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Soybean quality loss during constant storage conditions

by

Ibni Hajar Rukunudin

A dissertation submitted to the graduate faculty

partial fulfillment of the requirement for the degree of

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Ames, Iowa

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For the Graduate College

DEDICATION

In the name of Allah, Most Gracious and Most Merciful

A Ph.D. degree represents the highest educational accomplishment in my career, a childhood dream that comes true.

There are no better individuals to dedicate this achievement than to

my father, Rukunudin (may Allah have mercy on him)

for instilling in me a sense of value in learning and the pursuit of knowledge;

my mother, Asma, for her constant prayer and all the pain in bringing me up;

my wife, Sabariah, for her support and love;

and my three little children, Ashraf, Fatin and Izzati, whom I hope will develop the passion to learn and desire to explore the frontier of knowledge.

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thanks for the happiness, joy and love that they have provided during those years. I must say that this accomplishment is as much as theirs as it is mine.

GENERAL INTRODUCTION

The development of an index of deterioration can provide valuable guidelines in minimizing the unnecessary quality loss during handling and storage of agricultural crops. Carbon dioxide evolution in a biological material, as a result of decomposition of its dry matter, can be used as an index of deterioration. Steele and Saul (1962) were earlier workers to use carbon dioxide as a measure of the quality of corn during handling and storage. They measured the amount of carbon dioxide produced from shelled corn during laboratory storage and standardized as per kg of dry matter. The equivalent dry matter loss was calculated using the familiar carbohydrate decomposition model described as the oxidation of hexose sugars ($C_6H_{12}O_6$). The shelled corn were graded according to the United States Grain Grades and Standards. Saul (1967) and concluded that a loss of more than 0.5% in dry matter affects the market grade. It has been further observed that a loss of 1.0% in the dry matter would make shelled corn almost sample grade.

The use of dry matter loss as an index of quality has since attracted many related studies, such as those of Saul and Steele (1966), Steele et al. (1969), Fernandez et al. (1985), Friday et al. (1989), Al-Yahya et al. (1993), and Dugba et al. (1996), which improved the definition of dry matter loss. Multipliers were incorporated into the dry matter loss model to account for the effects of parameters such as breakage, temperature, moisture content, corn hybrid and fungicide. Dry matter loss prediction model was incorporated as a subroutine in an ambient temperature drying model for corn developed by Thompson et al. (1968). The subroutine is to predict the dry matter loss of the top layer of the drying corn and determine

whether drying could be completed before the grain loses quality. Another useful contribution made from several of these studies was the development of an allowable storage time (AST) table (MWPS, 1980). The values read from the table, at any particular moisture content-temperature combination, will give the length of time (days) that shelled corn can be safely stored before it loses 0.5% of dry matter. It is assumed that corn losses some quality within those limits of dry matter loss, but maintains its market grade.

The usefulness of the study to the corn industry has prompted the idea of establishing similar information for the soybean industry.

Soybeans, *Glycine max (L) Merill*, is one the oldest food crops grown by man. Grown first and for several years primarily as a forage and pasture crop and soil improvement purposes, it became a crop of worldwide economic importance only in the last 40 to 45 years to the United States and few other countries like Brazil, Argentina and China. During the last 25 years, world production increased by 350%, while U.S. production rose over 300%, making soybean industry one of the world's fastest growing agricultural sectors. In the United States, soybeans is second only to corn in terms of production value.

Soybeans account for about 50% of the international trade of major oilseeds in 1984/85-1988/89 (Crowder and Davidson, 1989), with the United States being the leading exporter of soybeans at more than 70% as of year 1987/88. The world supply of soybeans is driven by the demand for oil and high protein meal, each contributing about 30.2 and 50.2%, respectively, to the world supply of vegetable oil and feed meal production as shown in figures 1 and 2 (Wynstra, 1980).

Oilseeds, such as soybeans, are at a much higher risk of loss during storage than cereal grains. Under the same environmental condition, the moisture content requirement for safe storage of soybeans is usually 1% lower than for starchy cereal grains such as corn (Sauer et al., 1992). A study in maintaining quality of soybean during handling and storage therefore is a relevant proposition.

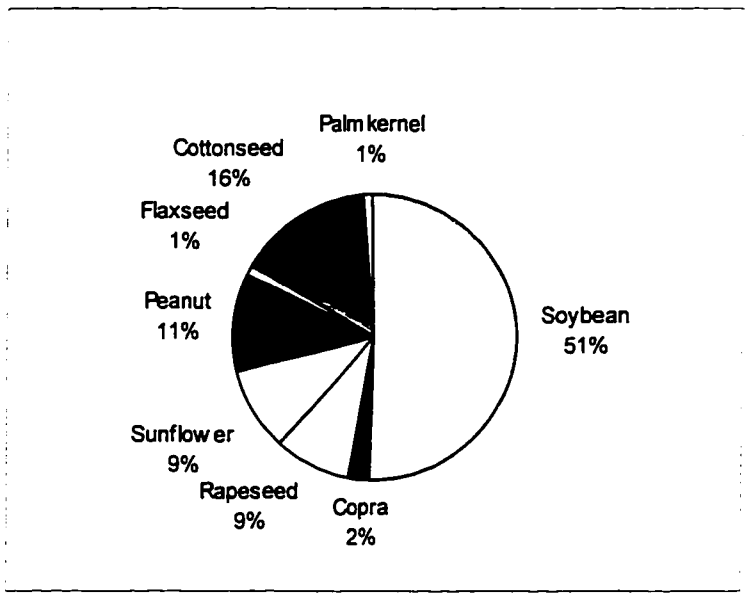

Figure 1. World Vegetable Oil Production

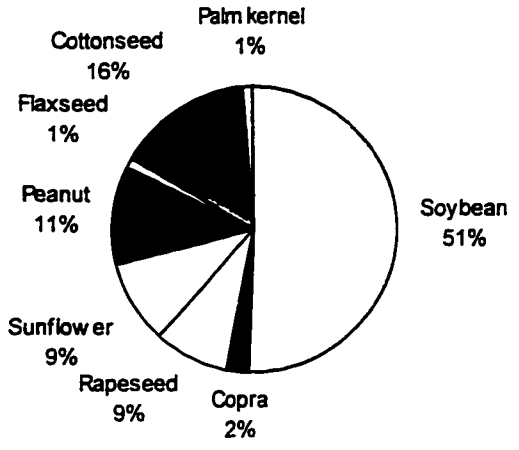

Figure 2. World Meal Production

A similar study of carbon dioxide production as a measure of physical quality of soybean during storage was initiated to relate dry matter loss with quality. Experiments for such a study require that soybeans be preserved at low temperature after harvest to facilitate a continuous supply and uniform quality of soybeans for future work. Low temperature preservation is known to maintain seed viability over a long period of time, but no information is available on the temperature-moisture-content-preservation period interaction effect with soybean quality parameters, such as damaged level and oil quality and the ability to produce carbon dioxide, when use in storage studies. It is therefore important that such information be established.

It is also felt that dry matter loss during storage of soybean be also evaluated with inherent quality such as its oil. One of the important criteria of soybean oil quality is its

percentage free fatty acids (FFA) content in the oil. Though a standard method exists for the analysis of FFA content in extracted oil (AOCS, 1989), adoption of the procedure is limited by the relatively large oil sample size. Laboratory storage experiments are usually carried out with a sample size of about 1-kg. A minimum amount of soybeans for any evaluation of FFA content would require an equivalent of at least 150 soybeans per analysis. Withdrawing such a quantity of soybeans for oil analysis coupled with the need for certain amounts of soybeans for other quality evaluations during the experiment may not be possible. A revised method for the determination of FFA on smaller sample size needs to be developed.

Objective

To develop a better understanding on the rate of deterioration of soybeans during storage and the impact of preservation of samples on the relative rates of quality loss during storage, the present research was conducted with the following objectives:

1. to revise a method of FFA analysis to use a smaller oil sample size

2. to define the rate of deterioration of soybean during storage as influenced by methods of handling and preservation

3. to define dry matter loss of fresh and preserved samples in term of FFA content and total damaged kernels

Explanation of dissertation format

This dissertation is written in manuscript format and consists of four papers. The papers were written in compliance with the formats required for the respective publications.

The first paper, "Effects of field and storage conditions on soybean quality: A review," will be submitted to Applied Engineering in Agriculture, an American Society of Agricultural Engineers (ASAE) publication. The second paper, "A modified method for determining free fatty acids from small soybean oil sample sizes," is to be submitted to the Journal of the American Oil Chemists' Society (AOCS). The third paper, "Carbon dioxide evolution from fresh and preserved soybeans," and the fourth, "Effects of preservation on quality of soybean during storage," are to be submitted to the Transactions of the American Society of Agricultural Engineers (ASAE). As required by those publications, the tables and figures are compiled at the end of each of the text.

Each manuscript contains an abstract, introduction, materials and methods, results and discussion and conclusions. The four manuscripts are preceded by a general introduction and are followed by general summary. The reference cited in the general introduction are listed after the appendices. The references cited in the introduction, materials and methods and results and discussion of each manuscript are listed at the end of that manuscript.

The dissertation also includes five appendices. Appendix A is an attachment describing the American Oil Chemists' Society recommended methods used during the course of the study. Appendix B presents the raw data from the AOCS and revised method of FFA determination experiments and its data analysis. Appendix C describes the calibration of

the carbon dioxide measuring system. Appendix D contains raw data from the carbon dioxide evolution studies, the rates of deterioration of soybean to 0.5 and 1.0% dry matter loss and data analysis. Appendix E is a compilation of raw data on soybean damage and quality and data analysis.

EFFECTS OF FIELD AND STORAGE CONDITIONS ON SOYBEAN QUALITY: A REVIEW

A paper to be submitted to the Applied Engineering in Agriculture

Rukunudin, I. H., and C. J. Bern

ABSTRACT

Field and storage conditions affects the quality of soybeans for processing. Soybean damage prior to harvest, which is influenced mostly by weather conditions, can caused up to 35% damage. This damage can be compounded by damage incurred during harvesting operation. Harvest damage is minimized by harvesting at 13 to 15% moisture contents. Field damaged soybeans are more difficult to store than a normal mature crop. During storage, damage could be inflicted by variables such as temperature, moisture of the microenvironment within the storage facility, and length of the storage period. Normal mature soybeans between 13.0 to 14.0% moisture content, can be stored for about nine months before they become sample grade. Deterioration of soybeans has a profound impact on the extracted oil quality than the meal. Oil extracted from deteriorated soybeans exhibited high FFA content than a normal bean. Damaged soybeans exhibited a faster rate of FFA increased during storage than sound soybeans. The high the FFA content results in high refining losses during extraction. The loss is generally 3 times the FFA content, but a loss of between 5 to 10 times the FFA content is no uncommon. Failure to minimize soybean deterioration at various points during handling results in financial losses to farmers, grain

storage operators, exporters and importers. The financial loss during refining as a result of high FFA content in soybean oil from a typical oil plant that produces 2,400 tanks cars of oil per year had been equated to be about $83,700.00.

INTRODUCTION

The soybean plant is an efficient protein producer and, in fact, yields more usable protein per hectare than any other commonly cultivated crop - at least three times more than rice, wheat or maize. It has been variously referred to as "The miracle golden bean," "Pearl of the orient," "The cow of China," and "The meat of fields". Regardless, soybeans is a proven and yet promising source of plant protein and edible oil, contributing about 50% and 30%, respectively, of the world's supply of feed meal and vegetable oil (Wynstra, 1980).

In the United States, the soybean crop is primarily crushed for oil and the meal is incorporated into animal feed. When raw soybeans are received at the processing plant, they are weighed and cleaned to remove foreign material. Drying is sometimes necessary if the beans are to be stored for a longer period of time or are to undergo a hot dehulling process. The soybean is then cracked into several pieces to facilitates further processing and enhance the separation of the hull from the rest of the bean. The dehulling process removes loose hulls from the cracked beans before flaking. It is in the flaked form that soybean oil is extracted.

The common method of oil extraction is the hexane solvent process. The crude oil separated from hexane is then subjected to a refining process. The principal steps in refining soybean oil are degumming, neutralization, bleaching and deodorization. After removal of

solvent, the spent flakes are ground to meal for use as animal feed. The use of meal for animal feed is not for the protein per se, but for the essential amino acids which are the building blocks for tissues and muscles. Other coproducts of soybean processing are lecithin and various soy protein products.

This paper summarizes field and storage factors affecting the rate of deterioration and quality of soybeans and their products during the time from physiological maturity through processing. Quality factors affecting soybean seed viability are only considered briefly.

SOYBEAN AND SOYBEAN PRODUCT QUALITY

USDA grade

One measure of soybean quality is the USDA grade. U.S. soybeans are traded on specific standards established by the USDA (table 1). The most important quality factors to the soybean processors are moisture, splits, foreign material and damage (Spencer, 1976). All of these contribute to successful storage of the crop for up to 10 months, and to commercially optimum processing yields, operating costs and product quality.

Presence of foreign material with stored soybeans can trigger an oxidative reaction and introduce undesirable pigments, such as chlorophyll. Some of these materials are high in moisture and may cause heat damage in storage. Broken soybeans, splitting and the damage occurring in storage can break natural barriers present in sound soybeans, and initiate enzymatic and biological processes that increase free fatty acids (FFA), nonhydratable phosphatides and metal concentration in the oil. The presence of fine materials has been

associated with increases in the concentration of foreign material along the spout line during loading. A 2% fine materials level can create a 50% concentration of fine materials along the spout line during loading (Christensen and Kaufman, 1975). The tightly packed spout line and the section above it may have minimum contact with the air during aeration; thus creating a favorable condition for fungal spoilage.

Free fatty acids

In general, the quality of soybeans will not affect the quality of soybean meal in terms of protein content and nutritional value as used in animal feed, but soybean quality does affect the quality of soybean oil in terms of free fatty acids (FFA), bleachability, phosphorus content and other factors (Erickson et at., 1993). Deterioration of grain or oilseeds is always accompanied by deteriorative changes in oils they contain. The oil may either be subjected to an oxidative reaction, resulting in typical rancid off flavors and odors or to hydrolysis, resulting in the production of FFA. Development of oxidative rancidity is rarely a problem during the storage of sound soybeans, as compared to hydrolysis of fats. The same observation has been made with raw wheat (Fellers and Bean, 1977). Figure 1 shows the typical reaction associated with the hydrolysis of fat, where fats (triglycerides) are broken down to FFA and glycerol, particularly when temperature and moisture content are high.

FFA level is not a soybean grade-determining factor, but high levels have been the focus of complaints lodged by foreign as well as domestic buyers. To soybean oil buyers, oil FFA content is a very important parameter since it indicates soybean quality and consequent

processing costs. If it is not removed from the crude oil, the end product will taste bitter. The economic effects of high FFA content in oil include higher refining losses, increased use of refining materials, and lower-quality refined oil.

According to Markley (1950), FFA in freshly extracted soybean oil may be present in the seed at the time of harvest or they may have developed in the seed after harvest or before processing. Soybean oils often contain 0.05 to 0.7% FFA (Hiromi et al., 1992) and those levels may increase during harvesting, handling and subsequent storage of the soybeans, especially in the case of abnormally high moisture content, which favors heating and enzyme activity. Hydrolysis of triglycerides is catalyzed by enzyme whose activity is increased by increased moisture.

Refining losses

Crude oil extracted from soybeans must be refined to remove impurities before use by food industries. The refining process yields what is termed "neutral soybean oil," along with undesirable components. The amount of impurities removed is referred to as the refining loss.

A simple way of estimating refining loss is to calculate the difference between the weight of crude oil entering the process and the weight of refined oil out. Specifically, refining loss is the sum of loss in weight of an oil during the removal of FFA and other impurities plus entrained neutral oil in soapstock during the normal alkali refining process. Refining losses, which involve substantial neutral oil loss, are influenced by the amount and kind of impurities in the oil.

FFA is used as an indicator of oil refining loss in soybeans and some other oilseeds. In kettle refining of coconut oils, for instance, the total loss usually does not exceed about 1.4 times the amount of FFA removed. In vegetable oils, such as cotton seed and soybean oils, the loss is generally 3.0 times the FFA content and the loss of between 5 and 10 times the FFA content is not uncommon. Several different formulae are used in estimating the percent refining loss. One simple example (Lusas et al., 1988) is given by the equation [1]:

$$\% \text{ Refining Loss} = 0.5 + \% \text{ phospholipids} * 1.5 + \% \text{ FFA} * 2.0 \quad \text{.....[1]}$$

The normal range of refining loss for soybean oil is between 1 and 1.5% (Robertson et al., 1973), and can be as high as 10% or even 15% for oils containing high percentage of phosphatides (Markley, 1950). Urbanski et al. (1980) calculated the dollars lost for a typical oil plant that produces 2,400 tank cars of oil per year. Using the industry dockage formula for neutral oil loss and a price of 9.75 cents per pound, an 18% increase in neutral oil represents an annual loss of $36,000.00. Based on the current market price of 23 cents per pound, the annual loss would be equivalent to $83,664.00.

Soybean oil compositions

During refining operation, concentrations of FFA together with phosphatides, unsaponifiable matter and trace metals are reduced. A typical composition of crude and refined soybean oils are shown in table 2. The refining process reduces FFA in the crude oil

by 83.3 to 92.8%. The maximum levels of FFA content, as set in the Yearbook and Trading Rules (1993-94) of the National Oilseed Processors Association (NOPA) are higher at 0.75% for crude degummed oil, 0.1% for once-refined soybean oil and 0.05% for fully refined soybean oil (NOPA, 1995).

Unsaponifiable material consists of compounds such tocopherols, sterols, phytosterols and hydrocarbons, ketones, alcohol which are not saponified by alkali but which are soluble in ether or petroleum ether. During typical oil processing operation, the concentrations of these compounds are reduced, however some are still remains in the final refined oil.

Lovibond color

Lovibond system of color measurement is one of the AOCS methods recommended for determination of color of the lighter refined oil and bleached oil and also of shortening and other oil and fat products. The presence of color in the crude oil may markedly affect subsequent processing. The determination of color is made by matching oils with the tinted glass in a tintometer, a 13.3 cm column of oil against red and yellow Lovibond color glasses. The corresponding red and yellow glasses scales that match the oil color is taken as the color of the oil. The method is not suitable for oils that are excessively dark or that contain colored substances other than red and yellow in high concentration The maximum Lovibond color for fully refined soybean oil is 20Y/2.0R.

PREHARVEST QUALITY

One of the most important considerations in storage is the quality of crop entering storage. High quality soybeans store well under relatively adverse conditions, while badly deteriorated seeds store poorly even though conditions are quite favorable. The most modern conditioned storage facility cannot really compensate for delayed or incautious harvesting, inadequate or improper drying, rough handling, and poor bulk storage.

Preharvest damage

Deterioration is traditionally associated with storage. But for some cereal grains and oilseeds, deterioration can begin prior to harvest, during the period following physiological maturity. This postmaturation preharvest period is considered by some to be the first segment of the storage period. The field environment during this period can be unfavorable for storage. Frequent and prolonged precipitation, high humidity and low temperature can cause what is termed weather damage. Weather damage refers to frost, freeze or field damage and also damage caused by drought and hail.

Weather damage

Severe deterioration of seeds can occur before they are even harvested, especially when harvest is delayed. Mature soybeans remaining in the field during prolonged wet weather can become field or weather damaged (Krober and Collins, 1948). The dampness can cause molds to grow rapidly, turning soybeans brown. During the harvest season, continued

wet weather, including heavy rain, has caused up to 35% field and storage damage to soybeans (Robertson et al., 1973).

The occurrence of cold weather during the growing period before full maturity can lead to frost damaged soybeans, rendering them green in color with low protein, low oil and high carbohydrate (Fulmer, 1988). Frost or freeze damage can occur when the temperature during the early harvesting season is low. Frost damage occurs when the ambient temperature drops to -2.2 to -1.7°C, and freeze damage occurs when the temperature drops to below -3.9°C (Sternberg et al., 1990). Indeed, green beans can lead to green salad oil if the processor doesn't employ extreme measures. Frost damaged beans were noted to have poor keeping quality. For example, surface-sterilized seeds from frost injured lots was found to have 18 to 40% *Alternaria* and other strains of fungi (Ramstad et al., 1942). With increased damage, there was an accompanying increase in the aerobic microfloral content. It is anticipated that immature and frost damaged soybeans would present a more serious storage problem than sound, high-grade soybeans. Frost injury might be expected to render the seed more susceptible to attack by microorganisms and result in increased internal microflora.

Weather damage to soybeans is also known to alter some chemical composition in the beans. The most widely cited quality loss due to damaged beans is the increase free fatty acids (FFA) content in the crude oil (Krober and Collins, 1948; Urbanski et al., 1980; Henderson, 1987). Other forms of quality parameters include Lovibond color (Robertson et al., 1973) and the lower flavor quality of refined oil (Sander, 1944; Hutchin, 1945). In a study to determine the extent of oil quality deterioration from field and storage damaged soybeans

in Georgia, Robertson et al. (1973) found that FFA content in field damaged samples was about 2.5% compared to 0.4% in undamaged beans. The Lovibond color of the extracted oil samples was 70Y-3.0R and 70Y-3.5R for undamaged and damaged soybean respectively. In a similar study by Urbanski et al. (1980), the same observation was made on the levels of FFA in field damaged, freeze damaged and undamaged soybeans. The FFA contents in field and freeze damaged beans were higher than in undamaged beans (table 3).

It has been reported that the FFA levels of the 1985-crop soybean, the year which initiated the tightening of the US grading standard, ranged from 0.92% to 4.57% (Henderson, 1987). The main processing concern of weather-damaged soybeans is that the oil is more costly to refine (Sanders, 1944) and even after refining it is often not of an edible grade. There was a suggestion that field-damaged soybeans should not be used for direct human consumption.

Soybeans damaged by early frost or storage also may contain increased amounts of non-hydratable phospholipids. These phospholipids are poorly recovered by hydration, and the resulting degummed oils have high levels of phosphorous containing compounds. These oils are difficult to process during later hydrogenation (Nash et al., 1984).

Effects of early harvest

Early harvesting, on the other hand, may increase the percentage of immature soybeans. Even during a normal year, harvested soybeans consist of not only mature sound soybeans but also immature or green soybeans. Yao et al. (1983) found that crude oils of

immature beans were greener in color and higher in FFA content than those of mature beans. Upon storage, the FFA content increased at a faster rate in the immature beans than in mature beans. However, the green color was more easily removed in refining than the red color caused by other field damage (Krober and Collin, 1948).

SOYBEAN HARVESTING

At the time of physiological maturity, the soybean has the highest quality for most uses.

Optimum harvest moisture

Since most grains attain maturity at moisture contents too high for efficient mechanical or even hand harvesting, they are, in effect, stored in the field from maturation to harvest. Soybeans are generally harvested at any time after the seeds are mature and the foliage is dry. At moisture contents higher than 18%, threshing is, however, difficult and harvesting can result in bruised and crushed soybeans. These damaged soybeans pose a problem during subsequent handling and storage, where fungi, discoloration and heating can develop in the crevices of machine damaged beans. Sound soybeans present a less hospitable medium for mold mycelial penetration and growth than do the highly damaged seeds (Milner and Geddes, 1946). Available evidence (Barger and Weber, 1949; Wilcke and Misra, 1984) suggests that the optimum range of moisture for combine harvesting of soybeans is between 13% and 15%. Below 13% moisture, seed cracking and splitting increases sharply as

moisture content decreases, while seed bruising and other less visible but still detrimental injuries increase at moisture contents above 15%.

Harvesting at 13% may also result in soybeans having the highest premium in the market, thus optimizing the financial return to a farmer. The U.S. soybean grade once used moisture content as one of the parameters of soybeans grade; 13% moisture was classified as Grade number one. Although moisture is no longer a criteria, allowing soybeans to dry down in the field to less than 13% causes weight loss which leads to a monetary loss.

Haugh and Bartch (1977) found moisture content and combine cylinder speed to be the most significant variables influencing mechanical damage, as evidenced from germination percentage. In a moisture range of between 10 to 15.5%, an average reduction of 6.1% in germination was caused by the gathering, feeding and primary threshing functions. Separation and cleaning accounted for an additional 3.3% decrease in viability. Invisible damage caused by mechanical handling resulted in abnormal seedlings from soybeans which appeared sound. Harvesting at a lower moisture content of 10% could also result in yield reduction due to shattering loss, which can be as high as 8% (Byg, 1969).

SOYBEAN HANDLING

Several studies have been conducted to determine physical and rheological properties of grain in order to predict the reaction of seed under actual handling circumstances. Soybeans may be handled 15 or more times before they reach the processors or crushing

plants, and 25 or more times before they reach a foreign buyer's end-use point (Paulsen, 1973).

Several studies found that soybeans exhibit optimum capacity to absorb compressive load in a moisture range of 11 to 14%. Bilanski (1966) found that under gradually applied loads, on the average, the force required to initiate seed coat rupture declined from 57.8 N (13 lb) at 11% moisture content to 44.4N (10 lb) at 16%. Paulsen (1978) confirmed these values when he found that the force to initiate seed rupture decreased as moisture content increased. With horizontal hilum position, the force of 144.0N was required to rupture 8.1% moisture soybean sample and only 38.2 N at 17% moisture content. Toughness, the energy absorbed by soybeans prior to seed coat rupture per unit of soybean volume, increased with moisture to a maximum value of 11 to 14%. At 1.0 mm/min deformation rate, the optimum compression energy absorbed by three soybean varieties under vertical and horizontal helum positions, range between 0.18 to 0.55 mJ/mm^3. Among Amsoy-71, Corsoy and Williams tested varieties, Corsoy appeared to have the highest capacity for absorbing compressive energy among the three varieties tested. Under impact, as in most handling situations, it was postulated that soybeans could withstand higher energy levels than those obtained in the study. Soybeans may be subjected to impact loading in threshing cylinders, centrifugal discharging of vertical bucket elevators, filling and discharging of screw conveyors, spouting and free-fall dropping. Damage is dependent upon the particle velocity immediately before impact and the surface against which impact occurs. Fiscus et al. (1971) concluded that

breakage of soybeans and corn increased exponentially with impact velocity. Equation [2] is a general equation developed for corn and soybeans.

$$B = cV^n \quad \text{.....[2]}$$

where B = percent breakage
V = velocity, in per s
c and n = constants varying with grain type, moisture and temperature

For soybeans between 11 to 11.5% moisture content at 0 to 3.9°C, the values of c and n are $2.6*10^{-6}$ and 2.2, respectively. At 12 to 12.5% moisture and 5 to 10°C, the values are $8.1*10^{-6}$ and 1.5, respectively.

SOYBEAN STORAGE

It is well known that in commercial practice, soybeans may normally be stored for periods of up to one year or longer before being processed into oil and meal. For seed, germination and seed viability are paramount but for soybeans that go into the commercial channels for crushing, FFA content is of prime importance.

There is a relationship between moisture content and storage period for market stock soybeans. Holman and Carter (1952), in their studies on the effects of initial soybean-moisture content on grade and other quality changes in farm storage, found that soybeans with an initial moisture content of less than 10% did not change in grade after nearly four years. A summary of the relationship is shown in table 4.

Soybean seed storage

Soybean seeds are known to be inherently short-lived (Delouche et. al., 1973).They deteriorate more rapidly than seeds of rice, corn, sorghum, wheat and many other types of seeds under the same conditions of production, harvesting, drying and storage. Of the major economic crops, only shelled peanut seeds are more short lived than soybean seed. According to Byrd and Delouche (1971), it is unusual to attempt to 'carry over' soybeans from one season to the next because of their relatively short storage life. Justice and Bass (1978) classify soybeans in the least storable group in their relative 'storability index'. Mills (1989) described and illustrated several cases of catastrophic losses of various kinds of seeds and other agricultural products. He regarded oilseeds as a much higher risk than cereals. There is a graph illustrating the relationship between temperature during storage and number of days of allowable storage time (Spencer, 1976). Soybean seeds at 22% moisture content can be stored for 2 days at 21.1°C grain temperature. At 14% moisture content and with the same temperature, the allowable storage time is 42 days.

There could be several reasons for its poor storability. The most interesting observation was of an earlier work of Zabolotskii and Barsukor (1932), original work was not examined but was cited by Markley (1950), that soybeans absorb moisture faster and retain it longer than other oil-bearing seeds.

Temperature and moisture effects

Temperature and moisture content are the two most important variables affecting deterioration of grain in storage. The significance of moisture content was demonstrated in great detail by Holman and Carter (1952) in one of the earlier studies, between 1944 to 1946, on storage of soybeans. Under diurnal air temperature fluctuation of between 22 to -6.7°C, they found that soybeans, when placed in storage bins at moisture contents of 10% or less, maintained their grade for four years. Germination, however, decreased moderately after the second year and rapidly after the third year. At 12 to 12.5% moisture content, the stored soybeans maintained grade for 3 years before they became musty. Germination, on the other hand, decreased considerably in the first year and was almost zero after three years. When stored at moisture contents of 13 to 14%, the soybeans became sample grade after 10 months; there was also a drastic drop in germination.

There is also an interaction between moisture and temperature for safe storage as emphasized by Sauer et al., 1992. At 14.0 to 14.5% moisture content, soybeans can be stored for several years without invasion by storage fungi and without reduction in quality if the temperature can be maintained at 5 to -6°C. At 30°C, and with the same moisture content, soybeans will be invaded by storage fungi within weeks. Although it may be economically infeasible for commercial storage application, soybeans can be safely stored below 5°C and below 11.5% moisture content for a considerable period of time (Kaufman, 1969). Other studies on the interaction of temperature and moisture during storage of soybeans also showed similar trend (Ramstad and Geddes, 1942; McNeal, 1966).

The interaction of temperature and moisture content means that there is a safe limit of moisture content for a particular temperature and duration. The goal of storage is to store soybeans as high a moisture content as is safe with zero deterioration, minimum grade changes, low overall cost and minimum handling.

Fungi in storage

It is the relative humidity of the intergranular atmosphere that influences storage life of stored soybeans. Microorganisms are the principal agents responsible for deterioration of stored soybeans having a moisture content above a certain critical limit (Markley, 1950; Christensen and Kaufman, 1969). Some of these fungi do not invade soybeans before harvest, but inoculum of these fungi is ever present and if storage conditions are favorable to the growth of the fungi, the soybeans will be invaded. The emphasis therefore must be, not on the avoidance of inoculum, but on the maintenance of conditions in storage that will not permit the storage fungi to develop. Infestation, growth and reproduction of both storage fungi and insects are strongly influenced by relative humidity of the microenvironment of the beans in the mass.

Since the soybean moisture content and intergranular atmospheric relative humidity are in equilibrium during storage, maintenance of a safe moisture content requires an average level of relative humidity in the storage environment no higher than that in equilibrium with the desired soybean moisture content.

A large quantity of literature has been written about the storage fungi and their relative growth in environments of different relative humidities. Christensen and Kaufman (1969) reported that fungi generally cannot grow at moisture contents below that in equilibrium with a relative humidity of approximately 65%. This is evident from the inability of drought-resistant storage fungi *A. restrictus* and *A. halophillian* to grow in seeds whose moisture content is below that in equilibrium with relative humidity of 65%.

Among the many fungi which naturally inhabit healthy soybeans, only a few are involved in storage deterioration within the moisture content range of 11 to 23%. Table 5 summarizes the ranges of relative humidities, moisture contents and the different species of fungi known to be involved in deterioration of stored products. *Aspergilus* and *Penicillium* are the most common species encountered in storage. Milner and Geddes (1946) attributed most of the deteriorative activity in moist stored soybeans to the growth of *A. glaucus* and *A. flavus*. Kennedy (1964) found *A. glaucus* to be the predominant fungus in 28 samples of soybeans collected from elevators in five states.

Milner and Geddes (1946) observed that at ordinary temperatures, there was marked acceleration in the respiratory activity of soybeans between 14 and 14.6% as contrasted to the very low and relatively constant respiration rate at moisture contents between 8.5 and 14%. They suggested that there was active biological activity occurring between the narrow range of 14.0% and 14.6%. The additional activity was found to be the fungal growth. The equivalent relative humidity at that range of moisture content is 74.0 and 76.2%. Based on

their observation and several other studies by investigators before them, a relative humidity of 75% was considered a minimum for fungi to grow at ordinary temperatures.

Ramstad and Geddes (1942) were the first to associate the respiration of fungi and their growth with decreases quality of stored soybeans. Extremely high respiration rates can occur in highly damaged seeds at moisture values favorable for fungal growth. The increased nutrient availability to the fungi, as might be expected to damage seeds, tends to decrease the humidity requirement for fungal growth. Thus, sound seeds, such as those of the high quality sample with their unbroken seeds coats, present a more inhospitable medium for mold mycelial penetration and growth than do the damaged seeds, in which the majority of the seed coats were ruptured. Bailey (1921) demonstrated that cracked, shrunken immature kernels respire more rapidly than sound grain of the same moisture content; the presence of foreign material and of sprouted, frost or heat-damaged kernels also was shown to increase respiration. Mechanically damaged corn deteriorated about 3.5 times faster than hand-shelled kernels (Sauer, 1992).

Insect in storage

Like fungi, infestation by storage insects is also encouraged by moisture and temperature. Continuous growth and development of insects results in spoilage and infestation of soybeans.

Most insects do not develop or feed on stored grain at temperatures below 10°C. At 0°C, the insects will eventually die. Most insects usually require a bulk temperature of above

20°C to reproduce rapidly (Mills, 1990). Insects cause little problem if moisture content was low enough for satisfactory storage and bins are made weathertight. Farm storage studies under Illinois condition by Holman and Cater (1952), concluded that insects are not a serious problem during storage of soybeans at 12% moisture content or less for a period of two or more years. With the exception of granary weevil (*Sitophilus granarius*) and the rice weevil (*Sitophilus oryza*), insects found in infested stored soybeans are generally the same as insects found in stored corn. The Indian meal *moth (Plotia interpunctella*) has been found to be present at any time during the study. Its feeding habits confined largely to split beans in the upper layer of the stores.

Changes in free fatty acid content during storage

FFA content of stored soybeans has long been used as a sensitive index of initial grain deterioration (Zeleny and Coleman, 1938). When soybeans are stored, oil quality continues to decrease, as measured by an increase in FFA. Holman and Carter (1952) sampled soybeans from 70 full-size farm-type bins and on analysis of FFA content, measured in term of acid values, showed some variation at different moisture contents during storage at ordinary temperatures (table 6). The highest FFA content recorded after one year of storage at the normal storage moisture content of 13.0 to 14.0% was 1.95%.

McNeal (1966) conducted a 12-month laboratory study using soybeans with different moisture contents and two storage temperatures (10 and 26°C (table 7). The increase in FFA content after 12 months period, was high in soybeans with high moisture content and high

storage temperature. There was a four-fold increase in the FFA content at 14.6% moisture content at 10°C as compared to 13-fold increased at 26°C.

A worst case example of FFA development in stored soybeans was reported by Robertson et al. (1973) where excessively damaged soybeans contained oil with an FFA of 47.1% and a Lovibond color of 70Y-20.5R compared to 70Y-3.0R. The rate of increase in FFA content in soybeans increases as the quality of soybean decreases. Urbanski et at. (1980) showed that there was a linear relationship between FFA increase with months of storage (figure 2), the rate was higher with badly damaged beans. Iverson and Koeltzow (1986) established a linear relationship between FFA and damaged kernels total (DKT) as described by equation [3]:

$$\%FFA = 0.159\ (\%DKT) + 0.915 \quad \text{.....[3]}$$

where FFA = Free fatty acid, %
DKT = Damaged kernels total, %

TRANSPORTATION

In the international marketing of oilseeds and their products, the greatest concern for quality preservation during shipment has been for the soybean itself. Few studies have been done on degradation of oilseeds and their products while being transported. This is a subject of current interest in many quarters. A study by the USDA Agricultural Research Service (ARS) on eight shipments arriving at five European destinations from the 1985 crop

(Henderson, 1987), revealed that the average FFA levels was at 0.82%. The marketing standard for soybean oil is 0.75%. The report also mentioned another random sampling, conducted in the same year, from foreign shipments of soybeans leaving U.S. ports. The FFA content, on the average, was 1.9%. Some European and Asian soybean processors have previously expressed concerned with U.S. soybean quality during the 1984 and 1985 crops where a number of European processors turned to South American soybeans (Mounts et al., 1990). Soybean crop reportedly yielded oil with FFA content of 1.2 to 1.6%. Destination soybeans also showed significant increases in nonhydratable phospholipid content in the extracted oil than the origin beans, contributing to a higher refining loss.

Many earlier studies have however been related to the quality problems in static land-based storage situations which can be applied or extrapolated to transportation containers. Contributing to the quality preservation problem of the soybean during shipment in international commerce has been the changing patterns of transportation modes and handling techniques in the past 15 years. Cargoes are loaded much faster, at rates up to 2,500 Mg/h. Vessels have increased in size from 1,500-18,000 Mg capacity to 45,000 and 60,000 Mg or larger. In today's modern cargo vessel with deep holds, it is not surprising, therefore, to find that the percentages of splits and broken bean pieces are greater at the discharge point than when loaded. The combined height of the loading spout and free fall into the hold of the vessel can approach a 30.5 m average for the entire cargo. The advent of large capacity self-trimming bulk carriers in recent years has further increased spout-line separation of splits and foreign material from whole soybeans.

In a transportation study conducted by Velasco and Abdul-Baki (1979) on soybeans in shipment to Japan, samples were taken from a 51,000 Mg shipment that arrived in Japan and sieved into four portion (wholes, halves, pieces and fines). The level of FFA expressed as percent of crude oil in the composite samples increased as breakage of the bean increased. The FFA in bean halves, pieces and fine increased 2.8, 4.7 and 14.6 times those of whole soybeans, respectively. The corresponding neutral oil loss from these fraction was 4.5% for whole beans, 4.62% for halves and 6.08% for pieces.

In another transportation study conducted during the year 1985 to 1988 crop (Mount et al., 1990), there was no difference in moisture content and test weight of U.S. soybeans on arrival in the importing countries. The only economic factor concerned was the fine material content because of its non-soybean material and its role in storability of soybeans. However, the FFA in the crude oil met the maximum limit of 0.75% allowed in the trading rules of the National Oilseed Processors Association.

CONCLUSIONS

The maintenance of soybeans during storage begins in the field after the postmaturation preharvest period. A prolong unfavorable weather condition can damage the crop manifesting itself in the percentage mold damage and the increase in chemically induced products such as FFA. The harvesting and handling contribute to deteriorative changes during storage by creating the potential sites for deterioration through increased breakage content in a soybean lot. Deterioration during storage is a function of moisture content of the

soybeans, temperature during storage, the length of storage and the inherent quality of soybeans entering storage. The quality of soybeans and its products depend on how much deterioration can be minimized from field to storage.

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Table 1. Official US Grades for soybeans (USDA, 1993)

Grade	Minimum test weight per bushel, lb.	Maximum limits of: Damage kernels: Splits, %	Heat damaged, %	Damage Total, %	Foreign material %	Brown, black and/or bicolored soybeans in yellow or green soybeans, %
US No. 1	56.0	10.0	0.2	2.0	1.0	1.0
US No. 2	54.0	20.0	0.5	3.0	2.0	2.0
US No. 3[2]	52.0	30.0	1.0	4.0	3.0	5.0
US No. 4[3]	49	40.0	3.0	8.0	5.0	10.0
US Sample Grade[1]						

[1] US Sample grade shall be soybeans which do not meet the requirements for any of the grades from US No. 1 to US No. 4, inclusive; or which are musty, sour, or heating; or which have any commercially objectionable foreign odor; or which contain stones; which are otherwise of distinctly low quality.

[2] Soybeans which are purple mottled or stained shall be graded not higher than US No. 3.

[3] Soybeans which are materially weathered shall be graded not higher than US No. 4.

[4] Moisture was removed from the grade standards on September 1, 1985.

Table 2. Summary of the compositions of crude and refined soybean oil (Perkins 1993).

Composition	Crude Oil	Refined Oil
Triglycerides (%)	95 - 97	>99
Phosphatides (%)	1.5 - 2.5	0.003 - 0.045
Unsaponifiable Matter (%)	1.6	0.3
FFA (%)	0.3-0.7	< 0.05
Trace Metals		
Iron (ppm)	1 - 3	0.1 - 0.3
Copper (ppm)	0.03 - 0.05	0.02 - 0.06

Table 3. Percentage of fatty acid in crude soybean oils (Urbanski et al., 1980).

Variety	Type of damage	FFA (%)
Williams variety		
	Field damaged	0.19
	Freeze damaged	0.28
	Undamaged	0.15
Clark 63 variety		
	Field damaged	0.23
	Freeze damaged	0.24
	Undamaged	0.12

Table 4. Relationship between moisture content and allowable storage period to avoid grade drop (Holman and Carter, 1952)

Moisture content (%)	Storage period
< 10	4 years
10.0 to 12.5	1 to 3 years
13.0 to 14.0	6 to 9 months
14.0 to 15.0	6 months

Table 5. Relative growth of fungi during soybean storage at various conditions (Sauer et al., 1992).

Relative Humidity (%)	Equilibrium Moisture Content[1] (%)	Fungi
65 - 70	11 - 12	*A. halophilian*
70 - 75	12 - 14	*A. restictus*, *A.glaucus* any above
75 - 80	14 - 16	*A. candidus*, *A. orchraceus*, any above
80 - 85	16 - 19	*A. flavus*, *Pennicilium* spp, any above
85 - 90	19 - 23	any above

[1] At 25°C

Table 6. Free fatty acids content (expressed as acid values) during on-farm storage (Holman and Carter, 1952).

Moisture Content (%)	Storage period	
	Initial	After 12 months
8.0 - 9.0	0.15	0.40
12.0 - 12.5	0.40	1.25
13.0 - 14.0	0.25	1.95
15.0	0.40	1.60[1]

[1] after 145 days

Table 7. Free fatty acid (%) development in soybeans during a 12-month laboratory storage (McNeal, 1966).

Moisture Content	10°C		26°C	
(%)	Initial	Final	Initial	Final
7.4	0.10	0.20	0.10	0.30
10.7	0.10	0.20	0.10	0.50
14.6	0.10	0.40	0.10	1.30
15.8	0.10	0.50	0.10	1.40
18.4	0.20	2.00	0.40	3.50

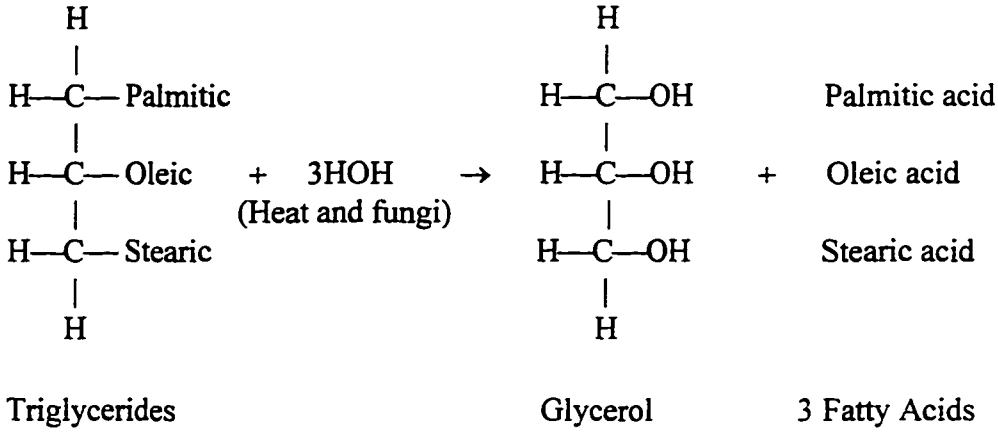

Figure 1. Hydrolysis of fat

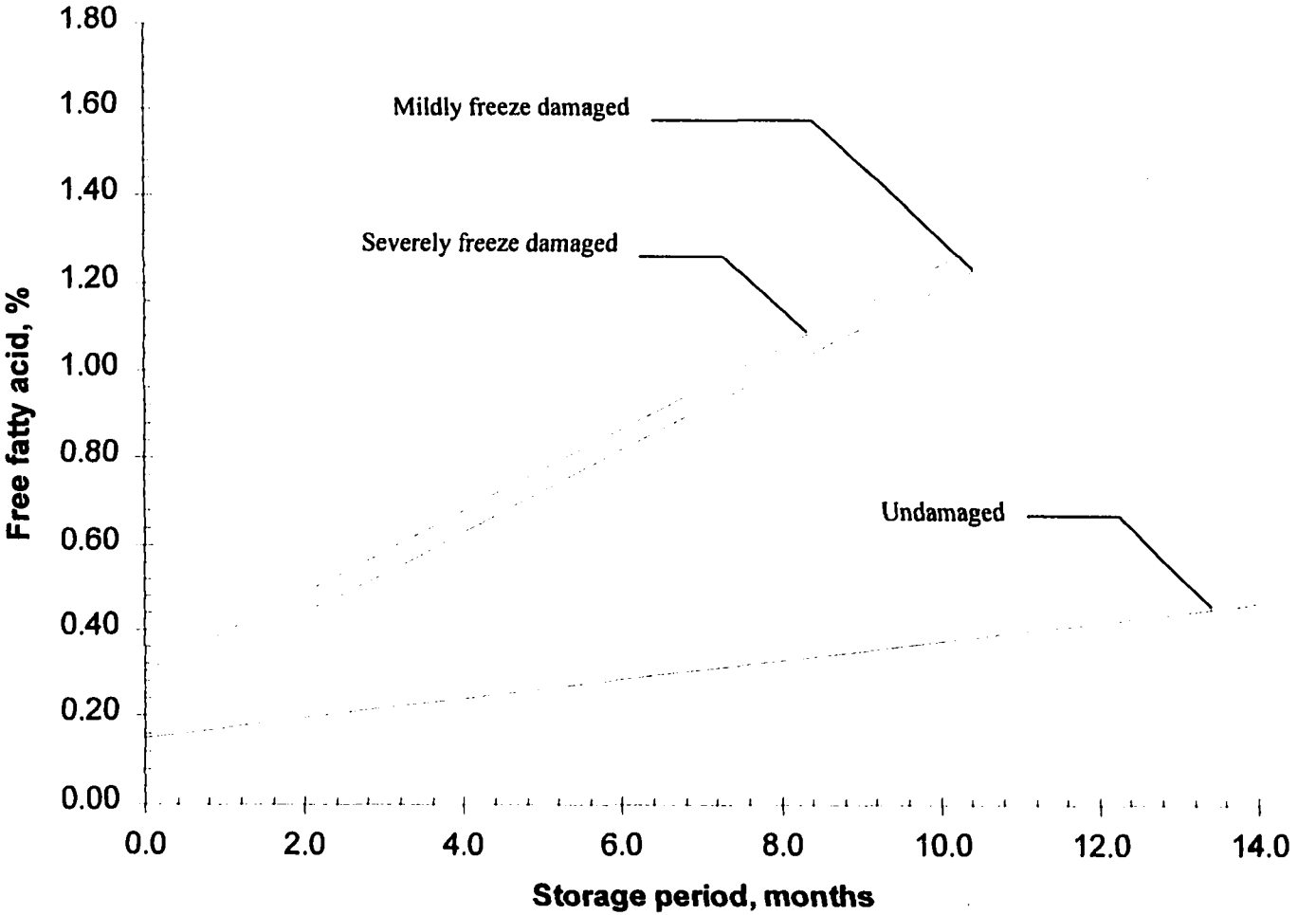

Figure 2. Free fatty acids during storage of Williams soybeans

A MODIFIED METHOD FOR DETERMINING FREE FATTY ACIDS FROM SMALL SOYBEAN OIL SAMPLE SIZES

A paper to be submitted to the Journal of the American Oil Chemists' Society

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ABSTRACT

A modification of the AOCS Official Method Ca 5a-40 for determination of FFA in 0.3 to 6.0-g samples of refined and crude soybean oil is described. The modified method uses only about 10% of the weight of oil sample, alcohol volume and alkali strength recommended in the Official Method. Standard solutions of refined and crude soybean oil of between 0.01 and 75% FFA contents were prepared by adding known weights of oleic acid. The FFA contents determined from small sample sizes using the modified method were compared with the % FFA of larger sample sizes described in the Official Method. The relationship between the modified and the official methods, both for refined and crude oils, can be described by a linear function. The relationship for refined soybean oil had an R^2 value of 0.997 and a slope of 0.99 ± 0.031. Crude soybean oil is defined by a line with R^2=0.9996 and a slope of 1.01 ± 0.013.

INTRODUCTION

In the soybean industry, the presence and development of any amount of free fatty acid (FFA) in soybean oil is undesirable. The tolerance is theoretically at zero percent.

During the entire time between harvest and processing, the FFA content of oil in soybeans indicates how well the beans have been treated. The eventual consequence of high FFA content is a monetary loss since processing soybeans with high FFA content results in more refining loss. Specifically, refining loss is the sum of weight loss of an oil during removal of FFA and other impurities plus the weight of entrained neutral oil in soapstock during the normal alkali refining process. The percentage loss of oil during refining can be estimated as a multiple of the FFA percentage. Norris (1982) estimates the potential loss at about three times the FFA content. Because of the economic impact of high FFA, it is important to determine the initial level of FFA in the oil, and also to monitor accurately the concentration of FFA by use of a reliable, simple and quick method.

The standard method for FFA determination in extracted crude and refined soybean oil samples is based on the acid-base titration technique in a nonaqueous system. The method commonly used is AOCS Official Method Ca-5c -40 (AOCS, 1989). The procedure prescribes the use of a maximum 56.4-g and a minimum 3.53-g oil sample for the titration, depending on the level of FFA in the oil sample (table 1). When oil samples are limited, as in some storage studies or when experimental use of oilseeds crops are evaluated, sample sizes of this magnitude is not possible. A simple and inexpensive method is needed which accurately quantifies FFA content while using a smaller sample size.

The use of smaller amount of chemicals will have an impact, especially to the developing countries, where the availability of chemicals are scarce and expensive. On the other hand, it is more prudent to use less of the resources particularly those hazardous to the

environmental. Hexane, a solvent use in the titration, is one of the hazardous compound, highly inflammable and its disposal can be harmful to the environment. A smaller size of oil samples will also results in the saving of storage space.

Lanser et al. (1991) developed a user-interactive computer-assisted Fourier transform infrared (FTIR) method to estimate of FFA in soybean oil samples. The method requires only one drop of soybean oil. The limitation of the method, and other similar spectrometric methods, such as that described by Canham and Pacey (1987), is the cost of the instruments; it is prohibitive for most laboratories. Lansel et al. (1991) also compared a modified version of the Official Method, which used between 6 and 7-g of oil, but no further explanation was given on the method. The correlation coefficient of the modified method was 0.999. The high correlation indicates that the officially recommended sample size can be scaled down without losing the accuracy of FFA determination.

The objective of this research was to develop and evaluate a method for the determination of FFA within the range of 0.01 to 75% FFA content in soybeans oil by using an oil sample of about 10% (between 0.3 and 6.0-g) of the weight recommended in the AOCS Official Method.

MATERIALS AND METHODS

Different amounts of oleic acid were added to the oil to make up the desired concentrations of FFA in the standard soybean oil solutions. The levels of the FFA in the

known oil standards were then determined by using of both the modified method and the AOCS Official Method.

Refined soybean oil sample

The refined bleached and deodorized (RBD) soybean oil of Hi-Vee[1] brand was purchased from a local grocery store. The percentage FFA content of the original RBD soybean oil was determined to be 0.035%±0.005 by using the AOCS Official Method Ca 5a-40 (Appendix A). The value is a mean from three replicates.

Crude soybean oil sample

The crude soybean oil used was obtained from Archer Daniels Midland Company[2], Decatur, Illinois. The FFA content of the crude oil was first determined by the AOCS Official Method Ca 5a-40 before further use. The average initial FFA content in the crude oil sample, from three replicates, was found to be 0.33%±0.037.

Oleic acid

Oleic acid of National Formulary (NF) and Food Chemicals Codex (FCC) grades was used, as purchased from Fisher Scientific[3], Fair Lawn, NJ to make up the required

[1, 2, 3] Mention of trader or manufacturer names is for the benefit of the readers only and does not imply an endorsement, recommendation, or exclusion by Iowa State University over other firms or similar products not mentioned.

concentrations of FFA in the refined and crude soybean oil samples. The oleic acid purity was not tested, but was assumed to be 100%, as described in the specification.

AOCS Official Method Ca-5a-40.

The FFA content of soybean oil is usually determined by titration with a standard alkali, NaOH, of specific strengths or normalities (N). A widely used method is AOCS Official Method Ca 5a-40 (AOCS, 1989). This method's recommended oil sample size, volume of alcohol (ethyl alcohol) and strength are listed in table 1. The NaOH solutions of various normalities were standardized according to AOCS Official Method H 12-52 (AOCS, 1989).

The FFA concentration in fats and oils is calculated as percent oleic acid. The expression (equation [1]) as given in AOCS Official Method Ca 5a-40 (AOCS, 1989) is:

$$\text{FFA, as oleic acid \%,} = \frac{\text{alkali volume (mL)} * \text{alkali Normality} * 28.2}{\text{sample weight (g)}}$$

Modified method

In the modified method, oil sample and reagent quantities proposed are about 10% of the values recommended in table 1. The titration procedure, however, remained the same.

Preparation of standard soybean oil solutions for titration

Refined and crude soybean oil samples of known initial FFA contents were each divided into five and four lots, respectively, in Erlenmeyer flasks. A set of five levels of FFA concentrations for refined soybean oil and four levels for crude soybean oil, representing approximately the average values of the five different ranges of FFA in the AOCS Official Method, were chosen (table 2). Oleic acid weights were then calculated, based on the estimated total weight of soybean oil to be used in the titration for a particular FFA level. The calculated weights of oleic acid were added to the respective soybean oil lots to give the predetermined FFA concentrations. The solutions were stirred for 3 to 5 min by use of magnetic stirrers. All standard oil solutions were stored at 2 to 5°C in stoppered flasks. Flask headspaces were flushed with nitrogen prior to closure.

Experimental design

The experiment was divided into two parts. Part one dealt with FFA determination in refined soybean oil. A total of 10 treatments combination were used consisting of two methods and five FFA levels (2 x 5 factorial). Part two involved use of crude soybean oil samples with two methods and four levels of FFA (2 x 4 factorial), giving eight treatments. Each treatment was replicated three times, resulting in a total of 30 and 24 observations, respectively, in the first and second parts. The experiment was a completely randomized block design (CRBD) with replications serving as blocks.

Statistical Analysis Software (SAS Institute, 1990) was used for analysis of data. Analysis of Variance (ANOVA), General Linear Model (GLM) and regression through the origin procedures were used. Coefficient of determinations, R^2, and the coefficients of variation (square root of the means square error divided by the mean of the measured values) were determined to evaluate suitability of the method. Hypothesis tests were conducted on the regression lines to find the best fit model. Significance was established at $P < 0.05$ unless otherwise indicated.

RESULTS AND DISCUSSION

Modified method

The preliminary determination of FFA in a refined soybean oil with a sample size of 2.82-g, (10% of the AOCS Official Method weight), showed that the concentration of NaOH needed to detect and quantify presence of FFA was about 0.0125N. Based on this estimation, NaOH concentrations to be used for the modified method at different ranges of FFA were then adjusted accordingly. Alcohol volume to be used in the modified method was also reduced to 10% of the AOCS Official Method volume (table 3). Although oil sample weights and alcohol volumes were one tenth of those recommended by the Official Method, NaOH normalities were slightly more than 10% of the recommended strengths. Table values need not be strictly followed and should only serve as guidelines in preparing the sample and reagents.

In the modified method, burette and Erlenmeyer flask sizes to be used need to be rationalized with the expected titer volume. The graduation of the commonly used burette (50-mL capacity) may not be small enough to accurately capture the reading. For the lower ranges of the FFA content, smaller burettes with capacities between 5 and 25 mL should be used. A 150-mL Erlenmeyer flask was used.

The procedure used in the modified method was the same as for Official Method, except the amounts were only about 10% of the original values. These smaller quantities will result in savings in reagent costs and in time spent if oil is to be extracted. The use of weaker strengths of NaOH, as in the revised method, allows a more accurate end point determination.

FFA determination

Refined soybean oil

Table 4 shows the FFA content determined by using the AOCS Official Method and using the modified method. Raw data is listed in Appendix B. Assuming the AOCS method as a reference, it can noted that within the range of this study, the modified method slightly underestimated the FFA content in the oil in the FFA range of 0.1 to 50.0%. The magnitude of underestimation ranged from 3.9 to 12.1%. But within the range of FFA content of 0.01 to 0.2%, which encompasses the normal range of FFA content allowed under the standard definition of refined soybean oil and trading values of soybean oil (NOPA, 1995), the deviation was small. Adoption of the modified method for the determination of FFA content in refined soybean oil destined for consumer markets would amount to 3.9% variation from

the AOCS Official Method. This amount can be considered small enough to risk any possible marked variation in the FFA values between the two methods during FFA determination based on the current refining capability. The difference in the FFA content is known to be a function of the processing steps. Typical variation is shown in table 5 (Sleeter, 1981), where each processing step results in removal of a fraction of FFA content. With the normal refining process, the FFA content was about 0.05% after refining and 0.03% after hydrogenation and deodorization.

Reinforcing the above argument statistically, an analysis of variance (ANOVA) showed that there was no significant difference between the means of FFA content for the two methods. The P value calculated by using SAS was about 0.54. There was a significant interaction effect between FFA contents and the methods ($P < 0.0014$). This observation could be explained by the underestimation of the FFA in the lower ranges of FFA (0% to 50%) and the overestimation of the FFA content from the modified method in the upper range (greater than 50% FFA content). The size of overestimation (1.6%) was small compared to when the method underestimated the % FFA content (50% and below).

Crude soybean oil

Determination of FFA in crude soybean oil samples was also carried out by using both methods. Table 4 shows the FFA contents in the four standard crude soybean oil solutions. The modified method underestimated FFA values over the range of FFA used. The magnitude of underestimation from the AOCS method was greater (11.2%) at a lower range

of FFA. The difference in FFA content measured by the modified method therefore becomes less when the FFA content in the soybean is greater. Unlike the refined soybean oil results, there was no interaction between concentrations and methods with crude soybean oil. There was no significant difference in the FFA mean values between the two methods ($P < 0.11$).

Generally, the modified method did better in estimating the FFA content in crude soybean oil than in refined oil, as was evident in the coefficients of variation of 1.1% and 2.11%, respectively.

Linear Regression Analysis

Refined soybean oil

Figure 1 shows a plot of the relationship between the Official Method and the modified method. Simple linear regression was conducted to correlate the two methods. Statistical analysis based upon the ANOVA tables generated by the GLM procedure showed first order regression to be a very good approximation in describing the relationship between the two methods over a wide range of FFA contents (0.01 to 75%). The coefficient of determination (R^2) was at 0.995, which means that approximately 99.5% of the variation in the values of the FFA content determined by using the AOCS Official Method was accounted for by the linear regression with the modified method. The linear relationship can be described as:

$$OM = \beta_0 + \beta_1 * MM \quad \text{.....Model [1]}$$

where: OM = Official Method, % FFA
MM = Modified Method, % FFA
$ß_0 = 1.04$
$ß_1 = 0.97 \pm 0.04$

The y intercept, $ß_0$, of 1.04, is the mean FFA value when the measured FFA value RM equals zero. The value can be quite misleading since RM =0 was not within the range of the experimental data and the predicted value cannot be 1.04 when the measured value is zero. Using of the linear model [1] may produce errors, especially at the very low values of FFA. For example, if the measured FFA content is 0.1, the predicted FFA is 1.11%. If the relationship of the revised method and official method is as desired, the slope and the intercept of the regression line should be 1.0 and 0.0, respectively.

Alternatively, a linear regression analysis 'through the origin' was performed on the data. Under the procedure, the regression will set the y intercept at zero. The regression procedure would therefore meet, in part, the expectation of the results. The 0.99 ± 0.031 slope is better than model 1 as it is very close to 1, the ideal slope (table 6). The R^2 (0.997) is slightly greater than that obtained in model [1] (figure 1). The full model is:

$$OM = \beta_1 * MM \quad \text{.....Model [2]}$$

where: $\beta_1 = 0.99 \pm 0.031$

Hypothesis testing was therefore made for the slope of the line ($ß_1$) with a null hypothesis, H_0: $ß_1 = 1$. The t-statistic is calculated from the expression:

$$t = \frac{\text{Estimate - Hypothesized value}}{\text{Standard error of estimate}}$$

The "Estimate" equals the slope from the regression analysis, the "hypothesized value" equals 1 and "standard error of estimate" was obtained from SAS output. Table 6 summarizes the results of the regression analysis. The calculated t of 0.7 infers an acceptance of the null hypothesis that the slope (0.99) is in fact 1.

Figure 1 shows the plot of model [2] and the line of equal values drawn to indicate how far off the models are from an ideal relationship. Model [2] is superior to model 1 in defining the relationship between the modified method and the Official Method.

Crude soybean oil

The plot of the FFA values in crude soybean oil measured by the two methods is shown in figure 2. Again, the correlation between the two methods can be best described by a linear function.

By following the same argument for the intercept, a regression analysis also was conducted by setting the intercept at 0 as default. The summary of the statistic is shown in table 6. The slope is 1.01 ± 0.013 with an R^2 value of 0.9996. The t-statistics of 1.7 (table 6) infers the acceptance of the null hypothesis that the slope β_1 for the crude oil also is 1. The regression line for crude soybean oil (R^2=0.9995) therefore presents better correlation than

the model developed for refined soybean oil (R^2=0.997). The final model that described the correlation between the modified method with the Official Method for crude soybean oil is:

$$OM = \beta_1 * MM \quad \text{.....Model [3]}$$

where: $\beta_1 = 1.01 \pm 0.013$

Figure 2 shows the best fit linear model relating the modified method with the Official Method for crude soybean oils. Linear model [2] for refined soybean oil and an ideal model also are superimposed on the plot for comparison.

Lanser's revised procedure versus revised method from the study

Lanser et al. (1991) published a set of FFA values in crude soybean oil established by the AOCS Official Method and their version of a modified method. Regression analysis, through the origin, on the FFA values between the two methods was carried out to compare with model [3] from the study. Lanser's data yield a line with an R^2 of 0.999 and a slope of 1.09 ± 0.025 as compared to a slope of 1.01 from model [3]. The regression line for Lanser's model is plotted in figure 3 along with the model [3] and the line of equal values. There is a 9 and 1% variation in Lanser's and model [3] from the ideal line, respectively. The variation is not unexpected as in any titration, the methods are not only a function of FFA content but also of all other components that will react with alkali. Variation in the samples used may have contributed to the difference. In addition, it should also be noted that titration end point is usually not distinct and is often subjective. Also, the range in FFA values used in Lanser's

study was small, 0.03 to 4.98%. As described earlier from this study, the greatest variation between the Official Method and the modified method occurs at the lowest range of FFA content.

CONCLUSIONS

The findings of the study indicate that the modified method for determining FFA in the refined and crude soybean oils is reliable and can be used as an alternative to the AOCS Official Method. This method is specific for FFA determinations in soybean oil and may be modified for other types of oils. The method may be applicable to industry and laboratories currently using the AOCS Official Method.

The relationship of the revised method to the AOCS Method for the refined and crude soybean oils, based on models [2] and [3] are close to the ideal relationship. The model that best describes the relationship between the revised and the official methods for refined and crude soybean oil is given by straight lines passing through the origin having slopes of 0.99 ± 0.031 and 1.01 ± 0.013, respectively. The modified method estimates the FFA content better in crude soybean oil than in refined soybean oil. The use of the modified method reduces the constraint in the preparation of oil samples, and greatly lowers the consumption of organic solvent and other reagents associated with the procedure.

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Table 1. Sample size and reagents used in AOCS Official Method Ca-5a-40 (AOCS, 1989)

Range of FFA (%)	Weight of oil sample (g)	Volume of ethyl alcohol (mL)	Normality of NaOH (N)
0.01 - 0.2	56.4	50.0	0.10
0.20 - 1.0	28.2	50.0	0.10
1.0 - 30.0	7.05	75.0	0.25
30.0 - 50.0	7.05	100.0	0.25 or 1.0
50.0 - 100.00	3.525	100.0	1.0

Table 2. FFA concentration of prepared standard soybean oil solutions

Ranges of FFA from AOCS Official Method (%)	Estimated FFA content prepared from refined soybean oil (%)	Estimated FFA content prepared from crude soybean oil (g)
0.01 - 0.20	0.1	-
0.20 - 1.00	0.6	0.6
1.00 - 30.00	15.0	15.0
30.00 - 50.00	50.0	50.0
50.00 - 100.00	70.0	70.0

Table 3. Sample size and reagent concentrations used in the modified method

FFA Range (%)	Weight of oil sample (g)	Volume of ethyl alcohol (mL)	Normality of NaOH (N)
0.01 - 0.2	5.64	5.0	0.013
0.20 - 1.0	2.82	5.0	0.013
1.00 - 30.0	0.70	7.5	0.031
30.00 - 50.0	0.70	10.0	0.13
50.00 -100.0	0.35	10.0	0.125

Table 4. FFA values from AOCS and modified methods using RBD and crude soybean oil

Method	FFA concentrations[1]					Overall Mean	CV
	[0.01 - 0.2]	[0.2 - 1.0]	[1.0 - 30.0] (%)	[30.0 - 50.0]	[50.0 - 100.0]	(%)	(%)
RBD:							
AOCS	0.103	0.603	15.60	51.45	70.95	27.74	3.64
Revised	0.099	0.57	13.71	49.10	72.10	27.51	3.67
% Difference from AOCS[2]	-3.90	-5.00	-12.10	-4.60	1.60		
Crude soybean oil:							
AOCS	na	0.89	15.53	50.90	71.20	27.76	2.05
Revised	na	0.79	14.20	49.80	70.90	27.28	2.09
% Difference from AOCS[2]	na	11.20	-8.60	-2.20	-0.40		

Standard Error of a mean : refined soybean oil = 0.58; crude soybean oil = 0.33

[1] average from 3 replications

[2] negative indicates underestimation, positive over estimation; na=not available

Table 5. Relationship of free fatty acid content as a function of processing steps (Sleeter, 1981)

Processing Steps	Free fatty acids from 2 different runs	
	I (%)	II (%)
Crude	0.61	0.53
Degummed	0.31	0.44
Refined	0.05	0.05
Deodorized	0.02	0.03
Hydrogenated and Deodorized	0.025	0.03

Table 6. Summaries of the linear regression statistic

Model	Slope $ß_1$	Standard error of estimate $s_{\beta 1}$	t for H_o: $ß_1 = 1$	Status of H_o	R^2
Refined soybean oil:					
2	0.99±0.031	0.0143	(0.99 - 1)/0.0143 =0.7	Accept H_o $ß_1$ =1	0.997
Crude soybean oil:					
3	1.01±0.013	0.006	(1.01 - 1)/0.006 =1.7	Accept H_o $ß_1$ =1	0.9996

Null hypothesis (H_o) is rejected if $t > t_{table}$ at $\alpha = 0.05$
Model 2: $t_{0.025,14} = 2.145$
Model 3: $t_{0.025,11} = 2.201$

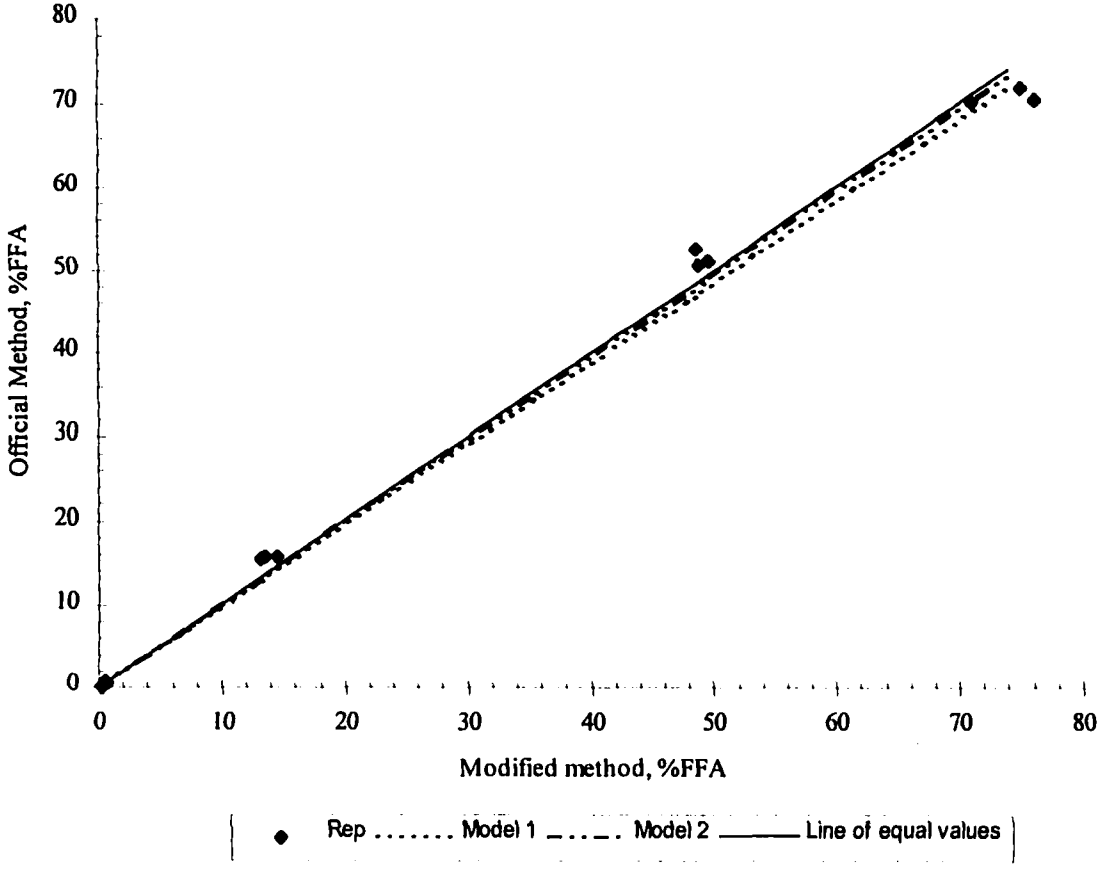

Figure 1. Relationship between the AOCS Official Method and the modified method based on refined soybean oil

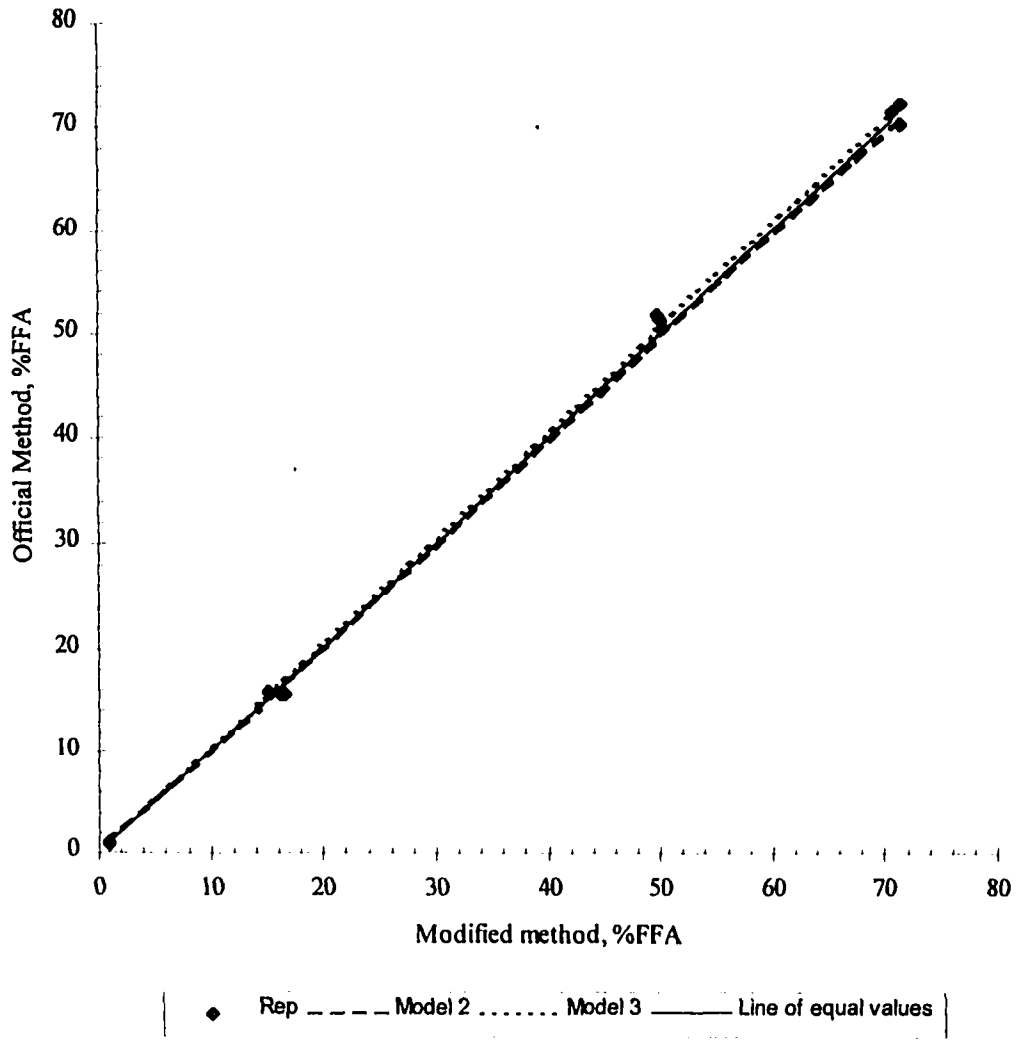

Figure 2. Relationship between models for crude (Models 3), refined (Model 2) soybean oil and line of equal values

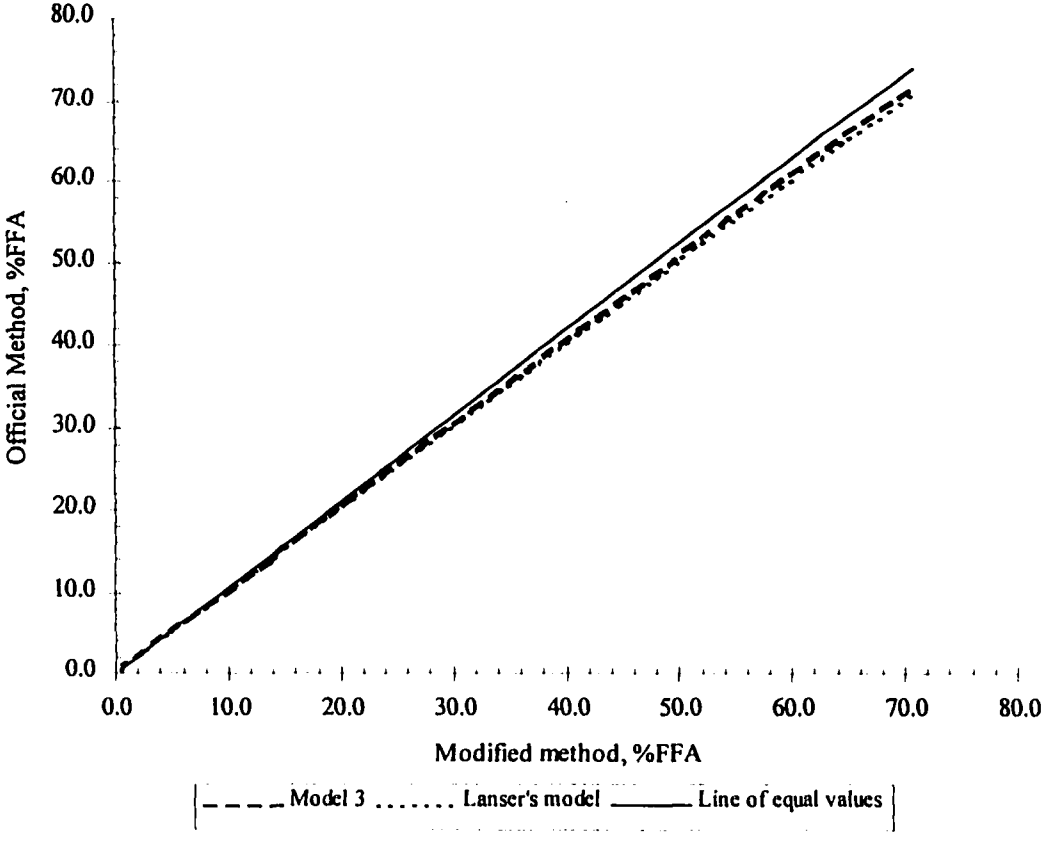

Figure 3. Relationship between model 3 and Lanser's model for crude soybean oil

CARBON DIOXIDE EVOLUTION FROM FRESH AND PRESERVED SOYBEANS

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ABSTRACT

A study was conducted on carbon dioxide evolution from stored soybeans using fresh and preserved soybean samples, manually and mechanically harvested. Preservation of samples was accomplished by holding 22 and 9% moisture content soybeans at -18 and 10°C for 26 and 48 weeks. The carbon dioxide produced during storage as a result of deterioration was recorded and the rate of deterioration was established as the number of days of storage before the sample lost 0.5 and 1.0% of its dry matter. Methods of harvesting and moisture content at harvest influenced the rate of deterioration during storage. Fresh machine-harvested soybeans at 13% moisture content, required 22.5 days to lose 0.5% dry matter, compared to about 11.5 days for the 22% moisture content sample. Fresh soybeans manually harvested at 20% moisture content took about 26.2 days to lose 0.5% dry matter. Preservation of soybean samples resulted in faster rates of deterioration during storage compared to fresh samples. The decline in the rate of deterioration as a function of preservation period can be explained by a linear model. Machine-harvested soybeans preserved at 9% moisture content lose 0.5% its dry matter during storage by about 0.21 ± 0.043 days per week of preservation.

Soybeans preserved at 22% almost maintained a uniform rate of deterioration throughout the preservation period.

INTRODUCTION

At the time an oilseed reaches physiological maturity, it is considered to be at its prime state in every aspect of quality. It then begins to deteriorate with time, slow at low moisture contents and temperatures but very rapid when they are high. Deterioration of most biological material is associated with the decomposition of carbohydrates as a result of respiration. The selective respiratory utilization of carbohydrates in soybeans is assumed to be similar to the oxidative combustion of typical carbohydrates such as hexose sugars (Ramstad and Geddes, 1942). Hexose sugars, or 6-carbon sugars, are monosaccharides or simple sugars. Examples of hexose sugars are glucose (also known as dextrose or glucose), fructose and galactose.

Carbohydrate decomposition during deterioration of soybeans is discussed by Milner and Geddes (1946a). They found that during this biological phase of respiratory behavior of seeds, the increased rate of respiration, a symptom of deterioration, was accompanied by a decrease in both reducing and nonreducing sugars. There was no change in the fat content during this phase. The protein content has been found to be slightly increased, but was not speculated to have any role during the decomposition process. The increase was, in fact, attributed to the decrease in the sample dry matter. A similar reduction in the sugar content of soybeans was observed by Howell et al. (1959) when they studied the respiration of ripening

soybean seeds. Wilson (1995) reported similar changes in protein and carbohydrates in fungus-damaged soybeans, but either no change or an increase in the oil concentration was observed.

The decomposition process which results in the loss of dry matter is usually modeled as a breakdown of simple sugars represented by the following equation:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2{,}835\ kJ \quad \text{.....[1]}$$

Following this equation, the evolution of 14.7g of carbon dioxide (CO_2) per kg dry matter is equivalent to a loss of 1.0% carbohydrate (dry matter).

Muir et al. (1985) treated the rising concentration of CO_2 in interseed air of stored wheat, rapeseed, barley and corn as a measure of quality. The analysis of samples from the locations that registered high CO_2 concentration indicated that the kernels had undergone spoilage.

But is was Steele et al. (1969) who demonstrated that decomposition of dry matter during deterioration of shelled corn can be determined by measuring the CO_2 produced. An equivalent dry matter loss was then calculated based on equation [1]. In the case of commercial soybeans, the loss of dry matter may result in a loss of grade, as is evident in the case of stored shelled corn (Saul and Steele, 1966). They evaluated the length of time that shelled corn can be stored before 0.5% of its dry matter is lost. The 0.5% was considered the

threshold value of dry matter loss in shelled corn before the grade is lowered because of an increase in total damaged kernels.

From the pioneering work of Steele et al. (1969), several corn storage experiments were later conducted using CO_2 evolution as a measure of quality (Fernandez et al., 1985; Friday et al., 1989; Al-Yahya et al., 1993; Aljinovic et al., 1995; Dugba et al., 1994). One of the useful contributions made from these studies was the development of an allowable storage time (AST) table for different combinations of corn moisture content and storage temperature range (MWPS, 1980).

This type of table would be valuable in understanding and minimizing losses during handling and storage of soybeans. But before such a table can be developed, several tests need to be conducted. The limitation of a laboratory setup to conduct such experiments requires that soybeans be preserved at harvest for later testing. Any changes in the characteristics of the state of soybeans during preservation which influences the CO_2 evolution during a test, if it is not accounted for, can introduce error in the final analysis.

In the case of shelled corn there was no significant difference in the CO_2 evolution for samples preserved at -10°C and 22% moisture content for a period of 100 days when compared to fresh harvested corn (Fernandez et al., 1985). Seeds preserved at low temperature, particularly at subfreezing temperatures, have been known to retain viability for several years.

Knowles (1967) presents a review of a few experiments on seed preservation at between -7 to -20 ° C. Soybean seeds, preserved at -10 ° C with 8 to 9% moisture content, still

germinate after 10 years of storage. Conifer seeds preserved better at -18 than -11 or-4 °C. Viability of vegetable and flower seeds was found to be higher at -20 than at 0 °C. Grass seeds had better retention of viability at -18 than at 1 or 20 °C.

Most of the studies looked only at the viability of particular seeds when they were allowed to germinate under laboratory conditions after a specific period of preservation. Better germination rates were reported from seeds preserved at lower temperatures. The effects of temperature and moisture content in maintaining the quality of stored soybeans, in terms of CO_2 evolution during the deterioration process when held near ambient air conditions, were not found in the literature.

OBJECTIVES

This study is therefore undertaken to define the impact of preservation in maintaining the initial freshness of soybeans as measured from CO_2 evolution.

The specific objectives of the study are:

1. to determine the effect of harvesting practices on the rate of CO_2 evolution in freshly harvested soybeans
2. to compare the effects of preservation temperatures on CO_2 evolution during storage
3. to compare the effects of preservation moisture contents on CO_2 evolution during storage
4. to compare the effect of preservation period on CO_2 evolution

MATERIALS AND METHODS

Soybeans

The soybeans used in the study were Kruger 2555[4] grown at the Iowa State University Agronomy and Agricultural Engineering Research Center, 15 km West of Ames. The soybean lots were combine or hand harvested in September 1995 at 22 to 20% (high) or 8 to 13% (low) moisture contents. The lots were cleaned by the use of a Dockage Tester (Carter Day Model XT3[5]). The cleaning was accomplished using a 13-mm (0.5-in.) square-hole, 8.5-mm (20/60-in.) round-hole and 7.9-mm x 19.1-mm (20/64-in. x 3/4-in.) slotted sieves. The slotted sieve removes splits from the whole soybean lot.

Preservation of soybean samples

Preservation of soybean lots at -18 and 10 °C involved only the machine-harvested soybean lots, harvested at 22 and 13% moisture contents. The cleaned soybean lot, machine-harvested at 22% (high moisture content), was packed in a polyethylene bag and preserved only at one temperature, -18 °C. High moisture soybeans were not preserved at 10 °C because they would become moldy in a short period of time. The 13% moisture content soybean lots were air dried to 9 to 10% moisture content before being preserved at -18 or 10 °C. Each bag contained about 1200g.

[4, 5] The mention of trader or manufacturer names is for the benefit of the readers only and does not imply an endorsement, recommendation, or exclusion by Iowa State University over other firms or similar products not mentioned.

For each of the preservation temperatures, three different freezer or cold room chambers were used, representing three replications. A total of six chambers were used and six bags of cleaned soybean samples were preserved in each of the chambers.

The three freezers (at -18°C) were located in the Davidson Hall Biomaterials Laboratory. The three 10°C cold rooms used were located at the Seed Science Center, the Agronomy Building and the Community for Agricultural Development (CAD) warehouse, respectively. The soybeans were preserved for a period of more than a year, with sampling done at 0, 26 and 48 weeks after harvest.

Carbon dioxide measuring system

A CO_2 measuring system (figure 1) similar to that described by Aljinovic et al. (1995) and Dugba et al. (1996) was used. A second solenoid valve was added to accommodate four more storage tubes to make a total of 12 tubes.

During the study, carbon dioxide produced by 1-kg soybean samples stored under constant aerated storage conditions was measured. The storage condition was 26 ° C, 95% relative humidity and 0.45 m^3/min/Mg aeration. Compressed air which has been filtered, stripped of CO_2 and conditioned to 95% relative humidity and 26°C was forced through the 3-foot soybean columns in recyclable glass tubes. Recycling was accomplished by autoclaving at 120 °C for 20 minutes before reuse. It should not be assumed that 26 ° C and 95% relative humidity is a recommended storage conditions for soybeans. This condition was chosen to accelerate the process of soybean deterioration during storage study.

The CO_2 produced by the soybean samples while in storage was trapped by the CO_2 absorbing section of the system (figure 1). The sulaimanite CO_2 absorbent agent (Al- Yahya. 1991), was packed in plexiglass tubes. The weight gain recorded every 24 hours was a measure of the amount of CO_2 produced during the period. The weight of the CO_2 gained was corrected to account for the residual CO_2 present in the air stream (refer to Appendix C for the equation). The tubes were changed when either the color of the sulaimanite changed from the initial dark gray to light gray or the indicating drierite packed at the far end of the tube changed to pink. Sulaimanite is a powdery mixture of vermiculite particles impregnated with potassium hydroxide solution and vacuum dried at 172.4 kPa, 80 °C for 12 hours to a dry state (Al-Yahya, 1991).

The 93.08 ± 2.7% relative humidity air was maintained by bubbling the incoming air stream through a 30-mm-long water column. The storage temperature of 25.82 ± 0.38°C was achieved by use of an air conditioner and a space heater.

The airflow rate through each tube was adjusted to 0.45 m^3/min/Mg (0.4 cfm/bu) by the use of individual valves and monitored by flowmeters (Matheson Model PM-1022[6]).

Experiment I: Fresh soybeans

The soybeans used in the experiment 1 were freshly harvested under two modes of harvesting and at two moisture contents. Harvesting was carried out manually, (hand

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harvesting followed by handshelling), and by combine. A total of 4 treatments were used (table 1).

Before the start of the experiment, the low moisture soybean samples were raised to about 21% moisture content by direct addition of calculated weight of distilled water. The approach used was quite similar to the method described by Milner and Geddes (1945), although Ramstad and Geddes (1942) earlier found this to be unsatisfactory with soybeans. They noted the problem in ensuring uniform distribution of moisture as some of the beans swelled very greatly and seed coats loosened. To ensure minimum swelling of the beans and uniform distribution of water, the addition of water to a particular bag of soybean was accomplished by use of a spray bottle. Then the bag was rotated by hand for 2 to 3 minutes to uniformly distribute the water. The water was added in 3 or 4 stages. Each stage is separated by storing the bags in a 4 to 5° C cold room for 6 to 12 hours. This prevented a sudden swelling of the beans. Samples were then kept at room temperature for about 12 hours before being used in the experiment. Most of the beans soon presented a normal appearance as the water was taken up by the cotyledon. The reconstitution of moisture was to ensure that measurement of CO_2 was made from the soybean samples with the same initial moisture content. No assumption had been made on the behavior of rewetted soybeans during storage to represent the naturally wet soybeans. The soybean samples were poured into the glass storage columns which were arranged randomly in the CO_2 measuring system.

Experiment II: Preservation of soybean samples

This experiment investigated the effects of moisture content, preservation temperatures and period of preservation on CO_2 evolution during storage at 26°C and 95% relative humidity conditions. There were three treatments:

1. Machine harvested at 21% moisture content and preserved at -18°C

2. Machine harvested at 13% moisture content, air dried to 9 to 10% moisture content and preserved at -18°C

3. Machine harvested at 13% moisture content, air dried to 9 to 10% moisture content and preserved at 10°C

Samples were drawn at 26 and 48 weeks after preservation and used in the CO_2 evolution studies.

The process of deterioration during storage was monitored by measuring CO_2 produced while samples were stored at a constant laboratory condition of 26°C and 95% relative humidity. The fresh soybeans combine harvested at 21% and 13% moisture content represented two types of control. The low moisture content soybeans were reconstituted with water to about 21% as described above before being used in the study. The soybeans were poured into the storage columns and arranged at random in the CO_2 measuring system.

Statistical analysis

Each treatment was replicated three times in a restricted randomization design. Complete randomization of the environmental chambers to each temperature could not be

achieved. Statistical analysis on the data was carried out using the Statistical Analysis Software (SAS Institute, 1990). Polynomial regression models to describe the CO_2 evolution with time of storage were established using the General Linear Model Procedure (Proc GLM) to the third order with zero intercept. The coefficients of the terms were included in the model if they were significant as indicated by t-statistics. Comparisons of the rates of deterioration between treatments were made by means of the Analysis of Variance (ANOVA) where the measurement for the sample preserved 26 and 48 weeks were considered repeated measures. Significance was established by calculating the least significant different (LSD) between the means (Steel et al., 1997). Unless otherwise stated, the significance was established at $P < 0.05$. Visible microbial growth and development during storage was also noted.

RESULTS AND DISCUSSION

Effect of harvesting practices on the CO_2 evolution from fresh soybeans

Machine harvested

Soybeans are usually harvested after field drying to about 13% moisture content or below and harvest at higher moistures is uncommon. The risk that soybeans will sustain mechanical damage at high moisture content is high. For comparison, figure 2 shows a family of curves describing the CO_2 evolution from fresh soybeans harvested mechanically at high (22%) and low (13%) and held at-18°C and 22% moisture. The curves were derived from third order polynomial regressions on the data of three replications (Appendix D). The

coefficients of the terms (table 2) were only considered as part of the model if their respective t-statistics were shown to be significant.

Table 3 shows the respective average storage times, defined as the number of days of aerated storage before soybeans lost 0.5% and 1.0% of their dry matter. LSDs of two treatment means were established at the two dry matter loss levels. Soybeans machine-harvested at high moisture content were found to lose 0.5% of its dry matter at twice the rate of soybeans harvested at low moisture content. Severe mechanical damage, as expected from combining high moisture content soybeans, no doubt contributed to the faster rate of deterioration. The rate of deterioration of soybeans harvested at 13% moisture content, which is within the range of 11 to 14% moisture content associated with optimum toughness (Paulsen, 1977), would therefore demonstrate the minimum rate of deterioration that could be achieved when soybeans are mechanically harvested. At 13% moisture content, it took 22.5 days for samples to lose 0.5% dry matter.

Hand harvested

The experiment also tracked the deterioration of hand-harvested soybeans at high (20%) and low (9%) moisture contents (Appendix D). The plots of CO_2 evolution measured during storage for the two treatments are shown in figure 2 with the equation of the models described in table 3. Soybeans at high moisture content took about 26.2 days before losing 0.5% dry matter (table 3). Soybean quality is considered at its prime level at physiological maturity, which is usually at 50 to 60% moisture content (Howell et al., 1959; Rose 1979).

According to Howell et al. (1959), and Hurburgh and Benson (1995), full maturity, that is when the pods are brown in color and ready to harvest, is reached at about 18 to 20% moisture content. However the practical harvesting moisture content is about 13% or below. Thus the deterioration curve of hand harvested soybeans at 20% may be for soybeans near highest quality.

Hand harvested soybeans at low (9%) moisture content, as expected, exhibited a significantly faster rate of deterioration than soybeans at high moisture content, losing 0.5% dry matter in 19.8 days. These aerated storage times, however, were also found to be significantly less than for soybeans mechanically harvested at 13%. This may be attributed to the low moisture content at harvest (9%) and delayed harvesting which may have been detrimental to quality. Prolonging field drying after soybeans have reached harvest moisture content (13%) should be viewed with caution. Any cracks due to overdrying developed in the hulls of those lots would render them more susceptible to microbial attack. According to Milner and Geddes (1946a), damaged seeds present a more hospitable medium for mold mycelial penetration and growth of microorganisms than undamaged beans. It is in these cracks and broken parts of the beans that mold growth first appears.

Carbon dioxide evolution curves of preserved samples

Polynomial regression analysis between the amount of carbon dioxide evolved per kg dry matter loss and storage time for the preserved samples yielded curves as shown in figure

3. (see Appendix D for complete data). The models describing the respective relationships are listed in table 4.

Effect of -18 and 10°C preservation temperatures on CO_2 evolution

In an attempt to determine how preservation temperatures affect the CO_2 evolution, soybeans machined harvested at low moisture content (9%) were preserved in -18 and 10°C chambers. Soybeans were taken out of the chambers after 26 and 48 weeks of preservation and were used in the aerated storage tests. Based on the corresponding carbon dioxide evolution curves, the average times for soybeans to lose 0.5 and 1.0% dry matter are summarized in table 5.

Analysis of variance (table 6) shows that aerated storage times of samples, averaged over preservation period for the two temperatures, shows no significant difference (P>F= 0.45). There is, however, a high level of significance of the effect of preservation period on the aerated storage times, averaged over temperatures, when the sample was used in the storage studies (P>F=0.00). No preservation period-by-temperature interaction effect can be detected (P>F= 0.64). Analysis of the linear component of the preservation period source of variance shows that the rate of deterioration, averaged over temperatures, is affected by the period (P>F= 0.00). The linear by temperature variance shows that the slopes between temperatures do not differ (P>F= 0.5), implying that a common slope is sufficient to explain the variation in the rate of deterioration in the 9% moisture content sample persevered at -18 and 10°C with reservation period. Although the deviation or the lack of fit component is

significant (P>F=0.1), the larger mean square (320.3) of the linear compared to the deviation (2.3), shows that a straight line is sufficient to explain the relationship.

A linear relationship between storage time, averaged over temperatures, and preservation period was established with a coefficient of determination, R^2, of 0.95, a slope of -0.214 ± 0.025 and an intercept of 22.8 (figure 4).

Generally, samples preserved at -18°C either at 26 or 48 weeks were found to have longer storage times to both levels of dry matter loss than samples preserved at 10°C (table 5). However the differences between the pairs were not significant as indicated by the LSDs (table 5). For an example, the 26 weeks samples preserved at -18°C deteriorated to 0.5% in 18.4 days as compared to 17.7 days at 10°C. The difference, 0.7 days, was insignificant since the LSD for this comparison is 1.34 days. The same observation can be made with 48 weeks samples. Clearly, the findings indicate that there was no significant effect on the rate of deterioration between 9% moisture content soybean preserved at -18 or 10°C. Preserving soybeans at -18°C, therefore, does not maintain freshness better than preservation at 10°C.

Effect of moisture contents during preservation in -18°C on CO_2 evolution

The effect of moisture contents during preservation in maintaining the freshness of soybeans was investigated by comparing soybean samples at 22 and 9% when preserved at -18°C (see Appendix D).

Based on the carbon dioxide evolution curves, the average deterioration times are summarized in table 7. Analysis of variance on the data at 0.5% dry matter loss (table 8)

shows there was a significant effect of soybean moisture content during preservation (P>F= 0.004) on the rate of deterioration, averaged over period, during the storage studies. There were also significant effects of preservation period (P>F= 0.00) and preservation period by moisture (P>F= 0.00) on the deterioration times for the samples. Analysis of the linear component averaged over all moisture contents indicated that the slope differs from zero (P>F= 0.0001) and linear by moisture component of (P>F= 0.0001) implies that slopes differ among moisture contents. Although the deviation from a straight line clearly do not fit to weekly average over the two moistures, as P>F= 0.6, the larger mean square for linear effect (72.5) compared with the deviation effect (0.2) justifies use of a straight line to explain the relationship between the rate of deterioration during storage and preservation period.

Regression analysis between rates of deterioration and preservation period shows a high coefficient of determination (R^2=0.95) for the 9% soybean samples, with a negative slope of -0.21 ± 0.043 days per week of preservation period and an intercept of 22.9 (figure 4). The linear characteristic is almost the same as rate averaged over temperatures. For 22% moisture content soybeans, there is almost no change in the rate for between fresh soybeans and those preserved for 48 weeks.

Soybeans with a low moisture content, therefore, demonstrated a decrease in rate throughout the preservation period. At 48 weeks, the rate was almost as high as for soybeans harvested at high moisture content. While the rate was maintained in soybean with high moisture content, preservation of low moisture bean resulted in a drastic impact on its ability to maintain initial rate of deterioration.

Observation on microbial growth

During the storage study, observations were made on the growth and development of visible fungi on the soybeans. Mycelial growth was visible after 4 to 13 days of storage. The growth was first spotted on soybeans at the bottom of the storage unit, at the end where the air enters. The range depends on the history of the sample. Samples preserved and machine harvested at high moisture were found to show faster visible mold growth than hand harvested at high moisture samples. The first and predominant mold to appear the during storage was the grayish growth of *Penicillium spp. Penicillium spp.* are common storage fungi which require relative humidity in the range of between 85 to 90% for minimum growth (Christensen and Saur, 1982). The dominance of the species is expected as the storage condition in the studies was maintained at 95% relative humidity. In the fresh samples, there were also visible spots of whitish cotton-like mold growing along side the grayish mold especially at the bottom section of the storage unit They can either be *Fusarium* or/and *Phombosis spp.*, both of which belong to a group of fungi collectively known as field fungi.

Growth of the grayish mold intensified within the 200 mm bottom portion of the storage unit at the initial storage, but eventually spread all over the entire storage unit. Depending on the history of the soybean, it took between 12 to 24 days to cover the entire storage unit. It was during this phase that spots of orange mycelial growth of *Aspergilus spp.* also became visible. In tubes containing fresh soybeans, there were also spots of black mold growth resembling of those of *Chaetomiun spp.*, another field fungi.

CONCLUSIONS

Based on the results of this studies, the following conclusion could be made:

1. Moisture content of soybeans during harvest has the greatest impact on the rate of deterioration during storage. A moisture content of 13% can be considered best for harvest, in terms of deterioration. Even soybeans manually harvested at 8% exhibited a higher rate of deterioration than the 13% machine-harvested sample.

2. Soybeans with a moisture content of 9%, when preserved at -18°C, had a slower rate of deterioration than soybeans preserved at 10°C. The difference in the rate, however, was not significant.

3. Soybeans at 20% moisture content maintained the same rate of deterioration as fresh beans when preserved at -18°C.

4. The rate of deterioration of soybeans at 9% moisture content preserved in -18°C and 10°C environments increases linearly with period of preservation. The rate is faster in soybeans preserved in a 10°C environment.

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Table 1. Treatments for experiment 1

Treatment #	History of sample
1	Machine harvested at 21% MC
2.	Machine harvested at 13% MC
3.	Hand harvested at 20% MC
4	Hand harvested at 9% MC

MC = moisture content

Table 2. Regression models for CO_2 evolution from fresh soybean samples

Treatment	General model: Y, g CO_2/kg dm = $c_1 t + c_2 t^2 + c_3 t^3$ Coefficients of the polynomials		
	c_1	c_2	c_3
Machine-harvested/high MC	0.195	ns	0.038
Machine-harvested/low MC.	ns	0.012	0.0001
Hand-harvested/high MC	0.034	0.006	0.0001
Hand-harvested/low MC	ns	0.018	ns

MC =moisture content; t = number of days, ns = not significant

Table 3. Average aerated storage times of freshly harvested soybeans

	Number of days to reach the respective percentage of DML[1]			
	Combine Harvest		Hand Harvest	
Moisture at Harvest	DML 0.5%	1.0%	0.5%	1.0%
High	11.5	17.8	26.2	37.1
Low	22.5	31.2	19.8	28.1

[1]Mean from three replicates;
$LSD_{\alpha=0.05}$ @ 0.5% DML = 2.134, $LSD_{\alpha=0.05}$ @ 1.0% DML = 2.69

Table 4. Regression models for CO_2 evolution from preserved soybean samples

Model	General model: Y, g CO_2/kg dm = $c_1 t + c_2 t^2 + c_3 t^3$ Coefficients of the polynomials		
	c_1	c_2	c_3
22%MC,-18°C,26 weeks	ns	0.056	0.001
9%MC,-18°C,26 weeks	ns	0.014	0.0003
9%MC, 10°C,26 weeks	ns	0.014	0.0005
22%MC,-18°C,48 weeks	ns	0.057	-0.001
9%MC,-18°C,48 weeks	0.244	0.026	ns
9%MC, 10°C,48 weeks	0.258	0.028	ns

MC=moisture content; t = number of days, ns = not significant

Table 5. Average aerated storage times of combine-harvested soybeans preserved at 9% MC

Preservation Period (Weeks)	Number of days to reach the respective % DML [1] 0.5% 9% MC in -18° C	0.5% 9% MC in 10° C	1.0% 9% MC in -18° C	1.0% 9% MC in 10° C
0	22.5	22.5	31.2	31.2
26	18.4	17.9	23.9	22.9
48	12.4	11.8	18.8	17.8

[1] Mean from three replicates; $LSD_{\alpha=0.05}$ @ 0.5% DML= 1.34 ;
$LSD_{\alpha=0.05}$ @ 1.0% DML = 1.62

Table 6. ANOVA on the aerated storage times of 9% soybean samples preserved in -18 and 10°C environments

Source	DF	MS	F	P>F
6. (a) Main ANOVA				
Temperature,T	1	0.9	0.7	0.45
Chamber (T)	4	1.32		
Period, P	2	161.3	320.0	0.0
P*T	2	0.24	0.5	0.6
P*C(T)	8	0.5		
	17			
6. (b) Subdivision of linear				
Linear, Lin	1	320.3	616.0	0.00002
Lin*T	1	0.3	0.5	0.5
Lin*C(T)	4	0.52		
6. (c) Subdivision of deviation components				
Deviation, Dev	1	2.3	4.6	0.1
Dev*T	1	0.22	0.45	0.5
Dev*C(T)	4	0.5		

Table 7. Average aerated storage times of combine-harvested soybean preserved at 22 and 9% MC in -18°C environments

Storage Period (Weeks)	Number of days to reach the respective % DML [1]			
	0.5%		1.0%	
	22% MC in -18°C	9% MC in 10°C	22% MC in -18°C	9% MC in 10°C
0	11.5	22.5	17.8	31.2
26	10.1	18.4	14.4	23.9
48	11.6	12.4	18.0	18.3

[1] Mean from three replicates; MC=moisture content;
$LSD_{\alpha=0.05}$ @ 0.5% DML= 0.86 ; $LSD_{\alpha=0.05}$ @ 1.0% DML = 1.18

Table 8. ANOVA on the aerated storage times during storage from 22 and 9% soybean samples preserved in -18°C environment

Source	DF	MS	F	P>F
8. (a) Main ANOVA				
Moisture, M	1	203.35	251.0	0.004
Chamber, C	2	0.8	1.0	0.5
C*M	2	0.8		
Period, P	2	36.4	173.1	0.0
P*M	2	42.2	200.9	0.0
P*C*M	8	0.21		
	17			
8. (b) Subdivision of linear				
Linear, Lin	1	72.5	72.5	0.0001
Lin*M	1	78.5	78.5	0.0001
Lin*C*M	4	0.26		
8. (c) Subdivision of deviation components				
Deviation, Dev	1	0.2	0.3	0.6
Dev*M	1	5.8	8.5	0.04
Dev*C*M	4	0.69		

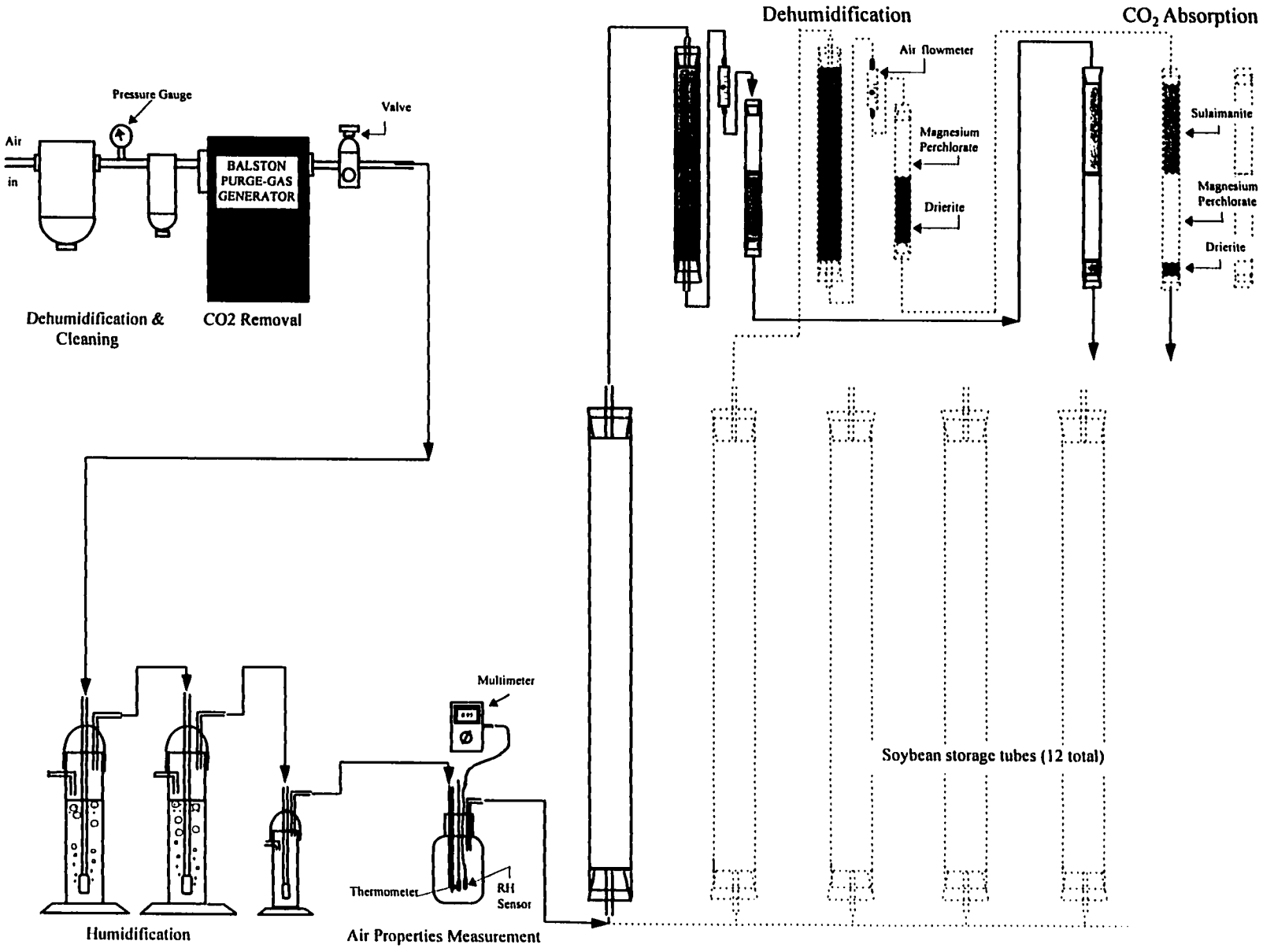

Figure 1. CO_2 measuring system

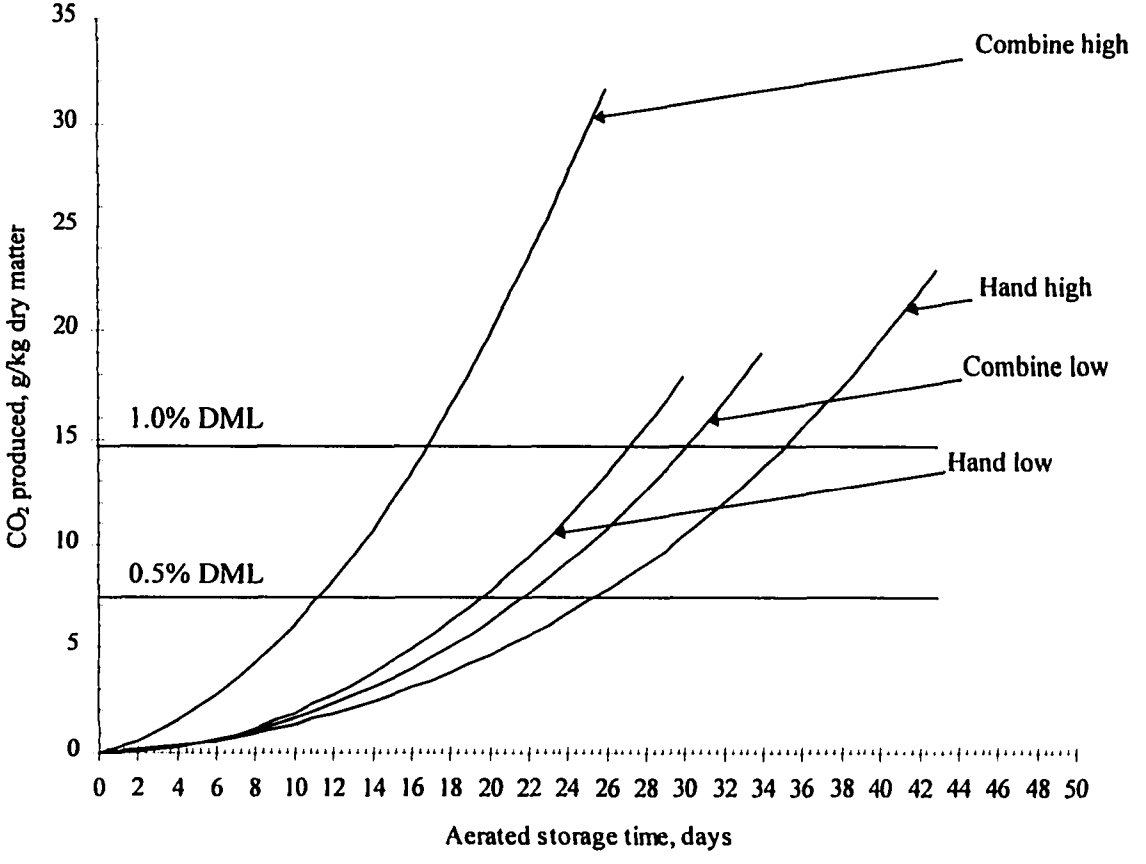

Figure 2. CO_2 evolution from freshly harvested soybeans under two harvesting modes and two moisture contents

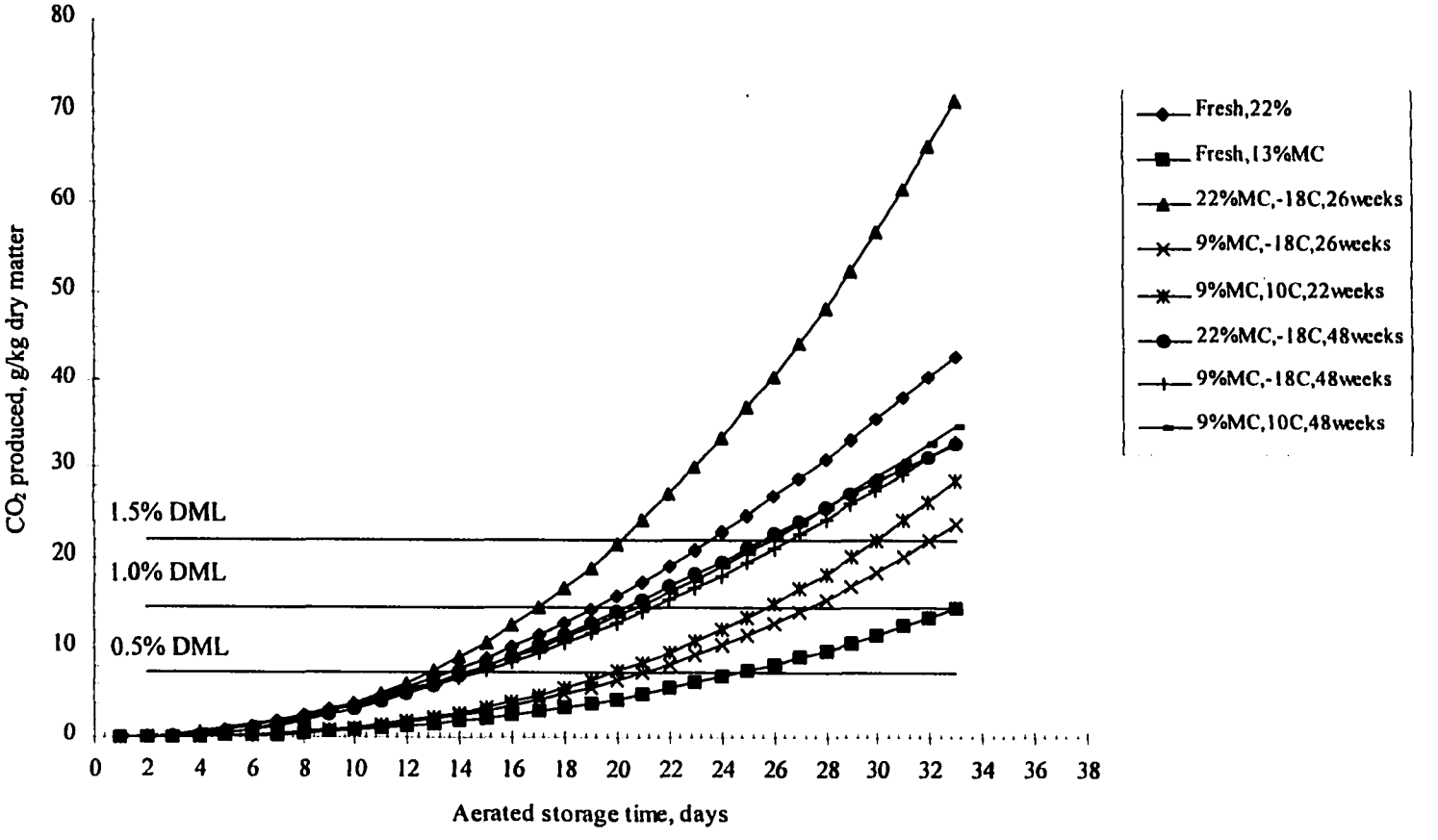

Figure 3. CO_2 evolution of soybeans under different preservation conditions

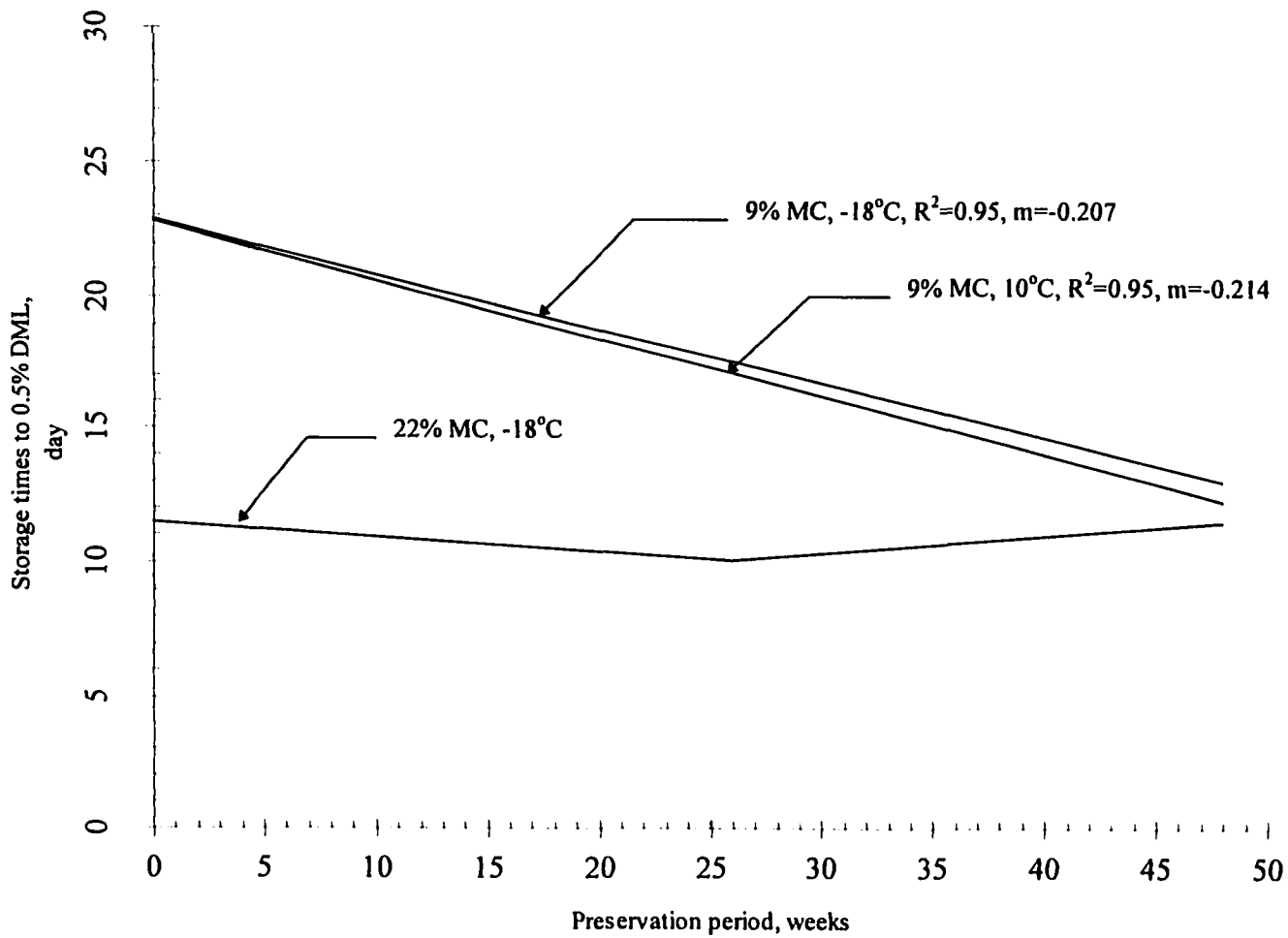

Figure 4. Rate of deterioration during storage as a function of preservation period

EFFECTS OF PRESERVATION ON QUALITY OF SOYBEAN DURING STORAGE

A paper to be submitted to the Transactions of the American Society of Agricultural Engineers (ASAE)

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ABSTRACT

Fresh soybean and soybean samples preserved at 22 and 9% moisture content in -18 and 10°C environments were used in the study of carbon dioxide evolution during storage. The effect of preservation on the quality of soybean were evaluated based on % free fatty acid (FFA) content and damaged kernels total (DKT). Soybeans preserved at 22% moisture content in -18°C exhibited a linear increase in the FFA with preservation period at a rate of increase 0.007 ± 0.004 % FFA per week of preservation with a coefficient of determination $R^2 = 0.7$. There was no effect of preservation temperature on the 9% moisture content soybeans. There was no clear evidence to indicate that the DKT is affected by preservation. The use of preserved soybean sample in carbon dioxide evolution studies were found to affect the % FFA development and DKT. Soybeans preserved at 22% moisture content and -18°C had a linear rate of % FFA increase during carbon dioxide evolution studies (R^2=0.82) at 0.02 ± 0.008 % FFA per week of preservation. Soybeans preserved at 22 and 9% moisture showed little variation in DKT through out the preservation period. There was however a tendency for the damage level to increase slightly during storage from samples preserved

over a long period of time. The slightly relationship between FFA and dry matter loss (DML) can be described by a straight line with R^2=0.8 and with a rate of increase (slope) of 0.72 ± 0.08 % FFA per unit of DML and an intercept of 0.15. The relationship between DKT can also be expressed by a straight line (R^2=0.7) with a rate of increase (slope) of 3.52 ± 0.5 % DKT per % unit DML.

INTRODUCTION

Deterioration of grain during handling and storage is associated with a loss of dry matter. Direct measurement of carbon dioxide produced as grain deteriorates during laboratory scale storage test can be related to dry matter loss (DML). Steele (1967) was one of the earlier researchers to use DML as a variable in a deterioration model developed for stored shelled corn. The amount of DML during deterioration of corn has also been linked with a decrease in the market grade of corn. A loss of more than 0.5% dry matter of shelled corn has found to lower the grade by one grade (Saul and Steele, 1969). The 0.5% DML becomes a threshold value and has been adapted as a basis of the widely used Allowable Storage Time (AST) table was formulated for corn (MWPS, 1980).

Different crops require the use of different sets of values and information before such tables can be developed. Paper 2 in the thesis reported the results of a study conducted to define the process of deterioration of soybeans as measured by the amount of carbon dioxide produced during laboratory storage of freshly harvested soybeans and soybeans that were preserved for a period of time before use in the storage studies.

The most direct and significant impact of deterioration during handling and storage of soybeans is the implication on the soybean marketable grade. While 0.5% DML level was established for shelled corn, the value cannot be directly applied to soybean. Further observation of corn after it has sustained a loss of 1% DML showed that it had became almost sample grade (Saul, 1967). Specific DML values and the grades associated with them are required to be developed for soybeans.

Since quality attributes of oilseeds also depend on end-use, the impact of deterioration on other important quality parameters relevant to the industry need to be evaluated concurrently. In the soybean industry, where the main products are oil and high protein meal, the maintenance of high quality soybeans is more critical in oil processing industry than in meal production. One of the few important quality attributes of oil highly regarded by the soybean oil industry is the free fatty acids (FFA) content of the oil. Low FFA content is correlated with high quality oil. Zeleny and Coleman (1938) found that fat acidity can be used as an index of deterioration of corn. They determined fat acidity in 252 corn samples and plotted the average percentage increase in fat acidity against the corresponding corn grades. FFA appeared to be a reliable index of soundness of grain.

During storage of soybeans, minimizing the loss of quality of soybean as they deteriorate in storage has to be achieved consistently, through minimum fluctuation in the damaged level and other quality parameters. Oil quality continues to decline during storage of soybeans, at a rate measured by an increase in FFA. The rate of increase in FFA content during storage of soybean was found to be higher in damaged than in sound soybeans

(Urbanski et al., 1980). Relating DML in soybeans to the FFA content in the oil may be useful in interpreting soybean quality.

It is a common practice to preserve samples in low temperature environments as storage experiments cannot be conducted all at the same time due to physical constraints or storage treatments requiring a different time frame. For shelled corn, preserving at -10°C freezer has been found to maintain freshness, in terms of its rates of deterioration (measured by the amount of carbon dioxide evolved per kg of DML), as a freshly harvested soybeans (Fernandez et al., 1985). Carbon dioxide production by samples preserved at 22% moisture content and 3°C environment for 70 days was significantly higher than fresh 22% moisture content corn. No information is available on the effect of preserved soybean samples on %FFA development when samples are used in carbon dioxide evolution studies.

The argument is that soybean may have been damaged somewhat during preservation and thus may have different rates of FFA development during storage than fresh soybeans and samples held at a shorter periods. There is therefore a need to establish the effect of preservation on the development of FFA during storage and how preservation itself, affects damage and FFA content in the preserved sample.

OBJECTIVE

This paper describes a study conducted to define the deterioration of soybeans, as measured by its DML, damage level and oil quality parameter. The specific objectives of the study are to determine:

1. the effect of preservation on FFA content
2. the effect of preservation on the damaged kernels totals (DKT)
3. the effect of fresh and preserved soybean on the FFA development during storage
4. the effect of fresh and preserved soybean on the DKT during storage
5. to define the equivalent soybean grades on the basis of its DML and FFA content

MATERIALS AND METHODS

Soybeans

The soybeans sample used in the study were Kruger 2555[4] variety. The samples were combine or hand harvested at high (20 - 22% moisture contents) and low (8 - 13%) moisture contents at the Iowa State University Agronomy and Agricultural Engineering Research Center, 15 km west of Ames. They were cleaned by using a Carter day Model XT 3 Dockage Tester[5]. The cleaning was done with 12.2 mm (0.5 in) square hole, 0.25 mm (20/60 in) round hole and 7.94 X 1.91 mm (20/64 X 3/4 in) slotted sieves. The composition of oil, protein and fiber of the soybeans, determined by a Near Infrared Reflectance (NIR), were 18.8%±0.3, 34.55%±0.4 and 4.8%±0.3, respectively.

[4, 5] Mention of trader or manufacturer names is for the benefit of the readers only and does not imply an endorsement, recommendation, or exclusion by Iowa State University over other firms or similar products not mentioned.

Preparation of samples

Fresh soybeans

Fresh soybeans, machine and hand harvested at high and low moisture contents were subjected to the laboratory storage tests. The experiment consisted of two treatments, each replicated three times. A carbon dioxide measuring system developed at the Department of Agricultural and Biosystems Engineering, Iowa State University (Dugba et al., 1996) was used.

The evolution of carbon dioxide was measured during storage of soybeans in an aerated units with air at 95% relative humidity and 26°C. The amount of carbon dioxide evolved during storage, in g of carbon dioxide per kg dry matter, was recorded on a daily basis until the experiment was terminated either at 14.5 g/kg (1% DML), 22.05 g/kg (1.5% DML) or at 29.4 g/kg dry matter (2.0% DML) and samples were taken. The allocation of the soybean samples to each storage unit in the carbon dioxide measuring system was made at random. 300 g samples were first withdrawn from the storage studies when an estimated 7.35 g per kg DML (0.5% DML) was produced. The remainder of the soybean sample was left in the storage unit until termination The samples at the two DML levels from the four treatments were analyzed for total damage kernels and FFA content.

Preservation of soybean samples

The scope of the storage experiment required that samples for subsequent experiments be preserved as all experiments could not be conducted at once.

Preservation of soybean samples were accomplished by packing them in sealed plastic bags at 22 and 9% moisture content in a -18°C environment (freezer) and at 9% moisture content in a 10°C environment (cold room). Three freezers and three cold rooms, representing three replicates of preservation temperatures, were used to hold samples. The initial damage levels, FFA contents and moisture contents were determined before the samples were placed in the environmental chambers. When samples were ready to be taken out for the storage experiment, the damage level, FFA content and moisture contents were again measured.

Preserved sample

During preservation, soybean samples were taken after 26 and 48 weeks of preservation and were subjected to storage experiments as described for fresh samples.

Samples were withdrawn first at the equivalent 0.5% DML and later when the experiment was terminated. Damage level and FFA content were determined. The data were compared with those of the fresh soybeans.

Damaged kernels total

Whole soybean samples of 160 g at the two DML levels were bagged, labeled and sent to the Central Iowa Grain Inspection Services, Inc., Des Moines, Iowa for damage testing. The damage test results were reported as DKT.

FFA analysis

Oil was extracted from each sample by using ethyl alcohol as described in AOCS Official Method (1989) Aa 4-38. The FFA content in the crude oil was determined by titration with a standard solution of NaOH according to a modified version of the AOCS official Method Ca 5a-40 (paper 2 of this thesis).

Moisture content

Soybean moisture contents were determined by the oven method. A 10 g whole soybean sample was heated in a forced draft oven for 4 hr at 130°C. Moisture content was expressed as percentage on wet basis, unless otherwise stated.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and general linear model (GLM) procedures of the Statistical Analysis Software (SAS Institute, 1990). Observations made on the samples at different time periods during preservation were treated as repeated measures. Corresponding testing of linearity and lack of fit were made to summarize the information. Specific differences between treatments were determined by means of either an F test or least significant different (LSD) with significance established at $P > F = 0.05$. Whenever appropriate, regression analyses were also carried out on the data to establish the relationship patterns between variables.

RESULTS AND DISCUSSION

Soybean quality during preservation

During the preservation of soybean samples, the influence of soybean moisture content and preservation temperatures on %FFA content, damage level and final moisture were monitored (see Appendix E for complete data).

Effects of moisture content and preservation temperature on FFA content during preservation

Effect of moisture content. The effect of initial moisture content of soybeans on the final FFA during preservation was determined by measuring FFA contents in the 22 and 9% moisture content soybean samples preserved at -18°C. The initial FFA content was determined and compared with FFA content from 26 and 48 week-old sample after preservation.

Linear regression analysis of FFA contents with preservation period for the two soybean moisture contents yielded slopes, intercept and coefficient of determination, R^2, shown in table 1. While the increase in the FFA content in the 22% moisture content samples during preservation can be best described by a straight line (R^2=0.73), the same is not true for soybean samples preserved at 9% moisture content.

ANOVA showed that there was not only a significant effect of moistures content (22 and 9%) on the FFA content during preservation at -18°C, with P>F = 0.001 (table 2a), but the preservation period and preservation period by moisture interaction effects were also

important (P>F = 0.00007 and P>F = 0.0001 respectively). Subdivision of the preservation period effect into linear components (table 2b) showed that FFA content averaged over moisture content had a slope that differs from zero (P>F = 0.009). Also, the slopes for the two moisture contents differed, as indicated by the linear by moisture P>F = 0.01. The implication of different slopes is that preservation period affects %FFA content in soybeans. It is clear from the level of significance of deviation changes, P>F = 0.004 (Table 2c), that most of the changes over preservation period is explained by the straight line and deviation from the straight line is minor by comparison. The deviation by moisture source measures the pattern deviation from one moisture to another. Since P>F = 0.009, the relationship between FFA and preservation period for individual moisture therefore can be expressed as a straight line. This was especially true with the 22 % moisture content sample where the coefficient of determination, $R^2 = 0.7$, and the rate of increase in FFA during preservation is about 0.007 ± 0.004 % of FFA per week of preservation and an intercept of 0.08. The 9% moisture content relationship is not only poorly explained by a linear model (R^2=0.06), but the rate of increase of 0.0004 was too small to have any influence on the overall soybean oil quality. Preserving soybeans at 9% moisture and in -18°C could be considered to have no influence on final FFA content.

Table 3 shows the mean FFA content of the soybean samples preserved at the two moisture contents and -18°C preservation chambers averaged over the preservation period. The % FFA, averaged over preservation period, for high and low preserved moisture content

soybean samples are 0.25 and 0.14%, respectively. The mean initial FFA content of the sample was about 0.17%.

Effect of preservation temperature. The effect of preservation temperature on the initial and final FFA was determined by analyzing FFA content in soybean samples preserved at 9% moisture content and -18 and 10°C preservation temperatures. ANOVA indicates that there was no significant difference (P>F = 0.62) in the FFA content for 9% moisture content soybeans when preserved at the two temperatures. The effects of preservation period and preservation period by temperature interaction were also not significant with p>F = 0.2 and 0.7, respectively. Preserving soybeans at 9% moisture content and either -18 or 10°C environment results in a minimal change in the FFA content over 48 weeks. The summary of the mean FFA content, averaged over preservation temperature for the three preservation periods is given in table 4. FFA content was found to increase from 0.133 to 0.17 % over 48 weeks of preservation. The LSD indicates that that there was no marked variation in FFA content during preservation.

Effects of moisture content and preservation temperature on DKT of soybean during preservation

Effect of moisture content. The level of DKT of the samples after preservation were compared based on the values determined by the Central Iowa Grain Inspection Service, Inc., Des Moines on 150-g samples. Total damage is assumed to reflect a determination of soybean quality that carried over into the processed oil.

The effect of moisture content of soybeans at 22 and 9% during preservation on DKT was found to be significant (P>F=0.008) when held at -18°C temperature. However, careful examination of the data revealed that the statistical significance should be viewed with caution (table 5). The bulk of the variation came from soybeans at 22% moisture content after 26 weeks of preservation, where the average damaged value was 0.47%, the initial was 0%. The mean value of damaged kernels from the same sample but after 48 weeks was 0.03%, and two of the three replicates had zero damage levels. Being a subjective measurement, the significant effect of moisture contents on the DKT during preservation may have been due to a human error and not a result of the treatment. It has been observed visually that soybean samples at high moisture content after 26 and 48 weeks of preservation had hulls slightly dull in color as compared to bright hull color for soybeans at 9% moisture. This dull color may have deceived the operator to treat the sample as damaged.

Effect of preservation temperature. The effect of preservation temperatures (-18 and 10°C) on DKT in samples preserved at 9% moisture content was not significant, P>F= 0.21. Although the period effect was significant, the slope of the line does not differ from zero (P>F= 0.37). The increase in FFA averaged over temperatures was not significant. The mean DKT levels averaged over temperature during preservation period is shown in table 6.

Therefore, DKT did not change during preservation at either 22 or 9% moisture content in an -18 or 10°C.

Effect of preservation on moisture content of soybean

The initial and final moisture content of soybeans before and after preservation were also monitored to determine if there was any variation in moisture content during preservation. ANOVA was performed only on data for 9% moisture content soybeans preserved at -18 and 10°C. There was no significant change in moisture content during preservation averaged over the two temperatures (P>F =0.83). There was a significant decrease in moisture content averaged over temperatures during the preservation period (P>F= 0.007), but evidence (P>F for linear by moisture interaction = 0.7) indicates that a common slope (P>F= 0.03) was sufficient to capture the preservation period effect. However a small slope (-0.01) and a coefficient of determination R^2 of 0.4 indicated that the increase was too small and a linear model would be inadequate to summarize the decrease in the moisture content during preservation. Table 7 shows the mean moisture content values averaged over temperatures for the three preservation periods. Observation of the high moisture content soybeans at -18°C showed very slight variation (0.8%) in the value over time from the initial. At 48 weeks moistures were at 22.32 and 22.5%, respectively.

Use of preserved soybean in storage studies

FFA and DKT development during storage studies were evaluated using fresh soybeans and soybeans after 26 and 48 weeks of preservation. Soybean samples were taken at different levels of DML during the carbon dioxide evolution studies and analyzed for FFA and DKT (Appendix E).

Effects of preservation on FFA development during soybean storage

Effect of moisture content. The effect of moisture content of soybeans during preservation on FFA development during storage was determined by analyzing stored soybean samples (fresh, and preserved 26 and 48 weeks) after about 0.5% of its dry matter was lost.

ANOVA (table 8) shows that there was a significant effect of preserved soybean moisture contents on the FFA when soybeans were used during storage studies (P>F = 0.005). There were also significant preservation period and preservation period by moisture effects on the FFA development while soybeans were in storage. The linear components of the preservation period effect shows that the slope of the line of FFA, averaged over moisture, with preservation period differs from zero at P>F = 0.003. The significance of the linear by moisture interaction, P>F = 0.001, indicates that the slopes between the two moistures (22 and 9%) differs. Although the significant level of deviation or lack of fit is big (0.06), it was sufficient to describe the increase in FFA during storage as a straight line model as the mean square of the linear (0.48) is very much higher than the deviation (0.09).

Separate regression analysis on the FFA content with preservation period from 22 and 9% stored soybean samples gave an output as shown in table 9. With R^2=0.82, a linear model with a slope of 0.02 ± 0.008 and intercept of 0.24 is sufficient to summarize the increase in the FFA during the storage experiment for samples preserved at 22%. The longer soybeans are preserved at 22% moisture content, the higher the % FFA content of the sample at 0.5% DML.

The R^2 value (R^2=0.6) for the 9% moisture content sample could explain a linear variation in 9% moisture content soybean where % FFA per week of preservation was found to decrease (slope = - 0.003), but is too small for consideration. The mean FFA content at 0.5% DML for 9% moisture content soybean samples at 26 and 48 weeks samples were 0.252 and 0.244%, respectively compared to 0.367% when fresh.

Effect of preservation temperature. The effect of preservation temperature (-18 and 10°C) on the rate of hydrolysis during storage was determined by analyzing FFA contents in the 9% moisture content samples. ANOVA shows that there was significant temperature effect, P>F = 0.03, on FFA content during storage when soybeans were preserved at 9% at -18 and 10°C (table 10). There was enough evidence (P>F =0.04) to show that the % FFA during storage averaged over temperatures was also affected by length of preservation. The interaction of preservation period by temperatures effect on FFA was too low to be of any significance (P>F= 0.06). Examination of the P>F for linear (0.01), linear by temperature (0.26) and lack of fit (0.32) indicate that there is lack of evidence to summarize the relationship linearly. In fact, regression analysis for a common slope yields a low R^2 value (0.2). Table 10 shows the variation in the FFA after storage between fresh and preserved samples. There is no increase in FFA after 26 weeks of preservation (0.37%) from the initial value (0.37%), but the value tended to decline after 48 weeks of preservation (0.26%).

Effects of preservation on DKT during soybean storage

Effect of moisture content. Similar analysis was also performed on the DKT of soybeans during storage (table 11). There was a significant effect of preserved soybean moisture content (P>F = 0.04) on the DKT (averaged over preservation period) during the storage studies. The preservation period and preservation period by moisture content interaction effects also were not significant (P>F= 0.17 and 0.81 respectively). The level of significance of the linear component shows that DKT does not vary with preservation period. A summary of DKT is shown in table 11. Soybeans preserved at 22% moisture content results in 3.5% DKT compared to 2.8% from the fresh sample. For 9% moisture soybeans, the range in DKT is 0.6 and 1.3% for fresh and preserved samples, respectively. There is a trend, though small, that the longer soybeans are preserved, the higher the DKT will be when it is used in the storage studies.

Effect of preservation temperature. The temperature (-18 and 10°C) during preservation of 9% moisture soybeans had no significant effect on DKT in the samples during storage (P>F= 0.9). There were also no evidence to say that preservation period and preservation period by temperature has any influence on the DKT during storage (P>F = 0.5 and 0.9, respectively). Examination of the raw data (table 12) shows that DKT at 0.5% DML from fresh soybean is 0.73 and 1.62% from preserved sample. The longer soybeans are preserved, the higher the DKT level after storage.

Relationship between FFA and DML

The FFA content at two DML levels were regressed with the corresponding DML. The relationships between FFA content and DML were established for fresh, 26- and 48-week preserved soybean samples by linear regression. The FFA content from the high moisture content sample at 48 weeks was first adjusted to account for the effect of preservation. Figure 1 shows the linear plot of the regression model describing the increase in the FFA content of fresh and samples preserved for 26- and 48-weeks with DML. The models were found to be sufficient to explain the increase in the FFA content with the DML with R^2 values of 0.7, 0.9 and 0.8 for fresh, 26- and 48-weeks samples respectively (table 13). The slopes of the lines indicate the rate of increase in FFA per unit change in DML. Fresh soybeans exhibited a slightly faster rate of increase in FFA (0.8) among three samples. Fresh soybeans may have carried a higher microbial load and wider range of fungi species than preserved samples. Being fresh from the field, the fungi were still thriving on the substrate and continued to thrive when the samples were put in the storage unit. Soybean highly infected with fungi are typically known to produce oils with higher FFA content (Wilson et al., 1995). The preserved samples have a rate of increase between 0.7 to 0.72%.

Relationship between DKT and DML

DKT was regressed with DML for each set of the data obtained from fresh, 26- and 48-week samples by using linear regression analysis. Since the DKT at zero DML is zero, the

regression analysis with no intercept option was performed. Figure 2 shows the linear plots of DKT against DML of the three models.

The coefficient of determination (R^2) of 0.52 for the fresh sample shows that linear correlation for the two variables can be considered as barely sufficient (table 13). Most of the observations of DKT data for the fresh sample at particular DML levels, were distinctly scattered below the 4% DKT level except three observations that had DKT values of more than 11.8%. The three observations represented soybean samples machine harvested at high moisture content (22%). Harvesting operations may have damaged the beans so badly that even though the samples were initially clean and splits were separated, the cracks and the bruises may have encouraged rapid growth of microflora, which was enough to inflict excessive kernel damage during deterioration. Regression analysis on the same data excluding the data for soybeans that were machine harvested at high moisture content improved the R^2 by about 30% (R^2= 0.67).

For the 26- and 48-week preserved samples, the total kernel damage were highly correlated with DML, with regression analysis yielding R^2 values of 0.85 and 0.71%, respectively. Considering the subjectivity of the total kernel damage determination, the coefficient of determination obtained can be considered high.

Examination of the slopes of the regression models indicated that the rate of increase in the damage for every unit of dry matter soybean losses was slower in fresh samples than in the preserved soybean samples. The rates of increase are 3.1 ± 1.2, 3.6 ± 0.62 and 3.9% ± 1.0

for fresh and 26- and 48-week preserved samples, respectively. The rate of increase in DKT shows an increasing trend as preservation period increases.

Interpretation of grade from DKT and FFA

DKT is one of the factors in the Official US Standards for soybeans that defines marketable grade for whole soybean. Table 14 shows the maximum levels of DKT allowed for a particular soybean grade.

Determination of DKT is to reflect deterioration of soybean quality at a level that is detrimental to the quality of the processed oil. The method is based on 'interpretive line slides', photographic illustration of the intensity of surface mold damage or discoloration to designate soybeans as damaged. The assessment is, however, less-than-perfect reflection of the inherent value of soybeans for processing into oil and meal. To correlate DKT, a subjective evaluation, to DML so as to provide a useful and comprehensive relationship would require as many observations as one could possibly obtain and from condition ranging as widely one would expect soybean to be exposed to. The scope of this research would not permit the gathering of such data possible, but considering the limited data currently available from the study, comments would still be appropriate.

Figure 3 is a repetition of the scatter plot of DKT (primary Y-axis) against DML, but superimposed with lines representing the minimum requirement of damage level for the four grades. The line passing diagonally through the points in the plot is a regression line of the

DKT versus DML using all observations irrespective of their initial background. The model:

$$DKT, \% = \beta_1 * DML \quad \text{.....[1]}$$

where: DKT = % damaged kernels total
DML = % dry matter loss
$\beta = 3.52 \pm 0.5$

was found to have a high coefficient of determination, R^2, of 0.7.

The rate of change of DKT with DML, the slope of the above model, shows that one depreciation of grade is likely to occur for every 0.5% increase in the DML, the same observation established for corn (Saul and Steele, 1969). The pattern can be clearly demonstrated as shown by the vertical dotted lines (fig 3).

The regression line of FFA (on secondary Y-axis) against DML (fig 3)was also established using all observations. The general model, with R^2 of 0.79, can be described as:

$$FFA, \% = \beta_0 + \beta_1 \cdot DML \quad \text{.....[2]}$$

where: FFA = % free fatty acids
DML.= % dry matter loss
$\beta_0 = 0.15 \pm 0.1$
$\beta_1 = 0.72 \pm 0.08$

Dropping vertical lines from the DKT model, at the respective points of intersection with the damaged lines, to the FFA model enables reading of corresponding FFA off the secondary Y-axis for each soybean grades. Column 3 of table 14 shows the FFA values at the

four damaged levels using the above relationships. Grade 1 and 2 would have FFA values of less than 0.8% and grades 3 and 4 are between 1 and 2%.

Iverson and Koeltzow (1986) established a relationship between FFA content in soybean oil with damage level. The equation:

$$\%FFA = 0.159\ (\%DKT) + 0.915 \quad \text{.....[3]}$$

predicted the values of FFA for the four soybean grades (column 4 of table 14). Grades 1 and 2 have FFA values of less than 1.0% while grades 3 and 4 are between 1 to 1.5% FFA content. The use of DML underestimates the FFA level of soybeans in grades 1 and 2, but overestimates in grades 3 and 4.

CONCLUSIONS

Based on the results of the study, the following conclusions are drawn:

1. There is a significant increase in FFA during preservation of 22% moisture soybean at -18 environment. The rate of increase was about 0.007% FFA per week.

2. Preserving soybean samples at 9% moisture content and -18 and 10°C has no effect on FFA content.

3. There is no clear evidence to show any difference in DKT in soybeans preserved at 22 and 9% moisture at -18°C. However, DKT of soybean preserved at 9% moisture content does not vary.

4. There is a 5% decrease in 9% moisture content soybeans during preservation between the initial and the 48-weeks sample.

5. Preserved soybeans at 22% moisture content at -18°C environment gives higher FFA content in soybeans than soybeans preserved at 9% moisture content.

6. Soybeans preserved at 22% moisture content in -18°C will result in higher DKT during storage studies than 9% moisture content soybean.

7. There is a positive linear correlation between FFA and DML and DKT and DML during storage of soybean.

8. Based on DML, the FAA content for grades 1 and 2 soybeans is less than 0.8% and between 1 to 2% for grades 3 and 4.

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Table 1. Characteristics of linear relationship between FFA during preservation and preservation period of samples preserved at 22 and 9% MC in -18°C environment

MC (%)	Slope	Intercept	R^2
22	0.007±0.004	0.08±0.13	0.73
9	0.0004	0.1	0.06

MC=moisture content

Table 2. ANOVA of FFA during the preservation of 22 and 9% MC soybean samples in -18°C environment

Source	DF	MS	F	P>F
2. (a) Main ANOVA				
Moisture, M	1	0.054	771.4	0.001
Chamber, C	2	0.003	42.9	0.02
C*M	2	0.00007		
Period, P	2	0.059	23.6	0.0004
P*M	2	0.044	17.6	0.001
P*C*M	8	0.0025		
	17			
2. (b) Subdivision of linear				
Linear, Lin	1	0.092	23.0	0.009
Lin*M	1	0.07	17.5	0.01
Lin*C*M	4			
2. (c) Subdivision of deviation components				
Deviation, Dev	1	0.027	37.3	0.004
Dev*M	1	0.017	22.7	0.009
Dev*C*M	4	0.00075		

MC=moisture content;

Table 3. FFA content during the preservation of 22 and 9% MC soybean samples in -18°C environment, averaged over period

MC (%)	FFA (%)
22	0.248
9	0.138

MC=moisture content; $LSD_{\alpha=0.05} = 0.03$

Table 4. FFA content during the preservation of 9% MC soybean samples in-18 and 10°C environments, averaged over temperature

Preservation period (Weeks)	FFA (%)		
	-18°C	10°C	Average over temperature
0	0.133	0.133	0.133
26	0.27	0.133	0.13
48	0.155	0.189	0.17
$LSD_{\alpha=0.05}$	-	-	0.07

MC=moisture content

Table 5. DKT during the preservation of 22 and 9% MC soybean samples in -18°C environment, averaged over MC

Preservation period (Weeks)	DKT (%)		
	22%MC	9%MC	Average over MC
0	0.00	0.0	0.00
26	0.47	0.1	0.283
48	0.03	0.03	0.032
$LSD_{\alpha=0.05}$	-	-	0.1

MC=moisture content

Table 6. DKT during the preservation of 9% MC soybean samples in-18 and 10°C environments

Preservation period (Weeks)	DKT (%)		
	-18°C	10°C	Average over temperature
0	0.00	0.00	0.00
26	0.10	0.17	0.133
48	0.00	0.07	0.033
$LSD_{\alpha=0.05}$	-	-	0.16

MC=moisture content

Table 7. Soybean MC during preservation at -18 and 10°C environments

Preservation period (Weeks)	Moisture content (%)		
	-18°C	10°C	Average over temperature
0	8.52	8.53	8.53
26	8.35	8.38	8.36
48	8.10	7.95	8.02
$LSD_{\alpha=0.05}$	-	-	0.38

MC=moisture content

Table 8. ANOVA of FFA content after storage to 0.5% DML from soybean samples preserved at 22 and 9% MC

Source	DF	MS	F	P>F
8. (a) Main ANOVA				
Moisture, M	1	0.86	215.0	0.005
Chamber, C	2	0.012	3.0	0.25
C*M	2	0.004		
Period, P	2	0.28	28.0	0.0002
P*M	2	0.46	46.0	0.00003
P*C*M	8	0.01		
	17			
8. (b) Subdivision of linear				
Linear, Lin	1	0.48	38.4	0.003
Lin*M	1	0.88	70.4	0.001
Lin*C*M	4	0.0125		
8. (c) Subdivision of deviation components				
Deviation, Dev	1	0.09	7.2	0.06
Dev*M	1	0.03	2.4	0.2
Dev*C*M	4	0.0125		

MC=moisture content;

Table 9. Characteristics of linear relationship between FFA during storage and preservation period

Moisture Content (%)	Slope	Intercept	R^2
22	0.02±0.008	0.24	0.82
9	-0.003±0.002	0.36	0.60

Table 10. FFA content at 0.5% DML from 9% MC soybean samples preserved at -18 and 10°C environments

Preservation period (Weeks)	FFA (%)		
	-18°C	10°C	Average over temperature
0	0.37	0.37	0.37
26	0.252	0.48	0.37
48	0.224	0.291	0.26
$LSD_{\alpha=0.05}$	-	-	0.13

MC=moisture content

Table 11. Mean DKT at 0.5% DML for samples preserved in -18 and 10°C.

Preservation period (%)	% DKT in 22% MC	9% MC	Averaged over MC
0	2.3	0.6	1.45
26	4.7	1.9	3.3
48	3.5	2.4	1.3
$LSD_{\alpha=0.05}$	-	-	2.9

MC=moisture content;

Table 12. DKT during storage studies from sample preserved at 9% moisture content.

Preservation period (weeks)	Mean DKT (%)
0	0.733
26	1.70
48	1.62
$LSD_{\alpha=0.05}$	2.76

Table 13. Characteristics of linear relationship between FFA and DML

Preservation period (weeks)	Slope	Intercept	R^2
a). FFA			
0	0.8±0.22	0.13±0.25	0.70
26	0.7±0.1	0.14±0.13	0.90
48	0.72±0.15	0.14±0.10	0.79
b). DKT			
0	3.1±1.2	0.0	0.50
26	3.6±0.62	0.0	0.85
48	3.9±1.0	0.0	0.70

Table 14. Minimal damage levels for soybean grades and the predicted FFA content

Grade	Minimal damage level (%)	FFA (%)	FFA Current line[1] (%)
1	2	0.54	0.90
2	3	0.76	0.97
3	5	1.16	1.12
4	8	1.79	1.34

[1] Iverston and Koeltzow (1986)

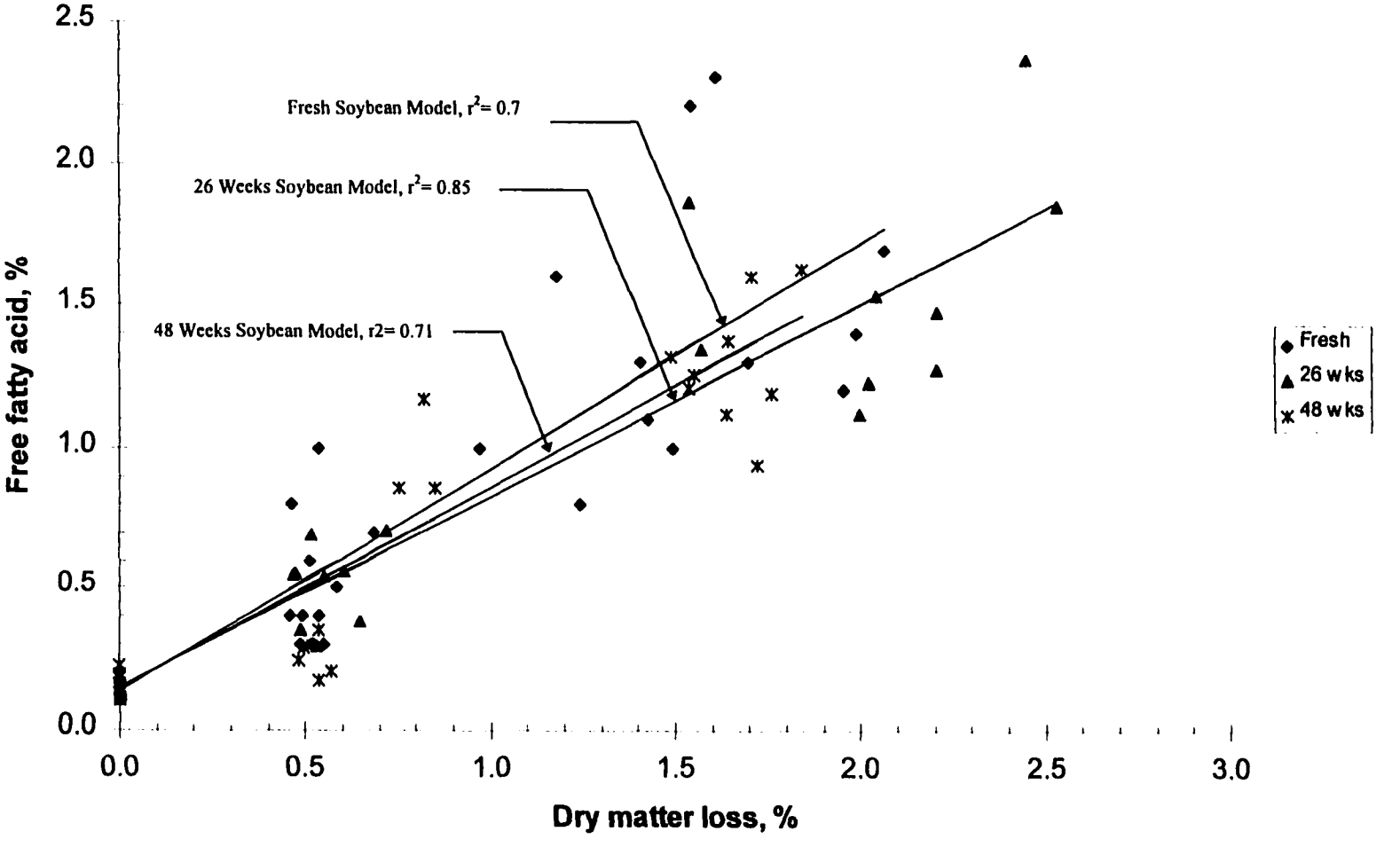

Figure 1. Regression of FFA on DML for preserved soybeans during storage

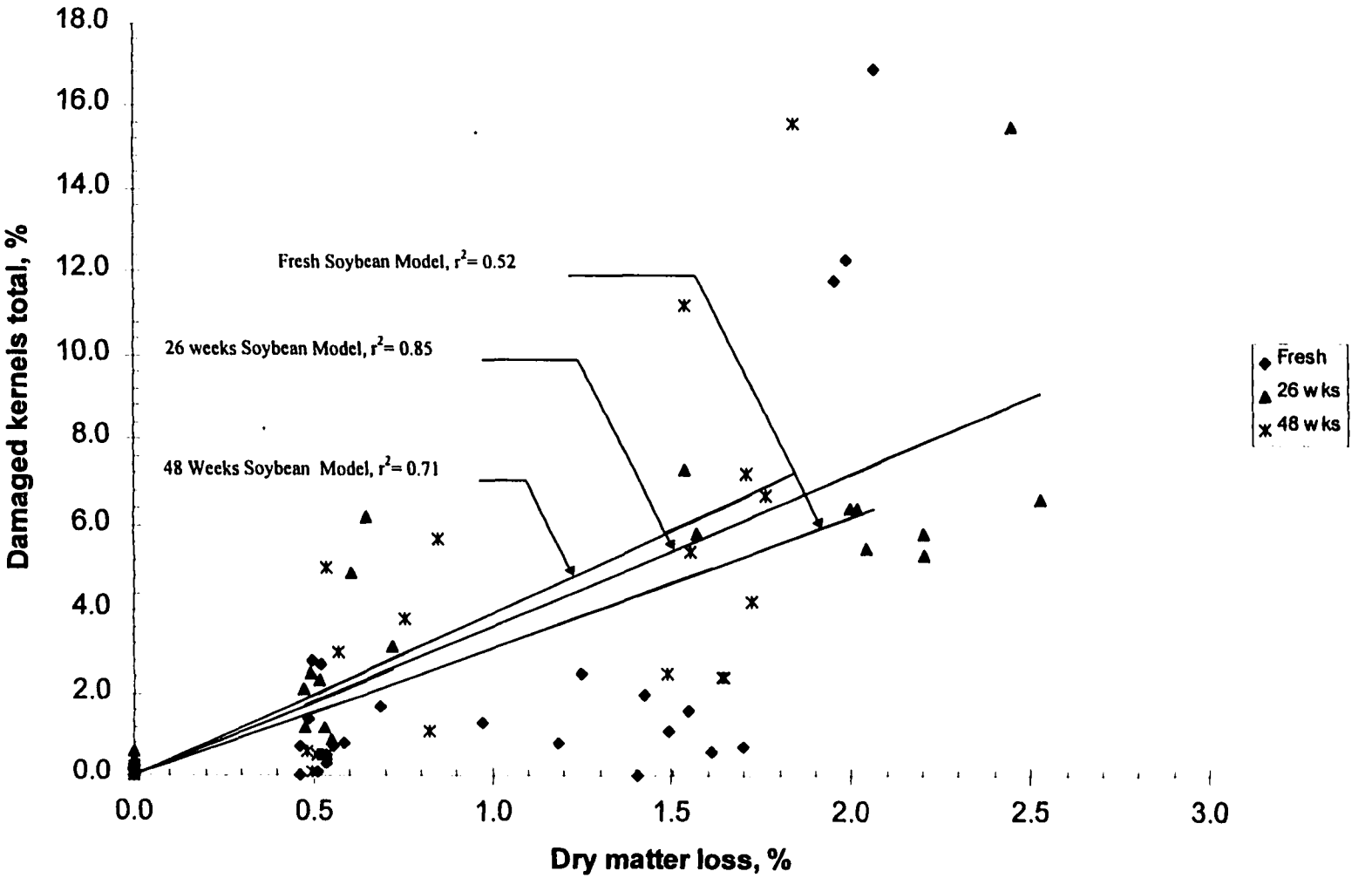

Figure 2. Regression of DKT on DML for preserved soybeans during storage

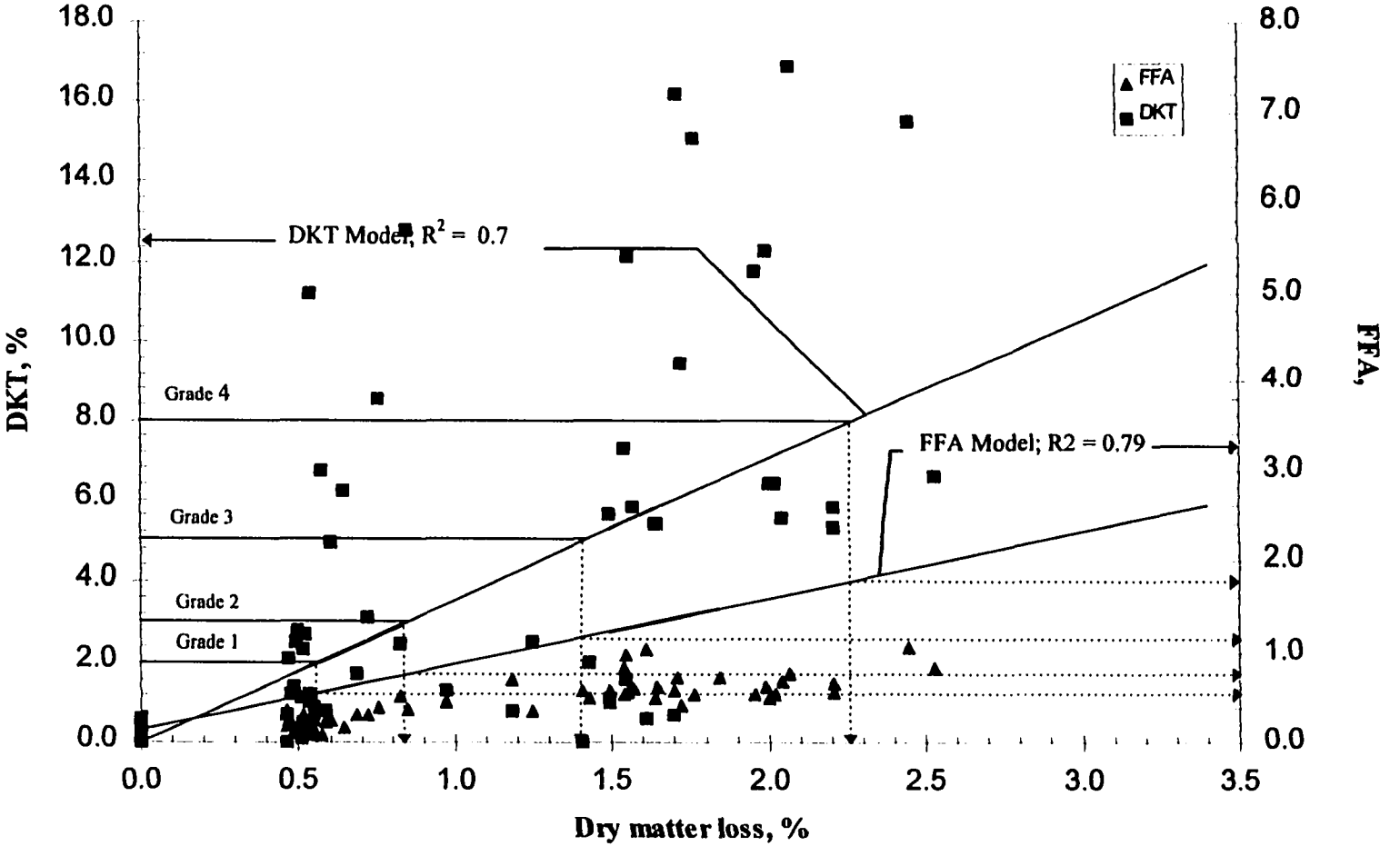

Figure 3. Regression of FFA and DKT on DML for soybeans during storage

GENERAL SUMMARY

A modified method for determining free fatty acid (FFA) in crude and refined soybean oils from 0.3 to 6.0 g oil sample size was developed. Good correlation between the modified and official methods was established for crude and refined soybean oils with R^2 of 0.9995 and 0.997, respectively. The correlations were explained by linear relationship with a slope of 1.01 and 0.99 for the crude and refined oil, respectively.

The number of days soybean lost 0.5 and 1.0% dry matter, for low and high harvest moisture content (MC) soybeans both mechanically and manually harvested, were determined. Soybeans at two different MC's were preserved at two low temperature environments and effects of preservation on the CO_2 evolution were established. Polynomials models describing the respective CO_2 evolution with storage time were obtained. Soybeans manually harvested at 20% MC were found to have the slowest time of deterioration (26.2 days) while machine harvested soybeans at the same MC were more than 50% faster. At optimum harvest MC (13-14%) where soybeans are considered to have high degree of toughness, 0.5% dry matter was lost in 22.5 days. This could be the slowest possible rate of deterioration that soybeans would have under normal harvesting operation as even manually harvested soybean at 8% MC have a faster rate of deterioration.

Preserved soybeans generally have a higher rate of deterioration during storage than fresh one. The decline in the rate of deterioration during storage for the 9% MC soybeans can be linearly described as a function of the preservation period, with rate declining at 0.21 day per week of preservation. There was however little variation in the 22% MC sample.

The effects of preservation on %FFA and damaged kernels total (DKT) during storage was also evaluated. Soybeans preserved at 22% MC at -18°C temperature exhibited a linear increase in the rate of %FFA content during storage with a slope of 0.2. There was no significant increase in the 9% preserved sample when averaged over the preservation temperatures. DKT during the storage of 22 and 9% preserved samples were not influenced by preservation, but there was a trend of increasing damaged levels, small though, in soybeans preserved for a longer period of time.

A relationship between %FFA content and DKT with dry matter loss (DML) during storage were also established. The variation can be summarized as linear functions, with R^2 of 0.8 and 0.7 for the %FFA and %DKT, respectively. The rates of increase in %FFA and DKT were 0.72 and 3.53 % per unit DML, respectively. The combination of the DKT and FFA models as a function of DML characterized grades 1 and 2 soybeans to have less than 0.8% FFA and between 1 and 2% for grades 3 and 4.

RECOMMENDATIONS FOR FUTURE WORK

The following list are areas of recommendation for future study:

1. A study on the effects of different breakage levels in soybean on the rate of carbon dioxide production during deterioration of stored soybean.

2. A study on the effects of different breakage levels on the development of percentages FFA content and DKT during deterioration of stored soybean.

3. A study on the effects of different storage conditions (temperatures and relative humilities) on the rate of carbon dioxide production during deterioration of stored soybean.

4. A study on the relative rates of carbon dioxide production between naturally and artificially wet soybean samples.

5. Microbiological study during deterioration of stored soybean.

6. Adaptation of the modified method for determining FFA in other oilseeds.

7. Exploring the possible use of DML to develop a new interpretative damage lines for determining soybean grades.

APPENDIX A. AOCS OFFICIAL METHODS

AOCS Official Method Ca 5a-40

Reapproved 1977
Revised 1987
Reapproved 1989

Free Fatty Acids

Definition: This method determine the fatty acids existing in the sample

Scope: Applicable to all crude oil and refined vegetable oil, marine oils and animal fats.

Apparatus:

1. Oil sample bottles, 115 or 230 mL (4 or 8 oz) or 250 mL Erlenmeyer flasks.

Reagents:

1. Ethyl alcohol, 95% (U.S.S.D. Formula 30 and 3A are permitted). See Notes, 1. The alcohol must give a definite, distinct and sharp end-point with phenolphthalein and must be neutralized with alkali to a faint, but permanent pink color just before using.

2. Phenolphthalein indicator solution, 1% in 95% alcohol.

3. Sodium hydroxide solution, accurately standardized. See AOCS Specification H 12-52. See Table 1 for the appropriate normality (N) of the sodium hydroxide solution, depending on the expected free fatty acid (FFA) concentration range in the sample.

Procedure:

1. Samples must be well mixed and entirely liquid before weighing, however, do not heat the sample more than 10°C over the melting point.

2. Use Table 1 above to determine the sample weight for various ranges of fatty acids. Weigh the designated sample size into an oil sample bottle or Erlenmeyer fl (see Notes, 2).

3. Add the specified amount of hot neutralized alcohol and 2 mL of indicator.

4. Titrate with standardized sodium hydroxide, shaking vigorously until the appearance of the first permanent pink color of the same intensity as that of the neutralized alcohol before the addition of the sample. The color must persist for 30 seconds.

Calculations:

1. The percentage of free fatty acids in most types of fats and oils is calculated as oleic acid, although in coconut and palm kernel oils it is frequently expressed as lauric acid and in palm oil in terms of palmitic acid.

$$\text{(a) Free fatty acids as oleic, \%} = \frac{\text{mL of alkali} \times \text{N} \times 28.2}{\text{Weight of sample}}$$

$$\text{(b) Free fatty acids as lauric, \%} = \frac{\text{mL of alkali} \times \text{N} \times 20.0}{\text{Weight of sample}}$$

$$\text{(c) Free fatty acids as palmitic, \%} = \frac{\text{mL of alkali} \times \text{N} \times 25.6}{\text{Weight of sample}}$$

2. The free fatty acids are frequently expressed in terms of acid value instead of % free fatty acids. The acid value is defined as the number of mg of KOH necessary to neutralized 1 g of sample. To convert % free fatty acids (as oleic) to acid value, multiply the % free fatty acids by 1.99.

Notes:

1. Isopropanol, 99%, may be used as an alternate solvent with crude ands refined vegetable oils.

2. Cap bottle and shake vigorously for one minute if oil has been blanketed with carbon dioxide gas.

3. See JAOCS 59:658 (1976) regarding the ruggedness of this method.

Table A1. FFA range, alcohol volume and strength of alkali.

FFA range, %	Grams of sample	ml of alcohol	Strength of alkali
0.00 to 0.2	56.4 ± 0.2	50	0.1 N
0.2 to 1.0	28.2 ± 0.2	50	0.1 N
1.0 to 30.0	7.05 ± 0.05	75	0.25 N
30.0 to 50.0	7.05 ± 0.05	100	0.25 or 1.0 N
50.0 to 100.0	3.525 ± 0.2	100	1.0 N

AOCS Specification H 12-52
Amended 1988
Reapproved 1989

Standard sodium hydroxide solution

Apparatus:

1. Erlenmeyer flask, 300 mL capacity.

2. Burette, accurately calibrated to meet National Bureau of Standards specifications. Because alkali will dissolve glass, to avoid volumetric errors it should not be stored in calibrated apparatus. Burettes used continuously should be recalibrated periodically.

3. Absorption tower or drying tube of adequate capacity packed with efficient absorbent for carbon dioxide such as Ascarite or soda lime.

Reagents:

1. Carbon dioxide free distilled water, prepared by one of the following methods.

   (1) Boil water for 20 minutes and cool with absorption tower protection against CO_2 absorption;
   (2) bubbles a vigorous stream of air, freed from CO_2 by passing through a tower with Ascarite or soda lime, through water for 12 hours (see Notes, 1).

2. Sodium hydroxide (1 + 1). To one part by weight of NaOH (containing less than 5% Na_2CO_3) in an alkali resistant flask or bottle add one part of H_2O. Swirl until solution is complete. Close the container tightly with a rubber stopper. Set aside until Na_2CO_3 has settled, leaving perfectly clear supernatant liquid (see Notes, Caution).

3. Acid potassium phthalate, $KHC_8H_4O_4$, National Bureau of Standards standard sample for acidimetry. Crush sample to a fineness of approximately 100 mesh and dry for 2 hours at 120 C. Allow to cool in an efficient dessicator.

4. Ethyl alcohol, 95% by volume (U.S.S.D. Formula 30 or 3A is permitted).

5. Phenolphthalein indicator. Dissolve 1.0 gram of phenolphthalein in 100 ml of alcohol.

Procedure:

1. The following table gives the approximate quantities of NaOH solution (1+1) necessary to make 10 liters of standard solution of the indicated normality:

Approximate normality	Sodium hydroxide solution to be diluted to 10 liters	
	Milliliters	Grams
0.1N	54	82
0.25N	135	205
0.5N	270	410
1.0N	540	820

2. Add the required quantity of NaOH solution (1+1) to enough CO_2 free distilled water to give a total volume of 10 liters and mix well. Protect the solution from CO_2 by stoppering tightly or by means of absorption tube or tower.

3. Weigh accurately sufficient dried $KHC_8H_4O_4$ to require 40 mL of the sodium hydroxide solution to be standardized and transfer it to a 300 mL flask that has been swept free from CO_2. Add 50 mL of cool CO_2 free H_2O. Stopper the flask and swirl gently until the $KHC_8H_4O_4$ is dissolved.

4. When the $KHC_8H_4O_4$ is in solution, add 3 drops of phenolphthalein indicator and titrate to the first persistent faint pink color with the solution to be standardized taking precautions to exclude CO_2.

5. Determine the quantity of sodium hydroxide solution by the following equation:

$$\text{Normality, N} = \frac{\text{Grams of } KHC_8H_4O_4}{\text{mL of NaOH} * 0.20444}$$

Notes:

Caution

As an alternate method for removing carbonate, a barium hydroxide solution may be added to the undiluted sodium hydroxide solution. If the barium hydroxide solution is used, make certain that the sodium hydroxide solution is cooled prior to the addition of barium hydroxide. Failure to do so will result in a violent splattering of the sodium hydroxide solution and could result in serious injury. See the Laboratory Safety section at the beginning of this Edition for precautions regarding the handling of strong alkalis.

Numbered Notes

1. When the standard sodium hydroxide is prepared as directed it is essentially free of carbon dioxide. It is therefore possible to correct for the amount of alkali required to produce the end point with phenolphthalein as described in the method. The resulting solution is expected to have true normality with respect to the hydrogen ion concentration.

APPENDIX B. RAW DATA ON DEVELOPMENT OF A MODIFIED METHOD FOR FFA DETERMINATION AND DATA ANALYSIS

Table B1. Raw data from free fatty acid analysis using AOCS Official Method and modified method on refined soybean oil

Obs	Rep	Conc	Meth	NAOH Normality (N)	Sample weight (g)	Alcohol volume (mL)	Titre volume (mL)	FFA (%)
1	1	1	1	0.0987	56.29	50.0	2.04	0.10
2	1	1	2	0.0125	5.66	5.0	1.58	0.098
3	1	5	1	1.01059	3.76	100.0	9.30	70.49
4	1	4	2	0.12274	0.74	10.0	10.62	49.67
5	1	3	2	0.032	0.78	7.5	11.41	13.19
6	1	2	1	0.0987	28.3	50.0	6.10	0.60
7	1	4	1	1.01059	7.0	100.0	12.55	51.09
8	1	5	2	0.1227	0.3	10.0	6.60	76.15
9	1	3	1	0.25594	7.31	75.0	15.70	15.50
10	1	2	2	0.0138	2.88	5.0	4.38	0.59
11	2	4	1	1.01059	7.07	100.0	13.05	52.60
12	2	2	2	0.0125	2.77	5.0	4.75	0.54
13	2	5	2	0.12274	0.36	10.0	7.4	71.15
14	2	1	2	0.0125	5.69	5.0	1.38	0.09
15	2	3	2	0.03197	0.81	7.5	12.95	14.41
16	2	2	1	0.09871	28.34	50.0	6.2	0.61
17	2	1	1	0.09871	56.50	50.0	2.1	0.10
18	2	5	1	1.01059	3.71	100.0	9.15	70.29
19	2	4	2	0.12274	0.75	10.0	10.55	48.69
20	2	3	1	0.25594	7.10	75.0	15.40	15.65
21	3	4	2	0.12274	0.79	10.0	11.15	48.85
22	3	2	2	0.0138	2.88	5.0	4.38	0.59
23	3	2	1	0.09871	28.10	50.00	6.10	0.60
24	3	1	1	0.09871	56.10	50.0	2.15	0.11
25	3	1	2	0.0138	5.65	5.0	1.62	0.11
26	3	4	1	1.01060	7.03	100.0	12.50	50.67
27	3	3	2	0.03197	0.73	7.5	10.95	13.52
28	3	3	1	0.25594	7.27	75.0	15.75	15.64
29	3	5	2	0.12274	0.42	10.0	9.1	74.99
30	3	5	1	1.0106	3.48	100.0	8.8	72.07

Obs=Observation; Rep=Replication; Conc=Concentration; Meth=Method (1=AOCS, 2=Modified)

Table B2. Raw data from free fatty acid analysis using AOCS Official Method and modified method on crude soybean oil

Obs	Rep	Conc	Meth	NAOH Normality (N)	Sample weight (g)	Alcohol volume (mL)	Titre volume (mL)	FFA (%)
1	1	5	2	0.124	0.32	10.0	6.25	71.53
2	1	2	1	0.101	28.57	50.0	8.5	0.85
3	1	3	2	0.028	0.66	7.5	12.6	15.09
4	1	4	2	0.124	0.75	10.0	10.65	50.06
5	1	3	1	0.247	7.05	75.0	16.05	15.85
6	1	2	2	0.011	2.45	5.0	7.8	0.96
7	1	4	1	0.99	7.12	100.0	13.15	51.58
8	1	5	1	0.99	3.13	100.0	7.9	70.50
9	2	5	1	0.99	3.71	100.0	9.65	72.57
10	2	2	1	0.101	28.72	50.0	9.15	0.91
11	2	3	2	0.028	0.66	7.5	13.85	16.49
12	2	4	2	0.124	0.77	10.0	11	50.36
13	2	4	1	0.99	7.20	100.0	13.05	50.62
14	2	5	2	0.124	0.51	10.0	10.25	70.85
15	2	3	1	0.247	7.06	75.0	15.9	15.68
16	2	2	2	0.011	2.34	5.0	7.15	0.92
17	3	4	1	0.99	7.26	100.0	13.5	51.94
18	3	5	2	0.124	0.47	10.0	9.6	71.54
19	3	3	2	0.028	0.73	7.5	15.05	16.19
20	3	2	1	0.101	28.57	50.0	8.9	0.89
21	3	2	2	0.028	2.451	5.0	7.4	0.91
22	3	3	1	0.247	7.07	75.0	15.9	15.66
23	3	4	2	0.124	0.75	10.0	10.6	49.82
24	3	5	1	0.99	3.18	100.0	8.25	72.48

Obs=Observation; Rep=Replication; Conc=Concentration; Meth=Method (1=AOCS, 2=Modified)

Table B3. Analysis of Variance of the two methods based on refined soybean oil

Source	df	SS	MS	F	P
Rep	2	0.68	0.34	0.33	0.72
Trt	9	25139.5	2793.3	2741.0	0.0001
Conc	4	(25110.7)	(6277.7)	(6160.0)	0.0000
Method	1	(0.4)	(0.4)	(0.4)	0.54
Conc*Method	4	(28.4)	(7.1)	(7.0)	0.0014
Error	18	18.3	1.02		
Total	29	25158.5			

Table B4. Analysis of Variance of the two methods based on crude soybean oil

Source	df	SS	MS	F	P
Rep	2	0.585	0.29	0.88	0.433
Trt	7	18693.9	2670.6	8092.73	0.0000
Conc	3	(18690.9)	(6230.3)	(18879.7)	0.0000
Method	1	(0.96)	(0.96)	(2.91)	0.110
Conc*Method	3	(2.07)	(0.69)	(2.09)	0.147
Error	14	4.65	0.33		
Total	23	18699.2			

Table B5. ANOVA procedure class level -experiment one

Class	Level	Value
TRT	10	1 2 3 4 5 6 7 8 9 10
REP	3	1 2 3
CONC	5	1 2 3 4 5
METHOD	2	1 2

Table B6. Mean values at treatment level-experiment one

Level of TRT	N	FFA Mean (%)	FFA SD
1	3	0.103	0.0058
2	3	0.099	0.0101
3	3	0.603	0.0058
4	3	0.573	0.0289
5	3	15.597	0.0839
6	3	13.707	0.6311
7	3	51.453	1.0150
8	3	49.070	0.5257
9	3	70.950	0.9751
10	3	74.096	2.6170

Table B7. Mean values at concentration level-experiment one

Level of CONC	N	FFA Mean (%)	FFA SD
1	6	0.101	0.008
2	6	0.588	0.025
3	6	14.652	1.111
4	6	50.262	1.492
5	6	72.52	2.468

Table B8. Mean values at method level-experiment one

Level of METHOD	N	FFA Mean (%)	FFA SD
1	15	27.741	29.563
2	15	27.509	30.382

Table B9. ANOVA procedure class level- experiment two

Class	Levels	Values
TRT	8	1 2 3 4 5 6 7 8
REP	3	1 2 3
CONC	4	1 2 3 4
METHOD	2	1 2

Table B10. Mean values at treatment level- experiment two

Level of TRT	N	FFA Mean (%)	FFA SD
1	3	0.883	0.031
2	3	0.930	0.026
3	3	15.730	0.104
4	3	15.923	0.737
5	3	51.380	0.682
6	3	50.080	0.271
7	3	71.850	1.170
8	3	71.301	0.396

Table B11. Mean values at concentration level- experiment two

Level of CONC	N	FFA Mean (%)	FFA SD
1	6	0.907	0.036
2	6	15.827	0.483
3	6	50.730	0.850
4	6	71.578	0.836

Table B12. Mean values at method level- experiment two

Level of METHOD	N	FFA Mean (%)	FFA SD
1	12	34.961	29.37
2	12	34.560	28.94

APPENDIX C. CALIBRATION OF CARBON DIOXIDE MEASURING SYSTEM

CALIBRATION OF CARBON DIOXIDE MEASURING SYSTEM

Rukunudin, I. H., and C. J. Bern

ABSTRACT

Verification of the level of carbon dioxide in the air entering the soybean storage units is an essential step to ensure correct estimation in the loss dry matter during storage of soybean. The rate of carbon dioxide accumulation from the residual carbon dioxide still presence in the air after passing through the gas generator was about 0.0276391 gm per day. The recorded carbon dioxide accumulation in the sulaimanite tube for a specific period of time therefore needs to be corrected.

INTRODUCTION

The development of an index of deterioration during storage of soybeans is built around the premise that the CO_2 produced from the stored soybeans is a measure of the loss in its dry matter. The CO_2 measuring system used in the study, as shown in figure 1 of paper number 2 of this thesis, requires that air from a compressor be filtered and free of CO_2 before being conditioned and passed through the stored soybean. Any CO_2 presence in the inlet air prior to passing through the store soybean mass would contributed to an erroneous reading of the index.

Similar but earlier studies on measuring CO_2 production during the storage of shelled corn (Steele, 1967; Fernandez et al., 1985; Al- Yahya et al., 1993) used potassium hydroxide (KOH) in solution as a medium to remove CO_2 from the air before passing through the storage unit. According to Al-Yahya (1991), the use of 25% KOH solution was found to be sufficient to removed CO_2 from the air as evident from the gas chromatography (GC) analysis of the air sampled from the system.

In a later CO_2 measuring system developed for shelled corn storage study (Dugba et al., 1994), a purge gas generator was used in place of the KOH solution to strip the CO_2 from the air. The manufacturer's specification claimed that the condition of the outlet air from the unit is CO_2 free.

OBJECTIVE

The objective of this experiment therefore is to verify the CO_2 level in the air leaving the gas generator unit and to propose a correction procedure if there appears to be some residual CO_2 left in the system.

MATERIALS AND METHODS

Purge Gas Generator Unit

The complete Purge Gas Generator unit consists of two dust filters connected in series and an FTIR purge gas generator model 74-45 (manufactured by Balston Inc.). The function

of the two filters is to remove dirt particles, oil and moisture from the air stream before it enters the unit. In the generator the air is dried and filtered off from any CO_2 is removed.

Experimental set-up

Figure 2 shows the set up of the experiment where sulaimanite tubes are connected in parallel to the air stream leaving the gas generator unit. Sulaimanite is a CO_2 absorbing agent made up of vermiculite particles impregnated with KOH. The material has the ability to absorb 100% of the CO_2 from the air stream (Al-Yayha, 1991).

During the experiment, the weight of the sulaimanite tube is weighed at about every 24 hours for eight days. Any weight gained by the tube is considered to be the amount of residual CO_2 still present in the air. The airflow was maintained at 0.45 m^3/min./ton (0.9 standard cubic feet per hour - scfh) throughout the experiment. Three replicates of sulaimanite tube are used in the experiment and are arranged at random.

RESULTS AND DISCUSSION

Table 1 shows the cumulative weight gained in the weight of the sulaimanite tubes as recorded in period of 8 days.

Linear regression through the origin is performed on the data to get the best fit model. A linear model is found to be sufficient, with an R^2 of 0.997, to represent the relationship between the CO_2 concentration (by weight) in the air leaving the generator and time. Figure 2 is the plot of the above model superimposed on the experimental data against time.

The model that best described the relationship at 0.45 m^3/min ton is:

$$CO_2, g = 0.0276391 * day$$

CO_2, g = g of CO_2 at any number days

day = number of days

CONCLUSIONS

There is still a trace of CO_2 in the air leaving the purge gas generator unit as indicated by the weight gained by the sulaimanite tube. The amount is therefore must be subtracted from the weight gained by the sulaimanite tube at any particular time during a storage study by an amount that is given by the model.

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Fernandez, A., R. Stroshine, and J. Tuite. 1985. Mold growth and carbon dioxide evolution during storage of high-moisture corn. Cereal Chem. 62(2.): 137-144.

Steele, J. L. 1967. Determination of damaged shelled corn as measured by carbon dioxide. Unpubl. Ph.D diss., Iowa State University, Ames, IA.

Table C1. Cumulative weight gained of the CO_2 absorbing agent with time

Cum time (hr)	Cum wt Rep 1 (g)	Cum wt Rep 2 (g)	Cum wt Rep 3 (g)	Avg. Cum (g)
0.00	0.00	0.00	0.00	0.000
0.23	0.02	0.03	0.02	0.023
42.25	0.03	0.03	0.04	0.033
72.75	0.07	0.08	0.08	0.077
103.25	0.11	0.11	0.11	0.110
125.25	0.16	0.14	0.17	0.157
149.00	0.17	0.17	0.18	0.173
172.75	0.2	0.19	0.21	0.200
199.00	0.23	0.22	0.24	0.230

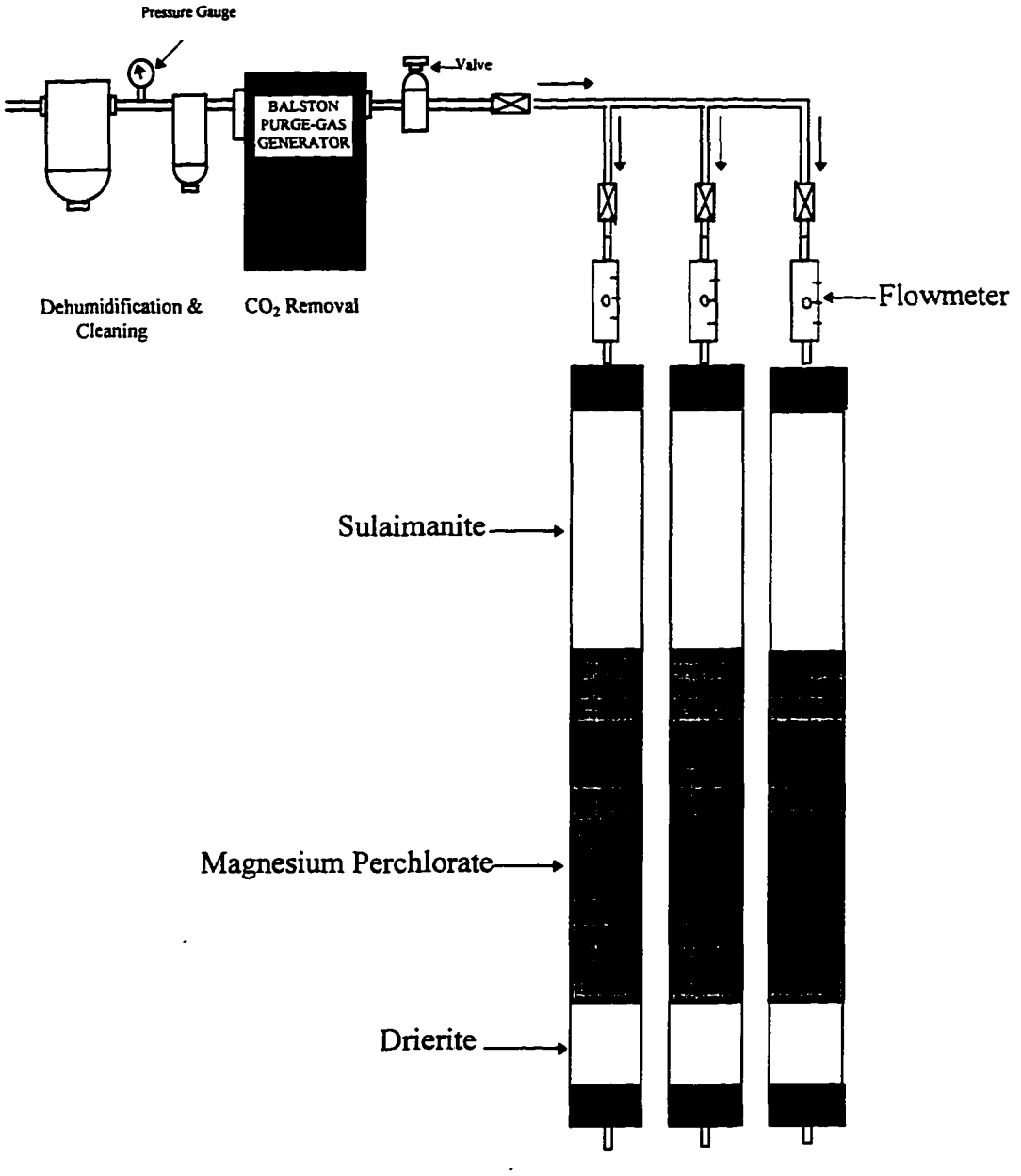

Figure C1. CO_2 calibration system

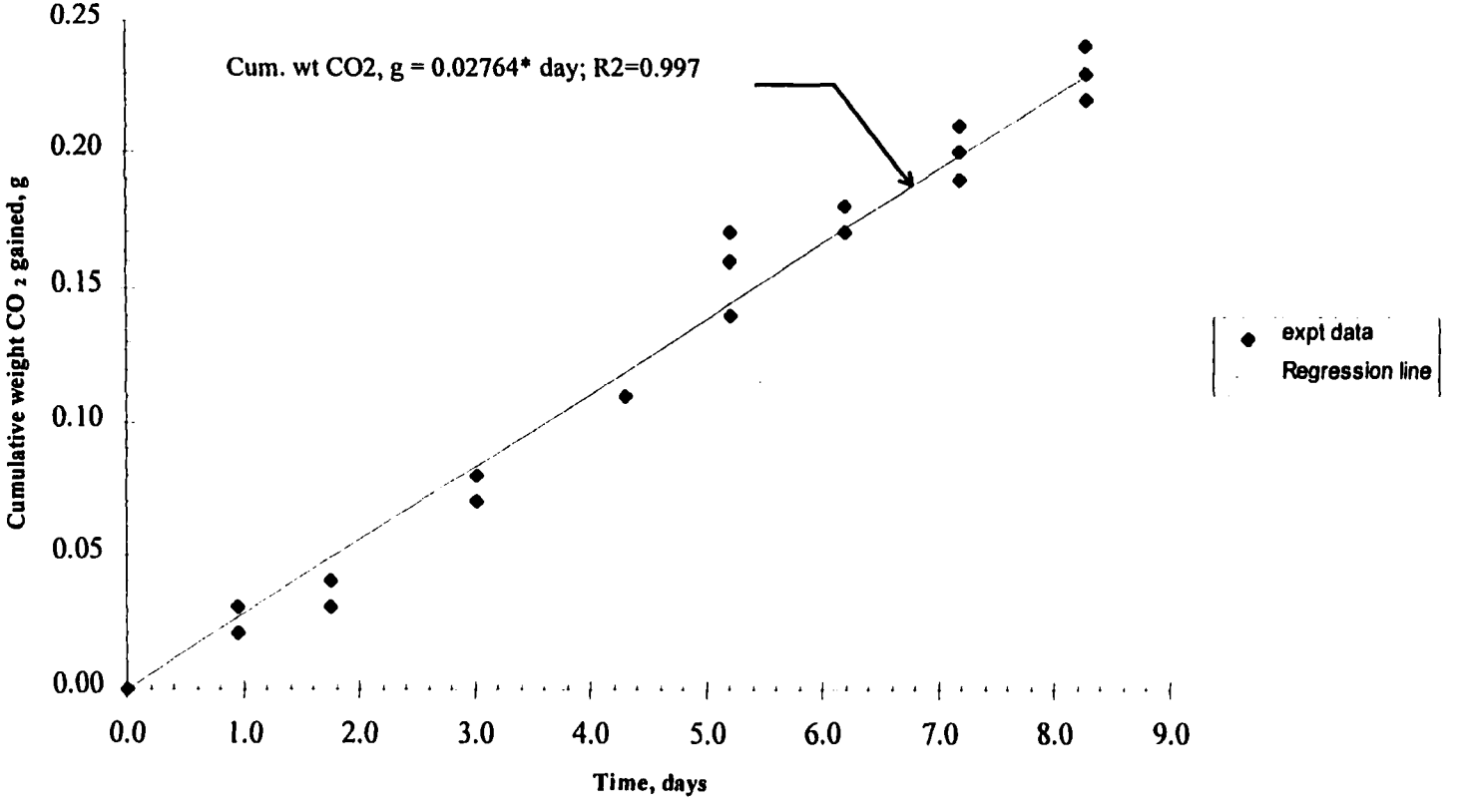

Figure C2. Cumulative weight of CO_2 gained by the sulaimanite with time

APPENDIX D. RAW DATA AND DATA ANALYSIS ON CARBON DIOXIDE EVOLUTION DURING AERATED STORAGE

Table D1. CO_2 raw data from Experiment I treatment 1

Hour	Rep I	Rep II	Rep III
0.00	0.00	0.00	0.00
20.75	0.32	0.34	0.33
38.75	0.49	0.59	0.46
62.75	0.72	0.75	0.71
87.75	1.00	1.07	0.97
114.50	1.41	1.47	1.36
137.00	1.91	2.00	1.88
157.00	2.49	2.59	2.50
183.50	3.52	3.69	3.55
206.75	4.49	4.75	4.52
225.00	5.22	5.57	5.30
251.00	6.21	6.62	6.30
276.25	7.15	7.67	7.29
302.25	8.10	8.74	8.26
322.00	9.02	9.70	9.16
347.25	10.24	11.14	10.40
374.50	11.57	12.65	11.75
393.50	12.44	13.63	12.69
424.50	14.04	15.34	14.34
447.25	15.35	1668	15.70
467.25	16.54	17.90	16.88
492.75	18.17	19.59	18.52
514.75	19.60	21.07	19.98
540.25	21.36	22.86	21.77
562.75	23.05	24.50	23.43
594.50	25.57	27.09	25.98
610.50	26.86	28.42	27.29
633.25	28.76	30.40	29.21

Rep=Replicate

Table D2. CO_2 raw data from Experiment I treatment 2

Hour	Rep I	Rep II	Rep III
0.00	0.00	0.00	0.00
22.75	0.05	0.05	0.05
42.75	0.15	0.13	0.12
68.25	0.29	0.21	0.25
90.25	0.40	0.30	0.33
115.75	0.57	0.41	0.47
138.25	0.67	0.51	0.59
170.00	0.89	0.68	0.83
186.00	1.00	0.77	0.91
208.75	1.18	0.91	1.07
235.50	1.43	1.11	1.30
257.25	1.64	1.31	1.52
268.25	1.73	1.41	1.67
290.50	1.98	1.65	1.90
303.75	2.10	1.76	2.02
334.00	2.62	2.14	2.44
359.25	3.20	2.52	2.90
378.75	3.66	2.85	3.28
403.80	4.29	3.34	3.83
410.25	4.46	3.46	3.99
431.50	5.03	3.94	4.52
456.00	5.71	4.51	5.17
484.25	6.54	5.16	5.99
504.25	7.14	5.65	6.55
528.25	7.89	6.28	7.27
546.00	8.60	6.79	7.87
573.50	9.42	7.38	8.57
593.00	10.04	7.85	9.13
619.75	10.96	8.53	10.01
642.80	11.79	9.20	10.87
665.00	12.66	9.92	11.74

Rep=Replicate

Table D2. (continue)

Hour	Rep I	Rep II	Rep III
689.25	13.69	10.69	12.79
714.25	14.84	11.52	13.86
723.50	15.31	11.84	14.31
738.25	16.05	12.38	14.96
763.50	17.02	13.35	16.22
785.95	18.30	14.27	17.39

Table D3. CO_2 raw data from Experiment I treatment 3

Hour	Rep I	Rep II	Rep III
0.00	0.00	0.00	0.00
22.50	0.12	0.12	0.12
42.50	0.18	0.19	0.16
69.00	0.29	0.31	0.27
92.25	0.39	0.41	0.36
110.50	0.45	0.48	0.42
136.50	0.53	0.56	0.50
161.75	0.63	0.66	0.59
187.75	0.77	0.80	0.71
207.50	0.89	0.92	0.83
232.75	1.07	1.10	1.01
260.00	1.25	1.29	1.19
279.00	1.35	1.38	1.29
310.00	1.55	1.62	1.49
332.75	1.74	1.84	1.68
352.75	1.91	2.05	1.88
378.25	2.20	2.38	2.18
400.25	2.46	2.65	2.43
425.75	2.82	3.05	2.75
448.25	3.18	3.44	3.07
480.00	3.79	4.13	3.64
496.00	4.15	4.49	3.94
518.75	4.67	5.07	4.45
545.50	5.37	5.80	5.10
567.25	5.90	6.38	5.62
578.25	6.41	6.72	5.99
600.50	6.94	7.42	6.64
613.75	7.52	7.86	6.80
644.00	7.87	8.40	7.32
669.25	8.48	9.02	7.94
688.75	8.99	9.51	8.40
713.80	9.68	10.14	9.04
720.25	9.92	10.33	9.21
741.50	10.49	10.90	9.78

Rep=Replicate

Table D3. (continue)

Hour	Rep I	Rep II	Rep III
766.00	11.14	11.58	10.44
794.25	11.89	12.33	11.20
814.25	12.45	12.98	11.76
838.25	13.16	13.76	12.46
856.00	13.78	14.45	13.05
883.50	14.76	15.61	13.99
903.00	15.58	16.36	14.74
929.75	16.81	17.45	15.82
953.00	17.92	18.48	16.85
975.00	19.07	19.56	17.88
998.95	20.50	21.20	19.11
1024.25	22.07	23.00	20.44
1033.50	22.72	23.69	20.95

Table D4. CO_2 raw data from Experiment I treatment 4

Hour	Rep I	Rep II	Rep III
0.00	0.00	0.00	0.00
22.75	0.11	0.10	0.13
49.50	0.28	0.23	0.29
71.25	0.38	0.33	0.39
82.25	0.45	0.40	0.42
104.50	0.53	0.70	0.79
117.75	0.54	0.71	0.80
148.00	0.70	0.86	0.98
173.25	0.91	1.05	1.18
192.75	1.04	1.19	1.38
217.80	1.24	1.40	1.60
224.25	1.32	1.46	1.67
245.50	1.50	1.66	1.95
270.00	1.80	1.91	2.37
298.25	2.20	2.24	2.74
318.25	2.54	2.55	3.12
342.25	3.03	2.99	3.65
360.00	3.46	3.37	4.16
387.50	4.22	3.96	5.02
407.00	4.79	4.47	5.73
433.75	5.63	5.25	6.83
457.00	6.39	5.96	7.85
479.00	7.18	6.69	8.88
503.25	8.12	7.58	10.09
528.25	8.81	8.24	11.14
537.50	9.88	9.38	12.18
552.25	11.11	10.61	13.36
577.50	12.33	11.58	14.83
599.95	13.81	12.81	16.31
627.25	15.18	14.03	17.78
648.50	16.55	15.20	19.25
676.50	18.17	16.52	20.82
695.25	20.20	18.20	23.00
724.50	21.97	20.67	24.97

Rep=Replicate

Table D5. CO_2 raw data from Experiment 2 treatment 1

Hour	Rep I	Rep II	Rep III
0.00	0.00	0.00	0.00
20.75	0.32	0.34	0.33
38.75	0.49	0.59	0.46
62.75	0.72	0.75	0.71
87.75	1.00	1.07	0.97
114.50	1.41	1.47	1.36
137.00	1.91	2.00	1.88
157.00	2.49	2.59	2.50
183.50	3.52	3.69	3.55
206.75	4.49	4.75	4.52
225.00	5.22	5.57	5.30
251.00	6.21	6.62	6.30
276.25	7.15	7.67	7.29
302.25	8.10	8.74	8.26
322.00	9.02	9.70	9.16
347.25	10.24	11.14	10.40
374.50	11.57	12.65	11.75
393.50	12.44	13.63	12.69
424.50	14.04	15.34	14.34
447.25	15.35	16.68	15.70
467.25	16.54	17.90	16.88
492.75	18.17	19.59	18.52
514.75	19.60	21.07	19.98
540.25	21.36	22.86	21.77
562.75	23.05	24.50	23.43
594.50	25.57	27.09	25.98
610.50	26.86	28.42	27.29
633.25	28.76	30.40	29.21

Rep=Replicate

Table D6. CO_2 raw data from Experiment 2 treatment 2

Hour	Rep I	Rep II	Rep III
0.00	0.00	0.00	0.00
22.75	0.05	0.05	0.05
42.75	0.15	0.13	0.12
68.25	0.29	0.21	0.25
90.25	0.40	0.30	0.33
115.75	0.57	0.41	0.47
138.25	0.67	0.51	0.59
170.00	0.89	0.68	0.83
186.00	1.00	0.77	0.91
208.75	1.18	0.91	1.07
235.50	1.43	1.11	1.30
257.25	1.64	1.31	1.52
268.25	1.73	1.41	1.67
290.50	1.98	1.65	1.90
303.75	2.10	1.76	2.02
334.00	2.62	2.14	2.44
359.25	3.20	2.52	2.90
378.75	3.66	2.85	3.28
403.80	4.29	3.34	3.83
410.25	4.46	3.46	3.99
431.50	5.03	3.94	4.52
456.00	5.71	4.51	5.17
484.25	6.54	5.16	5.99
504.25	7.14	5.65	6.55
528.25	7.89	6.28	7.27
546.00	8.60	6.79	7.87
573.50	9.42	7.38	8.57
593.00	10.04	7.85	9.13
619.75	10.96	8.53	10.01
642.80	11.79	9.20	10.87
665.00	12.66	9.92	11.74

Rep=Replicate

Table D6. (continue)

Hour	Rep I	Rep II	Rep III
689.25	13.69	10.69	12.79
714.25	14.84	11.52	13.86
723.50	15.31	11.84	14.31
738.25	16.05	12.38	14.96
763.50	17.02	13.35	16.22
785.95	18.30	14.27	17.39

Table D7. CO_2 raw data from Experiment 2 -22% MC, -18°C, 26 weeks

Hour	Rep I	Rep II	Rep III
0.00	0.00	0.00	0.00
20.00	0.19	0.20	0.24
44.00	0.45	0.44	0.49
66.00	0.87	0.86	0.94
91.00	1.23	1.09	1.22
114.00	1.72	1.51	1.71
133.25	2.25	1.99	2.29
163.75	3.55	3.13	3.62
185.75	4.77	4.11	4.70
209.50	6.29	5.28	5.88
233.25	7.09	6.43	7.01
259.50	8.88	7.64	8.30
281.50	10.55	8.85	9.46
306.50	11.27	10.04	10.61
333.00	13.91	12.50	12.96
349.00	16.57	14.97	15.31
380.25	19.20	17.42	17.65
402.50	21.85	19.88	19.99
429.25	24.49	22.34	22.34
453.50	27.14	24.80	24.68
473.00	29.79	27.26	27.03
493.00	32.44	29.73	29.38

Rep=Replicate

Table D8. CO_2 raw data from Experiment II - 9% MC, -18°C, 26 weeks

Hour	Rep I	Rep II	Rep III
0.00	0.00	0.00	0.00
20.50	0.06	0.03	0.09
47.50	0.26	0.17	0.19
68.00	0.29	0.20	0.23
94.25	0.35	0.32	0.35
110.00	0.42	0.40	0.43
141.75	0.61	0.57	0.62
166.00	0.82	0.75	0.80
190.25	1.04	0.94	1.03
214.50	1.31	1.22	1.30
240.00	1.68	1.56	1.70
262.75	2.08	1.98	2.10
286.75	2.58	2.43	2.63
312.50	3.17	3.00	3.22
333.50	3.78	3.54	3.83
357.00	4.51	4.12	4.50
379.75	5.29	4.73	5.17
410.50	6.55	5.67	6.28
423.00	7.05	6.21	6.72
442.75	7.81	6.94	7.61
469.50	9.27	7.61	8.26
495.50	10.91	8.43	8.90
520.25	12.62	9.30	9.81
531.25	13.36	9.69	10.85
545.75	14.30	10.25	11.20
566.75	15.70	11.03	11.73
590.00	17.16	11.92	12.57
613.50	18.84	12.81	13.55
623.50	19.61	13.23	14.00
638.00	20.74	13.68	14.46
667.25	22.90	14.82	15.63

Rep=Replicate

Table D8. (continue)

Hour	Rep I	Rep II	Rep III
675.00	23.78	15.30	16.12
697.25	25.81	16.31	17.19
719.00	30.18	18.61	19.52
737.25	31.94	19.57	20.48
758.00	34.25	20.76	21.64
782.25	37.24	22.59	23.07

Table D9. CO_2 raw data from Experiment II - 9% MC, 10°C, 26 weeks

Hour	Rep I	Rep II	Rep III
0.00	0.00	0.00	0.00
20.50	0.11	0.12	0.05
47.50	0.20	0.21	0.19
68.00	0.27	0.24	0.20
94.25	0.38	0.35	0.27
110.00	0.46	0.43	0.35
141.75	0.65	0.59	0.50
166.00	0.85	0.79	0.70
190.25	1.07	1.01	0.91
214.50	1.36	1.29	1.20
240.00	1.74	1.70	1.59
262.75	2.05	2.16	2.02
286.75	2.63	2.94	2.62
312.50	3.41	3.35	3.16
333.50	4.18	4.00	3.83
357.00	5.10	4.77	4.60
379.75	5.99	5.52	5.35
410.50	7.52	6.69	6.53
423.00	8.13	7.15	6.98
442.75	9.26	8.49	7.35
469.50	10.82	9.95	8.70
495.50	12.51	11.47	10.25
520.25	14.23	12.98	11.64
531.25	14.82	13.42	12.10
545.75	15.88	14.33	12.96
566.75	17.43	15.73	14.36
590.00	19.30	17.06	15.69
613.50	21.22	18.45	17.08
623.50	22.16	19.32	17.95
638.00	23.49	20.44	19.10
667.25	26.25	22.51	21.14
675.00	27.39	23.48	22.02
697.25	29.99	25.47	24.04
743.00	35.87	30.90	28.88
761.25	36.02	32.41	30.00

Table D10. CO_2 raw data from Experiment II - 22% MC, -18°C, 48 weeks

Hour	Rep I	Rep II	Rep III
0.00	0.00	0.00	0.00
22.75	0.26	0.29	0.17
47.50	0.42	0.54	0.33
68.25	0.64	0.76	0.51
91.25	0.91	1.05	0.74
116.25	1.17	1.39	1.02
141.25	1.60	1.89	1.47
165.75	2.22	2.57	2.06
185.50	2.94	3.31	2.75
206.25	3.91	4.22	3.62
233.50	5.22	5.52	4.82
259.00	6.60	6.78	6.01
286.50	7.97	8.08	7.27
306.25	9.02	9.00	8.10
332.50	10.45	10.30	9.33
355.25	11.38	11.13	10.11
376.25	12.47	12.12	11.05
406.25	14.38	13.82	12.56
432.25	15.83	15.05	13.61
454.50	17.18	16.15	14.55
478.50	18.47	17.36	15.57
502.00	19.92	18.67	16.65
521.40	21.26	19.94	17.65
550.25	23.54	21.87	19.37
581.75	26.34	24.43	21.85
590.25	27.11	25.13	22.58

Rep=Replicate

Table D11. CO_2 raw data from Experiment II - 9% MC, -18°C, 48 weeks

Hour	Rep I	Rep II	Rep III
0.00	0.00	0.00	0.00
23.75	0.44	0.33	0.68
45.75	0.73	0.55	0.90
67.75	1.05	0.77	1.12
76.75	1.25	0.91	1.22
91.50	1.43	1.08	1.36
115.50	1.65	1.43	1.67
146.25	2.47	2.10	2.33
162.75	2.73	2.32	2.77
187.75	3.69	3.04	3.58
209.50	4.96	4.13	4.65
241.75	5.45	4.78	5.27
261.50	6.22	5.47	5.95
282.50	7.15	6.25	6.61
287.75	7.37	6.42	6.80
310.25	8.37	7.47	7.89
314.25	8.49	7.59	8.06
331.75	9.24	8.20	8.75
360.50	10.93	9.55	10.46
386.75	12.13	10.51	11.93
414.25	13.20	11.54	12.79
437.90	14.51	12.66	14.17
453.75	15.72	13.68	15.38
480.75	17.33	15.20	17.03
505.75	19.13	16.71	18.66
533.25	21.35	18.45	20.58
548.75	22.72	19.52	21.73
571.75	24.90	21.30	23.63
577.75	25.33	21.85	24.12

Rep=Replicate

Table D12. CO_2 raw data from Experiment II - 9% MC, 10°C, 48 weeks

Hour	Rep I	Rep II	Rep III
0.00	0.00	0.00	0.00
23.75	0.45	0.35	0.36
45.75	0.69	0.59	0.67
67.75	1.00	0.85	0.91
76.75	1.21	0.99	1.05
91.50	1.48	1.22	1.27
115.50	1.90	1.33	1.39
146.25	2.90	2.21	2.22
162.75	3.38	2.71	2.69
187.75	4.29	3.60	3.53
209.50	5.43	4.81	4.77
241.75	6.15	5.54	5.54
261.50	6.90	6.22	6.32
282.50	7.74	7.07	6.87
287.75	7.90	7.26	7.07
310.25	8.94	8.37	8.25
314.25	9.11	8.55	8.53
331.75	10.03	9.45	9.29
360.50	11.57	10.74	10.38
386.75	13.06	12.12	11.53
414.25	14.53	13.49	13.16
437.90	16.18	15.21	14.79
453.75	17.67	16.52	15.80
480.75	19.74	18.48	17.61
505.75	21.73	20.40	19.39
533.25	24.21	22.81	21.49
548.75	25.90	24.24	22.78

Rep=Replicate

Table D13. CO_2 production at 0.5 and 1.0% dry matter loss of a freshly combine-harvested soybean

	Number of days to reach			
	0.5% Dry Matter Loss		1.0% Dry Matter Loss	
Rep	Combine-harvested at 22% MC	Combine-harvested at 13% MC	Combine-harvested at 22% MC	Combine-harvested at 13% MC
1	11.7	21.3	18.1	29.6
2	11.1	23.9	17.3	33.3
3	11.5	22.2	17.9	30.6
Avg	11.5	22.5	17.8	31.2

Table D14. CO_2 production at 0.5 and 1.0% dry matter loss of a freshly hand-harvested soybean

	Number of days to reach			
	0.5% Dry Matter Loss		1.0% Dry Matter Loss	
Rep	Hand-harvested at 20% MC	Hand-harvested at 13% MC	Hand-harvested at 22% MC	Hand-harvested at 13% MC
1	25.7	20.2	36.7	29.0
2	25.02	20.7	35.9	30.0
3	27.8	18.6	38.7	25.4
Avg	26.2	19.8	37.1	28.1

Table D15(a). CO_2 production at 0.5% dry matter loss from 22 and 9% MC soybean when preserved at -18° C

	Number of days to reach 0.5% Dry Matter Loss					
	At 0 week storage		After 26 weeks storage		After 48 weeks storage	
Rep	Combine harvested at 22% MC	Combine harvested at 13% MC	Combine harvested at 22% MC and stored	Combine harvested at 13% MC and stored at 9% MC	Combine harvested at 22% MC and stored	Combine harvested at 13% MC and stored at 9% MC
1	11.7	21.3	9.8	18.0	11.5	12.0
2	11.1	23.9	10.5	18.9	11.4	12.8
3	11. 5	22.2	10.0	18.4	12.0	12.5
Avg	11.5	22.5	10.1	18.4	11.6	12.4

Table D15(b). CO_2 production at 1.0% dry matter loss from 22 and 9% MC soybean when preserved at -18° C

	Number of days to reach 0.5% Dry Matter Loss					
	At 0 week storage		After 26 weeks storage		After 48 weeks storage	
Rep	Combine harvested at 22% MC	Combine harvested at 13% MC	Combine harvested at 22% MC and stored	Combine harvested at 13% MC and stored at 9% MC	Combine harvested at 22% MC and stored	Combine harvested at 13% MC and stored at 9% MC
1	18.1	29.6	14.1	23.0	17.2	18.4
2	17.3	33.3	14.5	25.2	17.7	19.6
3	17.9	30.6	14.3	23.5	19.0	18.5
Avg	17.8	31.2	14.3	23.9	18.0	18.3

Table D16(a). CO_2 production at 0.5% dry matter loss of 9% MC soybean preserved in -18° C and 10° C environments

	Number of days to reach 0.5% Dry Matter Loss				
	At 0 week storage		After 26 weeks storage	After 48 weeks storage	
Rep	Fresh Soybean	Stored in -18°C with 9% MC	Stored in 10° C with 9% MC	Stored in -18° C with 9% MC	Stored in 10° C with 9% MC
1	21.3	18.0	17.0	12.0	11.3
2	23.9	18.4	17.5	12.8	12.0
3	22.2	18.9	18.5	12.5	12.2
Avg	22.5	18.4	17.7	12.4	11.8

Table D16(b). CO_2 production at 1.0% dry matter loss of 9% MC preserved in -18° C and 10° C environments

	Number of days to reach 0.5% Dry Matter Loss				
	At 0 week storage		After 26 weeks storage	After 48 weeks storage	
Rep	Fresh Soybean	Stored in -18°C with 9% MC	Stored in 10° C with 9% MC	Stored in -18° C with 9% MC	Stored in 10° C with 9% MC
1	29.6	23.0	22.0	18.4	17.3
2	33.3	25.2	23.0	19.6	18.0
3	30.6	23.5	23.8	18.5	18.2
Avg	31.2	23.9	22.9	18.8	17.8

Table D17. ANOVA on aerated storage times of 22 and 9% preserved soybean samples at 0.5 % dry matter loss

Source	DF	MS	F	P>F
(a) Main ANOVA				
Moisture, M	1	203.35	251.0	0.004
Chamber, C	2	0.8	1.0	0.5
C*M	2	0.8		
Period, P	2	36.4	173.1	0.0
P*M	2	42.2	200.9	0.0
P*C*M	8	0.21		
	17			
(b) Subdivision of linear				
Linear, Lin	1	72.5	72.5	0.0001
Lin*M	1	78.5	78.5	0.0001
Lin*C*M	4	4.6		
(c) Subdivision of deviation components				
Deviation, Dev	1	0.2	0.3	0.6
Dev*M	1	5.8	8.5	0.04
Dev*C*M	4	0.7		

Table D18. Aerated storage time, averaged over period, of soybean preserved at -18°C environment at 0.5% dry matter loss

Level of M (%)	N	Storage time Mean (days)	SD
22	9	11.1	0.776
9	9	17.8	4.4

Table D19. Aerated storage time, averaged over MC, of soybean preserved in -18°C environment at 0.5% dry matter loss

Level of P (weeks)	N	Rate Mean (days)	SD
0	6	17.0	6.1
26	6	14.3	4.6
48	6	12.0	0.5

Table D20. ANOVA on aerated storage times of 9% MC soybean samples preserved in -18 an 10°C environments at 0.5% dry matter loss

Source	DF	MS	F	P>F
(a) Main ANOVA				
Temperature,T	1	0.9	0.7	0.45
Chamber (T)	4	1.32		
Period, P	2	161.3	320.0	0.0
P*T	2	0.24	0.5	0.6
P*C(T)	8	0.5		
	17			
(b) Subdivision of linear				
Linear, Lin	1	320.3	616.0	0.00002
Lin*T	1	0.3	0.5	0.5
Lin*C(T)	4	0.52		
(c) Subdivision of deviation components				
Deviation, Dev	1	2.3	4.6	0.1
Dev*T	1	0.22	0.45	0.5
Dev*C(T)	4	0.5		

Table D21. Aerated storage time, averaged over period, of 9% MC soybean preserved in -18 and 10 °C environments at 0.5% dry matter loss

Level of T (°C)	N	Rate Mean (days)	SD
-18	9	17.8	4.4
10	9	17.3	4.7

Table D22. Aerated storage time of soybean, averaged over MC, preserved in -18 and 10°C environment at 0.5% dry matter loss

Level of P (weeks)	N	Rate Mean (days)	SD
0	6	22.5	1.2
26	6	18.1	0.7
48	6	12.1	0.5

Table D23. Aerated storage time, averaged over period, of soybean preserved in -18°C environment at 0.5% dry matter loss

Level of M (%)	N	Rate---------- Mean (days)	SD
22	9	16.7	1.86
9	9	24.6	5.49

Table D24. Aerated storage time, averaged over MC, of soybean preserved in -18°C environment at 0.5% dry matter loss

Level of P (weeks)	N	Rate Mean (days)	Rate SD
0	6	24.5	7.4
26	6	19.1	5.3
48	6	18.4	0.86

APPENDIX E. RAW DATA ON % FFA, DKT AND DATA ANALYSIS

Table E1. FFA content in soybeans during preservation

Rep	Fresh	26 weeks			48 weeks		
		Preservation temperature					
		-18°C		10°C	-18°C		10°C
		Moisture content					
		22%	9%	9%	22%	9%	9%
1	0.2	0.182	0.12	0.112	0.537	0.148	0.226
2	0.2	0.137	0.132	0.183	0.291	0.124	0.181
3	0.1	0.130	0.129	0.104	0.416	0.193	0.159

Rep=replicate

Table E2. DKT of soybean after preservation

	Rep	Fresh	26 weeks	48 weeks
	1	0.0	0.4	0.0
22%	2	0.0	0.4	0.0
-18°C	3	0.0	0.6	0.1
	1	0.0	0.0	0.0
9%	2	0.0	0.1	0.0
10°C	3	0.0	0.2	0.0
	1	0.0	0.3	0.0
9%	2	0.0	0.1	0.0
10°C	3	0.0	0.1	0.2

Rep=replicate

Table E3. Moisture content during soybeans preservation

Rep	MC	Fresh	26 weeks			48 weeks		
			Preservation temperature					
			-18°C	10°C		-18°C	10°C	
			Moisture content					
			22%	9%	9%	22%	9%	9%
1	High	22.48	23.32	na	na	22.6	na	na
2	High	22.57	23.53	na	na	21.07	na	na
3	High	21.9	24.02	na	na	23.93	na	na
1	Low	8.33	na	8.05	8.23	na	7.8	7.7
2	Low	8.80	na	8.40	8.48	na	7.94	8.2
3	Low	8.44	na	8.59	8.42	na	8.56	7.95

Rep=replicate; MC=moisture content; na=not available

Table E4. FFA and DKT of fresh soybean samples during storage

Obs	Treatment	Rep	DML %	FFA %	DKT %
1	Initial	1	0.00	0.1	0.00
2	Initial	2	0.00	0.1	0.00
3	Initial	3	0.00	0.2	0.00
4	Initial	1	0.00	0.1	0.00
5	Initial	2	0.00	0.1	0.00
6	Initial	3	0.00	0.1	0.00
7	Initial	1	0.00	0.2	0.00
8	Initial	2	0.00	0.1	0.00
9	Initial	3	0.00	0.2	0.00
10	Initial	1	0.00	0.2	0.00
11	Initial	2	0.00	0.1	0.00
12	Initial	3	0.00	0.2	0.00
13	Machine/High	1	0.49	0.30	1.40
14	Machine/High	2	0.52	0.30	2.70
15	Machine/High	3	0.50	0.40	2.80
16	Machine/Low	1	0.59	0.30	0.80
17	Machine/Low	2	0.46	0.40	0.70
18	Machine/Low	3	0.54	0.40	0.30
19	Hand/High	1	0.51	0.60	0.10
20	Hand/High	2	0.53	1.00	0.50
21	Hand/High	3	0.46	0.80	0.00
22	Hand/Low	1	0.55	0.30	0.70
23	Hand/Low	2	0.52	0.30	0.50
24	Hand/Low	3	0.69	0.70	1.70
25	Machine/High	1	1.96	1.20	11.80
26	Machine/High	2	2.07	1.70	16.90
27	Machine/High	3	1.99	1.40	12.30
28	Machine/Low	1	1.24	0.80	2.50
29	Machine/Low	2	0.97	1.00	1.30
30	Machine/Low	3	1.18	1.60	0.80

Table E4. (continue)

Obs	Treatment	Rep	DML %	FFA %	DKT %
31	Hand/High	1	1.55	2.20	1.60
32	Hand/High	2	1.61	2.30	0.60
33	Hand/High	3	1.43	1.10	2.00
34	Hand/Low	1	1.49	1.00	1.10
27	Hand/Low	2	1.41	1.30	0.00
35	Hand/Low	3	1.70	1.30	0.70

Obs=Observation; DML=Dry matter loss; Rep=Replicate

Table E5. FFA and DKT of 26-week preserved soybean samples during storage

Obs #	Treatment (Temp, °C/ MC, %)	Rep	DML	Oil sample weight	Titre volume	Normality N	FFA	DKT
			%	(g)	(mL)		%	%
1	-18/22	1	0	2.87	1.55	0.012	0.182	0.40
2	-18/22	2	0	2.71	1.10	0.012	0.137	0.40
3	-18/22	3	0	2.85	1.10	0.012	0.130	0.60
4	-18/9	1	0	2.80	1.00	0.012	0.120	0.00
5	-18/9	2	0	2.81	1.10	0.012	0.132	0.10
6	-18/9	3	0	2.88	1.10	0.012	0.129	0.20
7	10/9	1	0	3.01	1.00	0.012	0.112	0.30
8	10/9	2	0	2.76	1.50	0.012	0.183	0.10
9	10/9	3	0	2.92	0.90	0.012	0.104	0.10
10	-18/22	1	0.72	2.75	5.75	0.012	0.704	3.10
11	-18/22	2	0.61	2.66	4.40	0.012	0.557	4.90
12	-18/22	3	0.65	3.03	3.45	0.012	0.383	6.20
13	-18/9	1	0.53	2.70	2.35	0.012	0.293	1.20
14	-18/9	2	0.47	2.76	5.00	0.011	0.246	2.10
15	-18/9	3	0.52	2.60	5.35	0.012	0.217	2.30
16	10/9	1	0.55	2.80	4.50	0.012	0.541	0.90
17	10/9	2	0.49	2.62	2.70	0.012	0.347	2.50
18	10/9	3	0.48	2.92	4.80	0.012	0.553	1.20
19	-18/22	1	2.20	2.94	11.10	0.012	1.271	5.80
20	-18/22	2	2.02	2.85	10.40	0.012	1.228	6.40
21	-18/22	3	2.00	2.82	9.40	0.012	1.122	6.40
22	-18/9	1	2.53	2.75	15.10	0.012	1.848	6.60
23	-18/9	2	1.54	2.66	14.75	0.012	1.867	7.30
24	-18/9	3	1.57	2.58	10.30	0.012	1.344	5.80
25	10/9	1	2.45	2.88	22.57	0.011	2.363	15.50
26	10/9	2	2.20	2.71	13.25	0.011	1.475	5.30
27	10/9	3	2.04	2.78	12.65	0.012	1.532	5.50

Obs=Observation; Temp=Temperature, MC=Moisture content; Rep=Replicate;
DML=Dry matter loss;

Table E6. FFA and DKT of 48-week preserved soybean samples during storage

Obs #	Treatment (Temp, °C MC, %)	Rep	DML	Oil sample weight	Titre volume	Normality N	FFA	DKT
			%	(g)	(mL)		%	%
1	-18/22	1	0	2.94	2.00	0.028	0.537	0.00
2	-18/22	2	0	2.98	1.60	0.028	0.431	0.00
3	-18/22	3	0	2.85	1.50	0.028	0.416	0.10
4	-18/9	1	0	2.66	0.50	0.028	0.148	0.00
5	-18/9	2	0	2.86	0.45	0.028	0.124	0.00
6	-18/9	3	0	2.86	0.70	0.028	0.193	0.00
7	10/9	1	0	2.80	0.80	0.028	0.226	0.00
8	10/9	2	0	3.28	0.75	0.028	0.181	0.00
9	10/9	3	0	2.98	0.60	0.028	0.159	0.20
10	-18/22	1	0.85	3.00	4.60	0.028	1.211	5.70
11	-18/22	2	0.82	2.92	5.40	0.028	1.460	1.10
12	-18/22	3	0.76	3.03	4.40	0.028	1.147	3.80
13	-18/9	1	0.57	2.87	0.75	0.028	0.206	3.00
14	-18/9	2	0.51	2.71	1.00	0.028	0.291	0.50
15	-18/9	3	0.54	2.94	0.65	0.028	0.175	0.50
16	10/9	1	0.54	2.73	1.20	0.028	0.347	5.00
17	10/9	2	0.50	2.75	1.00	0.028	0.287	0.10
18	10/9	3	0.48	2.80	0.85	0.028	0.240	0.60
19	-18/22	1	1.84	2.75	6.90	0.028	1.981	15.60
20	-18/22	2	1.71	2.76	6.15	0.028	1.759	7.20
21	-18/22	3	1.54	3.06	5.80	0.028	1.497	11.20
22	-18/9	1	1.72	2.69	3.20	0.028	0.940	4.20
23	-18/9	2	1.49	2.88	4.80	0.028	1.316	2.50
24	-18/9	3	1.64	2.84	4.03	0.028	1.120	2.40
25	10/9	1	1.76	2.76	4.35	0.028	1.193	6.70
26	10/9	2	1.65	2.93	5.10	0.028	1.374	2.40
27	10/9	3	1.55	2.85	4.42	0.028	1.251	5.40

Obs=Observation; Temp=Temperature, MC=Moisture content; Rep=Replicate;
DML=Dry matter loss;

Table E7. ANOVA of FFA content in 22 and 9% MC soybean samples in - 18°C environment

Source	DF	MS	F	P>F
(a) Main ANOVA				
Moisture, M	1	0.054	771.4	0.001
Chamber, C	2	0.003	42.9	0.02
C*M	2	0.00007		
Period, P	2	0.059	23.6	0.0004
P*M	2	0.044	17.6	0.001
P*C*M	8	0.0025		
	17			
(b) Subdivision of linear				
Linear, Lin	1	0.092	23.0	0.009
Lin*M	1	0.07	17.5	0.01
Lin*C*M	4			
(c) Subdivision of deviation components				
Deviation, Dev	1	0.027	37.3	0.004
Dev*M	1	0.017	22.7	0.009
Dev*C*M	4	0.00075		

Table E8. Mean FFA values in -18°C environment at moisture content level

Level of Moisture (%)	N	FFA Mean (%)	FFA SD
22	9	0.248	0.167
10	9	0.138	0.036

Table E9. Mean FFA values in -18°C environment at period level

Level of Period (weeks)	N	FFA Mean (%)	FFA SD
0	6	0.133	0.051
26	6	0.138	0.022
48	6	0.308	0.174

Table E10. ANOVA of FFA content of 9% MC soybean samples in -18 and 10°C environment

Source	DF	MS	F	P>F
(a) Main ANOVA				
Temperature,T	1	0.0008	0.3	0.62
Chamber (T)	4	0.0027		
Period, P	2	0.003	2.1	0.2
P*T	2	0.0005	0.6	0.7
P*C(T)	8	0.0014		
	17			
(b) Subdivision of linear				
Linear, Lin	1	0.0044	11.6	0.03
Lin*T	1	0.0009	2.4	0.2
Lin*C(T)	4	0.00038		
(c) Subdivision of deviation components				
Deviation, Dev	1	0.0016	0.7	0.5
Dev*T	1	0.0009	0.04	0.8
Dev*C(T)	4	0.0024		

Table E11. Mean FFA values in -18 and 10°C environment at temperature level

Level of Temperature (°C)	N	FFA Mean (%)	FFA SD
-18	9	0.138	0.036
10	9	0.152	0.049

Table E12. Mean FFA values in -18 and 10°C environment at period level

Level of P (weeks)	N	FFA Mean (%)	FFA SD
0	6	0.133	0.052
26	6	0.130	0.028
48	6	0.172	0.036

Table E13. ANOVA of DKT analysis in 22 and 9% MC soybean samples in -18°C environment

Source	DF	MS	F	P>F
(a) Main ANOVA				
Moisture, M	1	0.067	119.6	0.008
Chamber, C	2	0.0172	30.7	0.03
C*M	2	0.00056		
Period, P	2	0.14	45.2	0.00004
P*M	2	0.067	21.6	0.0006
P*C*M	8	0.0031		
	17			
(b) Subdivision of linear				
Linear, Lin	1	0.003	1.8	0.25
Lin*M	1	0.0	0.0	1.0
Lin*C*M	4	0.0017		
(c) Subdivision of deviation components				
Deviation, Dev	1	0.29	111.5	0.0005
Dev*M	1	0.134	51.5	0.002
Dev*C*M	4	0.0026		

Table E14. Mean DKT values in -18°C environment at moisture content level

Level of MC (%)	N	DKT Mean (%)	DKT SD
22	9	0.167	0.235
9	9	0.044	0.073

Table E15. Mean DKT values in -18°C environment at period level

Level of Period (weeks)	N	DKT Mean (%)	DKT SD
0	6	0.000	0.000
26	6	0.283	0.223
48	6	0.033	0.052

Table E16. ANOVA of DKT analysis of 9% MC soybean samples in -18 and 10°C environments

Source	DF	MS	F	P>F
(a) Main ANOVA				
Temperature,T	1	0.009	2.25	0.21
Chamber (T)	4	0.004		
Period, P	2	0.029	4.0	0.06
P*T	2	0.0022	0.61	0.7
P*C(T)	8	0.0072		
	17			
(b) Subdivision of linear				
Linear, Lin	1	0.0033	1.0	0.37
Lin*T	1	0.0033	1.0	0.37
Lin*C(T)	4	0.0033		
(c) Subdivision of deviation components				
Deviation, Dev	1	0.0054	0.49	0.5
Dev*T	1	0.0011	0.1	0.8
Dev*C(T)	4	0.011		

Table E17. Mean DKT values in -18 and 10°C environments at temperature level

Level of Temperature (°C)	N	DKT Mean (%)	DKT SD
-18	9	0.033	0.071
10	9	0.078	0.109

Table E18. Mean DKT values in -18 and 10°C environments at period level

Level of Period (weeks)	N	DKT Mean (%)	DKT SD
0	6	0.000	0.000
26	6	0.133	0.103
48	6	0.033	0.082

Table E19. ANOVA of moisture contents of 9% MC soybean samples preserved in -18 and 10°C environments

Source	DF	MS	F	P>F
(a) Main ANOVA				
Temperature,T	1	0.0064	0.06	0.83
Chamber (T)	4	0.115		
Period, P	2	0.4	10.0	0.007
P*T	2	0.015	0.38	0.7
P*C(T)	8	0.04		
	17			
(b) Subdivision of linear				
Linear, Lin	1	0.77	10.4	0.03
Lin*T	1	0.02	0.27	0.63
Lin*C(T)	4	0.074		
(c) Subdivision of deviation components				
Deviation, Dev	1	0.02	*	*
Dev*T	1	0.01	*	*
Dev*C(T)	4	0.0		

Table E20. Mean MC values in -18 and 10°C environments at temperature content level

Level of Temperature (°C)	N	MC Mean (%)	MC SD
-18	9	8.323	0.330
10	9	8.286	0.299

Table E21. Mean MC values in -18 and 10°C environments at period level

Level of Period (weeks)	N	MC Mean (%)	MC SD
0	6	8.528	0.166
26	6	8.362	0.193
48	6	8.023	0.308

Table E22. ANOVA of FFA contents in 22 and 9% MC preserved soybean samples during storage at 0.5% DML

Source	DF	MS	F	P>F
(a) Main ANOVA				
Moisture, M	1	0.86	215.0	0.005
Chamber, C	2	0.012	3.0	0.25
C*M	2	0.004		
Period, P	2	0.28	28.0	0.0002
P*M	2	0.46	46.0	0.00004
P*C*M	8	0.01		
	17			
(b) Subdivision of linear				
Linear, Lin	1	0.48	38.4	0.003
Lin*M	1	0.88	70.4	0.001
Lin*C*M	4	0.0125		
(c) Subdivision of deviation components				
Deviation, Dev	1	0.09	7.2	0.06
Dev*M	1	0.03	2.4	0.2
Dev*C*M	4	0.0125		

Table E23. Mean FFA values of 22 and 9% MC preserved sample at moisture content level during storage at 0.5% DML

Level of Moisture (%)	N	FFA Mean (%)	FFA SD
22	9	0.718	0.443
9	9	0.281	0.080

Table E24. Mean FFA values of 22 and 9% MC preserved sample at period level during storage

Level of Period (weeks)	N	FFA Mean (%)	FFA SD
0	6	0.350	0.055
26	6	0.400	0.193
48	6	0.748	0.585

Table E25. ANOVA of FFA contents of 9% MC preserved soybean samples during storage at 0.5% DML

Source	DF	MS	F	P>F
(a) Main ANOVA				
Temperature,T	1	0.044	11.0	0.03
Chamber (T)	4	0.004		
Period, P	2	0.024	4.8	0.04
P*T	2	0.02	4.0	0.06
P*C(T)	8	0.005		
	17			
(b) Subdivision of linear				
Linear, Lin	1	0.036	18.0	0.01
Lin*T	1	0.0034	1.7	0.26
Lin*C(T)	4	0.002		
(c) Subdivision of deviation components				
Deviation, Dev	1	0.011	1.3	0.32
Dev*T	1	0.038	4.5	0.1
Dev*C(T)	4	0.0085		

Table E26. Mean FFA values of 9% MC preserved sample at temperature level during storage at 0.5% DML

Level of temperature (°C)	N	FFA Mean (%)	FFA SD
-18	9	0.281	0.080
10	9	0.379	0.108

Table E27. Mean FFA values of 9% MC preserved sample at period level during storage at 0.5% DML

Level of Period (weeks)	N	FFA Mean (%)	FFA SD
0	6	0.367	0.052
26	6	0.367	0.147
48	6	0.258	0.063

Table E28. ANOVA of DKT of 22 and 9% MC preserved soybean samples during storage at 0.5% DML

Source	DF	MS	F	P>F
(a) Main ANOVA				
Moisture, M	1	22.9	21.2	0.04
Chamber, C	2	0.72	0.7	0.6
C*M	2	1.08		
Period, P	2	5.14	2.2	0.17
P*M	2	0.51	0.22	0.81
P*C*M	8	2.3		
	17			
(b) Subdivision of linear				
Linear, Lin	1	2.9	1.1	0.35
Lin*M	1	0.19	0.07	0.8
Lin*C*M	4	2.6		
(c) Subdivision of deviation components				
Deviation, Dev	1	7.4	3.8	0.12
Dev*M	1	0.84	0.43	0.54
Dev*C*M	4	1.94		

Table E29. Mean DKT values of 22 and 9% MC preserved sample at moisture content level during storage at 0.5% DML

Level of Moisture (%)	N	DKT Mean (%)	DKT SD
22	9	3.522	1.790
9	9	1.267	0.963

Table E30. Mean DKT values of 22 and 9% MC preserved sample at period level during storage at 0.5% DML

Level of Period (weeks)	N	DKT Mean (%)	DKT SD
0	6	1.450	1.067
26	6	3.300	1.890
48	6	2.433	2.103

Table E31. ANOVA of DKT for 9% MC preserved soybean samples during storage at 0.5% DML

Source	DF	MS	F	P>F
(a) Main ANOVA				
Temperature,T	1	0.03	0.03	0.9
Chamber (T)	4	0.92		
Period, P	2	1.71	0.8	0.5
P*T	2	0.31	0.1	0.9
P*C(T)	8	2.1		
	17			
(b) Subdivision of linear				
Linear, Lin	1	2.34	1.0	0.37
Lin*T	1	0.24	0.1	0.76
Lin*C(T)	4	2.34		
c) Subdivision of deviation components				
Deviation, Dev	1	1.1	0.56	0.5
Dev*T	1	0.38	0.2	0.68
Dev*C(T)	4	1.95		

Table E32. Mean DKT values of 9% MC preserved sample at moisture content level during storage at 0.5% DML

Level of Moisture (%)	N	DKT Mean (%)	SD
22	9	1.311	0.921
9	9	1.389	1.505

Table E33. Mean DKT values of 9% MC preserved sample at period level during storage at 0.5% DML

Level of Period (weeks)	N	DKT Mean (%)	SD
0	6	0.733	0.052
26	6	1.700	0.678
48	6	1.617	0.959

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