gas impermeable containers, may add considerably to the shelf life of these powders. The results of Boggs, et al., (1964), and Burr, et al., (1969), are in agreement with this conclusion.

In the future, the use of some instrument that has more versatility than the polarographic oxygen electrode in measuring other gases would also be advantageous. This instrument should quantify such things as CO_2 liberation that might occur in a

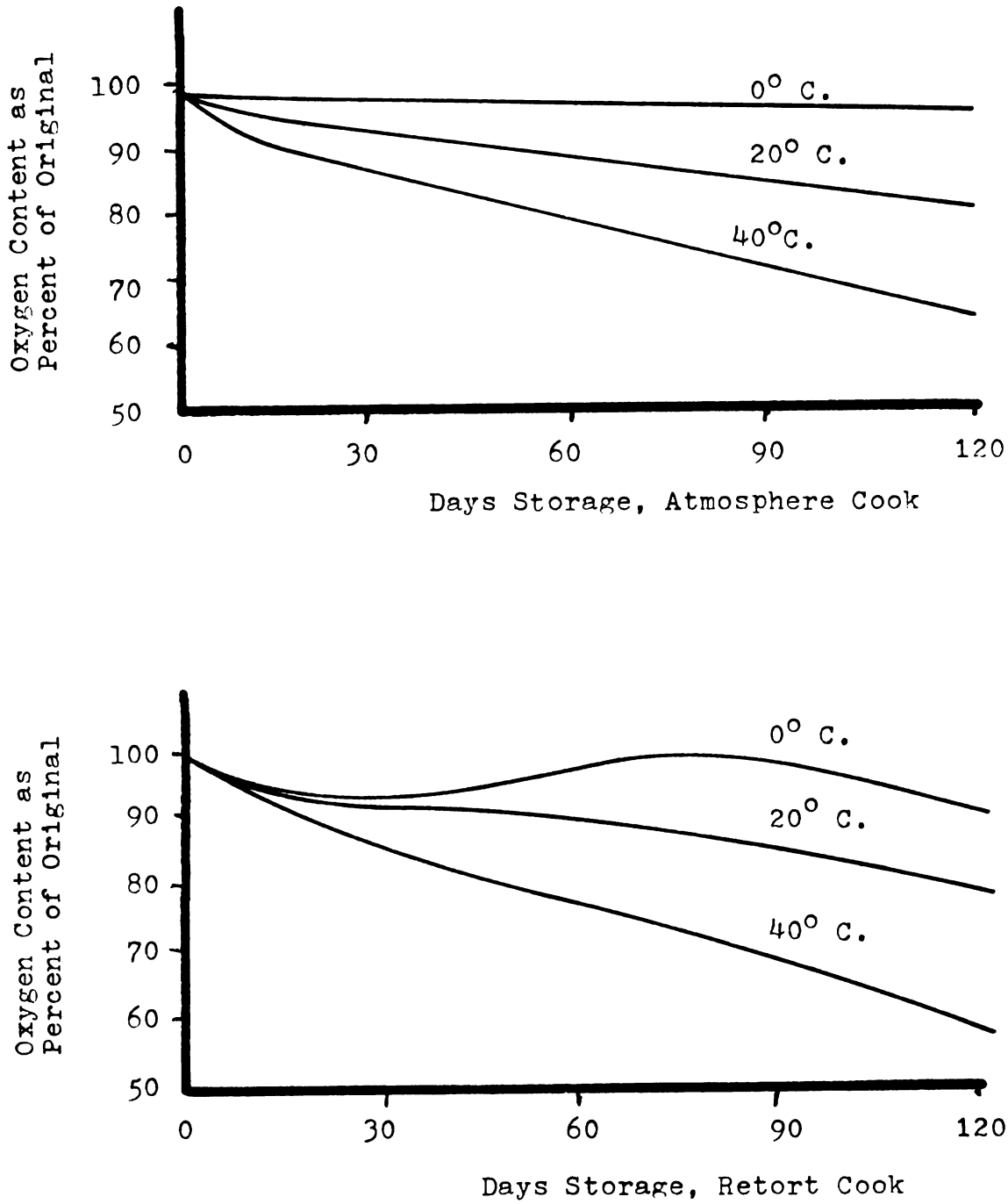


Figure 1. -- The effect of storage time and temperature on the oxygen in the head space. (Data shown represents mean values of 5 replicates)

non-enzymatic browning reaction (Cole, 1967), as well as loss of oxygen.

Chemical Evaluations

Lipid Oxidation: Fat acidity values (Table I) showed a significant loss at the 5% level with time. As one expects an increase in fat acidity with time, it would seem to suggest that the free fatty acids, instead of accumulating, were in fact degrading or becoming unavailable for this determination. In low moisture content powder as is present here, this may be the case, as one would not expect much hydrolysis to occur. This test does indicate that there was a difference between the two cooks at the 5% level, the atmosphere cook powders having higher values.

Table 1. -- Free Fatty Acid Values in Lipids, in mg. KOH/100 g. Powder (data shown represent mean value of 5 replicates)

	Retort Cook					Atmosphere Cook				
	Time									
Temperature	0 Days	30 Days	60 Days	90 Days	120 Days	0 Days	30 Days	60 Days	90 Days	120 Days
0° C.	70.0	68.1	62.3	62.5	62.1	68.7	65.8	65.8	69.4	67.6
20° C.	70.0	63.5	61.9	62.3	61.6	68.7	62.8	65.3	67.4	66.2
40° C.	70.0	64.5	61.2	61.2	62.3	68.7	63.6	67.0	70.8	66.4
Mean	70.0	65.4	61.8	62.0	62.0	68.7	64.0	66.0	69.2	66.7
	70.0	65.4	62.0	62.0	61.8	5%	69.2	68.7	66.7	66.0	64.0	5%

In comparing values calculated in this test with those given by Baker, et al., (1957), for raw beans, it becomes obvious that a large amount of hydrolysis of the lipids must occur during soaking and cooking. Koch, (1962), confirms this, suggesting that in preparation of food materials for dehydration, as was done here, derangements of the natural protective equilibrium of fats may occur, including rupture of lipid micelles and smearing of fat upon new surfaces. It is also to be noted that no large changes in values occurred in this test with changes in time and temperature, and as it is a highly empirical procedure, chances are there would be greater differences between different technicians and laboratories than there would be tetween a wholesome and degraded product.

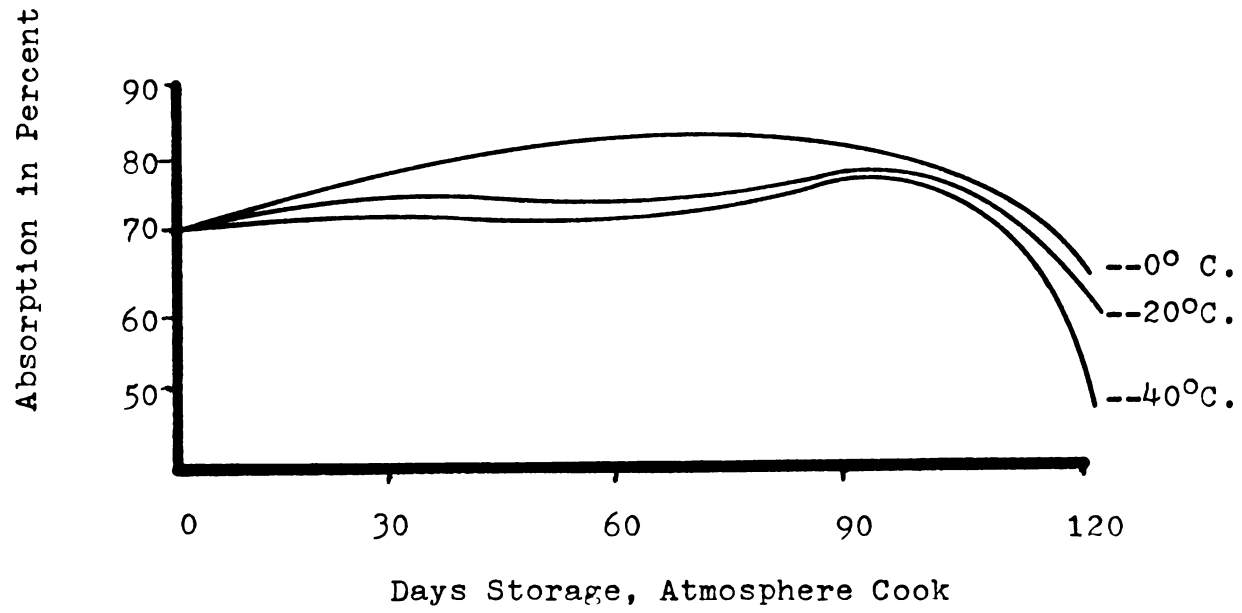
The TLC procedure used in conjunction with the above fat acidity test to confirm the accumulation of free fatty acids was not at all satisfactory. Results were variable, with some unexplainable changes in R_f values occurring between some samples and not in others. As the lipid samples spotted were a composite of all materials soluble in chloroform/methanol, and the test was sensitive only to groups or classes of glycerides (ie, mono-, di-, tri glycerides, and free fatty acids), this variability may have been due to contaminants within the solvent extraction. One other problem was that the majority of the lipids present in these legume seeds are phospholipids (White, et al., 1964) and they did not migrate from where they were spotted. If any additional TLC work is to be done on these powders, cleaning up of the lipid extract and investigation of

the phospholipids would be highly desirable. The phosphatides should be of particular interest because of their implication in acting as anti-oxidants, or as a first component to be oxidized, as was suggested by Koch, (1962). Because of the unsatisfactory results of the TLC procedure, no results of this procedure will be reported here.

According to Corliss, (1968), oxidation of polyunsaturated fatty acids is accompanied by increased ultra-violet absorption in the 232 mu range due to the formation of conjugated diene and triene hydroperoxides. The higher the absorption, the greater has been the exposure to oxygen. Both Figure II and Table 2 show there was not only a significant change in the U. V. absorption with time and temperature, but also a significant difference between cooks.

Table 2. -- Percent Absorption of Conjugated Diene and Triene Lipid Components at 242 mu

	Retort Cook						Atmosphere Cook					
	Time											
Temperature	0 Days	30 Days	60 Days	90 Days	120 Days	Mean	0 Days	30 Days	60 Days	90 Days	120 Days	Mean
0° C.	67	64	72	63	59	65.0	69	71	73	80	69	72.5
20° C.	67	68	80	73	69	71.4	69	80	84	83	67	76.7
40° C.	67	63	74	75	64	68.6	69	74	74	79	57	70.7
Mean	67.0	65.0	75.4	70.4	64.0		69.0	75.0	77.0	80.7	64.4	
Time	75.4	70.4	67.0	65.0	64.0	5%	80.7	77.0	75.0	69.0	64.4	1%
Temperature	71.4		68.6		65.0	5%	76.7		72.5		70.7	5%



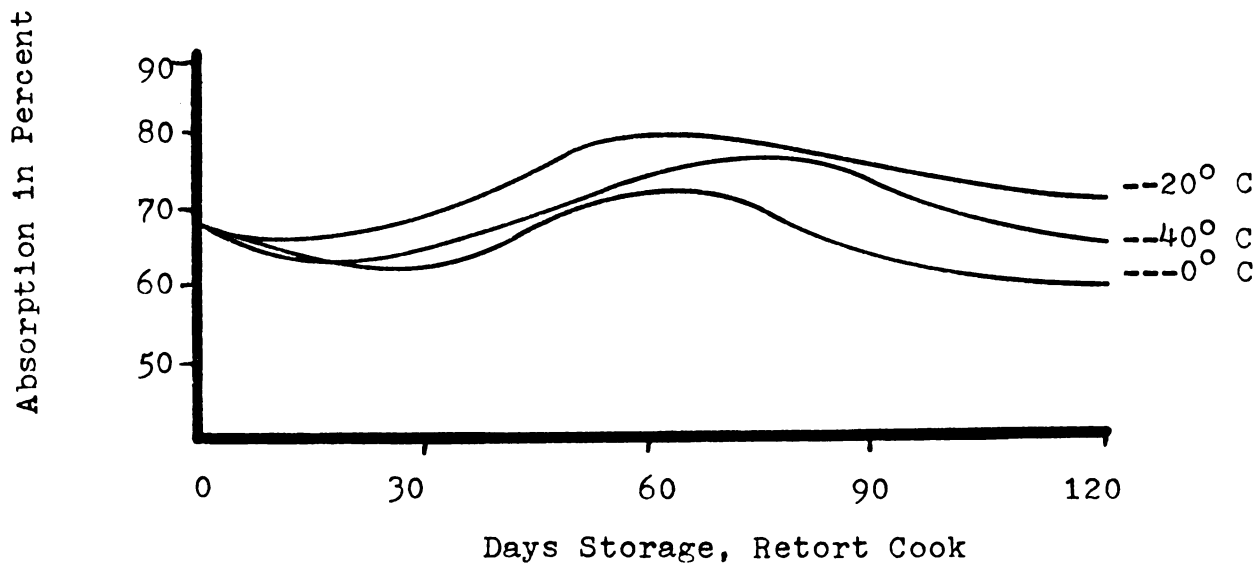


Figure 2. --The effect of storage time and temperature on the ultraviolet absorption of conjugated diene and triene lipid components at 242 mu.

The change in time and temperature may be predicted (Koch, 1962, and Feustel, et al., 1964), but the change in cooks needs further study. It was one of several indications (oxygen uptake, fat acidity, and carbonyl values) that there was a change in the lipid systems due to each of two methods of cooking, and the taste panel results indicated the atmosphere cook was slightly less stable than the retort cook, by a margin of up to 30 days. The panelists uniformly rejected the atmosphere cook 40°C. storage temperature at a period of 90 days, while this phenomenon did not occur in the retort cook until 120 days of storage.

There may be several possibilities that contribute to this difference in cooks: (1) an increase in the oxidation and/or hydrolysis of one cook compared to another; (2) because of the higher heat of the retort cook, sulfhydryl groups may be released (as is observed in high temperature treated milk products, Coulter and Jenness, 1964) and influence the stability of the lipids in this cook; and (3) the possibility that the cooking water had a higher iron concentration during the day of the atmosphere cook compared to the water on the day of the retort cook, due to the variable quality of the water available in the processing laboratory.

Further confirmation that the cooks caused a change in lipid structure, when one is compared to the other, is also presented in Figure III showing the results of the carbonyl determination. There was again a highly significant difference between cooks, with a variation occuring in both saturated and

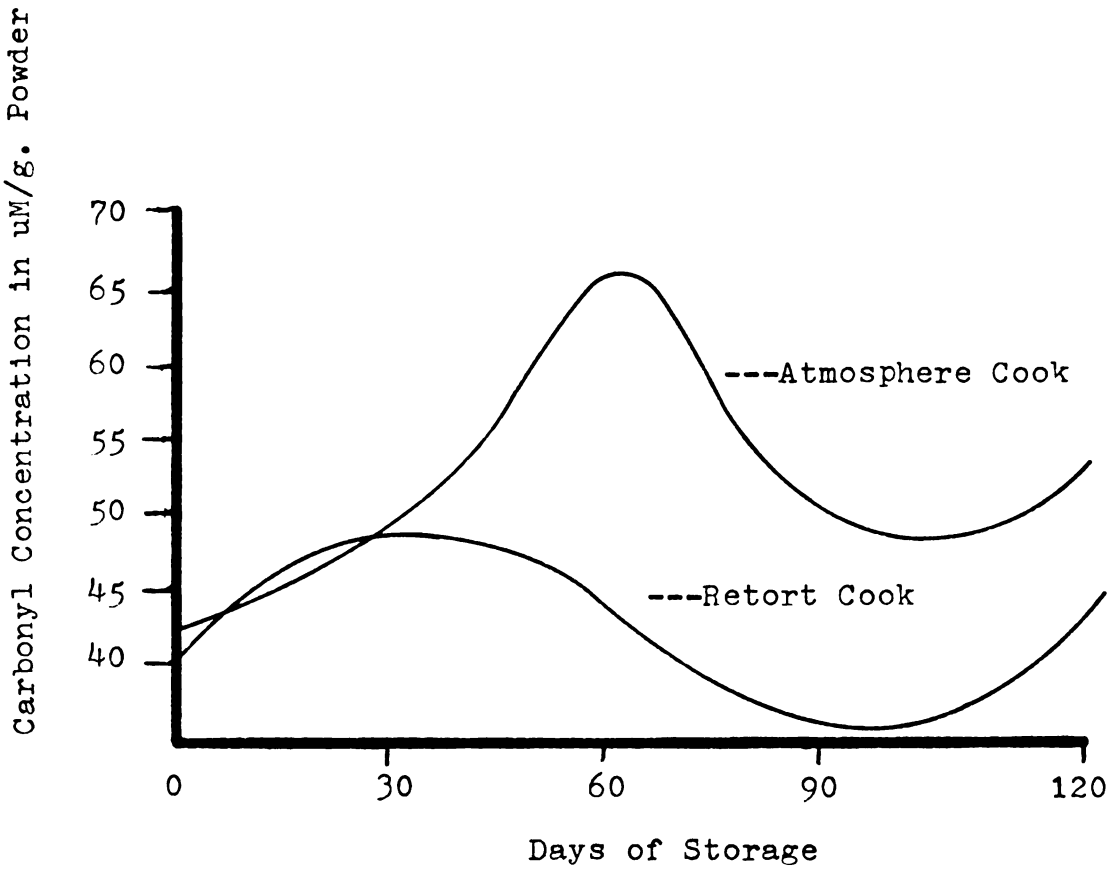


Figure 3. -- The effect of storage time on the quantity of total carbonyls present. (Data shown represents mean values of 15 replicates)

unsaturated carbonyls (see Appendix B) even at time zero, showing that a change in lipid and other carbonyl structures did occur between the two methods of cooking. Unfortunately, these values dropped (after reaching an initial high around 60 days) back to near "time zero" values. This eliminated this test as a method of showing storage degradation for quality control procedures.

The results of the free amino group analysis in the lipid fraction, showed (as did the fat acidity and carbonyl determinations) a decrease in value with storage time. This is in further agreement with Feustel, et al. (1964), that oxidation is somewhat insensitive to temperature. Corliss, (1968), suggests that free amino groups in lipids is involved in phospholipid oxidation, the free amino group value of certain phosphatides being reduced significantly during oxidation, indicating a possible Maillard-type browning and/or interaction with carbonyl groups. However, no statistical correlation between carbonyls, peptide bonding, or free amino groups was found.

Table 3. -- Micrograms Free Amino per Gram of Powder (Data shown represent mean values of 5 replicates)

Temperature	Retort Cook					Atmosphere Cook				
	Time									
	0 Days	30 Days	60 Days	90 Days	120 Days	0 Days	30 Days	60 Days	90 Days	120 Days
0°C.	141.0	142.8	140.4	140.2	140.4	136.7	142.2	139.0	138.0	137.2
20°C.	141.0	142.1	138.4	139.7	139.3	136.7	141.8	137.9	139.5	138.7
40°C.	141.0	141.0	141.8	139.9	140.9	136.7	146.8	142.6	144.9	138.3
Mean	141.0	142.0	140.2	140.0	140.2	136.7	143.6	140.2	140.8	138.1

Atmosphere Cook	143.6	140.8	140.2	138.1	136.7	5%

Non-Enzymatic Browning

The water soluble protein content decreased with time of storage (Figure 4). There was also a difference between cooks with the retort cook being slightly low. The most interesting feature of the Biuret test was that the change during storage showed a positive correlation at the 1% level ($r = 0.959$) with the oxygen level in the head space gas analysis. On the first thought one might think that there was an oxidation occuring of the peptide bonding (which the Biuret test measures) with time, but after some deliberation, one must conclude this could only be a minor contribution at best. The decrease in peptide bonds available to react with the Biuret reagent then, must come about as a result of this bonding being less available to the reagent. As a polar solvent is used in extracting the proteins (1 N. NaOH/ 8 m. urea), the

most plausible explanation was that the proteins were becoming less soluble in polar solvents with time. There are several possible reactions that commonly decrease the solubility of proteins in biological systems: (1) the condensation of (lipid) carbonyls with amino acids, (Braverman, 1963 and Jevons, 1964); and (2) the formation of hydrophobic lipid-protein bonds, (Cornwell and Horrocks, 1964); and (3) the formation of protein disulfide crosslinks (Wall, 1964). The effect of the condensation of reducing sugars and amino acids will be discussed under the boiling water extracted sugar test, and results indicated not much of this occurred in these powders.

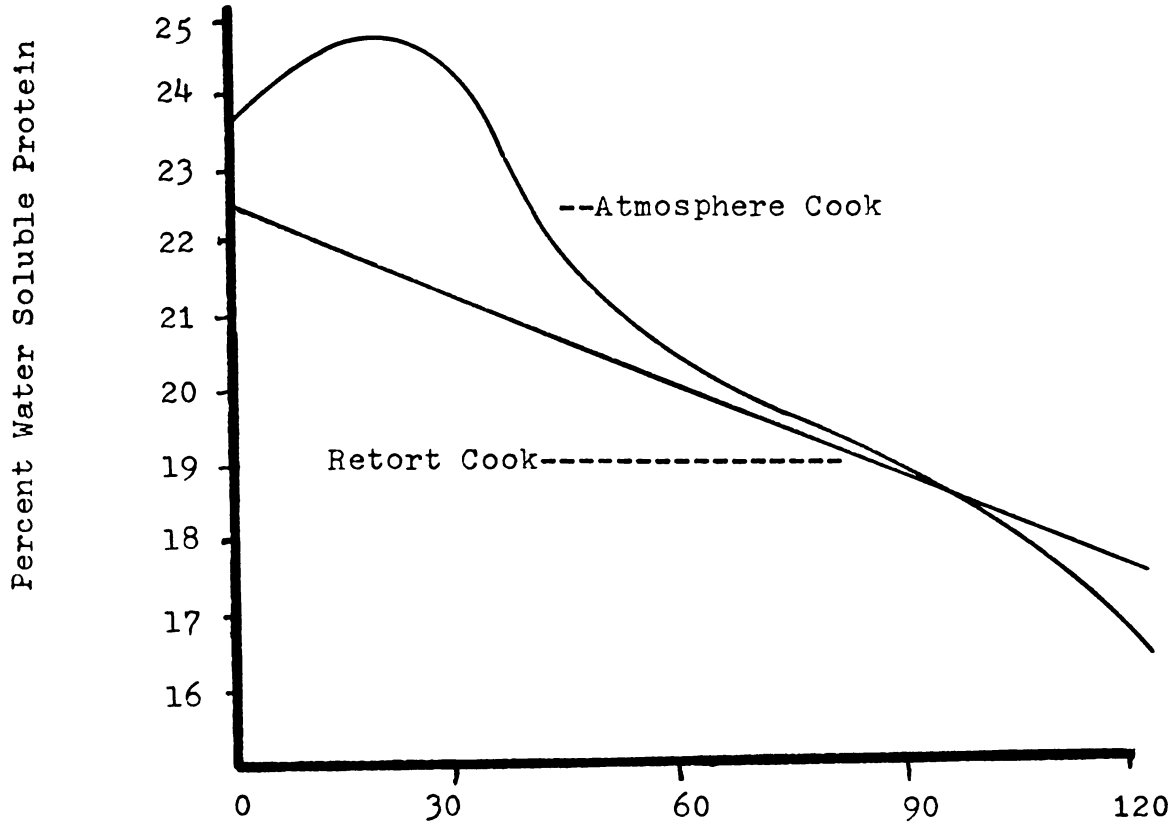


Figure 4. -- The effect of storage time on the values of the Biuret Test (Data shown represents mean values of 15 replicates)

Because of the probable decrease in availability of the peptide bonds, this test has very limited value in measuring non-enzymatic browning in these powders. The use of more sensitive and dependable protein tests are recommended in the study of future browning reactions.

There was one redeeming feature of this test. It may be an indirect indicator of oxidation. However, work will have to be done to improve precision of this method before results (see Appendix A for raw data) between technicians and laboratories could be meaningfully compared.

The boiling water extracted sugars were significantly lower in the atmosphere cooked powders than in the retort cooked powders, as might be expected, due to probable excessive leaching in the atmosphere cook (table 4). However there was no significant change with time or temperature which would indicate non-enzymatic browning. As this is a highly sensitive test (DuBois et al., 1956), one must conclude that sugar-amino acid condensation did not occur to any marked degree. Perhaps other more specific tests for reducing sugars (ie, Ting, 1956, as modified by Furoholmen, et al., 1964) may be more applicable to these powders.

Table 4. -- Milligrams of Boiling Water Extracted Sugar per Gram of Powder. (Data shown represents mean values of 5 replicates)

	Retort Cook						Atmosphere Cook					
	0 Days	30 Days	60 Days	90 Days	120 Days	Mean	0 Days	30 Days	60 Days	90 Days	120 Days	Mean
0° C.	89.8	94.8	88.0	91.7	90.3	91.0	80.7	77.7	81.2	82.0	80.8	80.5
20° C.	89.8	100.3	86.2	86.0	86.8	89.9	80.7	80.0	83.9	85.4	82.3	82.5
40° C.	89.8	93.3	88.1	83.8	85.9	88.2	80.7	76.1	80.6	83.1	82.3	80.6
Mean	89.8	96.2	87.5	87.2	87.7	89.7	80.7	78.0	81.9	83.5	81.8	81.2

No data is reported for the 5-hydroxymethylfurfural test as it was only applied in the last two storage periods and no differences were noted at this time. It is suggested that this test may have application in future browning reaction determination, if one were able to start at time zero. Braverman, (1963), has shown that HMF does absorb at 245 mu, and this product may also have been measured in the ultra-violet absorption spectra discussed earlier.

Sensory Flavor Evaluation

The sensory panel of untrained laboratory personnel was one of the variables more difficult to control in this study. As there was a lapse of 30 days between taste periods, and the panelists available in the building were not always the same, the results were somewhat variable. None the less, it can be said that the atmosphere cooked 40° C. storage sample was no longer "acceptable" at 90 days, and the retort cooked 40° C. storage sample was no longer "acceptable" at 120 days. As there was a statistically significant chemical difference (in the carbohydrate, protein and lipid tests) between the cooks in addition to the above mentioned difference in storage stability, it is a valid statement to say there was a difference in cooks, with the beans from the retort cook being slightly more stable.

It must also be noted that at the 120 day storage period, the atmosphere cook sample held at 0° C. storage temperature was also showing signs of off flavors. One panelist stated that the difference between sample 1 (0° C. storage) and

sample 3 (40° C. storage) was only a matter of degree; both samples had an unpleasant taste. This comment reflected somewhat the other panelists opinions. For additional data, refer to Appendix C.

It is recommended that in future panels only one reference be used throughout the test, and have it made from a sample stored at -20°C. or lower.

General Discussion

It was hoped that this research project would accomplish two objectives: (1) point out the chemical nature of product degradation, and thus suggest which systems (lipid, carbohydrate, and/or protein) contributed most to off flavors, and which controls might best be employed in maintaining quality in the powders; and (2) to find a valid quality control procedure that with further refinement could be used to monitor the quality of a given powder sample.

Where possible, quantitative procedures were used in the measurements to try to show changes with time and temperature. However, most of the tests employed, although showing statistically significant differences with time, probably could not be used as quality control procedures to determine degradation of these powders, because the changes were: (1) relatively small, as with the free fatty acid analysis, and differences between technicians and laboratories would be more significant than those between wholesome and degraded powders; (2) the powders degraded in a "bell-shaped" curve, as with the carbonyl determination, and by the time the flavor panel showed a marked

off flavor, the values of the variable being measured were back close to those of the new wholesome powder; or (3) the test itself had a high coefficient of variation, as with the Biuret determination.

In considering the relative merits of the atmosphere and retort cooks, one must keep in mind that from a point of view of plant overhead and continuous operation, the atmosphere cook has many advantages over the retort cook. These advantages probably more than offset the disadvantages of being lower in carbohydrate, higher in carbonyls, and slightly less stable with time.

SUMMARY AND CONCLUSIONS

This research project showed there were differences in the powders caused by the two methods of cooking. The retort powders not only showed significantly different lipid and protein characteristics, but also a slight trend in increased storage stability over the atmosphere cook.

Oxidation did occur and in significant amounts, as was shown by the oxygen headspace gas, fat acidity, ultraviolet absorption, carbonyl, and free amino group values. This would implicate the lipid system as one of the prime factors in storage degradation, and judging from the change shown in all these chemical tests with time, there is where one should concentrate to stabilize the powders in storage. In this vein, the use of anti-oxidants and inert gas packs should be thoroughly explored if these powders are to be marketed commercially.

Because the lipid fraction is probably involved in storage degradation, further studies should also be directed in this area. The exact nature of the lipid degradation should be investigated, with added emphasis placed on the phospholipids.

Since the protein-peptide bonding showed changes with time (probably due to decreased solubility in polar solvents) and correlation with oxygen uptake, the use of more sensitive techniques such as different forms of chromatography and infra-red spectrophotometry should be employed to elucidate their role in flavor changes. During the storage time of 120 days reducing sugar-amino acid condensation did not seem to play a major role in off flavors. With the use of anti-oxidants and particularly inert gas packs to increase shelf life, this type of browning may become a problem. The use of sulfur dioxide in dehydrated products controls this (Eskew, 1967, and Hendel, 1955), and its use in these legume powders should be considered.

Attention should also be directed towards improving and developing new methods to monitor storage degradation. The one method in this study that showed the most promise is that of head space gas analysis, both to monitor oxidation and as a quality control procedure. One type of instrument available that is more sensitive than the oxygen electrode used in this experiment is a gas partitioner (Moeller, 1967). This is an inexpensive gas chromatograph which employees a single thermal conductivity detector and short columns specific for

the gasses in which one is interested. This instrument could be set up to monitor carbon dioxide content, as well as oxygen, and thus a relative indication on non-enzymatic browning would also be present, (Cole, 1967).

The use of distilled water in soaking and cooking these powders in the future is also recommended to eliminate the possibility of pro-oxidant metals being present in one sample and not in another. This is one variable in this study that was not taken into account, and may have very marked effects on the two different cooks involved.

PROPOSALS FOR FURTHER RESEARCH

New research on these bean powders should include:

(1) The absolute confirmation of the optimum moisture content (mono-molecular layer) at which these powders are most stable.

(2) The study of the use of antioxidants and inert gas packs to increase shelf life of these powders.

(3) The use of a gas partitioner in head space gas analysis to determine quantitatively oxygen and carbon dioxide contents of these powders during storage.

(4) A detailed study of the lipids, including phosphatides, and their role in bean powder degradation.

(5) The use of more sensitive techniques in determining changes in these powders.

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APPENDICES

APPENDIX A

HUNTER COLOR DIFFERENCE METER VALUES

Hunter Color Difference Meter Values *

Storage Time	Storage Temperature	Retort Cook			Atmosphere Cook		
		L Value	a Value	b Value	L Value	a Value	b Value
0 Days	0° C.	78.0	-0.3	14.3	78.7	-0.5	13.6
	20° C.	78.0	-0.3	14.3	78.7	-0.5	13.6
	40° C.	78.0	-0.3	14.3	78.7	-0.5	13.6
30 Days	0° C.	78.2	-0.1	14.4	78.6	-0.5	13.6
	20° C.	78.1	-0.2	14.5	78.8	-0.5	13.7
	40° C.	78.3	-0.2	14.8	78.8	-0.5	13.7
60 Days	0° C.	78.0	-0.0	14.5	78.7	-0.6	13.8
	20° C.	78.2	-0.0	14.5	78.9	-0.6	13.9
	40° C.	78.2	-0.2	15.0	78.8	-0.8	14.2
90 Days	0° C.	77.8	-0.2	14.4	78.6	-0.8	13.6
	20° C.	78.1	-0.3	14.4	79.0	-0.8	13.7
	40° C.	77.8	-0.3	15.1	78.8	-0.7	14.1
120 Days	0° C.	78.0	-0.5	14.2	78.8	-1.0	13.5
	20° C.	78.1	-0.5	14.3	78.8	-1.1	13.7
	40° C.	78.1	-0.6	15.0	78.0	-1.1	14.1

*Data represent mean values of 5 replicates

APPENDIX B

SATURATED, UNSATURATED, AND TOTAL CARBONYLS

Carbonyl Values in Micro-Moles per Gram Bean Powder

Storage Time*	Storage Temperature	Retort Cook**			Atmosphere Cook**		
		Unsaturated	Saturated	Total	Unsaturated	Saturated	Total
0 Days		8.78	30.97	39.75	5.70	38.81	43.51
30 Days	0° C.	7.92	33.11	41.03	12.73	31.97	44.69
	20° C.	2.36	52.63	54.99	16.88	46.39	63.27
	40° C.	3.50	50.17	53.67	13.54	29.35	42.89
60 Days	0° C.	9.27	32.00	41.27	14.75	55.72	70.47
	20° C.	9.03	36.67	45.69	13.36	54.28	67.64
	40° C.	8.12	36.89	45.01	11.28	50.73	62.00
90 Days	0° C.	5.31	32.84	38.15	11.57	39.01	50.58
	20° C.	9.35	29.28	38.63	9.95	44.06	54.01
	40° C.	10.74	21.04	31.78	11.78	35.88	47.66
120 Days	0° C.	12.18	30.43	42.61	13.24	36.97	50.21
	20° C.	12.89	34.56	47.45	16.35	35.57	51.92
	40° C.	13.86	26.05	39.91	17.24	37.80	55.04

Data Represent Mean Values of 5 Replicates

*Denotes significance at 5% level

**Denotes significance at 1% level

APPENDIX C

FLAVOR PANEL SUMMARY

Flavor Panel Summary

Cook	Time 30 Days	60 Days	90 Days	120 Days
Atmosphere	3.04*±0.34	2.8 ± 0.38	3.01**± 0.38	2.8** ± 0.38
Retort	3.12 ±0.27	2.94**± 0.37	2.76* ± 0.37	2.73**± 0.42

Temperature	30 Days		60 Days		90 Days		120 Days	
	Atmos-phere	Retort	Atmos-phere	Retort	Atmos-phere	Retort	Atmos-phere	Retort
0° C.	3.45*	3.25	3.12	3.54**	3.16**	3.62**	3.16**	3.62**
20° C.	2.95	3.16	2.62	2.66*	3.08	2.62*	3.00*	2.70
40° C.	2.70*	2.83	2.63	2.62**	2.12**	2.50*	2.25**	1.87**

* Denotes significance at 5% level

**Denotes significance at 1% level