AN ABSTRACT OF THE THESIS OF

LUIS ALBERTO SAYAVEDRA-SOTO for the degree of Master of Science in Food Science and Technology presented on July 28, 1980

Title: EFFECT OF MOISTURE, TEMPERATURE AND SULFUR DIOXIDE ON COLOR OF DRIED APPLES.

Abstract APPROVED: _____
Dr. Morris W. Montgomery

When sulfur dioxide is used in commercial drying of fruits in combination with low storage temperatures, browning and microbial spoilage can be inhibited. However, the relationship between moisture level, storage temperature, and sulfur dioxide content on the color of dried fruit is not known; therefore the purpose of this research was to determine the influence of temperature, sulfur dioxide content, and moisture level on the color of dried apples during storage.

Dices (3/4" x 1/2" x 1/4") of Golden Delicious apples were dried after a 90 sec dip in aqueous solutions of 2500, 5000, or 7500 ppm of sulfur dioxide obtained from sodium bisulfite. The apples were dried to five different levels of moisture (13, 18, 22, 24, and 26% wet basis) and stored in controlled temperature rooms at 1°, 21°, and 38°C. Periodically the samples were analyzed for color (color index (CI) defined as Hunter L x a_L x b_L), total and free sulfur dioxide, moisture and water activity (a_W), to appreciate the changes of quality during 385 days of storage.

Sulfur dioxide level was directly influenced by storage temperature. As temperature increased, the sulfur dioxide level in the dried apples decreased following a negative exponential curve. At 1°C nearly no variation in sulfur dioxide level was observed during the experiment. Loss of free sulfur dioxide followed the same pattern as total sulfur dioxide. The concentration of free sulfur dioxide was a larger proportion of the total as the concentration of total sulfur dioxide was increased.

Moisture content of the dried apples decreased during storage at 38°C, but at 21°C moisture content decreased in the first 40 days reaching a level that remained constant until the end of the experiment. The constant level was approximately 85% of the initial moisture level. No appreciable change of water content occurred at 1°C. Water activities of the samples ranged between 0.40 and 0.85 and the optimal levels for color retention at the lower concentrations of total sulfur dioxide were approximately 0.75 a_w. This corresponded to a moisture of approximately 20%.

Total sulfur dioxide, moisture and CI analysis of the dried apples were used to derive three equations, one for each temperature of storage. From these equations, the following relationships were evident.

CI described the changes in color during the 385 days of the experiment. At 1°C sulfur dioxide and moisture influenced the changes of color approximately the same extent. Moisture content of 20% at all levels of sulfur dioxide at 1°C was optimal for maximum color preservation. Samples stored at 1°C retained an acceptable color longer

than those stored at 21°C or 38°C. The approximation of the equation derived from regression analysis of the data of changes in color at 1°C was 63%.

Changes in color at 21°C occurred faster than at 1°C. Acceptable colors were found until the 188 day samples. The water content of the samples had more influence on the color changes than the total sulfur dioxide content as determined by the regression equation. The regression equation describes 88% of the variations in color. Concentrations higher than 1500 ppm of total sulfur dioxide were necessary to maintain samples of acceptable colors for periods up to 188 days with moisture levels close to 20%. Storage temperature of 21°C was satisfactory for samples that do not require storage periods longer than 200 days and contain 1500 ppm of total sulfur dioxide and 20% moisture.

Very rapid changes in the parameters studied were observed at 38°C. The samples were very dark after 40 days and none had an acceptable color after 101 days of storage. The regression equation derived from the data described 87% of the variations of color. The temperature of 38°C is not recommended under any condition for storage of dried apples.

It was concluded that temperatures lower than 21°C and concentrations of approximately 1500 ppm of sulfur dioxide in the fruit tissue could preserve acceptable colors in samples of dried apples for periods of 200 days. Longer storage periods would be possible as temperature approaches 1°C.

Using the equations obtained in this experiment, an estimation can be made of the storage life of dried apples. The response surface

diagrams obtained are useful for visual comprehension of the influence of temperature, sulfur dioxide and moisture on color throughout the time of storage.

Effect of Moisture, Temperature, and Sulfur Dioxide on Color of Dried Apples

by

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INTRODUCTION

Research for better methods of food preservation has been one of the main activities of many food scientists. This research has resulted in many methods of food preservation. Some of these methods have been used for centuries with little change since man began to study these processes to preserve foods during storage, e.g., dehydration of food, use of low temperatures, or use of substances such as sodium chloride, sucrose, vinegar, wood smoke, or sulfur dioxide.

Sulfur dioxide is used in many food products to preserve quality. It is a controversial additive and its mode of action is not well known. Actually much research has been done to try to explain the mechanism of action of sulfur dioxide, however no one has been completely successful in describing the exact mechanism by which sulfur dioxide acts as a preservative in its diverse applications.

When sulfur dioxide is used in dried fruits in combination with low temperatures, browning and microbial spoilage are inhibited. Optimal levels of sulfur dioxide to use and the influence of storage temperature and moisture level on the preservation of the quality of dried apples are not well documented in the literature.

Annually large quantities of apples are available for marketing, other than in the fresh product. These apples are made into juices, purees, cider, and various canned and dried apple products. Industrialization of apple processing by sulfuring and drying of diced apple

portions has been highly developed. These diced apples are mainly used in the manufacture of desserts, such as cakes, pies, or snacks.

In the industry today, dehydration of 200 tons per day is not an uncommon operation. Much of the apple dehydration operation is carried out empirically with little regard to the optimal levels of moisture and sulfur dioxide. These estimations sometimes fail, causing shorter storage life than expected.

After dehydration the apples are stored in large warehouses for marketing throughout the year. Generally, the dehydrated apples are packaged in cartons and/or plastic bags. Most warehouses do not have controlled storage conditions and temperatures as high as 40°C on the roofs during the hottest days of summer are not unusual. These conditions increase the loss of sulfur dioxide and moisture, causing subsequent deterioration of the product. Some measures to avoid this deterioration are the use of controlled humidity in the warehouses to prevent changes of moisture content in the product, together with resulphuring in the cartons; the use of more efficient packages which are more resistant to the interchange of moisture or sulfur dioxide (Adsule and Anand, 1977); incorporation of sulfur dioxide into the tree fruits before processing (Dahlenburg, 1976); studies to understand the metabolism of sulfur dioxide in fruits (Kalus, 1978); computer predictions of food storage (Karel et al., 1971) and other possible remedial methods that may be applied in the food industry.

The purpose of the research presented here was to determine a method to estimate the storage life of dried apples if the initial

levels of moisture and sulfur dioxide, as well as the temperature of storage are known. The importance of storage temperature, loss of moisture, loss of sulfur dioxide on the rate of browning and some considerations in the interaction of the above parameters on storage of dried apples are discussed.

REVIEW OF THE LITERATURE

Sulfur Dioxide

The earliest use of sulfur dioxide was apparently the treating of wines in Roman times. Sulfur dioxide, long known for its purifying ability, was prepared on the spot by burning sulfur and has been used since the time of Homer for fumigating houses or for sanitizing wine vessels (Anon., 1975).

Although no human ailment or untoward effect resulting from such use has been recognized, concern over possible hazard caused by foods containing sulfur dioxide also goes back a considerable length of time, to an article published by Kionca in 1896 on the possible toxicity of sulfites (Anon., 1975).

The apparent absence of any risk associated with the use of sulfur dioxide added to food as sodium sulfite has led to the widespread acceptance in the food industry of this additive (Anon., 1974). The earliest reference to its use as a food preservative that could be found in the literature was a suggestion in the 17th century that casks should be filled with cider while they still contained sulfur dioxide, produced by burning sulfur in them (Roberts and McWeeny, 1972).

Chemistry of Sulfur Dioxide

Sulfur dioxide is a colorless non-flamable gas, has a strong suffocating odor, and is intensely irritating to eyes and respiratory tract. Its solubility in water is 17.7% at 0°C, 11.9% at 15°C, 8.5% at 25°C and 6.4% at 35°C (Merck Index, 1976). Odor detection level of sulfur

dioxide is as low as 3 ppm, it causes throat irritation at 8 ppm and coughing and eye irritation at 20 ppm. Some people can detect the presence of sulphur dioxide in certain foods quite easily by its rather metallic flavor, by odor, by a sensation in the nose similar to the onset of a cold, or by sneezing when the food is ingested (Green, 1976).

Sidwick (1952) suggested the structure, $S\overset{\leq O}{\underset{O}{}}$, for sulfur dioxide. It dissolves readily in water and the resulting solution possesses well known acidic properties, conducts an electric current, and behaves as an acid, presumably H_2SO_3. However, although the ions H_3O^+, HSO_3^-, and $SO_3^=$ are indeed present in the solution, the free acid has never been isolated or shown to exist. Nevertheless, existence of the free acid has been taken for granted by most chemists (Sidwick, 1952). Falk and Giguère (1958) in their studies found by infared spectra that sulfur dioxide molecules are not strongly hydrated in aqueous solutions and do not form the compound H_2SO_3. Unstable H_2SO_3 molecules might be involved as short-lived intermediates in the formation of HSO_3^- ions in the general equilibria of sulfur dioxide with water at different pH levels (Fig. 1) expressed as follows (Green, 1976):

$$H_2O + SO_2 \rightleftarrows H_2SO_3 \rightleftarrows HSO_3^- + H^+ \rightleftarrows SO_3^= + 2H^+$$

$\longleftarrow$ pH $\longrightarrow$

Decrease Increase

however, these ions can also be formed by another mechanism (Falk and Giguère, 1958):

$$2H_2O \rightleftarrows OH^- + H_3O^+ \quad ; \quad SO_2 + OH^- \rightleftarrows HSO_3^-$$

and the overall equilibria would be:

$$SO_2 + 2H_2O \rightleftarrows HSO_3^- + H_3O^+.$$

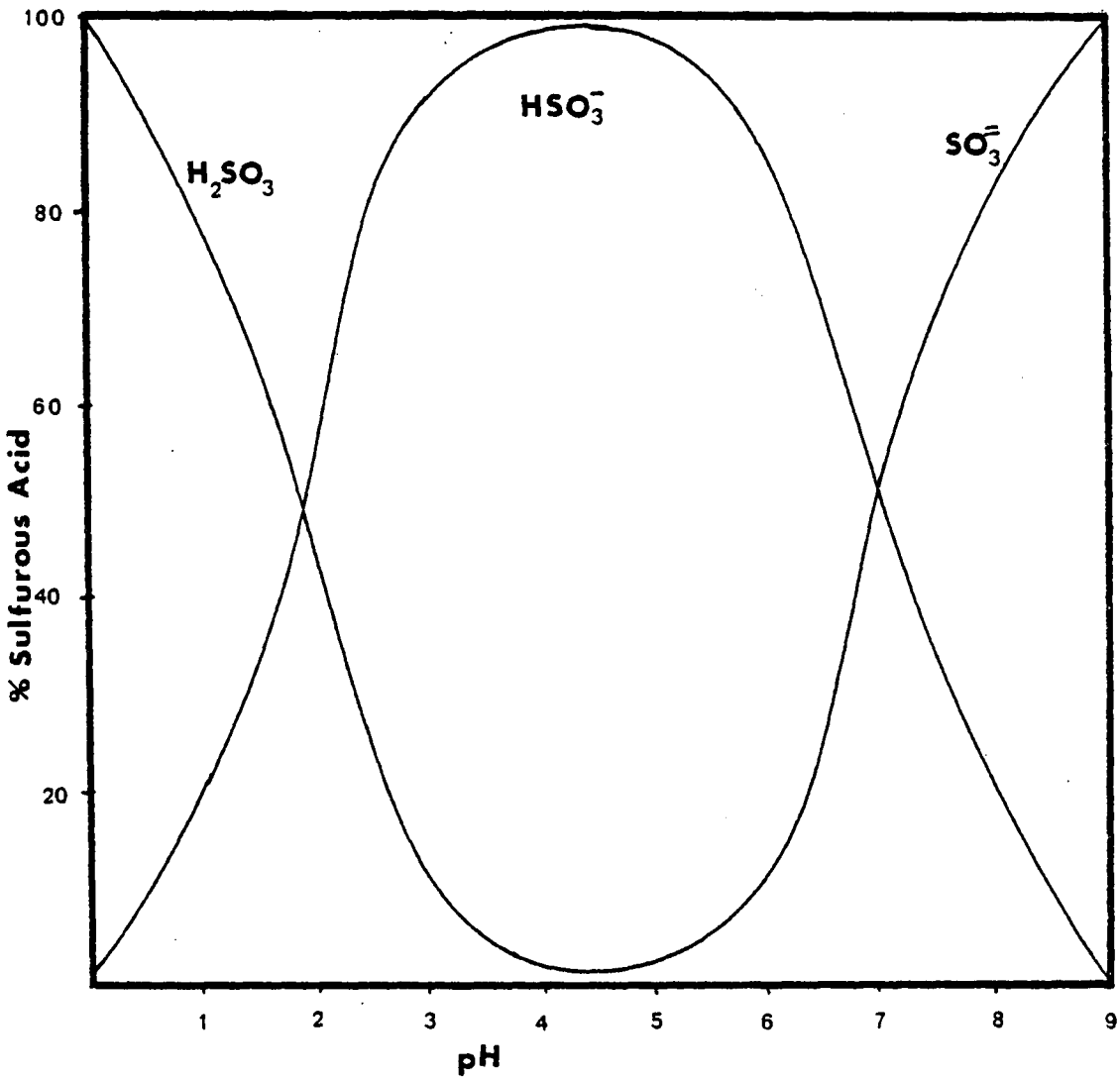


Figure 1. Distribution of various forms of sulfurous acid at various pH values (Joslyn, 1970).

Another possible mechanism might be that the molecule H_2SO_3 is not present in detectable concentrations even in solution.

The term sulfur dioxide is often used to determine all sulfites, hydrogen sulfites and metabisulfites. Sulphur dioxide can be obtained by any of the three groups of substances in different yields according to Table 1 due to the equilibria shown above.

When sulfur dioxide is used as an antioxidant it is reduced to elemental sulfur, i.e.:

$$2FeSO_4 + 2SO_2 \rightleftharpoons Fe_2(SO_4)_3 + S,$$

as in the enhancement of the oxidation of orange oil by sulfur dioxide (Beard et al., 1972).

Sulfur dioxide, when acting as a reducing agent, produces sulfate, i.e.:

$$2Na_2SO_3 + O_2 \rightleftharpoons 2Na_2SO_4.$$

The reversible process may occur and, therefore, account for some free sulfur dioxide in some processes.

Toxicity of Sulfur Dioxide

Ingestion of food containing sulfur dioxide at the levels established by the World Health Organization has been shown to be nontoxic, however sulfur dioxide is not recognized as an innocuous food additive. Numerous investigations of the toxicity of sulfite have failed to indicate toxicity and have tended rather to clear it of any toxic suspicion. The levels of sulfite introduced into the body by ingestion of food protected with sulfur dioxide, or by breathing air polluted with sulfur dioxide, are low relative to the capacity of the body to oxidize sulfur

Table 1. Available sulfur dioxide content from various sources which are permitted to be used as preservatives in foods[a].

Chemical	Formula	Theoretical yield (%)	Actual yield (%)	Approximated solubility (g/100ml)
Sulfur dioxide	SO_2	100.00	100.00	11 at 20°C
Acid sulfurous[b]	H_2SO_3	6.00	6.4-6.8	--
Sodium sulfite anh.	Na_2SO_3	50.82	--	28 at 40°C
Sodium sulfite heptahydrate	$Na_2SO_3 \cdot 7H_2O$	25.41	--	24 at 25°C
Potassium sulfite[b]	K_2SO_3	33.00	36.00	100 at 20°C[c]
Calcium sulfite[b]	$CaSO_3 \cdot 1\frac{1}{2}H_2O$	23.00	43-45	.0043 at 20°C
Sodium hydrogen sulfite	$NaHSO_3$[d]	61.56	--	300 at 20°C
Potassium bisulfite[b]	$KHSO_3$	53.31	--	Soluble[c]
Sodium metabisulfite	$Na_2S_2O_5$	67.39	61.00	54 at 20°C
Potassium metabisulfite[b]	$K_2S_2O_5$	67.43	52.00	25 at 0°C

[a]Green (1976) and Joslyn (1970)

[b]Joslyn (1964)

[c]CRC Handbook of Chemistry and Physics (1979)

[d]"There is some doubt whether sodium hydrogen sulphite exists in the solid state and the materials so described is either sodium metabisulphite or a mixture of bisulphite and metabisulphite" (Smith and Stevens, 1972).

dioxide. These very low levels of sulfite probably do not cause a toxic effect (Anon., 1974).

Some uses of sulfur dioxide in food processing are the following: to control microbial spoilage in low pH products (Scholey and Rawlingson, 1974); to increase the shelf life of some meat products (allowed only in some countries (Kidney, 1974), to prevent non-enzymic browning in a wide variety of fruits and juices including wine (Burroughs, 1974); and in the preservation of dry fruits to inhibit enzymic, non-enzymic browning or microbial spoilage (McWeeny, 1979). Sulfur dioxide can also be used to improve the quality of the dough of bakery products, or for modification of flours by reversible cleavage of the disulfide bond of cysteine in gluten by the following reaction (Roberts and McWeeny, 1972):

$$\begin{array}{l} S\cdot CH_2CH(NH_2)COOH \\ | \qquad\qquad \xrightarrow{SO_2} \\ S\cdot CH_2CH(NH_2)COOH \end{array} \qquad \begin{array}{l} HSCH_2CH(NH_2)COOH \\ \\ HSO_3SCH_2CH(NH_2)COOH \end{array}$$

The rate of loss of vitamin C during processing and storage of fruits was decreased with the use of sulfur dioxide (Bolin and Stafford, 1974). Sulfur dioxide prevented the oxidation of lipids in lipid-protein-emulsions, such as sausages by acting as an antioxidant agent; or prevents the development of the grey discoloration of minced meats by serving as a reducing agent in the reaction (Roberts and McWeeny, 1972):

$$(Fe^{2+})\text{Myoglobin} \xrightarrow{O_2} (Fe^{2+})\text{Oxymyoglobin} \xrightarrow{O_2} (Fe^{3+})\text{Metmyoglobin}.$$

With all the benefits and the very low toxicity of sulfur dioxide, mutagenicity has been suggested because sulfur dioxide has been shown to react with nucleic acids *in vitro*. Shapiro and Braverman (1972) showed by revertive studies with *Escherichia coli* mutants that sodium bisulfite

specifically induced mutations in only those mutants which were cytosine-guanine at the mutant site. Hayatsu and Miura (1970) noted that high concentrations of sodium bisulfite produced mutations in phage λ and proposed that the mutagenesis was related to the cytosine derivative specific reaction of sodium bisulfite. Valencia et al. (1972) found in fruit flies, that ingestion of 5120 ppm of sulfur dioxide caused a moderate mutation rate that did not differ significantly from the control. On the other hand, long term animal feeding studies failed to show any tumorigenic effect attributable to sulfite or any alteration in the descendants of the three generations treated. A non-toxic effect of doses of 0.35% $Na_2S_2O_5$ in the diet of pigs for 48 weeks was established (Valencia et al., 1972).

When sulfur dioxide was added to food containing thiamine the latter reacts to give compounds (Fig. 2) which possess no vitamin activity (Anon., 1975). Bhagat and Lockett (1964) showed that a stored diet containing sulfite caused toxic effects in rats which could be attributed to thiamine deficiency, but was probably caused by changes in the fats during the storage (Anon, 1975).

A group of human volunteers consumed a thiamine-deficient diet (120 µg of thiamine/1000 kcal) for 50 days and during the last 25 days of that period, received 400 mg of sulfur dioxide plus sodium glucose sulfonate. Exhaustive clinical and biochemical examinations of the subjects revealed no demonstrable effects attributable to a sulfite intake (Anon., 1975).

Because there are no data that show any immediate toxic effects, all the agents (sodium sulfite, sodium bisulfite, sodium metabisulfite,

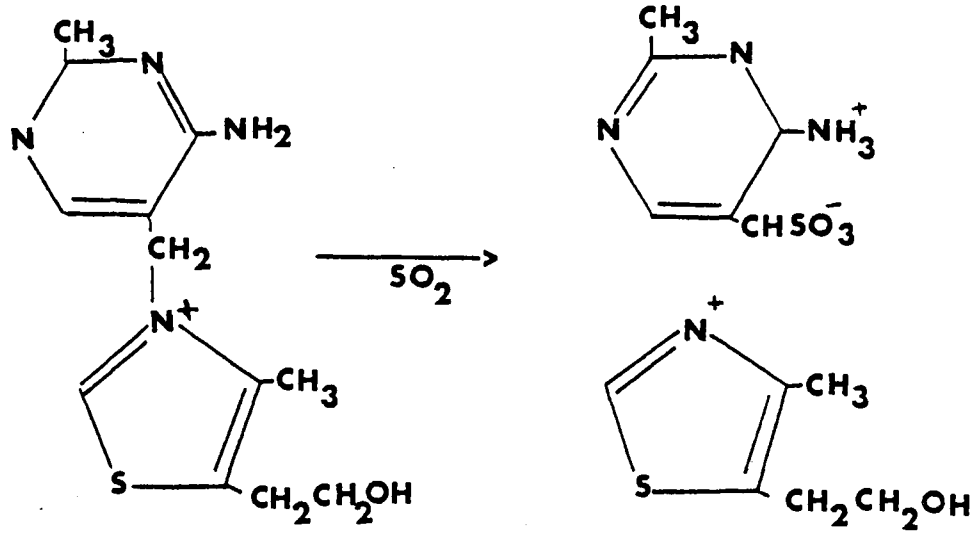


Figure 2. Cleavage of thiamine by sulfur dioxide to yield compounds with no vitamin activity.

potassium metabisulfite, potassium bisulfite and alkali sulfites) continue to be considered generally recognized as safe by the Food and Drug Administration.

Reactions of Sulfur Dioxide

General Reactions

Browning is a common accompainment of many organic reactions that are promoted by heat or dehydration and is of particular importance when such reactions occur in foods. Non-enzymic browning in foods is the consequence of complex chemical reactions in which sugars and their derivatives play an important role (Ingles, 1966).

Sulfur dioxide inhibits undesirable browning in some foods, i.e., in the sulfuring of portions or slices of fruit, which are to be dried. Applied in all active forms, sulfur dioxide maintains the desired quality of the food during processing and storage. Despite the variety and complexity of non-enzymic browning reactions in different food systems and the different reactive intermediates that may be formed during processing or storage, sulfur dioxide appears to be an effective inhibitor of this browning. Hodge (1953) reviewed the chemistry of browning in model systems applied to dehydrated foods and recognized three broad types of browning reactions in food technology: first, the most common type carbonyl reactions, includes the reactions of aldehydes, ketones, and reducing sugars with amines, peptides and proteins; the second type, caramelization, occurs when polyhydroxycarbonyl compounds (sugars or polyhydroxycarboxylic acids) are heated to relatively high temperatures

in the absence of amino compounds; and third, a broad type of browning frequently encountered by the food processor in the group of oxydative reactions, which, for example, convert ascorbic acid and polyphenols into de- or poly-carbonyl compounds. These oxidative reactions may or may not be enzyme catalyzed. Compounds that cause browning usually contain a carbonyl or a potential carbonyl grouping. Polyhydroxy compounds and sugars in which the carbonyl function is blocked do not give rise to browning and Hodge (1953) concluded that the most important model systems for studies of browning reactions were those in which α-hydroxy carbonyl (or α-amino carbonyl) compounds were transformed into unsaturated colored polymers.

Reynolds (1965) reviewed the procedures available to the food scientist for the inhibition of non-enzymic browning in foods and included control of the moisture content of the product, control of temperature of storage, removal of any active constituent of the foodstuff, and the addition of bisulfite.

The physical methods of inhibition of browning, such as the reduction of moisture content of a dehydrated food to 2% or reduction of the temperature of storage to 20°C, sometimes are not affordable economically or technically. This leads to a more feasible method such as the use of chemicals. Chemical methods are able to attack at any of the following three stages: the formation of ketose amines, the decomposition of ketose amines, or the condensations of sugar degradation products (Reynolds, 1965).

Burton et al. (1962a) studied the effect of sulfites on the aldose-amino reaction using ^{35}S as a radiochemical tracer and postulated that the initial uptake of sulfite occurred at carbonyl groups but that it

later became transferred to other sites. Sulfite migration is known to occur in these systems. This has been demonstrated with the lithium sulfonates of citral and cinnamic acid aldehyde. Under normal conditions citral forms a carbonyl bisulfite addition compound which readily loses sulfite on hydrolysis. Citral may subsequently form mono and dehydrosulfonic acid compounds with liberation of the carbonyl group. This carbonyl group can then undergo normal carbonyl reactions. Burton et al. (1962b) found that the nature, as well as the rate of reaction of intermediates produced in aldose-amino-reactions and the rate of inactivation of sulfur dioxide as a browning retardant depend on the pH of the reaction.

Burton and McWeeny (1963) showed that sulfite produced a more effective retardation in chromophore development when added initially (at time zero) than when added at a later stage. In their work, sugar stability in non-enzymic reactions was also considered and these authors found that aldopentoses reacted with glycine and amino compounds more rapidly than did aldohexoses to yield chromophores. The degree of browning developed from hexoses in ascending order was: glucose, mannose, and galactose. The pentoses arabinose and xylose resulted in more color than the hexoses. Burton and McWeeny (1963) suggested that the earliest effect of sulfite appeared to be on sugar stability.

Burton et al. (1963a), in their work on non-enzymic browning of phenols and its inhibition with sulfur dioxide, concluded that whether solid state or in weakly acid solutions, the non-enzymic browning of phenolic compounds in the presence of a nitrogenous function was faster than non-nitrogen browning and that sulfite was effective in retarding

the onset and development of such browning. Effective browning retarding agents are both carbonyl group binding reagents and reducing reagents. Sulfite may act at more than one point in the chain of reactions where sugars were the main source of the carbonylic groups required for the carbonyl-amino-reactions (Burton et al., 1963b).

Ingles and colleagues (1959, 1960, 1961, 1962), from studies in non-enzymic browning concluded that sulfite participated in five main types of reactions: (1) the formation of carbonyl bisulfite compounds with reducing sugars; (2) oxydation of reducing sugars to the corresponding aldonic acids with simultaneous reduction of sulfite to sulfur; (3) the esterification of the sugars to give, for example, glucose-6-sulfate; (4) reactions leading to the formation of β-sulfopropionic acid and; (5) sulfonation of deoxyhexoses.

Reactions with Carbonyl Groups

A large number of additional compounds of sulfur dioxide with carbonyl groups have been established, both with salts and with organic substances. The most important being, perhaps, the combination of sulfur dioxide with aldehydes and ketones (Green, 1976):

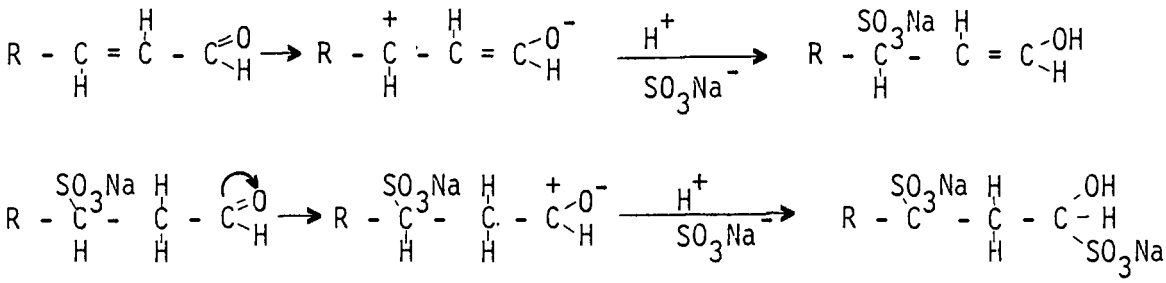
$$R - \overset{H}{\underset{}{C}} = O + NaHSO_3 \rightleftharpoons R - \overset{H}{\underset{OH}{C}} - SO_3Na$$

$$\begin{matrix} R_1 \\ R_2 \end{matrix} \!\!> C = O + NaHSO_3 \rightleftharpoons \begin{matrix} R_1 \\ R_2 \end{matrix} \!\!> \overset{}{\underset{OH}{C}} - SO_3Na$$

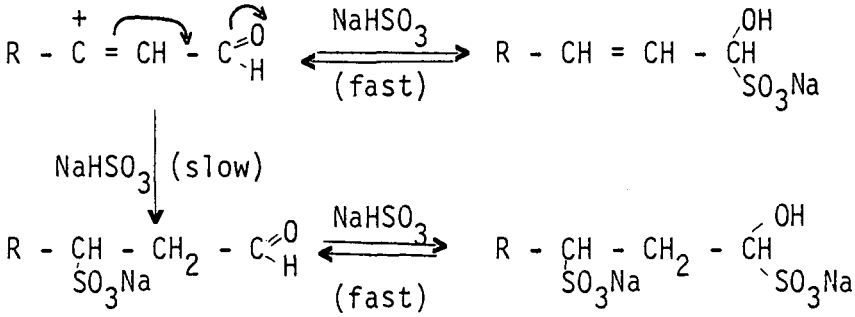
which could arise from the mechanism:

$$R - C \!\!<\!\! \begin{matrix} O \\ H \end{matrix} \longrightarrow R - C \!\!<\!\! \begin{matrix} {}^{+}O^{-} \\ H \end{matrix} \longrightarrow R - \overset{OH}{\underset{SO_3Na}{C}} - H .$$

The formation of doubly sulfonated compounds from α, β-unsaturated might be expected to proceed in two stages:



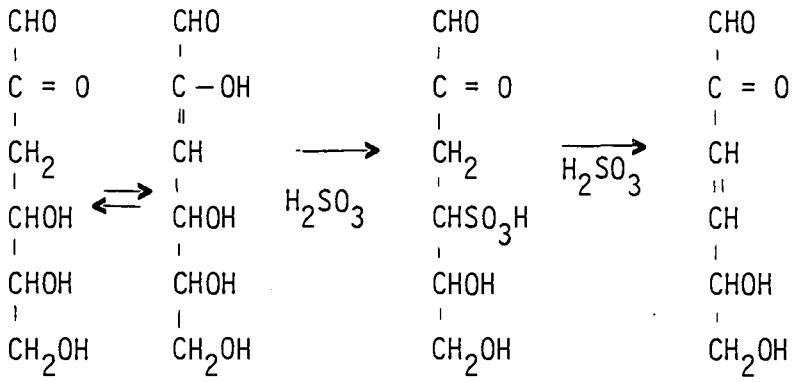
in a simplified form, the general reaction may be visualized as (Burton et al., 1963b):



Ingles (1966) studied the reactions of sulfur dioxide at different pH levels. The reaction that occurred at pH 4.0 was the formation of bisulfite addition compounds with aldose sugars, such as the cyrstaline addition compounds isolated by Ingles (1959). These products were after reacting glucose, galactose, mannose, xylose and arabinose with potassium bisulphite. Ingles (1961) isolated bisulfite addition compounds from glucosone, glycerosone, reductone, 3-deoxy-glucosone and dehydro-ascorbic acid, which are sugar compounds that have been implicated in various browning systems (Hodge, 1953).

Green (1976) in his review wrote that at pH of 4 to 5: (1) maximum amount of sulfur dioxide was in the combined form; (2) a relatively fast equilibria was attained between D-glucose and sulfur dioxide and (3) free sulfur dioxide was predominantly in the form HSO_3^- with less than 1% as a H_2SO_3, and the remainder as $SO_3^=$.

Sulfonic acid derivatives were formed when glucose was heated with sulfite at pH 6.5:



The sulfonic acid derivatives formed were very stable compounds. The derivatives were not broken in either acid or alkaline systems, nor in the Monier-Williams distillation used for the estimation of combined sulfur dioxide (Ingles, 1966).

From the above results Ingles (1966) concluded that when sulfur dioxide was added to a food at about pH 4.0 bisulfite addition compounds were formed rapidly. However, on storage or heating of the food, the available sulfur dioxide was diminished under these pH conditions and sulfur dioxide shifted disproportionately to sulfate and free sulfur. At higher pH levels losses were due to the binding of sulfur dioxide in the form of sulfonic acid groups attached to sugar molecules or their derivatives.

Gilbert and McWeeny (1976) in their preliminary investigation of sulfite balance on dehydrated vegetables concluded that only 35-45% of sulfite incorporated into vegetables was still measurable as a sulfur dioxide shortly after processing and dehydration.

Inhibition of Maillard Reaction

The major features of the Maillard reaction are (McWeeny, et al., 1974): first, in the initial condensation of glucose with glycine (a reversible reaction) the equilibria tend to be to the right in low moisture systems, therefore, favoring formation of the resultant glycosylamine. Second step is the Amadori rearrangement of the glycosylamine to ketoseamine as shown in Fig. 3. The rearrangement requires acid catalysis and the amino-acid function acts as its own catalyst. The ketoseamine is formed immediately. The next step is the degradation of the amino sugars to amino and non-amino containing compounds which are believed to be reactive intermediates leading to the production of brown colors and/or aromas (Fig. 4).

The effectiveness of sulfite in controlling these complex reactions probably lies in the number of quite distinct chemical reactions in which it can participate with the carbonyl intermediates and can be illustrated in the following scheme (McWeeny et al., 1974):

reducing sugar $\rightleftharpoons$ sugar hydroxysulfonate

simple carbonyl $\rightleftharpoons$ carbonyl hydroxysulfonate

di-carbonyl $\rightleftharpoons$ di-carbonyl hydroxysulfonate

α, β-unsaturated carbonyl $\longrightarrow$ sulfonated carbonyl

pigment (melanoidins) $\overset{?}{\rightleftharpoons}$????

McWeeny et al. (1974) explained the mechanism of browning inhibition under the following assumptions: there was evidence from the changing ratio of free and combined measurable sulfur dioxide that some fairly stable adducts were formed (McWeeny et al., 1969), and also there was evidence that these were di-adducts of α-dicarbonyls (such as

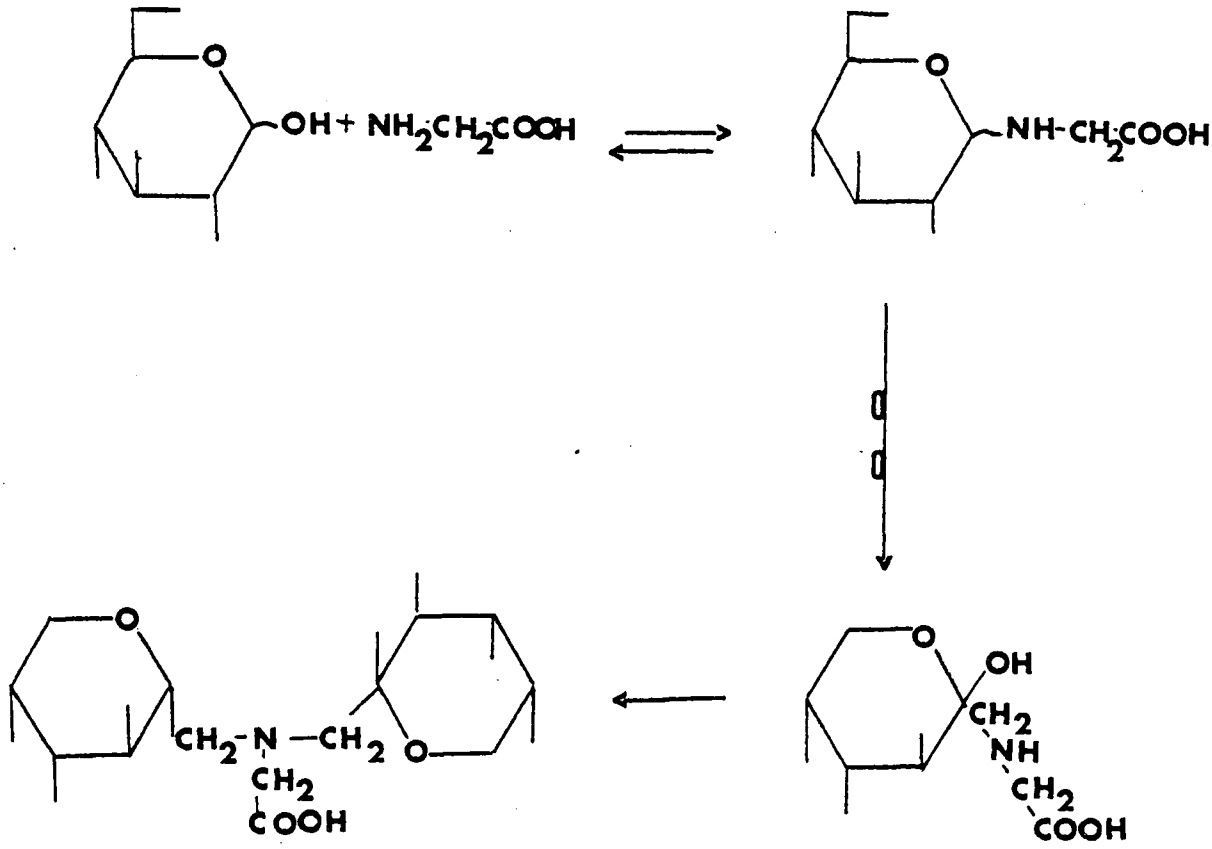


Figure 3. Initial stages of the Maillard reaction.

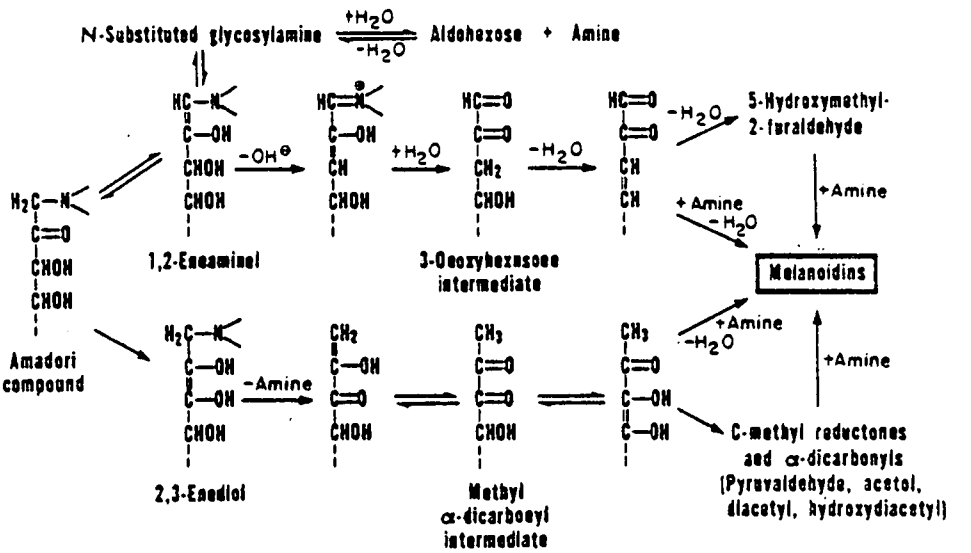
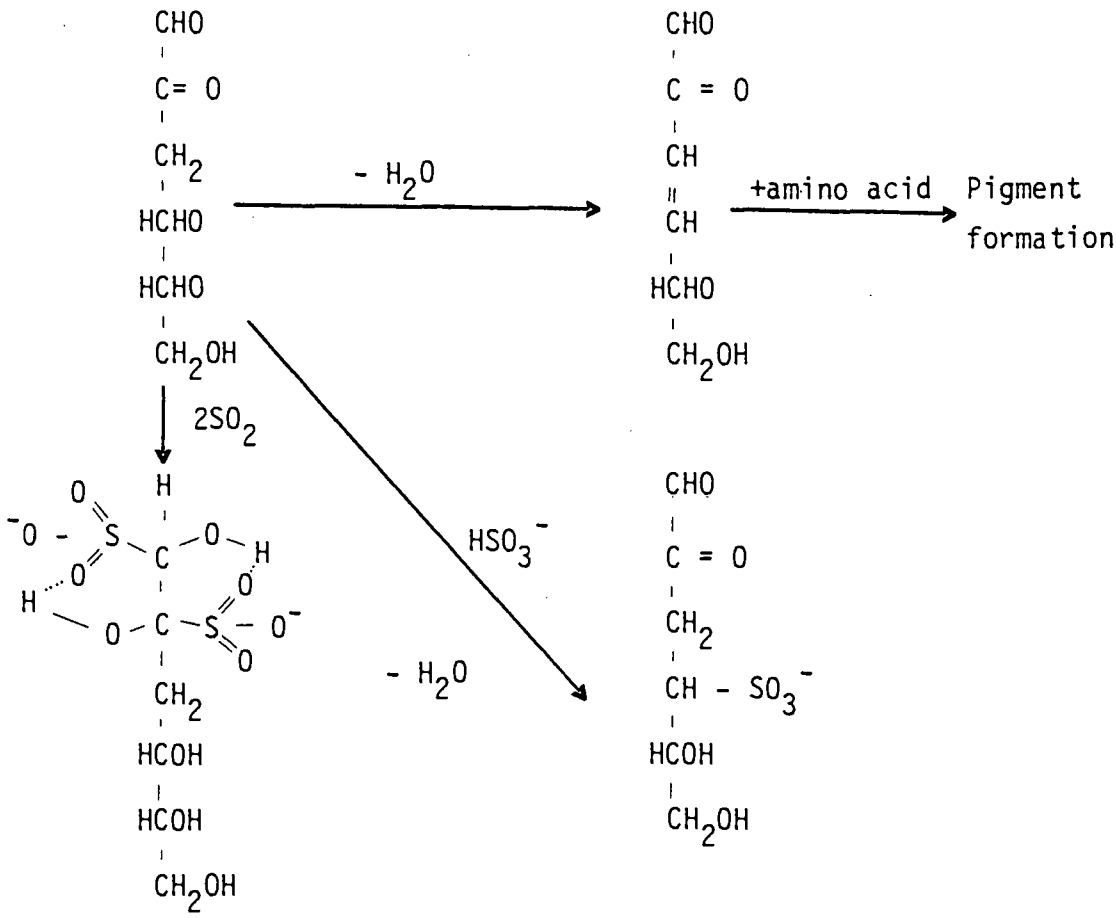


Figure 4. Sugar-amine (Maillard) browning reactions: two pathways to melanoidins and byproducts (Hodge, 1967).

3-deoxyosuloses) which were established by hydrogen bonding between the two adjacent hydroxysulfonate groups (Knowels, 1971). It also has been proposed that the 4-hydroxyl group was displaced to give 4-sulfo-osulose (Ingles, 1962). This compound could no longer undergo dehydration to give unsaturated osulose which browns readily



Anet and Ingles (1964) and Burton and McWeeny (1964) proposed that after dehydration to the unsaturated osulose has occurred, bisulfite addition to the olefinic bond resulted facilitated by the conjugated carbonyl group. The two last reactions led to a compound from which sulfur dioxide could not be removed.

McWeeny (1979) in a recent review of food additives mentioned that sulfur dioxide and the salts of sulfurous acid in many commodities were accompanied by a significant loss in the amount of measurable sulfur dioxide. The mechanisms involved in the loss and the magnitude of the observed effects are worth considering and can be summarized as follows: (1) Physical loss of sulfur dioxide; this is only possible in commodities with pH less than 4, since above this pH, sulfur dioxide is present entirely as non-volatile ionic and covalent species. (2) Loss of chemically measurable sulfur dioxide can be substantial, e.g., 200-300 mg sulfur dioxide/kg in dehydrated potato and much higher in commodities in which higher sulfur dioxide levels are permited. This loss can be provoked by the reactions mentioned above, i.e., formation of sulfonic acid of unidentified structure or sulfonated carbonyl compounds that are reported as the product of inhibition of non-enzymic browning by sulfur dioxide. (3) The loss by oxidation to sulfate is not substantial, e.g., 15-20% of the loss of sulfur dioxide in dehydrated vegetables (Gilbert and McWeeny, 1976).

Enzyme Inhibition by Sulfur Dioxide

Examples of the involvement of sulfur dioxide in the reversal or inhibition of various enzymic changes in plant tissues were reviewed by Haisaman (1974). These are shown in Table 2.

Sulfite inhibited the off-flavor formation in peas, probably due to inhibition of lipoxygenase or the formation of addition compounds with the carbonyl groups of the molecules responsible for the off-flavor (Bengtsson et al., 1967).

Table 2. Examples of the effect of sulfur dioxide on enzymic reactions in plant tissues (Haisaman, 1974).

Reaction	Content	Enzyme	Level of SO_2 found to prevent reaction (ppm)
Oxidation of ascorbic acid	Tomato	Peroxidase (suspected)	800
	Green Pepper and tomato	not specified	20-40
	Red pepper	" "	100
	Cauliflower	Ascorbate oxidase	1000
	Potato	" "	1000
	Pumpkin	" "	1000
Browning	Mushroom	Phenolase	64
	Potato	"	32
	Potato	"	1280
Browning/Blackening	Apple (frozen)	"	300
	Mushroom	"	100
	Apple (refrigerated)	"	1000-2000
Browning	Grape	Peroxidase	5580
	Turnip	"	320
Fermentation off-flavor	Peas	Lipoxygenase	1000
	Grape	Pyruvate descarboxilase	15

The degradation of ascorbic acid was entirely prevented by boiling the tissue or by using sulfur dioxide before maceration of the fruit. Work reported by Ponting and Joslyn (1948) indicated that sulfur dioxide inhibited ascorbate oxidase.

The inhibition of enzymic browning probably is one of the most important uses of sulfur dioxide. Joslyn and Braverman (1954) pointed out that sulfur dioxide disappeared rapidly when added to a macerated fruit. If the fruit had been blanched or if the sulfite has been added in excess, no loss occurred. These authors suggested that the sulfite was consumed by an enzymic reaction which could be inhibited by an excess of sulfite.

The oxidation of tyrosine to 3,4-dihydroxy phenylalanine (DOPA) in potatoes is an important example of the mono-phenol enzymic function (Fig. 5). Further oxidation of the DOPA leads to the formation of a quinone, which rapidly undergoes cyclization and further oxidation to form various indole derivatives. These derivatives condense to melanine. This is a typical example of the diphenol enzymic function, although the later steps in the sequence are generally regarded as non-enzymic. The levels of sulfite required to prevent enzymic browning or blackening obviously depends on the enzymic activity in the tissue and the types and levels of phenolic substrates (Haisaman, 1974). In both apples and pears, treatment with sulfur dioxide results in an undesirable softening of the tissue during storage. The texture could be improved by the addition of calcium salts to the dip. Color of the fruit pieces was also improved (Payne et al., 1968; Ponting et al., 1971, 1972).

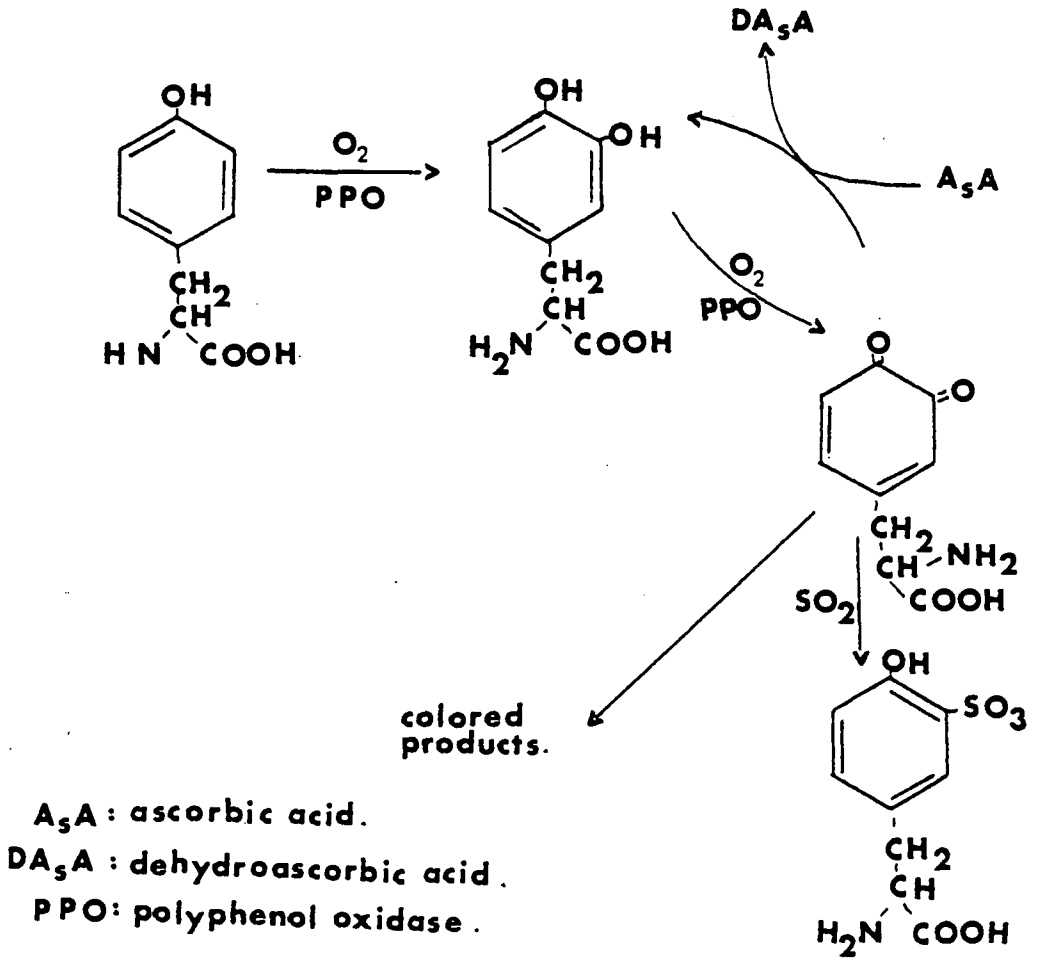


Figure 5. Some reactions illustrating enzymic browning and inhibition by a coupled reaction with a reducing agent (Haisaman, 1974).

Analysis of Sulfur Dioxide

Joslyn and Braverman (1954) wrote an extensive review about the available methods then known for the analysis of sulfur dioxide of treated fruit and vegetable products. These methods can be segregated into those designed to measure the free sulfur dioxide and those for total sulfur dioxide. The latter group may be subdivided into two groups: (1) those in which the bound sulfur dioxide is liberated by distillation from acid; and (2) those in which the combined sulfur dioxide is liberated by treatment of the liquid product or extract with excess alkali at room temperature and subsequent acidification to prevent recombination. The free or total sulfur dioxide may be quantified volumetrically, gravimetrically or colorimetrically. As Joslyn and Braverman (1954) mentioned in their review

> "..determination of free and combined sulfurous acid in foods is not likely to give reliable results unless the conditions under which the analysis is conducted are such that no appreciable alteration of equilibrium can occur during the determination.."

When sulfur dioxide reacts with the carbonyl groups of sugars, acids or aldehydes, it is fixed or bound. The unbound sulfur dioxide is called free sulfur dioxide (Joslyn, 1964).

The extensive use of sulfur dioxide, as the gas or in the form of sulfite solutions, for the preservation of foodstuffs has led to a variety of methods for its quantitative estimation. The Monier-Williams method, based on the oxidation of liberated sulfur dioxide with 3% hydrogen peroxide and titration of the resultant acid with standard

alkali, has been adopted and is still in use with small modifications as an official method by the Association of Official Analytical Chemists (Shipton, 1954; Thrasher, 1966; AOAC, 1980).

Suzuki et al. (1978) made a comparison of five methods of analysis currently in use for foods and suggested that for routine analysis the method by Ripper (1892) yielded fast and reliable results. However, for a more accurate technique the method by Monier-Williams gave better results, while the colorimetric method was better for low concentrations (less than 50 ppm).

There are many other methods that are not official techniques because their limited range of estimation, difficulty, and time to complete the analysis (Brun et al., 1961; Barnet, 1975; Nagaraja and Manjrekar, 1971).

Direct Methods Not Involving Distillation

The measurement of free sulfur dioxide by methods not involving distillation are based on the direct titration method with a standard solution of iodine (Ripper, 1892). The oxidation reaction is as follows:

$$H_2O + H_2SO_3 + I_3^- \longrightarrow SO_4^{=} + 3I^- + 4H^+.$$

For the determination of total sulfur dioxide, hydrolysis of the carbonyl sulfurous acid is necessary. This is accomplished in the Ripper method by using a strong alkali, acidyfing and titrating immediately with a standard solution of iodine using a solution of starch as an indicator.

In the preparation of extracts, as from dried fruits, losses of sulfur dioxide by oxidation as well as changes in the distribution of sulfur dioxide occur. The loss due to volatilization is more important than that caused by oxidation (Joslyn and Braverman, 1954).

Additional errors to this method were listed by Monier-Williams (1927). The errors were (1) the action of iodine on substances other than sulfur dioxide and by recombination of sulfur dioxide with acetaldehyde or any other carbonyl compounds and (2) in the direct titration of some juices or extracts by iodine using starch as an indicator, the end point tends to drift. This drift is caused by either excess of iodine reacting with other reducing substances present in the sample, or the slow decomposition of combined sulfur dioxide in the determination of free sulfur dioxide. To correct for iodine reducing substances other than sulfur dioxide, the iodine titration should be carried out, both in the original sample to measure the total reducing power including sulfur dioxide and in an aliquot to which has been added an excess of a bisulfite binding agent to measure the reducing power of the sample by itself (Joslyn and Braverman, 1954).

Distillation Methods

The most accurate method suggested by Joslyn (1964) was to hydrolize with a strong acid and distill the sulfur dioxide from the sample into a solution of iodine and back titrate the residual iodine with thiosulfate. The following reactions are involved:

$$SO_2 + I_2 + 2H_2O \longrightarrow 2HI + H_2SO_4$$

$$I_2 + 2Na_2S_2O_3 \longrightarrow Na_2S_4O_6 + 2NaI.$$

The distillation methods can be subdivided into two groups: (1) distillation with phosphoric acid and (2) distillation with chlorhydric acid. Schuller and Veen (1967) presented data which show that the results obtained by distillation with chlorhydric acid were higher than those obtained with phosphoric acid. However, the reproducibility of the results was greater with the phosphoric acid method.

The sulfur dioxide distilled from the acidified sample may be collected in a suitable receiving solution and titrated with iodine, as outlined above with standard alkali if the Monier-Williams modified method is used (AOAC, 1980), or using colorimetric methods (Nury et al., 1959).

When alkalimetric methods are used, sulfur dioxide is oxidized to form sulfuric acid with hydrogen peroxide and the amount of acid formed is measured with a standard alkaline solution. The sulfate can be determined gravimetrically by precipitation as barium sulfate and the excess of barium ion can be determined by the formation of a complex.

Colorimetric Methods

The colorimetric method most used is based in the well known reaction of Schiff for detecting aldehydes (Taylor et al., 1961) and a description of the method is explained by Nury et al. (1959). Three mechanisms of this reaction appear in the literature (Nauman et al., 1960): (1) formation of the Schiff's base,

$$R - NH_2 + HCHO \xrightarrow{SO_2} R - N = CH_2 + H_2SO_3;$$

(2) formation of an amino sulfinic acid followed by the addition of the aldehyde,

$$R - NH_2 + SO_2 \longrightarrow R - NH - SO_2H$$

$$R - NH - SO_2H + H_2C = O \rightarrow R - NH - SO_2 - CH_2OH$$

(3) formation of a sulfonic acid

$$R - NH_2 + SO_2 + H_2C = O \xrightarrow{H_2O} R - NH - CH_2 - SO_3H$$

In these reactions R $-NH_2$ is basic fuchsin, a dye which supposedly consists of a roughly equal mixture of pararosaniline and rosaniline (Pate et al., 1962).

Basic fuchsin, sulfur dioxide, and formaldehyde react to form a purple colored compound which has been used as the basis of a colorimetric method for determining formaldehyde as well as sulfur dioxide. According to Nauman et al. (1960) this colored compound is pararosaniline methylsulfonic acid.

Burroughs (1975) and Buechsenstein and Ough (1978) have been working extensively on the determination of sulfur dioxide in ciders and wines. These techniques are not partially applicable to dried fruits and therefore beyond the purpose of this review.

Absorption of Sulfur Dioxide in Fruit

McBean (1967) adapted a method for determining free sulfur dioxide in cider to observe changes in the levels of free and total sulfur

dioxide in sulfured apricots. He observed the increase of absorbed gaseous sulfur dioxide during exposures from 1 to 8 hours. He concluded that the rates of increase of combined sulfur dioxide were slightly higher than the corresponding rates of increase of free sulfur dioxide in the fruit.

The absorption of sulfur dioxide by fruit tissue is important and must be known in order to avoid darkening of fruits by underexposure or bleaching and flavor changes if the concentration applied is excessive (McBean et al., 1964). Bleaching of anthocyanins by sulfur dioxide is frequently encountered in the fruit industry. This bleaching may be reversible or irreversible. The most plausible explanation of the reaction between anthocyanins and sulfur dioxide according to Markakis (1974) is shown in Fig. 6.

McBean et al. (1965) discussed the influence of fruit characteristics of dried fruit on sulfur dioxide retention during dehydration. Conditions favoring rapid loss of the gas to the atmosphere exists immediately after sulfuring since the penetration of the gas is retarded by the cell damage caused during cutting or drying of the fruit (McBean et al., 1964). The amount of sulfur dioxide that remained in the fruit after sulfuring was directly influenced by the type, variety, and condition of fruit that was treated. They defined sulfur retention (SR) as:

$$SR = \frac{SO_2 \text{ in the dried fruit}}{SO_2 \text{ in the sulfured fruit}}$$

and found using this relation that pears, with the lowest absorption rate, had the highest SR values, ranging between 0.35 and 0.40, followed

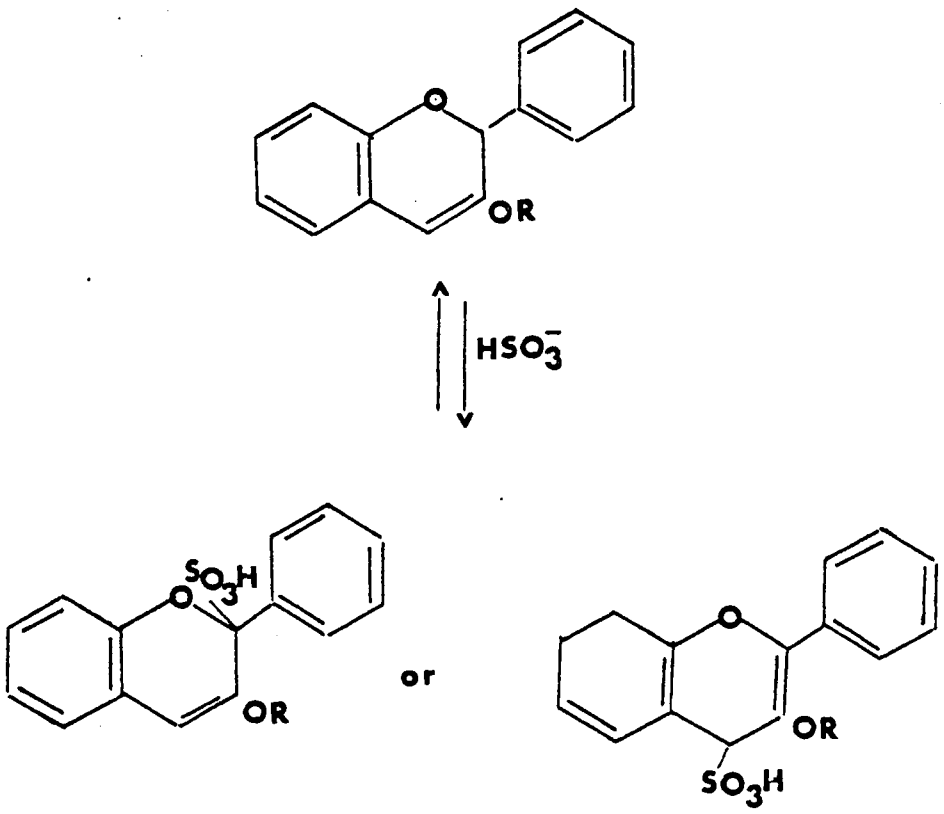


Figure 6. Bleaching of anthocyanins by sulfur dioxide (Markakis, 1974)

by peaches in the range of 0.30 and 0.40. Apricots, with the fastest absorption rate, retained the least sulfur dioxide.

When samples from the same orchard and of similar size and maturity were sulfured and dried together, SR values were quite similar. The retention of sulfur dioxide was less in smaller fruit. For the same reason, smaller fruit would be expected to lose relatively greater amounts of sulfur dioxide during drying. Also they showed that sulfur dioxide absorption during sulfuring decreased slightly as fruit maturity advanced. However, best-quality dried fruits were obtained by using only fully mature raw material.

McBean et al. (1965) observed that sulfur dioxide disappeared from fruit tissue during holding for 24 hours in a humid atmosphere. They also mentioned that during the early stages of drying, loss of sulfur dioxide from the fruit into the atmosphere was rapid. As drying proceeded, however, the rate of loss decreased markedly, probably because diffusion pathways were sealed in the dry tissue and some chemical fixation had occurred. Sulfur dioxide retention was only affected slightly by temperature of the drying air and air speed during drying but was greatly improved by drying at low relative humidities. This was presumably because diffusion pathways were sealed more quickly. Retention of sulfur dioxide in dried fruit was also influenced to some extent by the conditions under which the sulfur dioxide was absorbed by the fruit. The time of exposure to sulfur dioxide was the most important variable. This time must be long enough to permit the sulfur dioxide to penetrate deeply into the tissue. When fruits

were exposed to a high concentration of sulfur dioxide for a short time, the fruit acquired a high level of sulfur dioxide in the outer layers which was rapidly lost during drying before diffusion pathways were closed. Factors which retarded absorption of sulfur dioxide by fruit during sulfuring, such as the inherent nature of the fruit tissue, should also retard loss of sulfur dioxide during drying and, hence, improve retention. Thus, McBean et al. (1965) concluded that sulfur dioxide retentions were higher in fruit stored before sulfuring, in fruit blanched before or after sulfuring, and in fruit sprayed with water after sulfuring.

Stafford and Bolin (1972) studied the absorption of aqueous bisulfite by apricots and noted that there was a linear relationship between the concentrations of the bisulfite dip solution and sulfur dioxide content of the treated apricots. Penetration of the bisulfite into the fruit continued during drying. He also noticed that there was an increasing absorption rate of bisulfite into apricots that were dipped into progressively lower pH solutions, due to the increasing proportion of sulfurous acid at lower pH levels. The absorption rate of this acid would be expected to be greater than the bisulfite ion and was confirmed by the greater sulfur dioxide content in the fruit treated with lower pH solutions. While there was an increase in the absorption rate as the pH of the bisulfite solution was lowered from pH 4.5, there was also a significant increase in the amount of sulfur dioxide lost to the atmosphere from the solution. Stafford and Bolin (1972) also mentioned the rapid surface absorption during the first few seconds and a rapidly diminishing rate thereafter.

Influence of Moisture and Temperature on Fruit Quality During Storage

Sulfur dioxide in combination with low temperatures and low moisture contents has been known to be effective against browning in fruits. Barger and Pentzer (1948) studied the influence of low temperatures on fruit preservation and found that at 0°C original color was retained in dried peaches and apricots for 15 months and at 4.3°C the results were almost as good. He also noticed the influence of water content in regard to mold spoilage and recommended storage humidities not higher than 50 to 60%. Barger and Pentzer (1948) also noticed that fruits started browning when 75% or more of the initial sulfur dioxide content was lost.

Non-enzymic browning increased as humidity was increased up to a maximum in the intermediate-moisture range and then decreased again (Labuza et al., 1970). Another limitation of dried fruits to stability was growth of microorganisms which occurred at high water activities (Labuza et al., 1970). Water activity had a dominant influence on the rate of browning in all carbonyl containing systems, whether the carbonyls were present in the initial system or were formed during storage. Browning increased with water content up to a maximum which depends on specific conditions. Increased water activity not only accelerated the browning reaction, but also shortened the induction period. Most dried fruits had a slow browning rate at low relative humidities, increasing to a maximum in the range of intermediate-moisture foods where the probelm was no longer a lack of diffussion, but rather a dilution of reactants as water content increased.

Non-enzymic browning was one of the main degrading reactions that often occurs at a maximum rate in intermediate moisture foods which had an a_W range of 0.6 to 0.85 (Karel and Labuza, 1969; Labuza et al., 1970; Labuza et al., 1976; Loncin et al., 1968). When a sufficient amount of intermediates for non-enzymic browning was present, the rate of pigment production followed a zero order kinetics reaction. Temperature of the reaction controlled the length of the induction period and the rate of pigment production. At higher temperatures the rate of pigment formation was increased. This rate was dependent of a_W. Warmbier et al. (1976) found in his model that the maximum non-enzymic browning pigment production occurred at a_W of 0.45 to 0.55. This was unlike that found in most solid food systems which show a maximum rate near a_W 0.70 to 0.80.

Loncin et al. (1968) studied the practical importance of a_W in the phenomena of drying rates of foods, growth and destruction of micro-organisms, enzymic action and chemical reactions. In most cases, as seen in Fig. 7, many factors compete to spoil the food. Fig. 7 redrawn from Oswin (1976) shows an idealized model and the influence of several factors that can spoil the food at different a_W and storage times. Quality of dried foods can be controlled by the moisture content or water activity (Warmbier et al., 1976). In the work of Bone (1969), bacterial degradation of foods was usually controlled by keeping the water activity of the food less than $a_W = 0.90$. Yeasts and mold growth were inhibited by maintaining the a_W less than 0.80 (Bone, 1969).

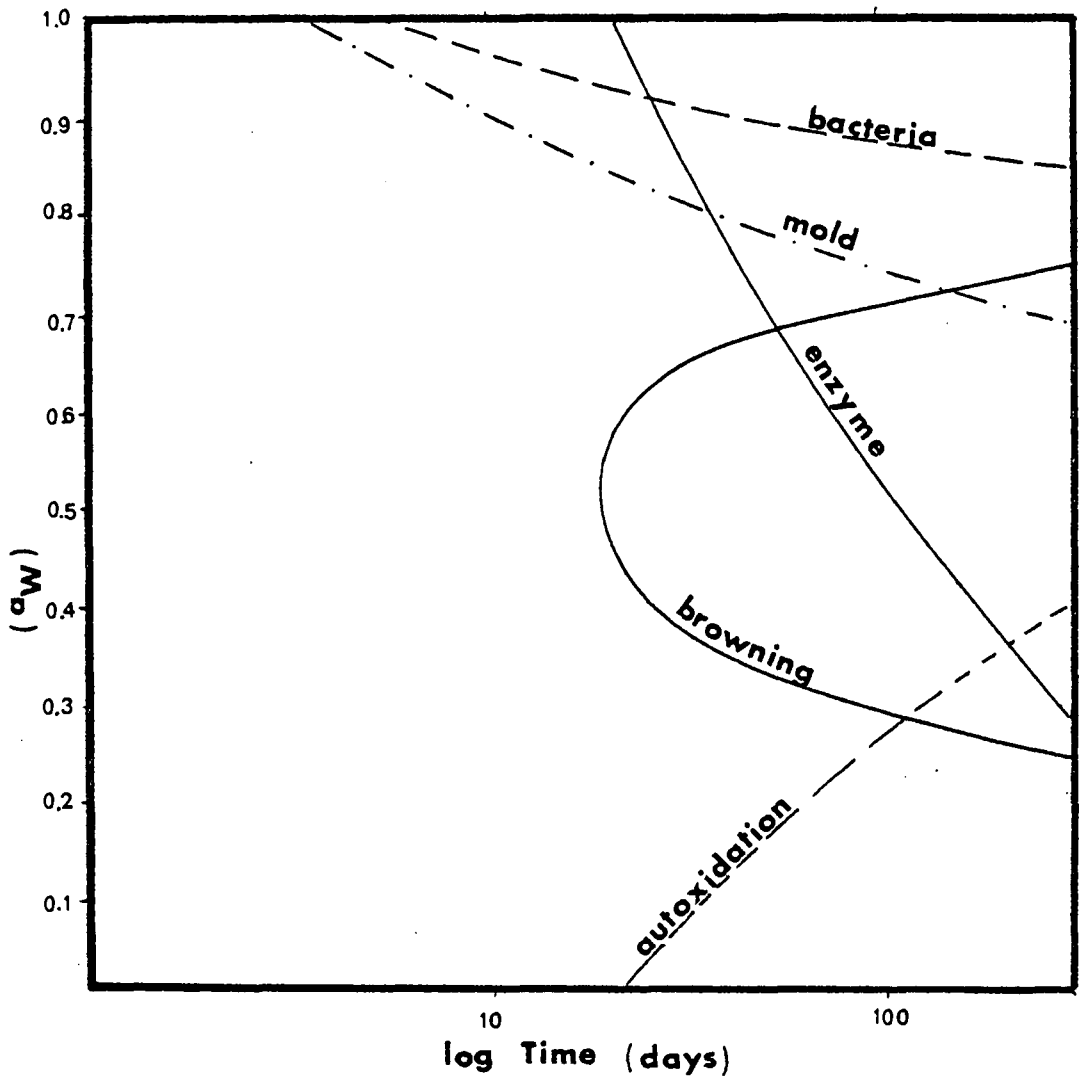


Figure 7. Some factors that compete to spoil food (redrawn from Oswin, 1976).

The drying rate by air at any instant was proportional to the difference between the vapor pressure at the surface of the product and the partial pressure of water in air (Loncin, 1961). It was also found that the most widespread enzymes such as the amylases, phenol oxidases and peroxidases were completely inactivated when a_w reaches 0.85 (Loncin et al., 1968).

Chirife and Iglesias (1978) mentioned in their review that equations for fitting isotherms in foods were of special interest in many aspects of food preservation by dehydration. Among them were the prediction of drying times (King, 1968) and shelf life of a dried product in a packaging material (Karel et al., 1971). Equilibrium relative humidity values or active water were more closely related to food product stability than total moisture content (Rockland, 1969). In this regard, Karel et al. (1971) developed several computer designs to predict storage stability of dried foods as a function of the extent of browning as related to duration of storage and moisture content, moisture content within the food as related to partial pressure of water, change of moisture content in the samples due to properties of the packaging material, or the food and the environment.

From the above discussion it can be seen that sulfur dioxide, moisture content of the product, temperature of the environment as well as the type of packaging material in which the food is stored are of great importance in the preservation of foods using dehydration.

Packaging of Dried Fruits

When foods containing sulfur dioxide are stored in sealed packages, sulfur dioxide may be lost, not only by the mechanisms mentioned above, but also by diffusion through the packaging material. Some sulfur dioxide accumulates as free gas in the package headspace and, if the container is permeable to the gas, loss will occur during storage. Permeation of gases and vapors is rarely a problem with glass and metal containers, but all containers made from plastic and cellulose have been shown to be permeable to some degree (Davis and Rooney, 1975). Davis and Rooney (1975) worked on permeation of sulfur dioxide through polymer films, from which polyethylene low density showed the highest permeability and PVDC[1]/regenerated cellulose/PVDC the lowest. They demonstrated that the package plays a major role in maintaining the good quality of the product, particularly dried foods treated with sulfur dioxide. Permeation of oxygen and sulfur dioxide and the presence of oxygen in the headspace all increase the rate of loss of sulfur dioxide. Thus materials for packaging of foods containing sulfur dioxide should have a low permeability to both oxygen and sulfur dioxide and the packages should be sealed with a minimum volume of headspace.

Some packages for dried food are designed to keep moisture out and to prevent volatilization of flavor and odor constituents (Sacharow and Griffin, 1970). In the packaging of sun dried fruit one of

[1]Copolymer of vinylidene chloride and vinyl chloride

the problems is insect infestation. These fruits must be thoroughly fumigated, or if practical, they may be heat treated. Packages should prevent infestation of insects and should be made from materials that insects will not penetrate. The package should also protect the dried product from moist environments; otherwise, the dried food may cake or molds may grow (Sacharow and Griffin, 1970).

Dried fruits are packaged in wooden boxes, cartons, spiral-wound paper canisters or large tins for shipment to the consumers. For retail consumption, dried fruits are packed in paper board folded cartons with foil laminated liners and/or overwraps. Cellophane-polyethylene-foil-polyethylene laminates have been commonly used for small packs of dried fruit used as a confection (Sacharow and Griffin, 1970).

Evaluation of Color Differences

A purely objective approach may be employed to define the color of an object. Burton et al. (1963b) made subjective visual assessments of color development on various carbonyl samples incubated in a glucose-glycine system to evaluate non-enzymic browning. Changes in color have also been appreciated by using spectrophotometric techniques to determine the change in one part of the spectrum, such as in the case of bleaching of pigments or browning. McWeeny and Burton (1962) used this approach to measure the intensity of browned samples by extracting the pigment with a mixture of ethanol and water and determining the absorbance at 282 nm. Walker (1962) used a wavelength of

490 nm. to measure the actual browning of apples after clarification of the tissue by centrifugation. Kahan (1977) used the change of absorption at 410 nm. to measure the browning of 4-methyl catechol in enzymic studies. Embs and Markakis (1965) measured the rate of browning caused by polyphenol oxidase and its inhibition by sulfur dioxide using the increase of absorption caused by the reaction at 420 nm. Wrolstad (1976) stated that

> "..browning is often measured and expressed in terms of absorbance units at a short wavelengths of the visible spectrum, 420, 440, 450, or 490 nm., all of which have been used by different authors."

Another criterium to evaluate food browning has been to measure the amount of product formed from such reaction i.e., melanoidin formation, glucose utilization, and/or loss of available lysine (Warmbier et al., 1976).

All of the above methods lack a point of reference to appreciate the real color of the samples. These techniques are only useful to determine the change in color and, if quantification is required, the change in color is often referred to a blank used in the same experiment.

With the exception of relatively few clear liquid foods such as oils and beverages, the color of foods is mainly a matter of reflection rather than transmission (DeMan, 1976). Fruit is best analyzed by reflectance color techniques (Nury et al., 1960).

Hunter (1958) developed a specialized photoelectric Color-Difference Meter, which has proved well adapted to the grading of a wide variety of products. Using a rectangular surface color solid devised by Adams (1942) where the spacings of surface colors are quite close to those of the Munsell color system which is perhaps the best known of

all the color order systems (Clydesdale, 1969). The Munsell system attempts to create painted colors to represent equal intervals of visual perception of color differences between adjacent samples and also specify these colors in terms of three coordinates: hue, value, and chroma (Clydesdale, 1969).

The instrument developed by Hunter (1948) produces results in terms of L that measures lightness and varies from 100 for perfect white to zero for black, approximately as the eye would evaluate lightness; and a_L and b_L that are the chromaticity dimensions and give designations of color as follows: a_L predicts redness if positive and greeness if negative, and b_L predicts yellowness if positive and blueness if negative. A diagramatic representation of the Hunter and Munsell solids is shown in Fig. 8. In the Hunter instrument, it is assumed that an intermediate signal switching stage exists between light receptors in the retina and the optic nerve which transmits color signals to the brain. In the switching mechanism, red responses are compared with green and result in a red to green color dimension. The yellow response is compared with blue to give a yellow to blue color dimension.

In a visual comparison between two samples, other variables such as gloss, texture, homogeneity of the surface, etc. must also be taken into account. Visible differences in these variables will be interpreted by the eye as color differences (DeMan, 1976). Irregularly colored foods can be placed in a spinner to expose a maximum of samples to a small aperture. In irregularly shaped foods the geometry of the food particle and orientation to the lens cause problems and give

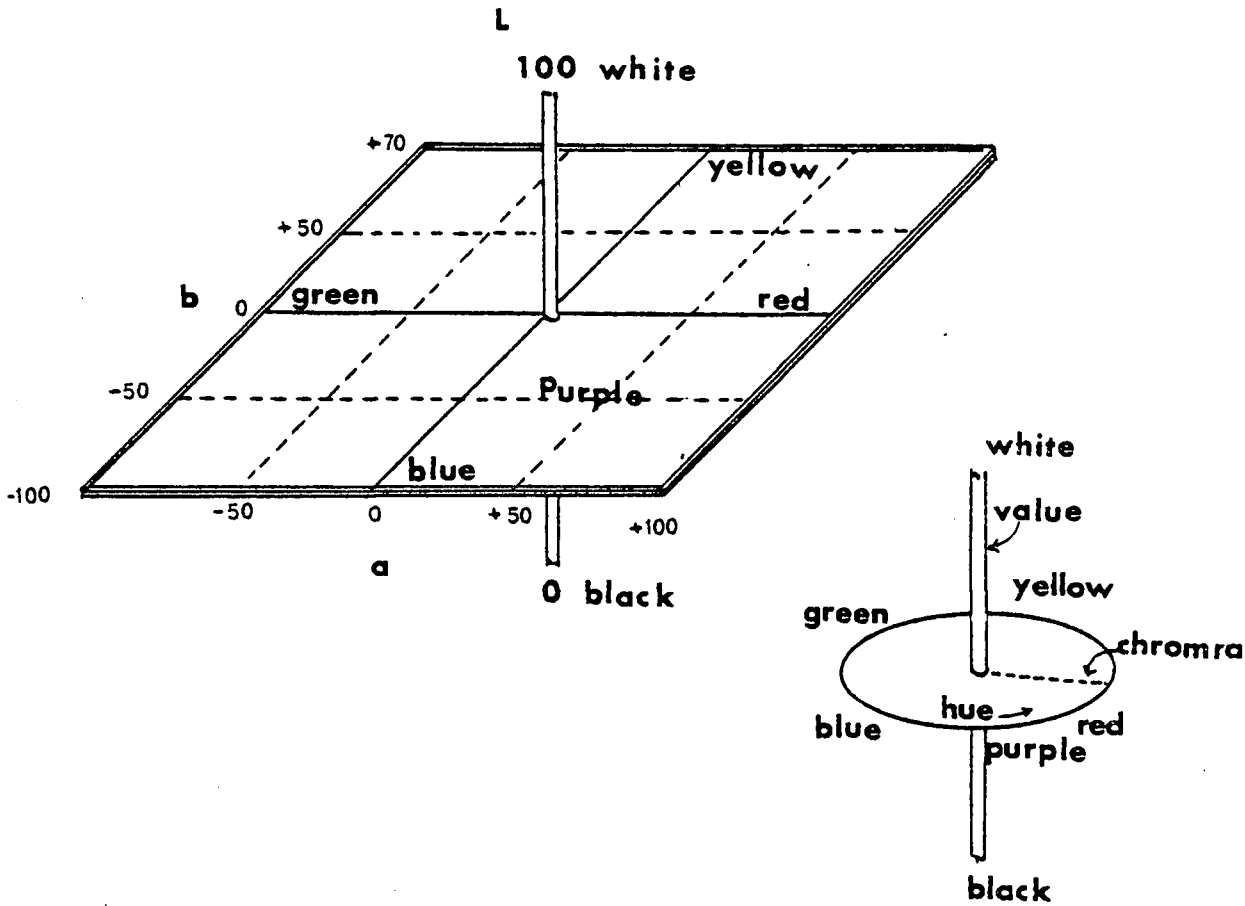


Figure 8. The Hunter and Munsell solids.

erroneous readings. Also physical modification of the light by the food may affect the evaluation of the color, i.e., reflection, refraction, transmission, diffusion or absorption (Clydesdale, 1972).

MATERIALS AND METHODS

Sample Preparation

Golden Delicious apples, which were peeled by dipping in aqueous 15% sodium hydroxide for 5 min and cored, were obtained from the inspection belt of the Tree Top, Inc. plant at Wenatchee, Washington, before exposure to sulfur dioxide. The apples were diced (3/4" x 1/2" X 1/4") and dipped in 10 gallon solutions of sodium bisulfite at the appropiate concentrations to yield 2500, 5000, and 7500 ppm of sulfur dioxide. The time of dipping was 90 seconds. A control was prepared using water as a dipping solution. The treated-diced apples were spread on drying trays, placed in an experimental dryer, and dried to a moisture content of 13, 18, 22, 24, or 26%. During the drying, the air temperature was maintained between 81 and 92°C for 1.5 to 2.0 hrs using an up flow air stream.

When the desired moisture content was achieved, the dried apples were coded and placed in plastic bags and allowed to stand for 10 days to equilibrate the mositure and sulfur dioxide in each of the batches. After this time of equilibration, mositure adjustments were made adding water or by further drying and zero time analyses were made before the samples were distributed to the respective constant temperature rooms. Table 3 shows the experimental design and coding system of the samples. Eight samples of 250 g were prepared for each of the specified storage conditions. One package of each treatment of moisture and sulfur dioxide at each storage temperature was drawn at the sampling times: 0, 40, 101, 188, 269, and 385 days. Evaluation of the quality changes

Table 3. Experimental Design

Temperature of Storage	Samples[a]														
	13-2	13-5	13-7	18-2	18-5	18-7	22-2	22-5	22-7	24-2	24-5	24-7	26-2	26-5	26-7
1°C			X	X	X		X	X	X			X	X	X	
21°C	X		X		X		X	X	X	X	X				X
38°C	X	X					X	X	X	X		X		X	X

[a]First number refers to the moisture content and second number to the concentration of sulfur dioxide dip solution (2-2500, 5-5000, and 7-7500).

were made at these intervals by measurement of color, moisture, and sulfur dioxide content. Samples with 22% moisture were also analyzed for free sulfur dioxide and water activity using the methods described below.

The bags used for packaging of the samples were of nylon-polyethylene copolymer. The film from which the bags were made was described by the manufacturer (St. Regis) as possessing low permeability, excellent side-seal strength, excellent heat stability, high optics and grease barrier in a low cost sheet. The oxygen permeability was specified as less than 1.0 cc/100m^2Atm/24 hr at 2.13°C. The bag size was 30 x 24 cm and 250 g of each sample were packaged with the least possible headspace.

Moisture Determination

The procedure used for determining moisture in dried fruits was that outlined by the AOAC (1980) in section 20.013. Samples of 5 to 8 g were weighed in aluminum weighing dishes of 8.5 cm of diameter previously cleaned and dried. The samples were dried in a vacuum oven for 6 hrs at 70°C. The difference in weight was expressed as a percent of moisture content.

Color Determination

A Hunter Lab color difference meter model D-25 P-2 was used. The sample was placed in a cubic glass cell of 5 cm on the side. Readings were taken using the refractive mode (arrangement No. 1) of the instrument, which was standardized against a standard white tile

No. DC 122, L = 94.02, a = - 0.9 and b = 1.2. The color of the samples was measured in three different places and the average was used for the analysis of the data; if the readings differed more than 20% from each other, a more representative number of readings were taken or a new sample was used. All materials other than dry apple were removed, i.e., seeds, dark spots, leaves, etc.

Water Activity Determination

An a_W value analyzer model 5803 manufactured by Lufft was used. For calibration, moistened filter paper with a saturated solution of barium chloride was placed into the analyzer. The analyzer was closed and allowed to equilibrate for three hours before setting the instrument to 0.90 a_W. All operations were performed in a room with controlled temperature previously set to 21°C and assuring that the temperature remained constant during calibration and determinations.

After calibration, the sheets of filter paper were removed and the sample was placed in the container, which was closed again and allowed to stand for at least 1 hr before readings were made.

Total Sulfur Dioxide

A modification of the method used by Potter (1954) and Pearson and Wong (1971) was used. A 60 g sample of dried apples was blended in 300 ml of water. The time of blending was 30 to 60 sec until completely homogenized. Homogenization times longer than 60 sec was not recommended to avoid excessive aeration and loss of sulfur dioxide by volatilization. If the sample required more than 60 sec to be

homogenized, a new sample was prepared using an extra amount of water and making the corrections for dilution.

From the homogenized sample, three equal parts of 60 g were placed in beakers of 400-500 ml. Five ml of a 20% sodium hydroxide solution and a pinch of $NaHCO_3$ (to expel the air) were added. Water was added to bring the total volume to 250 ml to make the sample less dense. All the above was mixed gently avoiding incorporation of air. Special attention was given to do all steps in the same time and sequence. The sample was allowed to stand for 20 min minimum but no more than 30 min to allow for dissociation of the bound sulfur dioxide in the sample. Occasionally the sample was gently stirred during this time.

To two of the beakers, one at a time, 5 ml of 25% HCL (v/v) solution was added mixing immediately to avoid high local concentration of acid followed by 10 ml of a 1% starch solution. This was titrated promptly with a standard iodine solution (0.1 N) within 30 sec to a definitive blue color that lasted for at least 20 seconds.

The third beaker was used as a blank in which the determination of the reducing material other than sulfur dioxide was measured. This was done by adding 5 ml of a 0.3% hydrogen peroxide solution after the addition of 5 ml of 25% HCl and mixing for 60 sec to oxidize the sulfur dioxide to sulfate. After this period of time, the titration was performed in the same manner as with the other two beakers.

In the samples without hydrogen peroxide a "fleeting" end point was observed, therefore, it was especially important that the titration of the samples was performed in the same length of time.

Free Sulfur Dioxide

A variation of the method described by Buechsenstein and Ough (1978) was used. Sulfur dioxide was displaced isothermically from the acidified sample with a stream of nitrogen, which carried the gas through a condenser into a hydrogen peroxide trapping solution. This solution contained an acid-base indicator composed of methylene red and methylene blue. The sulfuric acid formed was titrated with a standard alkali solution.

Samples of 15 g were blended in 200 ml of water for 30 to 60 sec, assuring proper homogenization. Times greater than 60 sec were avoided to prevent loss of sulfur dioxide by volatilization. From this homogenate 40 ml were taken and placed in a 500 ml round-bottomed flask, having two side inlets. A magnetic stirrer was placed under the flask, a nitrogen tank was connected to one of the side inlets and a condenser to the central neck. The second side inlet was used to place the sample into the flask. Two trapping tubes were attached to the exit of the condenser. The 10 ml of the trapping liquid used in each tube were made of a 0.3% hydrogen peroxide solution containing four drops of an indicator mixture (Fig. 9). This mixture was prepared with 0.1 g of methylene red and 0.05 g of methylene blue in 50% ethanol-water to make a total volume of 100 ml.

The displacement time for the free sulfur dioxide was 60 min using a nitrogen flow of approximately 1000 ml/min as was recommended by Burroughs and Sparks (1964). A single period of displacement was used (McBean, 1967). The 60 min period of displacement was chosen after

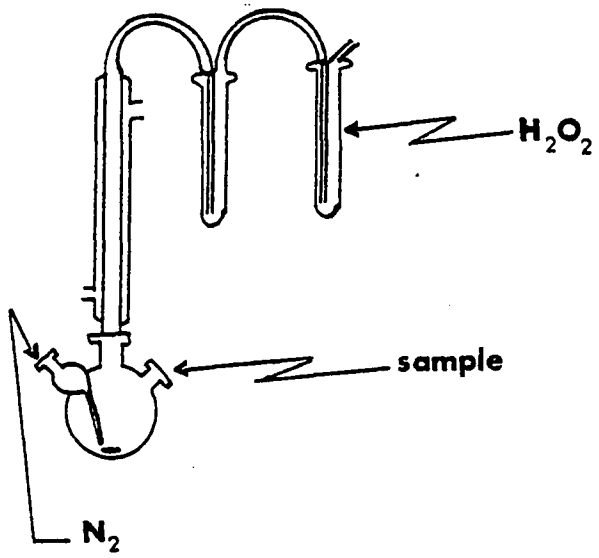


Figure 9. Apparatus for free sulfur dioxide determination.

observations were made that this time gave the more reproducible results for the samples of dried apple in the conditions tested in this work.

Special attention was paid in making all determinations under the same conditions, especially temperature of analysis, because temperature affects the equilibria of free and bound sulfur dioxide.

RESULTS AND DISCUSSION

Total Sulfur Dioxide Changes

The loss of total sulfur dioxide during the time of storage at the three temperatures used in this work are presented in Fig. 10. Sulfur dioxide levels remained reasonably constant in samples stored at 1°C, decreased moderately in samples stored at 21°C and decreased sharply in the first 100 days of the samples stored at 38°C. Regression equations were calculated to describe the loss of sulfur dioxide. Davis et al. (1973) indicated that the residual sulfur dioxide concentration followed a negative exponential curve with time which was described by the following equations:

$$\% SO_2 = a\, e^{-bt} \qquad \text{or} \qquad \log_e(\%SO_2) = \log_e a - bt$$

where a is the initial concentration, b is the slope representing the rate of change of $\log_e(\%SO_2)$ with time, and t is the time of storage. An arbitrary constant of 1 can be added to $\%SO_2$ if zero values are used for the concentration of sulfur dioxide in order to avoid having values of $-\infty$ (Davis et al., 1973).

The exponential curve calculated in this work for the samples stored at 1°C describes very poorly the changes of sulfur dioxide during storage. This was probably due to the low variability of the gas at this temperature with time and the values larger than 100%. These values can be attributed to experimental error caused by varitions of the end point during the iodometric titration to measure total sulfur dioxide. The equation found was:

$$\% SO_2 = 108.25\, e^{.00033\, t};\ r^2 = 0.43$$

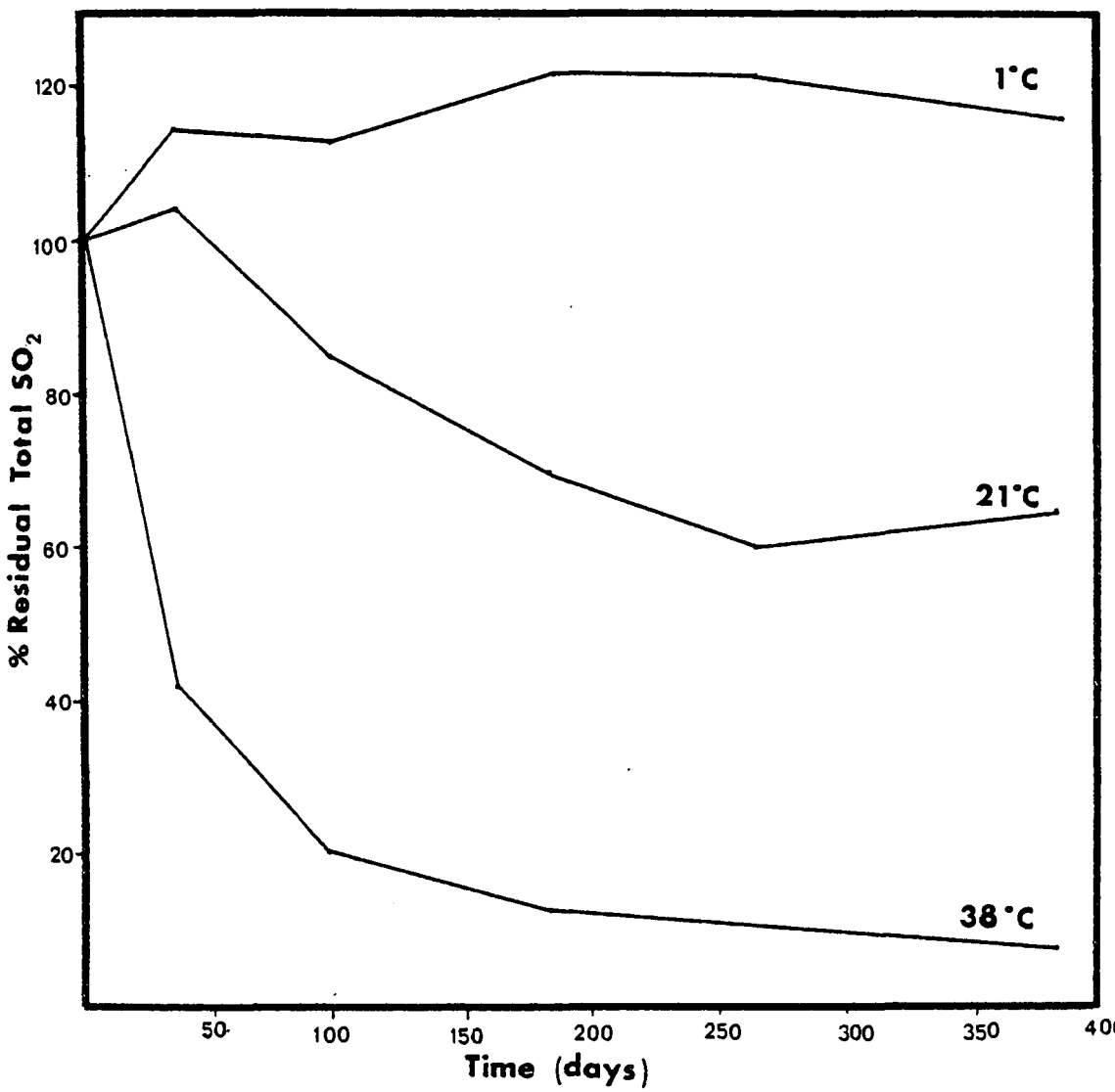


Figure 10. Changes of total sulfur dioxide during storage.

which is an exponential curve with positive slope that shows slight increases of $\%SO_2$ during the time of storage. This result was not logical because the equation describes a gain of total sulfur dioxide which is not possible under the conditions of this experiment. Results from other workers (Payne et al., 1968; Davis et al, 1973) demonstrated that when a negative exponential curve was fitted with their data, the equation described the loss of sulfur dioxide from the samples during the storage.

The equation calculated from the data obtained from the samples stored at 21°C was:

$$\% SO_2 = 100.17\ e^{-.0015\ t};\ r^2 = 0.84$$

and for the samples stored at 38°C (omitting from consideration the point at 269 days) the equation was:

$$\% SO_2 = 58.09\ e^{-.00637\ t};\ r^2 = 0.84$$

The term r^2 is called the coefficient of determination and can be interpreted as the proportionate reduction of total variation associated with the use of the independent variable x (time). In practice, r^2 is not likely to be 0 or 1, but rather somewhere in between these limits. The closer it is to 1, the greater is said to be the degree of association between x (time) and y (total sulfur dioxide) (Neter and Wasserman, 1974).

From the equations and Fig. 10 one can appreciate the very marked decrease of sulfur dioxide that occurred in the first 40 days of storage at 38°C. The decrease at 21°C was less marked and no considerable change was experienced at 1°C for the entire storage period. This

demonstrates the importance of temperature in maintaining the sulfur dioxide in the sample to prevent browning.

Much of the absorbed sulfur dioxide is lost during drying and, of that which remains, 80 or 90% is in the combined form (McBean, 1967). Drying also stabilizes the amount of sulfur dioxide held in the fruit tissue and a further slow loss of the preservative occurs during storage time. This loss is directly influenced by the temperature of storage as is demonstrated in this work. The loss of sulfur dioxide was cuased by any or all of the mechanisms explained in the literature review.

Davis et al. (1973) mentioned that the rate of loss of sulfur dioxide may also be influenced by the method and time of drying the fruit. Furthermore, in samples with higher levels of total sulfur dioxide, the ratio of free sulfur dioxide to total sulfur dioxide increased as the total sulfur dioxide level increased (Fig. 12). This may also influence the rate of loss of sulfur dioxide by volatilization.

The oxygen permeability of films is not a useful guide of their sulfur dioxide permeability (Davis and Rooney, 1971). Both loss of total sulfur dioxide by volatilization and the loss due to irreversible combination with the fruit constituents are important in determining the storage life of the fruit.

Changes of Free Sufur Dioxide

Similar results for free sulfur dioxide to those obtained in changes of total sulfur dioxide were expected. Instead, erratic results were obtained at 1°C and straight lines were obtained at 21°C

and 38°C (Fig. 11).

The Cause for the erratic results at 1°C may be due to the very small changes of total sulfur dioxide that took place during the time of storage. The pouches used in this experiment for packaging of the samples were also permeable to sulfur dioxide, and since samples with high content of sulfur dioxide were stored in the same cartons with the samples of low sulfur dioxide content, an interchange among samples may have taken place. This would have raised the content of free sulfur dioxide of some samples and caused the erratic behavior of the readings made between the periods 101 and 188 days.

The changes of free sulfur dioxide from day 40 to the end of the experiment at 21°C were almost a perfect fit to a straight regression line with negative slope described by the following equation:

$$\% \text{ Free } SO_2 = 109.53 - 0.27\ t; \quad r^2 = 0.9971.$$

Free sulfur dioxide in samples stored at 38°C dropped almost to zero or to undetectable amounts by storage day 101. These observations are in accord with the marked decrease of total sulfur dioxide (Fig. 10) and with the relation of free sulfur dioxide and total sulfur dioxide (Fig. 12).

The influence of temperature in the loss of total sulfur dioxide and free sulfur dioxide was the same, i.e., loss of sulfur dioxide increased as temperature increased. The loss of sulfur dioxide also depends on the permeability of the package to the gas.

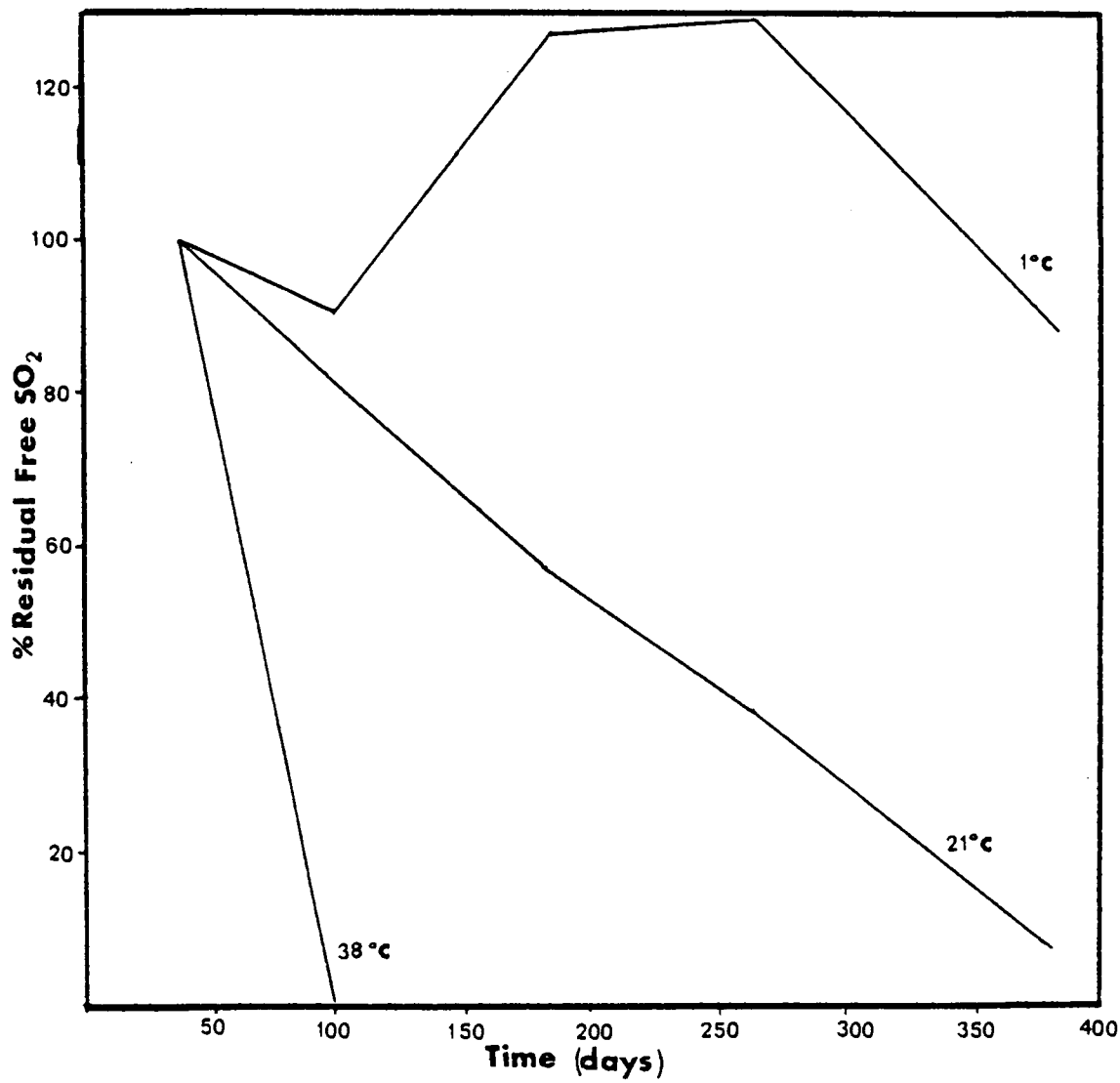


Figure 11. Changes of free sulfur dioxide during storage.

Amounts of Free Sulfur Dioxide Related to Total Sulfur Dioxide

The relationship of free to total sulfur dioxide is presented in Fig. 12. When the concentration of total sulfur dioxide increased, the concentration of free sulfur dioxide increased to a larger proportion. As the capability of the sample to bind sulfur dioxide was saturated, the amounts of free sulfur dioxide increased at rates to form a power curve in between zero and 2000 ppm. The following regression equation was calculated from the data:

$$\text{Free } SO_2 = .00228 \ (\text{Total } SO_2)^{1.7}; \quad r^2 = 0.88$$

The inhibition of browning depends on the availability of free sulfur dioxide in the sample and the amount of free sulfur dioxide depends on the binding power of the sample and on the quantity of total sulfur dioxide. McBean (1967) found that the increase of combined sulfur dioxide was slightly greater than the increase of free sulfur dioxide as the time of exposure to gaseous sulfur dioxide was increased.

Burroughs and Sparks (1973) studied the influence of pH in the binding power of wines and ciders for sulfur dioxide and mentioned that samples with high binding power required larger amounts of total sulfur dioxide to have the same preservative power.

The equation derived from the data in Fig. 12 can be useful in predicting the amounts of free sulfur dioxide in a sample when the concentration of total sulfur dioxide is known. This is an indirect method to determine if there is sufficient sulfur dioxide to prevent

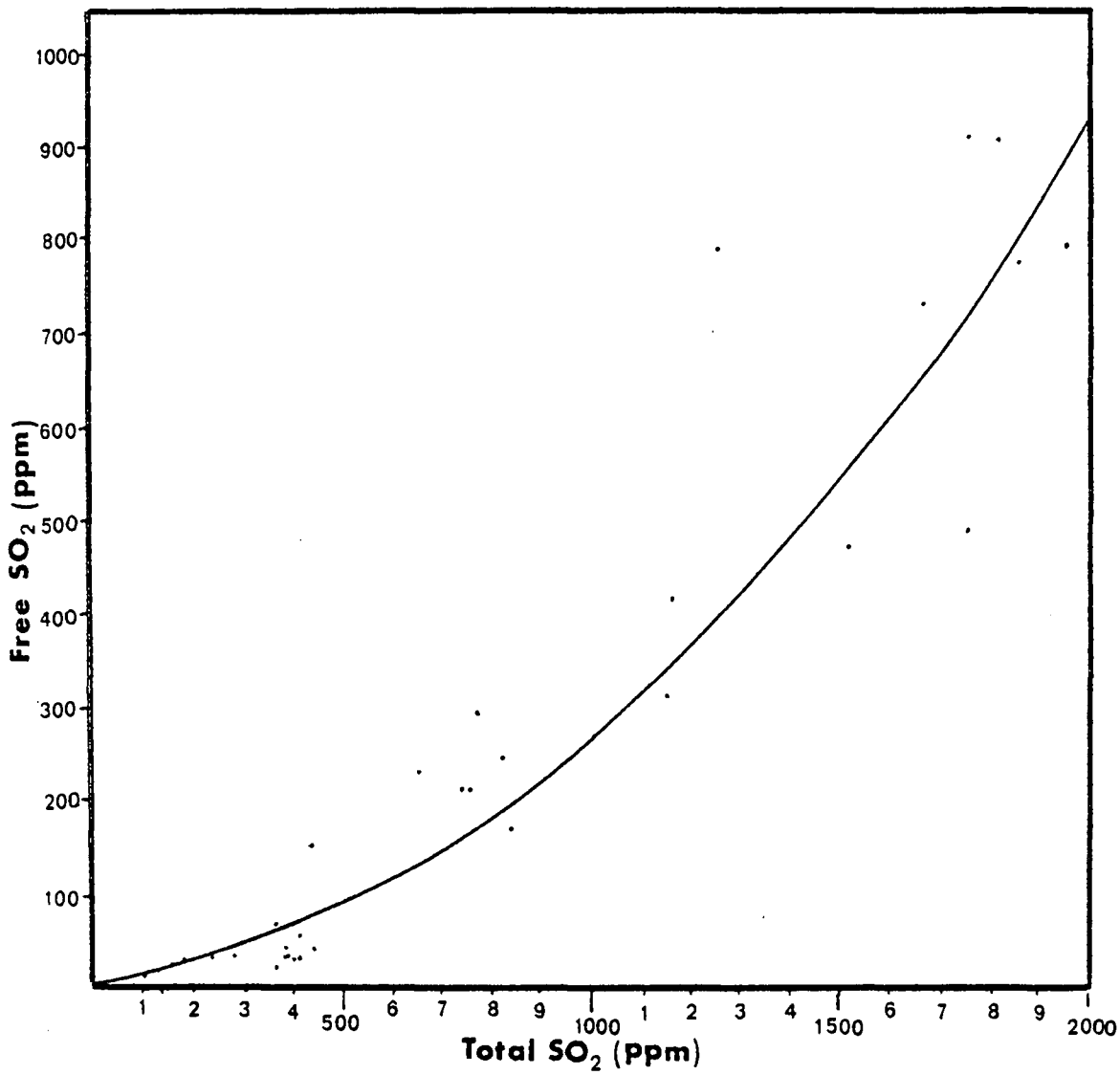


Figure 12. Relationship of free sulfur dioxide to total sulfur dioxide.

browning. Since the determination of total sulfur dioxide is faster than that for free sulfur dioxide, by using this relation an estimation of storage life of dried apples is possible.

Moisture Changes

Permeability of the film of pouches in which the dried fruit was stored can be in both directions, resulting in either a gain or a loss of moisture content. The direction in which the interchange of water takes place depends on the relative humidity of the surrounding environment.

When loss of total sulfur dioxide occurred, increase in other samples was not possible unless an excess of sulfur dioxide was applied to the exterior of the pouch. Moisture interchange could be in either direction as the samples stored at 1°C showed by the slight increase in moisture at 188 and 269 days (Fig. 13).

Because relative humidity of the rooms of storage was not controlled during the experiment, very rapid loss of moisture occurred at 38°C. To this point, it can be said that as relative humidity of the environment surrounding the sample is higher than the a_w of the sample, the loss of moisture can be described by a negative exponential curve. This is demonstrated at the temperature of 38°C, in which the relative humidity of the room was approximately 40%:

$$\% \text{ Moisture} = 93.71\ e^{-.00044\ t};\quad r^2 = 0.99,$$

where t is time in days.

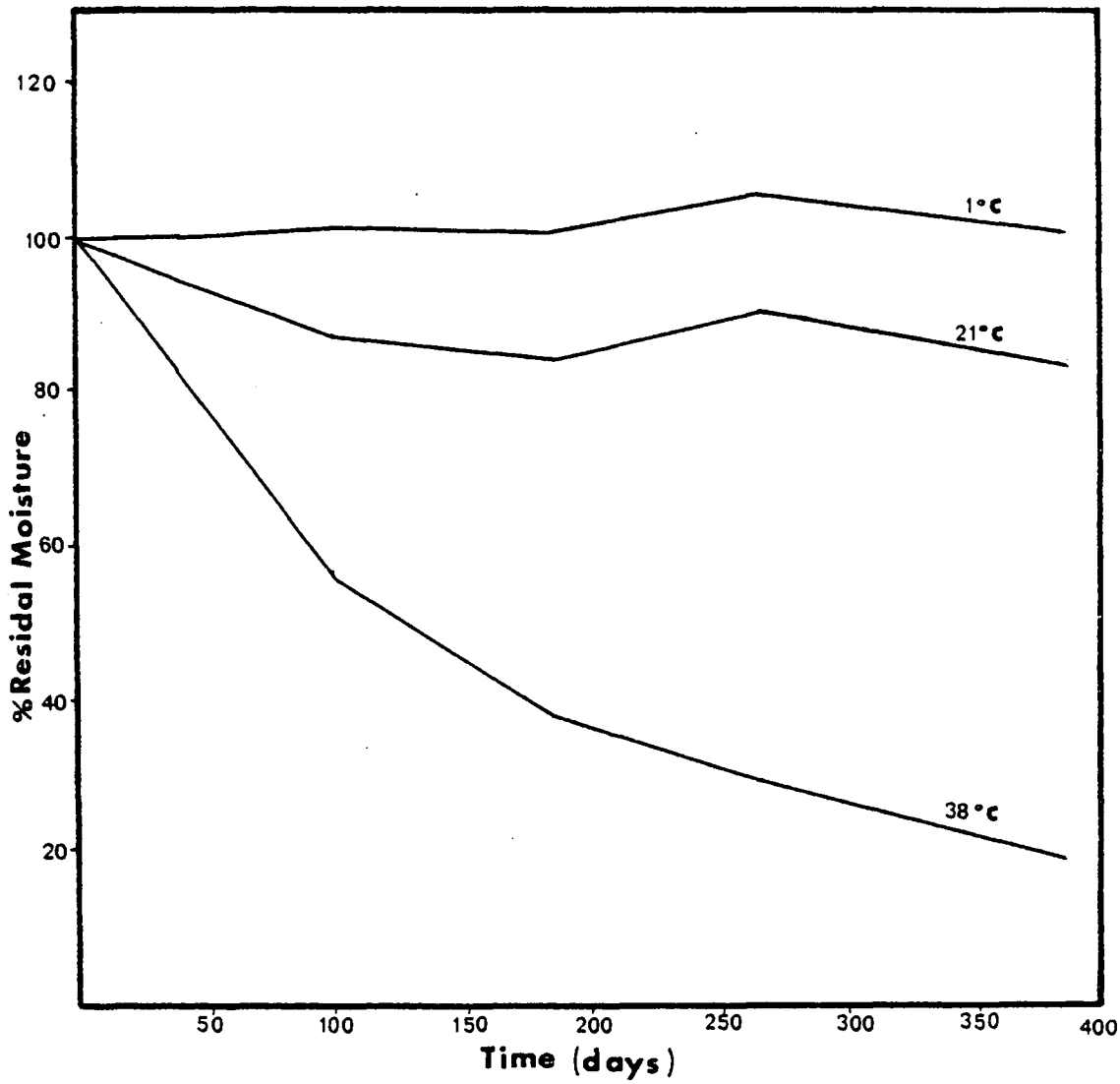


Figure 13. Changes of moisture content during storage.

Humidity of the room had a direct influence on the rate of loss of moisture from the samples, together with the type of package and temperature of storage.

Changes in Water Activity

Water vapor pressure can be reduced by the addition of solutes, and is a property defined for ideal solutions by Raoult's Law. This relation can be described as follows:

$$\frac{p}{p_o} = \frac{n_2}{n_1 + n_2}$$

where p and p_o are the vapor pressures of the solution and the pure solvent, respectively, and n_1 and n_2 refer to the number of moles solute and solvent. By definition, water activity (a_w) of a solution is equal to p/p_o. The ratio, p/p_o, also is equivalent to the relative humidity (R.H.) usually expressed as a percentage.

Because of the importance of moisture, a_w was determined in all samples containing 22% initial moisture content. A regression line (Fig. 14) was fitted in the range of a_w = 0.45 to a_w = 0.85 yielding the following equation:

$$\% \text{ Moisture} = 29.012 + 32.652 \ln a_w; \quad r^2 = 0.84$$

Moisture content of foods soon adjusts to the humidity (R.H.) of the environment. The availability of this moisture for deteriorative reactions is now known to be numerically equal to the surrounding R.H. expressed as a fraction or water activity (a_w) (Caurie, 1970). Of the many equations in the literature to fit the relation of moisture content

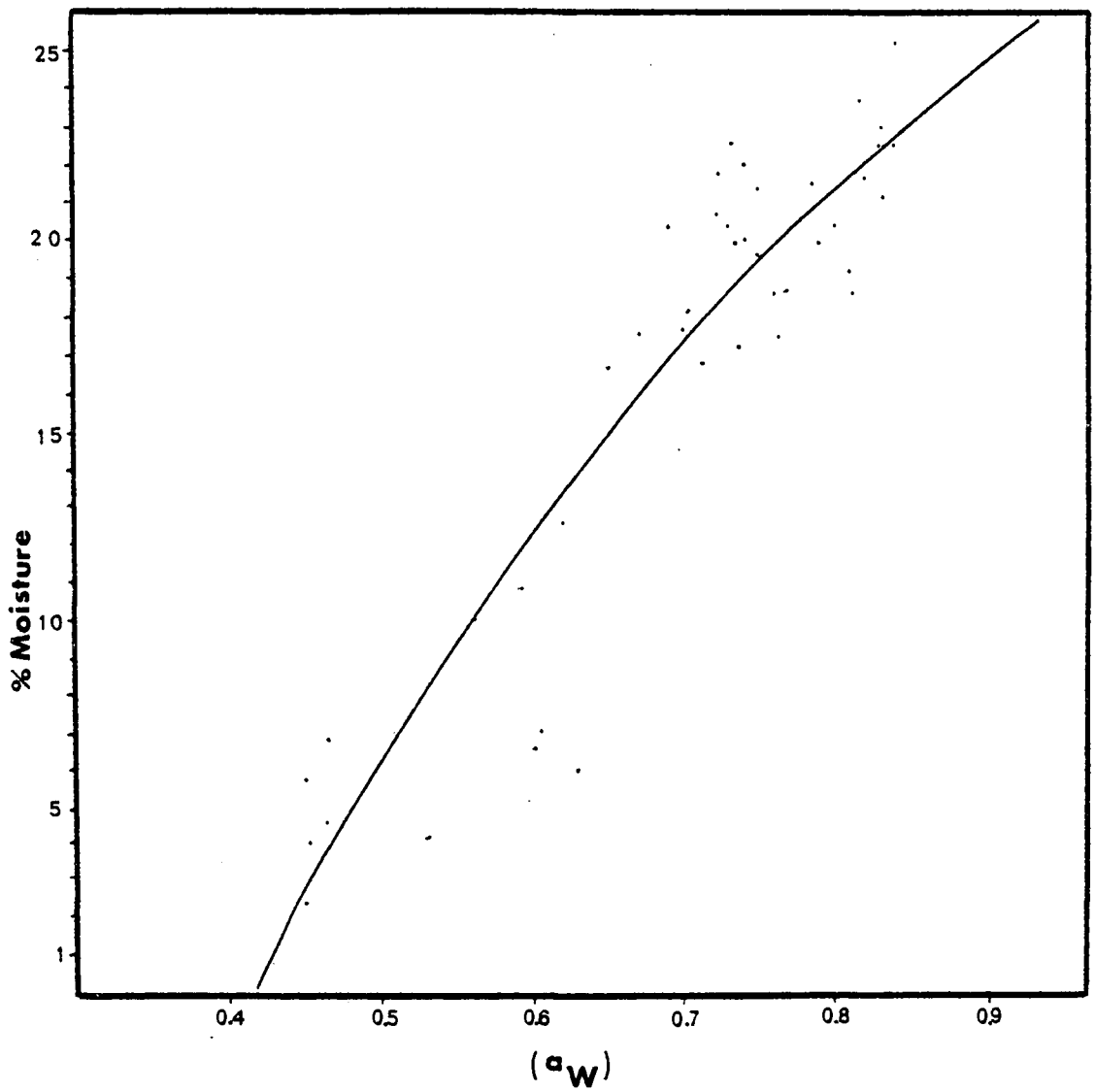


Figure 14. Relationship of water activity to moisture content.

and a_W, none has been found which is entirely satisfactory to describe the complex and variable nature of the physical and chemical properties of dehydrated food substances and natural products (Chirife and Iglesias, 1978).

The equation presented in this work may be useful for some practical situations with dried apples, such as the prediction of storage life, where only a small range of a_W is considered, i.e., a_W from 0.40 to 0.85. All a_W of dried apples in this work were within this range.

Color Changes

All samples were evaluated using the Hunter color difference meter. The terms L, a_L, and b_L were obtained for each sample at the times specified in the section on materials and methods.

Several methods reported in the literature for evaluation of changes in color were tested (MacKinney and Little, 1962; Clydesdale, 1969) and it was found that the criterion:

$$\text{Color Index (CI)} = L \times a_L \times b_L$$

could reduce the data to a single value. The CI expressed the color differences between samples, had a close approximation for the description of the changes in color, and was the simplest color relationship for dried apples under the conditions of this work.

It was observed that most samples having an acceptable color (judged with the help of Charlotte Deuel, Tree Top, Inc., who was able to classify visually those samples not commercially acceptable) had negative CI values and, as the samples became browner, the CI became

more positive. Also noticed was that the samples that had CI values between -1500 and 500 were difficult to evaluate for an acceptable color. This region was defined border line to separate acceptable samples from the unacceptable ones. Samples that fell in the border line region could have been marketed, depending on the use in the food industry.

To illustrate the changes in color during the 385 days of storage, the average color for all samples at each temperature was used to draw Fig. 15. From these data one can see that the storage temperature had a major influence on the rate of the change of color and the time that the samples remained in the acceptable region.

The range of CI values found in this work was from -4500 to 5000 in most samples. This range was computed from L values between 40 and 60 with very few samples having values of 35 or 65; from a_L values of -3.5 to 5.0 with extreme cases up to 9.0; and from b_L values that decreased from 27 to 17 with extraordinary cases of 10 as the brown color developed.

When the CI values of the samples were between 4000 and 7000, the samples were very dark. Some samples with CI values above 4000 had similar CI values but had different brown colors. This was due to low L and b_L values and is illustrated in the following example:

	Samples at 385 Days	
Value	22-2-70	22-7-100
L	58	36
a_L	5	10
b_L	21	17
CI =	6090	6120

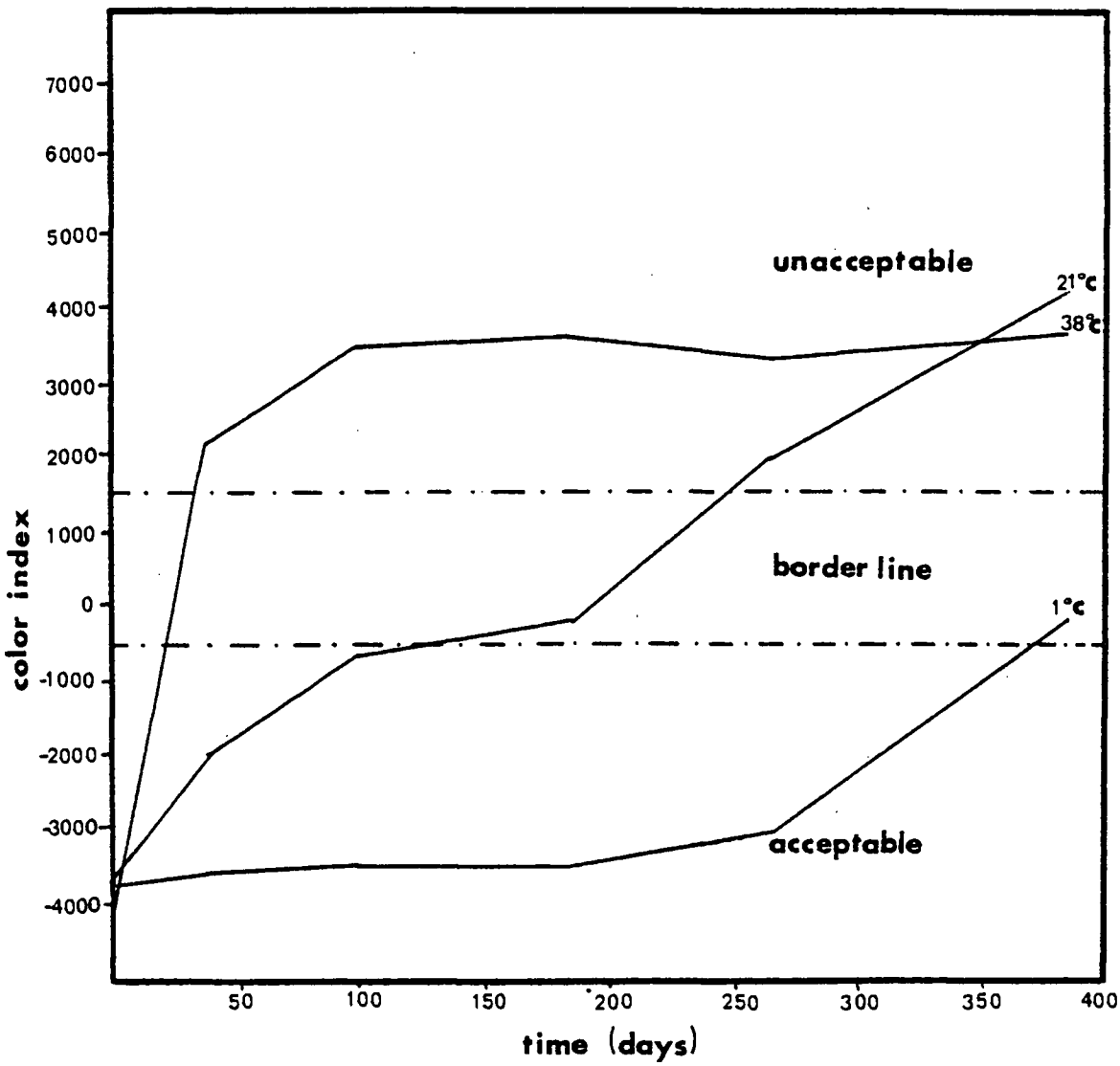


Figure 15. Changes of color during storage at 1°C, 21°C, and 38°C.

Only a few of these cases occurred and were not a factor in this experiment because any samples that had a CI value greater than 4000 were unacceptable. Color Index values lower than 4000 were representative of the intensity of the brown color in the dried apple samples. The use of the CI ($L \times a_L \times b_L$) was valid for samples in the range from negative values to 4000 for dried apples.

Since the storage temperature resulted in a large difference in change of color (Fig. 15), the data for the samples stored at the different temperatures will be treated separately.

Using the data obtained from the analysis of total sulfur dioxide, mositure, and color during the 385 days of storage, three equations were calculated, one for each of the three temperatures of storage (1°C, 21°C, and 38°C).

The equation found for samples stored at 1°C was:

$$CI^{1^\circ} = 10891.4 - 13.6327\ t - 7.09813\ S - 1053.62\ M - 0.01829\ t^2 + 0.001142\ S^2 + 21.4216\ M^2 + 0.1533\ SM + 0.0003036\ tSM + 0.000075\ t^2S + 0.003623\ t^2M - 0.0000043\ t^2SM$$

where t = time in days, S = total sulfur dioxide at time t in ppm and M = moisture at time t expressed as a percent. The r^2 was 0.6335 which, according to Rowe (1980), was an acceptable description of the changes in the color of dried apples at this temperature.

In the same manner, equations for 21°C and 38°C were calculated using the same method and nomenclature. The equation for 21°C storage temperature is:

$$CI^{21°} = 7394.04 + 14.4763\ t - 3.2477\ S - 1035.35\ M - 0.2116\ t^2 + 0.0022\ S^2 + 30.9710\ M^2 - 0.1462\ SM + 0.0003853\ tSM + 0.00003129\ t^2S + 0.02533\ t^2M - 0.00000005\ tS^2 - 0.0002\ t^2M^2.$$

The r^2 of 0.8811 obtained implies a good fit of the equation to the data obtained (Rowe, 1980).

The equation for 38°C was:

$$CI^{38°} = -606.634 + 44.685\ t - 9.5209\ S + 161.139\ M - 0.04481\ t^2 + 0.006752\ S^2 - 0.3690\ M^2 - 0.2243\ SM + 0.004427\ tSM - 0.0000075\ t^2S - 0.01156\ t^2M.$$

The r^2 of 0.8733 of this equation was also a good description of the color changes at 38°C (Rowe, 1980).

In each of the three equations presented above, 0, 40, 101, 188, 269, and 385 were substituted for the times (t) of analysis. Six new equations for each temperature of storage of the following form were generated:

$$CI^{t°} = B_0 - B_1S + B_2M + B_3S^2 + B_4M^2 + B_5SM$$

where B_0 to B_5 are the regression coefficients. These regression coefficients for 1°C, 21°C, and 38°C are presented in Tables 5, 7, and 9, respectively. Each of these new six equations generated for each of the three temperatures can give a response surface diagram where one can appreciate the change in color and the influence of temperature and moisture at each time of analysis.

The diagrams of Figs. 17, 18 and 19 are portions of the response contour diagrams (Fig. 16 c) that resulted from the response surface

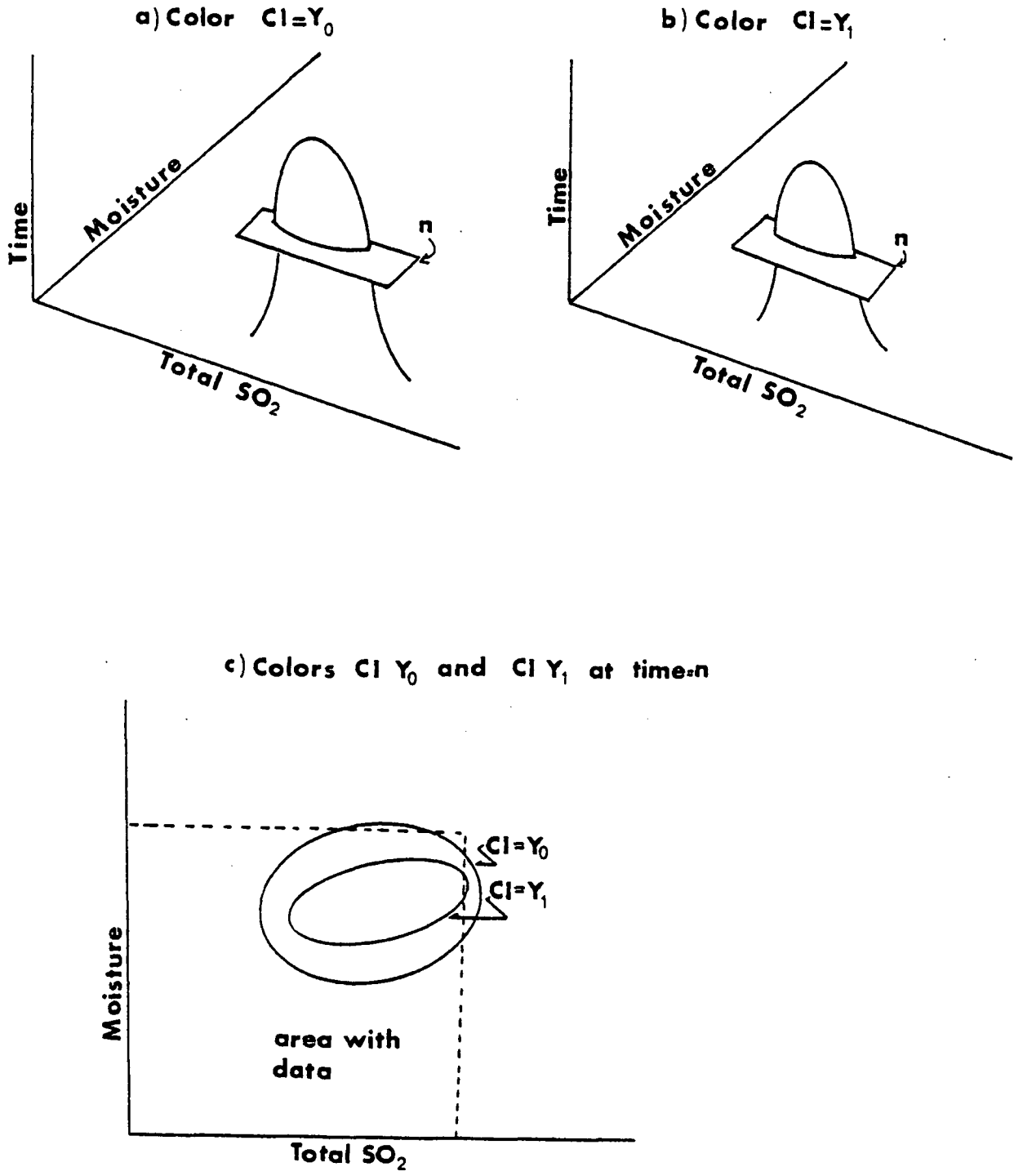


Figure 16. Response contour diagram (c) generated at time n from the response surface diagrams of colors Y_0 and Y_1 (a and b) in the limits of the experiment.

diagrams (Fig. 16 a and 16 b) at each of the times that were assayed. Each line in the response contour diagrams represents a color (CI) at each time of analysis. To draw these lines the CI values were chosen at equal intervals for a clear interpretation of the changes of color under different concentrations of total sulfur dioxide and moisture content at the temperatures of 1°C, 21°C, and 38°C (Fig. 17, 18, and 19) during the six times of anlaysis.

The three response contour diagrams for the time zero that resulted from the three temperatures had no meaning because all samples at time zero had the same color approximately. However, these are useful for comparison with the other diagrams (Fig. 17, 18, and 19).

Changes at 1°C

At 1°C practically no change in moisture (Fig. 13) and sulfur dioxide (Fig. 10 and 11) was observed between time zero and 101 days and only a slight change of any of these two variables could be noticed during this period. A small increase in mositure was observed at 269 days and sulfur dioxide remained almost constant during the time of the experiment.

At 1°C the influence of moisture content and total sulfur dioxide in the sample had approximately the same influence in the preservation of color as can be seen from the t values shown in Table 4. The t statistic value is a statistical test used for small sample tests of hypotheses. In the case of these experiments the t value determines the influence of the variables used to calculate the equation. The t statistic values were calculated from the equation:

$$t = \frac{b_k}{S_{b_k}}$$

where b_k is the regression coefficient b of the variable being tested k and S_{b_k} is the standard error of the regression coefficient b_k. All variables for which the absolute value of the t statistic exceeds a predetermined level were retained in the search procedure to determine the "best" set of independent variables for the equation. In the set of variables chosen, those having the highest absolute t value were the ones that had more influence in the description of the phenomena for which the equation was calculated (Neter and Wasserman, 1974).

Comparing the diagrams C and D of Fig. 17, one can see that higher levels of sulfur dioxide were necessary at 188 days to preserve the same colors that were present with less sulfur dioxide at periods shorter than 101 days.

It also can be seen in Fig. 17 E that color having lower values than -3500 in periods up to 269 days required higher amounts of sulfur dioxide to maintain acceptable colors than at shorter periods of time. Color in dried apples with a CI value between -3500 and -1500 still had acceptable color to be delivered to the market and they contained lower amounts of sulfur dioxide than samples with lower CI values (Fig. 15). In any case, color indexes below -4000 were rare and required very high amounts of sulfur dioxide which may have affected the flavor of the product.

Table 4. Equation of the relationship of color index to temperature, sulfur dioxide, and moisture; standard error of regression coefficients; and t statistic for the temperature of storage of 1°C.

Regression Coefficients	Variable	Standard Error of Regression Coefficients	t Statistic
$CI^{1°}$ =			
10891.4	(constant)	9764.5	1.115
-13.6327	t	9.9649	-1.368
-7.09813	S	5.8095	-1.222
-1053.62	M	782.04	-1.347
-.182985E-01	t^2	.51700E-01	- .354
+.114276E-02	S^2	.10544E-02	1.084
+21.4216	M^2	15.438	1.388
+.153368	SM	.25633	.598
+.303637E-03	tSM	.52459E-03	.579
+.751610E-04	t^2S	.48681E-04	1.544
+.362315E-02	t^2M	.21347E-02	1.697
+.428787E-05	t^2SM	.25161E-05	-1.704

Where t is time in days; S is total sulfur dioxide in ppm; M is moisture in percent; and E is 10 to the exponent on the right.

Table 5. Equations calculated for $CI^{1°}$ at the different times of analysis.

Time zero		**Time 40 days**	
$CI^{1°}$ = 10891.4	(constant)	$CI^{1°}$ = 10316.8	(constant)
-7.09813	S	-6.97787	S
-1053.62	M	-1047.83	M
+.001140	S^2	+.001140	S^2
+21.4216	M^2	+21.4216	M^2
+.153370	SM	+.158650	SM
Time 101 days		**Time 188 days**	
$CI^{1°}$ = 9327.83	(constant)	$CI^{1°}$ = 7681.71	(constant)
-6.33141	S	-4.44164	S
-1016.66	M	-925.563	M
+.001140	S^2	+.001140	S^2
+21.4216	M^2	+21.4216	M^2
+.140290	SM	+.058900	SM
Time 269 days		**Time 385 days**	
$CI^{1°}$ = 5900.10	(constant)	$CI^{1°}$ = 2930.51	(constant)
-1.65940	S	+4.04261	S
-791.445	M	-516.578	M
+.001140	S^2	+.001140	S^2
+21.4216	M^2	+21.4216	M^2
-.075230	SM	-.36530	SM

Where S is total sulfur dioxide and M is moisture in percent.

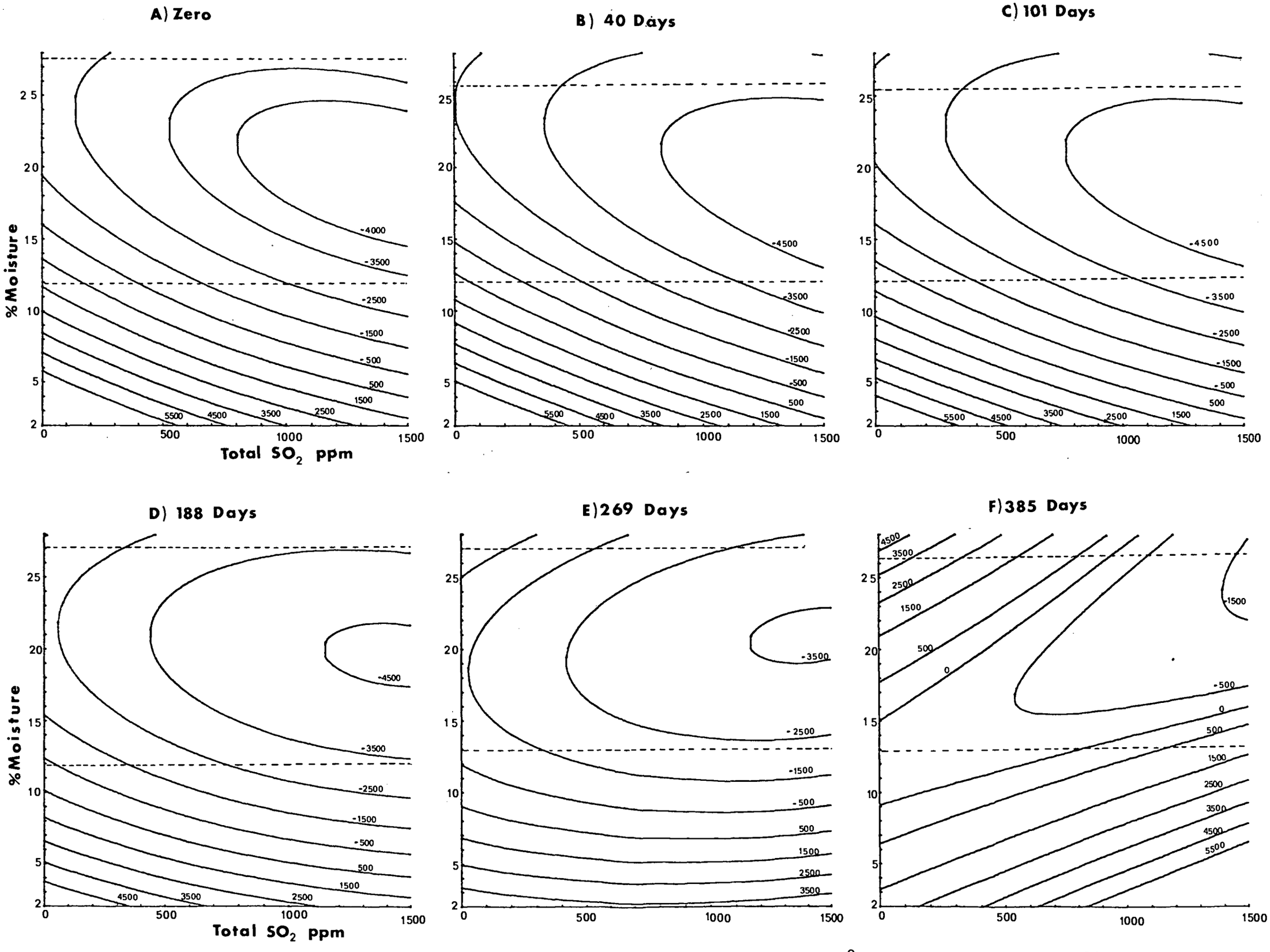


Figure 17. Response contour diagrams for the change in color of dried apples stored at 1°C. Numbers in the contour lines are the color indexes. The area within the dotted lines is described by the regression equation calculated from the data.

The concentration of sulfur dioxide to maintain a color with a CI value of -3000 up to 269 days was approximately 800 ppm (200 ppm of free sulfur dioxide, Fig. 12) and 20% moisture. Samples that maintained the moisture content of approximately 20% and the level of sulfur dioxide of 1500 ppm, still had an acceptable color for periods of storage up to 385 days.

In contrast to the prediction of the equation and the model in Fig. 17, some samples with sulfur dioxide levels lower than 800 ppm had acceptable colors for periods of storage up to 385 days. This failure of prediction by the calculated equation was because the coefficient of determination was 0.63. This low coefficient of determination was due to some variables different from sulfur dioxide, moisture, and time of storage which were not considered in the calculation of the equation.

In the diagrams of Fig. 17, 18, and 19 a region within the dotted lines shows where the data fell, which was used to calculate the equation. All points outside of these lines have a larger error in the description of what was happening during the change in color under the influences of moisture and sulfur dioxide through the experiment.

Water activities of most samples that had acceptable colors showed values above 0.75. The low temperature and high water activities are conditions that do not favor Maillard reaction, but it should be clear that some browning at very slow rates can occur in periods of storage longer than 200 days.

Changes at 21°C

The changes of color at 21°C were more notorious than those at 1°C (Fig. 15). The loss of sulfur dioxide was greater (Fig. 10 and 11) and moisture during the first days of storage decreased rapidly becoming constant at approximately 85% of the original level during the last part of the storage period (Fig. 13). The change of total measurable sulfur dioxide followed a similar curve as the one described by Davis et al. (1973).

At this temperature an appreciable deterioration of the product was observed at 101 days of storage (Fig. 18 C). Compared to the storage at 1°C, the conditions to maintain color index values of -2500 for periods of 101 days at 21°C were 800 ppm of total sulfur dioxide and 18% moisture content; the same conditions, approximately, for periods of storage of 269 days at 1°C. At 21°C faster rates of browning occurred and higher levels of sulfur dioxide were necessary to preserve the same acceptable CI values than at 1°C (Fig. 17 D and 18 D).

Moisture had a slightly higher influence in the preservation of color in these samples according to the t statistic in Table 6.

At day 188 of storage, 30% of the original content of sulfur dioxide was lost by any or all of the mechanisms mentioned above in the literature review (Fig. 10). This implies that, if dried apples with acceptable CI values are desired at 21°C, the initial amount of sulfur dioxide in the sample at time zero should be a least 2100 ppm to allow 1500 ppm of sulfur dioxide for periods of storage up to 188 days at 18% moisture content. An alternative could be to make periodic addi-

Table 6. Equation of the relationship of color index to temperature, sulfur dioxide, and moisture; standard error of regression coefficients; and t statistic for the temperature of storage at 21°C.

Regression Coefficients	Variable	Standard Error of Regression Coefficients	t Statistic
$CI^{21°}$ =			
7394.04	(constant)	3680.7	2.009
+14.4763	t	6.9827	2.073
-3.24771	S	2.3651	-1.373
-1305.35	M	397.73	-2.603
-.211696	t^2	.67113E-01	-3.154
+.222265E-02	S^2	.10985E-02	2.023
+30.9710	M^2	11.427	2.710
-.146253	SM	.11662	-1.254
+.385379E-03	tSM	.51188E-03	.753
+.312923E-04	t^2S	.33615E-04	.931
+.253378E-01	t^2M	.86549E-02	2.928
-.499517E-07	t^2S^2	.26374E-07	-1.894
-.720192E-03	t^2M^2	.26505E-03	-2.717

Where t is time in days; S is total sulfur dioxide in ppm; M is moisture in percent; and E is 10 to the exponent on the right.

Table 7. Equations calculated for $CI^{21°}$ at the different times of analysis.

Time Zero		Time 40 days	
$CI^{21°}$ = 7394.07	(constant)	$CI^{21°}$ = 7634.37	(constant)
-3.24771	S	-3.19764	S
-1035.35	M	-994.895	M
+.002223	S^2	+.002140	S^2
+30.9710	M^2	+29.8169	M^2
-.146253	SM	-.13840	SM
Time 101 days		**Time 188 days**	
$CI^{21°}$ = 6696.63	(constant)	$CI^{21°}$ = 2633.40	(constant)
-2.92850	S	-2.14171	S
-776.879	M	-139.810	M
+.001710	S^2	+.000460	S^2
+23.6243	M^2	+5.51653	M^2
-.107330	SM	-.073800	SM
Time 269 days		**Time 385 days**	
$CI^{21°}$ =-4030.36	(constant)	$CI^{21°}$ =-18411.2	(constant)
-.983370	S	+1.39059	S
+798.118	M	+2720.34	M
-.001390	S^2	-.005180	S^2
-21.1428	M^2	-75.7794	M^2
-.042590	SM	-.002120	SM

Where S is total sulfur dioxide in ppm and M is moisture in percent.

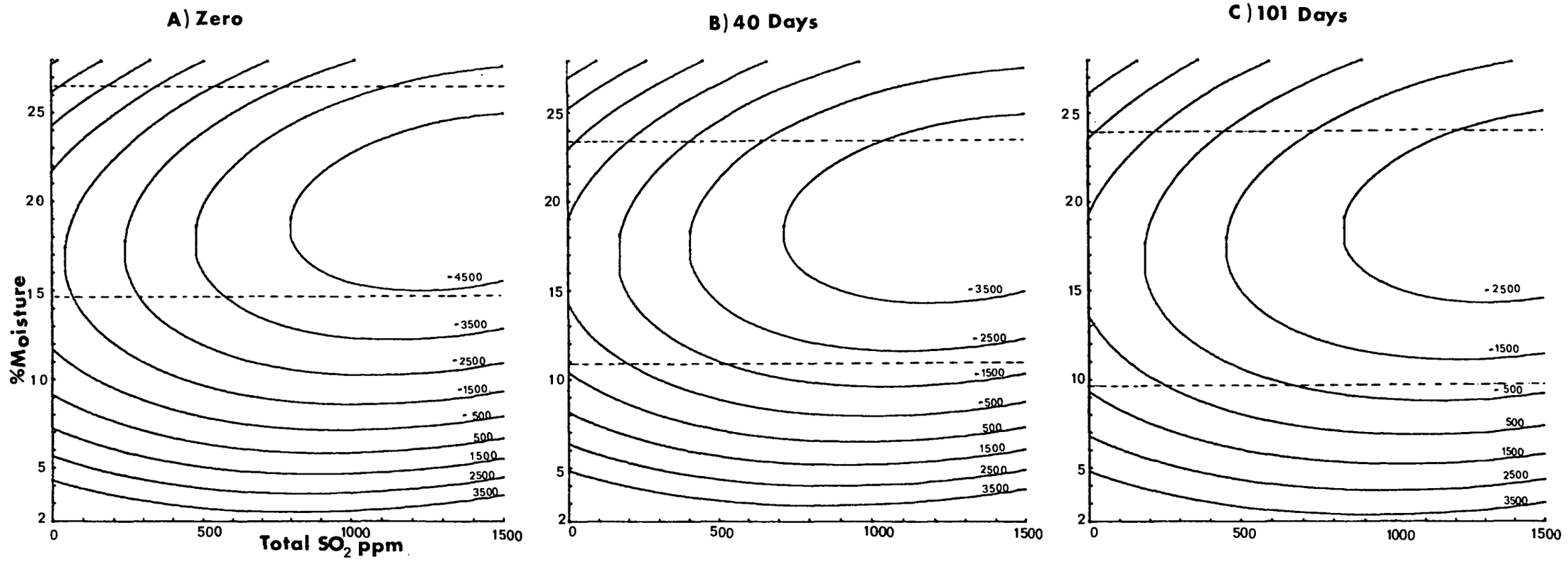


Figure 18. Response contour diagrams for the change in color of dried apples stored at 21°C. Numbers in the contour lines are the color indexes. The area within the dotted lines is described by the regression equation calculated from the data.

tions of the gas to keep a constant level of sulfur dioxide at 1500 ppm in the sample.

The conditions that were present at 21°C as compared to storage at 1°C for the development of browning in the dried apple, i.e., faster rates of loss of sulfur dioxide and faster decrease of mositure content during the storage periods were favorable for browning development.

In periods longer than 269 days, samples with unacceptable color were obtained at any level of sulfur dioxide used, according to the equations used to prepare Fig. 18 E (Table 7). However, some samples with acceptable colors close to the border line were found. The samples with acceptable color and not described by the equation were rare. These samples may be the part of the experiment that the equation cannot predict (r^2 = 0.88).

When the samples with low L values and low +a values (redness) were visually evaluated without a reference standard, the samples appeared acceptable. When a reference was used for visual judgment, it was difficult to classify the samples with low values of lightness, but when these samples were measured by the color difference meter and the CI calculated, the samples were clearly in the rejection region.

Changes at 38°C

Samples that were stored at 38°C developed a very deep brown color by storage day 101 (Fig. 19C). Almost all free sulfur dioxide was lost by this time and levels of moisture also drop very rapidly (Fig. 10, 11, and 13). At this temperature the permeability of the package was very critical, due to the high temperature and low relative humidity (40%)

of the room in which the samples were stored. All conditions for browning development were favorable, with almost no sulfur dioxide available to prevent browning.

In the diagrams of Fig. 17, 18, and 19 a region limited by dotted lines is shown. In Fig. 19 these regions became smaller because all samples were concentrated in a small range of total sulfur dioxide and mositure content and not spread over the range of moisture and sulfur dioxide levels as the samples stored at 1°C and 21°C. This was due to the fast decrease of sulfur dioxide and mositure content.

The equation calculated had a r^2 of 0.87 (Table 8). The samples that are located outside of the dotted region cannot be described by the equations with the same accuracy (Table 9). One must extrapolate outside of the dotted region to have an idea of how samples with better color might have behaved at this temperature for the time of storage of 385 days under the conditions of this work. Therefore, this extrapolation and the use of the model at 38°C is not recommended due to the short range of the variables used during the calculation.

Sulfur dioxide content played a more important role than the mositure level in the preservation of acceptable colors according to the t statistic shown in Table 8. Under any circumstances, a storage temperature of 38°C is not recommended for storage of dried apple because of the very fast rate of browning that developed.

Table 8. Equation of the relationship of color index to temperature, sulfur dioxide, and moisture; standard error of regression coefficients; and t statistic for the temperature of storage of 38°C.

Regression Coefficients	Variable	Standard Error of Regression Coefficients	t Statistic
$CI^{38°}$ =			
-606.634	(constant)	3259.8	- .186
+44.6885	t	15.282	2.924
-9.52093	S	4.4124	-2.158
+161.139	M	335.12	.481
-.448123E-01	t^2	.20768E-01	-2.158
+.675212E-02	S^2	.29933E-02	2.256
-.369009	M^2	11.767	- .031
-.224357	SM	.22853	- .982
+.442780E-02	tSM	.17992E-02	2.461
-.757069E-05	t^2S	.50775E-04	- .149
-.115635E-01	t^2M	.30206E-02	-3.828

Where t is time in days; S is total sulfur dioxide in ppm; M is moisture in percent; and E is 10 to the exponent on the right.

Table 9. Equations calculated for $CI^{38°}$ at the different times of analysis.

Time Zero		**Time 40 days**	
$CI^{38°}$ = -606.634	(constant)	$CI^{38°}$ = 1109.20	(constant)
-9.52093	S	-9.53304	S
+161.139	M	+142.637	M
+.006752	S^2	+.006752	S^2
-.369009	M^2	-.369009	M^2
-.224357	SM	-.047250	SM
Time 101 days		**Time 188 days**	
$CI^{38°}$ = 3449.77	(constant)	$CI^{38°}$ = 6210.95	(constant)
-9.59816	S	-9.78851	S
+43.1797	M	-247.561	M
+.006752	S^2	+.006752	S^2
-.369009	M^2	-.369009	M^2
+.222850	SM	+.222850	SM
Time 269 days		**Time 385 days**	
$CI^{38°}$ = 8171.90	(constant)	$CI^{38°}$ = 9956.13	(constant)
-10.0687	S	-10.6431	S
-675.607	M	-1552.86	M
+.006752	S^2	+.006752	S^2
-.369009	M^2	-.369009	M^2
+.966720	SM	+1.48035	SM

Where S is total sulfur dioxide in ppm and M is moisture in percent.

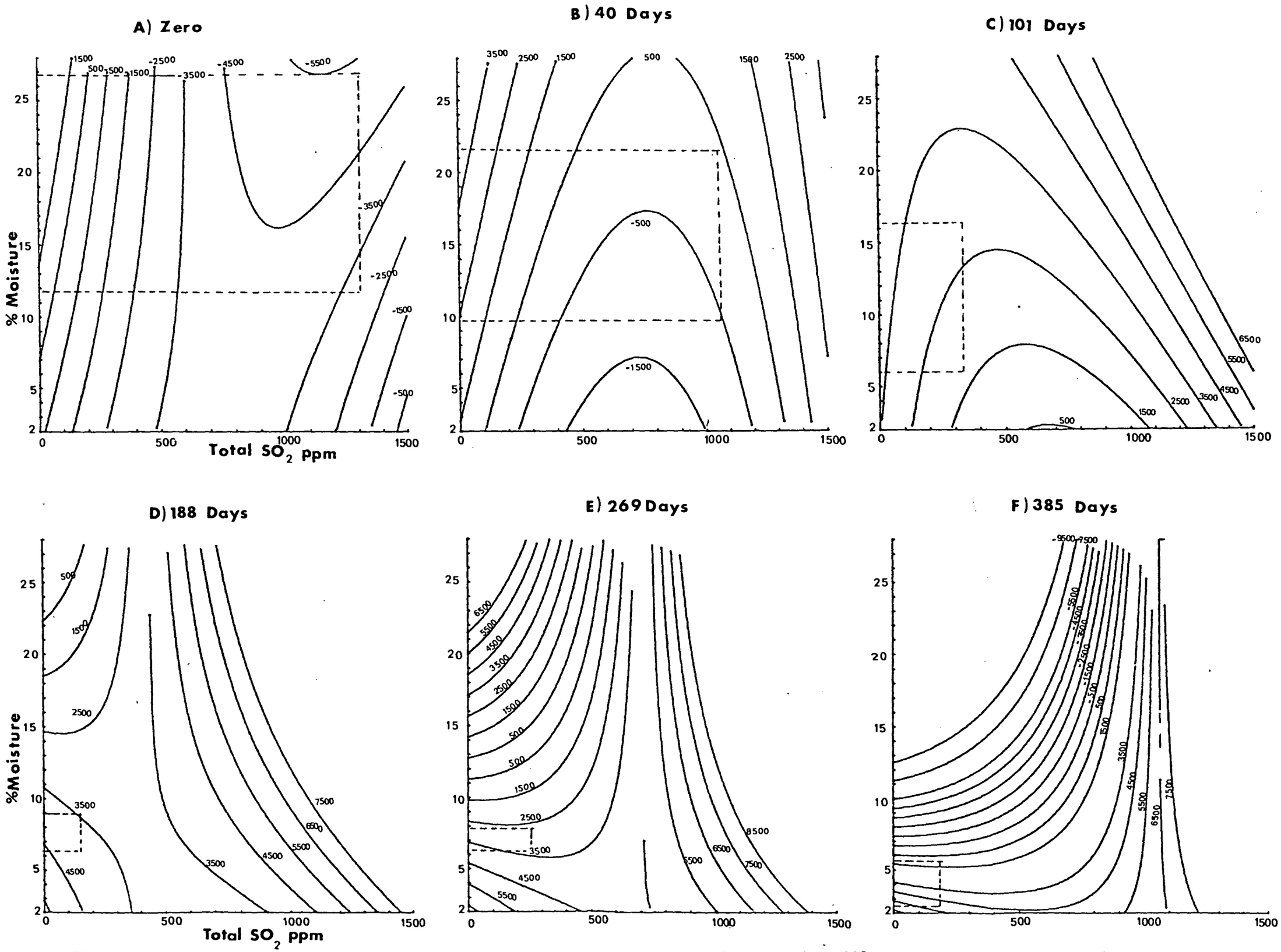


Figure 19. Response contour diagrams for the change in color of dried apples stored at 38°C. Numbers in the contour lines are the color indexes. The area within the dotted lines is described by the regression equation calculated from the data.

CONCLUSION

Sulfur dioxide and low temperatures were effective in the retarding of browning in dried apples. This observation agrees with that of other workers (Bolin et al., 1976; Roberts and McWeeny, 1972; Green, 1976).

Temperatures of storage had a major influence in the preservation of acceptable colors in dried apples. Free and total sulfur dioxide were lost at higher rates as temperature of storage increased showing a 90% loss at 38°C, 45% loss at 21°C, and almost no loss at 1°C during the 385 days that the samples were stored.

As the concentration of sulfur dioxide decreased, the rate of browning was accelerated. Moisture of the sample changed with time in relationship to the relative humidity of the storage room. At 1°C storage the moisture increased, a steady level was maintained at 20°C, and at 38°C there was a rapid loss of moisture content in the samples. This could have been provoked by the very low relative humidity of the room at 38°C.

The ratio of free sulfur dioxide to total sulfur dioxide increased as concentration of the total sulfur dioxide increased resulting in more color preservation, but it may have caused flavor problems.

Water activities of the samples with acceptable colors in the experiment were approximately 0.75, which corresponds to the levels of moisture of 20% wet basis. At this moisture content the levels of sulfur dioxide necessary to preserve acceptable colors were the lowest in the statistical model.

Color Index (CI) described the changes in color in dried apples within the conditions of this experiment.

The equations found for each of the three storage temperatures tested can be used to predict any color of dried apple under the conditions, levels of moisture, and sulfur dioxide used in this experiment.

No appreciable color changes were observed at 1°C during 269 days of storage, but changes of color were observed at the day 101 in samples stored at 21°C and a very rapid rate of browning was noticed inthe first 40 days of storage at 38°C.

Sulfur dioxide and moisture had almost the same influence in the preservation of acceptable colors for the samples stored at 1°C according to the t test of the statistical model. If moisture levels were approximately 20%, 1200 ppm of total sulfur dioxide were sufficient to preserve acceptable colors for 269 days.

Changes of color at 21°C were more marked than at 1°C. The acceptable colors with the lowest amount of sulfur dioxide were found at approximately 18% moisture content in samples stored at 21°C. Higher levels of sulfur dioxide were necessary to preserve CI values of -1500 for 188 days in dried apples at 21°C than at 1°C. Unacceptable samples were obtained at periods of time longer than 188 days at any levels of sulfur dioxide and moisture according to the statistical model used in this experiment. Poor statistical description was obtained at the storage times of 269 days and 385 days at the temperature of 21°C due to the lack of samples with good color. This was more notorious in the samples stored at 38°C where no samples with acceptable colors were present after storage for 40 days.

The temperature of 21°C was an acceptable temperature of storage for samples that were stored for periods shorter than 188 days, if a level of 1200 ppm of total sulfur dioxide was present in the fruit tissue at 20% moisture content.

At 38°C very rapid changes of color were observed during the first 40 days of storage and, according to the statistical model, acceptable colors cannot be preserved longer than 40 days under the conditions of the experiment. Poor description of the changes of color was obtained in the regions where samples were not located due to the fast decrease of total sulfur dioxide and moisture contents. Extrapolations were done to have an approximation of how the samples might have behaved.

The influence of the manner of storing the packages and the type of packaging material used had a definitive influence in the loss of sulfur dioxide and changes in moisture from the sample and, as a consequence, also in the rate of browning.

BIBLIOGRAPHY

Adams, E.Q. 1942. J. Opt. Soc. Am. 32, 168. Cited in: Hunter, R.S. 1958 Photoelectric Color Difference Meter. J. Opt. Soc. Am. 48 (12), 985.

Adsule, P.G. and Anad, J.C. 1977. Studies on Plastic Containers for Packing Fruit Products I. Changes Occurring in Model Solutions. Indian Food Packer 31 (6), 23.

Anet, E.F.J.L. and Ingles, D.L. 1964. Mechanism of Inhibition of Non-enzymic Browning by Sulphite. Chem. Ind. July 18, 1319.

Anon. 1974. Sulphur Dioxide and Foods. CSIRO Food Res. Q., 34, 80.

Anon. 1975. Sulfites as Food Additives. Food Technol. 29(10), 117.

AOAC. 1980. Official Methods of Analysis of the Association of Official Analytical Chemists. William Horowitz Editor. Published by the AOAC, Washington, D.C. 20040.

Barger, W.R. and Pentzer, W.T. 1948. Low Temperature Storage Retains Quality of Dried Fruit. Food Industries, March F1-F3.

Barnett, D. 1975. Electrodes for Estimating Sulphur Dioxide. CSIRO Food Rs. Q. 35,68.

Beard, J.H., Fletcher, B.C., and VanBerestein, E.C.H. 1972. The Enhancement of Orange Oil Oxidation by Sulfur Dioxide. J. Sci. Food Agric. 23, 207.

Bengtsson, B.L., Bosund, I., and Rasmussen, I. 1967. Hexanal and Ethanol Formation in Peas in Relation to Off Flavor Development. Food Technol. 21, 478.

Bhagat, B. and Lockett, M.F. 1964. The Effect of Sulphite in Solid Diets on the Growth of Rats. Food Cosmet. Toxicol. 2, 1.

Bolin, H.R. and Stafford, A.E. 1974. Effect of Processing and Storage on Provitamin A and Vitamin C in Apricots. J. Food Sci. 39, 1034.

Bolin, H.R., Stafford, A.E., and Yanase, K. 1976. Color Stability of Dried Peaches. Food Prod. Dev. 10 (8), 74.

Bone, D. 1969. Water Activity: Its Chemistry and Applications. Food Prod. Dev. 3 (8), 81.

Brun, P., Gasquet, C., Stoutz, S., and Nicoli, A. 1961. Application of Amperometry to the Determination of Sulfur Dioxide in Wine. Ann. Falsif. Fraudes 54, 412.

Buechsenstein, J.W. and Ough, C.S. 1978. Sulfur Dioxide Determination by Aeration Oxidation, a Comparation with Ripper. Am. J. Enol. Vitic.
29 (3), 161.

Burroughs, L.R. and Sparks, A.H. 1964. The Determination of the Free Sulphur Dioxide Content of Ciders. Analyst 89, 55.

Burroughs, L.F. and Sparks, A.H. 1973. Sulphite Binding Power of Wines and Ciders. J. Sci. Food Agric. 24, 187.

Burroughs, L.F. 1974. Browning Color in Fruit Juices and Wines. Chem. Ind. Sept. 21, 718.

Burroughs, L.F. 1975. Determining Free Sulfur Dioxide in Red Wine. Am. J. Enol. Viticult., 26 (1), 25.

Burton, H.S., McWeeny, D.J., and Biltcliffe, D.O. 1962a. Sulphites and Aldose-Amino Reactions. Chem. Ind. February 3, 212.

Burton, H.S., McWeeny, D.J., and Biltcliffe, D.O. 1962b. The Influence of pH on SO_2-Aldose-Amino-Reactions. Chem. Ind. September 22, 1682.

Burton, H.S. and McWeeny, D.J. 1963. Non-enzymic Browning Reactions: Consideration of Sugar Stability. Nature 197, 266.

Burton, H.S., McWeeny, D.J., and Pandhi, P.N. 1963a. Non-enzymic Browning: Browning of Phenols and Its Inhibition by Sulphur Dioxide. Nature 199, 659.

Burton, H.S., McWeeny, D.J., and Biltcliffe, D.O. 1963b. Non-enzymic Browning: The Role of Unsaturated Carbonyl Compounds as Intermediates and SO_2 as an Inhibitor of Browning. J. Sci. Food Agric. 14, 911.

Burton, H.S. and McWeeny, D.J. 1964. Non-enzymic Browning: Routes to the Production of Melanoidins from Aldoses and Amino-Compounds. Chem. Ind. March 14, 462.

Caurie, M. 1970. A Practical Approach to Water Sorption Isotherms and the Basis for the Determination of Optimum Moisture Levels of Dehydrated Foods. J. Food Technol. 6, 85.

Chirife, J. and Iglesias, H.A. 1978. Equations for Fitting Water Sorption Isotherms of Foods. Part I-A Review. J. Food Technol. 13, 157.

Clydesdale, F.M. 1969. The Measurement of Color. Food Technol. 26, 45.

Clydesdale, F.M. 1972. Measuring the Color of Foods. Food Technol. 26, 45.

CRC Handbook of Chemistry and Physics. 1979. Weast and Astle Editors. 60th Ed. CRC Press, Boca Raton, Florida.

Dahlenburg, A.P. 1976. Sulphuring of Tree Fruits for Drying. Food Technol. in Australia 28 (5), 168.

Davis, E.G. and Rooney, M.I. 1971. Kolloidzeitschrift 249, 1043. Cited in: Davis, E.G., McBean, D.McG., Rooney, M.L., and Gipps, P.G. 1973. Mechanisms of Sulphur Dioxide Loss From Dried Fruits in Flexible Films. J. Food Technol. 8, 391.

Davis, E.G., McBean, D.McG., Rooney, M.L., and Gipps, P.G. 1973. Mechanisms of Sulphur Dioxide Loss From Dried Fruits in Flexible Films. J. Food Technol. 8, 391.

Davis, E.G. and Rooney, M.L. 1975. Permeability of Polymer Films to Sulfur Dioxide at Low Concentration. J. App. Pol. Sci. 19, 1829.

DeMan, J.M. 1976. Color. In "Principles of Food Chemistry", AVI Publishing Co., Westport, Conn.

Embs, R.J. and Markakis, P. 1965. The Mechanism of Sulfite Inhibition of Browning Caused by Polyphenol Oxidase. J. Food Sci. 30, 753.

Falk, M. and Giguère, P.A. 1958. On the Nature of Sulphurous Acid. Can. J. Chem. 36, 1121.

Gilbert, J. and McWeeny, D.J. 1976. The Reactions of Sulphur Dioxide in Dehydrated Vegetables. J. Sci. Food Agric. 27, 1145.

Green, L.F. 1976. Sulphur Dioxide and Food Preservation. A Review. Food Chem. 1, 103.

Haiasman, D.R. 1974. The Effect of Sulfur Dioxide on Oxidasing Enzyme Systems in Plant Tissues. J. Sci. Food Agric. 25, 803.

Hayatsu, H. and Miura, A. 1970. The Mutagenic Action of Sodium Bisulfite. Biochem. Biophys. Res. Comun. 39 (1), 156.

Hodge, R.F. and Rist, C.E. 1953. The Amadori Rearrangement Under New Conditions and Its Significance for Non-enzymatic Browning Reactions. Am. Chem. Soc. J. 75, 316.

Hodge, J.E. 1953. Dehydrated Foods, Chemistry of Browning in Model Systems. J. Agric. Food Chem. 1 (15), 928.

Hodge, J.E. 1967. Origin of Flavor in Foods, Non-enzymiatic Browning Reactions. In "The Chemistry and Physiology of Flavors" Symposium on Foods. Schultz Eds. AVI Publishing Co., Westport, Conn.

Hunter, R.S. 1948. Photoelectric Color-Difference Meter. J. Opt. Soc. Am. 38, 661.

Hunter, R.S. 1958. Photoelectric Color Difference Meter. J. Opt. Soc. Am. 48 (12), 985.

Ingles, D.L. 1959. Chemistry of Nonenzymic Browning V.-The Preparation of Aldose Potassium Bisulfite Addition Compounds and Some Amine Derivatives. Aust. J. of Chem. 12, 97.

Ingles, D.L. 1959. Chemistry of Non-enzymic Browning VIII. The Hydrolytic Reactions of Aldose Bisulphite Addition Compounds. Aust. J. of Chem. 12, 288.

Ingles, D.L. 1959. Chemistry of Non-enzymic Browning VI. The Reaction of Aldoses with Amine Bisulphites. Aust. J. of Chem. 12, 275.

Ingles, D.L. 1959. Reduction of Bisulphite to Elemental Sulphur by Reducing Sugars. Chem. Ind. 12, 1045.

Ingles, D.L. 1960. Reactions of Aldoses with Bisulphite and Hydroxysulphite. The Formation of Sugar Sulphates. Chem. Ind. 13, 1159.

Ingles, D.L. 1960. Chemistry of Non-enzymic Browning XI. The reactions of Bisulphite with Reducing Sugars. Aust. J. of Chem. 13, 404.

Ingles, D.L. 1961. Reactions of Reducing Sugars with Sulphite. The Formation of β-sulphopropionic Acid. Chem. Ind. 14, 1312.

Ingles, D.L. 1962. The Formation of Sulphonic Acids from the Reactions of Reducing Sugars with Sulfites. Aust. J. of Chem. 15, 342.

Ingles, D.L. 1966. Prevention of Non-enzymic Browning. CSIRO Food Preserv. Q. 26 (2 and 4), 39.

Joslyn, M.A. and Saywell, L.G. 1934. Determination of Sulphur Dioxide in Fruit and Vegetable Products in "Laboratory Examination of Wine and Other Fermented Products," The AVI Publishing Co., N.Y.

Joslyn, M.A. and Braverman, J.B.S. 1954. The Chemistry and Technology of the Pretreatment and Preservation of Fruit and Vegetable Products with Sulfur Dioxide and Sulfites. In "Advances in Food Research" Vol. 5. Mark Eds. Academic Press, N.Y.

Joslyn, M.A. and Amerine, M.A. 1964. Dessert. Appetizer and Related Flavored Wines. The Technology of Their Production. University of California, Division of Agricultural Sciences.

Joslyn, M.A. 1970. Chemical Preservatives. In "Methods in Food Analysis, Physical, Chemical and Instrumental Methods of Analysis. 2nd Ed. Academic Press, N.Y.

Kahan, V. 1977. Latency Properties of Polyphenol Oxidase in Two Avocado Cultivars Differing in Their Rate of Browning. J. Sci. Food Agric. 28, 233.

Kalus, W.H. 1978. Metabolism of Sulfur Dioxide in Apples. Zetschrift Fur Lebensmittel-Untersuchung Undferschung 166 (6), 346. Abstract FSTA 10 11J 1615.

Karel, M. and Labuza, T.P. 1969. Optimization of Protective Package of Space Foods. Final Report USAF. School of Aerospace Medicine. Brooks Air Force Base, Texas. Contract #41-609-68-C-0015.

Karel, M., Mizrahi, S., and Labuza, T.P. 1971. Computer Prediction of Food Storage. Mod. Packag. August, 54.

Kidney, A.J. 1974. The Use of Sulphite in Meat Processing. Chem. Ind. September, 717.

King, C.J. 1968. Rates of Moisture Sorption and Desorption in Porous Dried Foodstuffs. Food Technol. Champaign 22, 509.

Knowels, M.E. 1971. Inhibition of Non-enzymic Browning by Sulphite: Identification of Sulphonated Products. Chem. Ind. August, 910.

Labuza, T.P., Tannenbaum, S.R., and Karel, M. 1970. Water Content and Stability of Low Moisture and Intermediate Moisture Foods. Food Technol. 24, 543.

Labuza, T.P., Acott, K., Tatini, S.R., and Lee, R.Y. 1976. Water Activity Determination: A Collaborative Study of Different Methods. J. Food Sci. 41, 910.

Loncin, M. 1961. Les Operations Unitaries du Genie Chimique. Edit. Dunod Paris, Desoer Liege.

Loncin, M., Bimbenet, J.J., and Lenges, J. 1968. Influence of the Activity of Water on the Spoilage of Foodstuffs. J. Food Technol. 3, 131.

Mackinney, G. and Little, A.C. 1962. Evaluation of Color Differences. In "Color of Foods:. The AVI Publishing Co., Inc., Wesport, Conn.

Markakis, P. 1974. Anthocyanins and Their Stability in Foods. CRC Critical Reviews in Food Technol. 4 (4), 437.

Merck Index. 1976. The Merck Index of Chemicals and Drugs. Windholz Ed. Published by Merck and Co., Inc. Rahway, N.J.

McBean D. McG., Johnson, A.A., and Pitt, J.I. 1964. The Absorption of Sulfur Dioxide by Fruit Tissue. J. Food Sci. 29, 257.

McBean, D.McG., Pitt, J.I., and Johnson, A.A. 1965. Retention of Absorbed Sulfur Dioxide in Fruit Tissue During Drying. Food Technol. 19, 1447.

McBean, D.McG. 1967. Levels of Free and Combined Sulfur Dioxide in Fruits During Sulfuring and Drying. Food Technol. 21, 1402.

McWeeny, D.J. and Burton, H.S. 1962. Development of Chromophores. Nature 196, 40.

McWeeny, D.J., Biltcliffe, D.O., Powel, R.C.T., and Spark, A.A. 1969. The Maillard Reaction and Its Inhibition by Sulphite. J. Food Sci. 34, 641.

McWeeny, D.J., Knowels, M.E., and Hearne, J.F. 1974. The Chemistry of Non-enzymic Browning in Foods and Its Control by Sulphites. J. Sci. Food Agric. 25, 735.

McWeeny, D.J. 1979. The Chemical Behavior of Food Additives. Proc. Nutr. Soc. 38, 129.

Monier-Williams, G.W. 1927. Determination of Sulfur Dioxide in Foods. Repts. Public Health and Med. Subjects No. 43, 1-56, Great Britain Ministry of Health, London.

Mukai, F., Hawryluk, I, and Shapiro, R. 1970. The Mutagenic Specificity of Sodium Bisulfite. Biochem. Biophy. Res. Comun. 39 (5), 983.

Nagarja, K.V., Manjrekar, S.P. 1971. Microcolorimetric Estimation of Sulfur Dioxide in Fruits and Fruit Products. J. AOAC 54 (5), 1146.

Nauman, R.V., West, W.P., and Tron, F. 1960. A Spectrophotometric Study of the Schiff Reaction as Applied to Quantitative Determination of Sulfur Dioxide. Anal. Chem. 32 (10), 1307.

Neter, J. and Wasserman, W. 1974. Regression Analysis of Variance and Experimental Design. Richard D. Irwin, Inc., Homewood, Illinois.

Nury, F.S., Taylor, D.H., and Brekke, J.E. 1959. Modified Direct Colorimetric Method for Determination of Sulfur Dioxide in Dried Fruits. J. Agr. Food Chem. 7 (5), 351.

Nury, F.S., Taylor, D.H., and Brekke, J.E. 1960. Research for Better Quality in Dried Fruits. Apricots. U.S. Dept. Agr. ARS-74-19.

Oswin, C.R. 1976. Food Packaging in Relation to Moisture Exchange. Chem. Ind. 18 December, 1042.

Pate, J.B., Lodge, J.P., and Wartburg, A.F. 1962. Effect of Pararosaniline in the Determination of Trace Amounts of Sulfur Dioxide. Anal. Chem. 34 (12), 1660.

Payne, C.H., Beavers, D.V., and Cain, R.F. 1968. The Chemical and Preservative Properties of Sulfur Dioxide Solution for Brining Fruit. Circular Information 628. March. Agricultural Experimental Station, Oregon State University, Corvallis.

Pearson, D. and Wong, T.S.A. 1971. A Direct Titrimetric Method for the Rapid Estimation of Sulfur Dioxide in Fresh Pork Sausage. J. Food Technol. 6, 179.

Ponting, J.D. and Joslyn, M.A. 1948. Ascorbic Acid Oxidation and Browning in Apple Tissue Extracts. Arch. in Biochem. 19, 47.

Ponting, J.D., Jackson, R., and Watters, G. 1971. Refrigerated Apple Slices: Effects of pH, Sulfites and Calcium on Texture. J. Food Sci. 36, 350.

Ponting, J.D. and Jackson, R. 1972. Refrigerated Apple Slices: Preservative Effects of Ascorbic Acid, Calcium and Sulfites. J. Food Sci. 37, 434.

Potter, E.F. 1954. A Modification of the Iodine Titration Method for the Determination of Sulfur Dioxide in Dehydrated Cabbage. Food Technol. 8, 269.

Reynolds, T.M. 1965. Chemistry of Nonenzymic Browning II. Inhibition of Browning. In "Advances in Food Research" Vol. 14. Chichester Eds., Academic Press, N.Y.

Ripper, M. 1892. Die Scwelfiege Saure im Weine und Deiren Bestimmung. J. Prakt. Chem. (N.F.) 45, 428; 46, 428.

Roberts, A.C. and McWeeny, D.J. 1972. The Uses of Sulfur Dioxide in Food Industry. A Review. J. Food Technol. 7, 221.

Rockland, L.B. 1969. Water Activity and Storage Stability. Food Technol. 23, 1241.

Rowe, K.E. 1980. Personal Communication. OSU Department of Statistics, Corvallis, Oregon.

Sacharow, S. and Griffin, R.C. 1970. Fruits and Vegetables in "Food Packaging". The AVI Publishing Co., Inc., N.Y.

Scholey, J. and Rawlinson, A.P. 1974. The Role of Sulphur Dioxide in Food Processing. Chem. Ind. 21 September, 716.

Schuller, P.L. and Veen, E. 1967. Preservatives: A Review of Methods of Analysis. J. AOAC. 50 (5), 1127.

Shapiro, R. and Braverman, B. 1972. Modificatio of Polyuridylic Acid by Bisulfite: Effect on Double Helix Formation and Coding Properties. Biomed. and Biophy. Res. Comun. 47 (3), 544.

Shipton, J. 1954. Estimation of Sulfur Dioxide in Dried Fruits. Food Preserv. Q. 14, 54.

Sidwick, N.V. 1952. In "The Chemical Elements and Their Compounds II, 897." University, Press, London, Oxford.

Smith, G. and Stevens, M.F.G. 1972. Sulphur Dioxide, Preservative and Potential Mutagen. Pharm. J. 12, 570.

Stafford, A.E. and Bolin, H.R. 1972. Absorption of Aqueous Bisulfite by Apricots. J. Food Sci. 37, 941.

Suzuki, H., Toyoda, M., Ito, Y., Iwaida, M., Nonogi, H., Fuke, T., Nagashima, C., Adachi, T., and Fujita, K. 1978. Comparison of Five Methods for Determining Sulphite in Foods. Shokuhim Eisegaku Zasshi 18 (3), 290.

Taylor, D.H., Miller, M.W., Nury, F.S., and Breeke, J.E. 1961. Temperature Effect on Colorimetric Determination of Sulfur Dioxide in Fruits. J. AOAC 44 (4), 641.

Thrasher, J.J. 1966. Sulphur Dioxide Determination by Modified Monier-Williams Method. J. AOAC 49 (4), 834.

Valencia, R., Abrahanson, S., and Wagoner, P. 1972. Annual Report, Food Res. Inst. University of Wisconsin, Madison p-108.

Walker, J.R.L. 1962. Studies on the Enzymic Browning of Apple Fruit. New Zealand J. of Sci. 5, 316.

Warmbier, H.C., Scinckels, R.A., and Labuza, T.P. 1976. Effect of Glycerol on Non-enzymatic Browning in a Solid Intermediate Moisture Model Food System. J. Food Sci. 41, 528.

Wrolstad, R.E. 1976. Color and Pigment Analysis in Fruit Products. State Bulletin 624, October. Agricultural Experiment Station, Oregon State University, Corvallis, Oregon.