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CHEMICAL STABILITY OF FREEZE-DRIED
APPLE SLICES (var. SCHONE VAN BOSKOOP)
AT VARIOUS WATER ACTIVITIES

presented by

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CHEMICAL STABILITY OF FREEZE-DRIED APPLE SLICES (var. SCHONE VAN BOSKOOP) AT VARIOUS WATER ACTIVITIES

By

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ABSTRACT

By

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"Schone van Boskoop" apple slices were freeze-dried to a final moisture content of 4.30% on dry basis. During storage the cells of freeze-dried slices were sensitive to humidity. Accordingly, chemical stability was studied in relation to water activity.

The freeze-dried slices were placed in desiccators of saturated salt solutions of known water activities. A sorption isotherm was prepared. Ascorbic acid, phenolics, volatiles and texture were measured.

There was a correlation between ascorbic acid and total phenolics retention.

1. At a_w = 0.22 the drop of total phenolics caused a drop in reduced ascorbic acid.

2. At a_w = 0.3 to 0.66 reduced ascorbic acid was degraded and the total phenolics changed very little.

3. At a_w = 0.7 the total phenolics decreased and browning began because ascorbic acid was no longer effective as an antioxidant.

The best storage was at $a_w \leq 0.24$. Also volatile components had the best retention; texture, flavor and color were good.

Dedicated to my late parents

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INTRODUCTION

The densest apple growing areas in Belgium are situated in the southern parts of Limburg. About 70% of the total apple production is Golden Delicious, but "Shone van Boskoop", the second most important variety, takes 20% of the total production.

Even though there is an over-production of apples in Belgium, there have been fairly large quantities of apples imported from other countries (France, South Africa, Italy) during the recent past, which pushes the price of apples grown in Belgium down to a very low level. The overall quality of the Golden Delicious grown in Belgium is not optimal so that this variety is not competitive with Golden Delicious imported from other countries. For this reason apple growers want to increase the production of other varieties adapted to the local agricultural conditions. "Shone van Boskoop" is one variety giving high production with good quality and this cultivar is good as a table fruit as well as for cooking. Along with the increased production of Boskoop, there is also a demand for new possibilities for processing of the fruit. The production of freeze-dried apple slices could be one of the possibilities.

Freeze-dried apple slices are materials of a physiological system, constantly undergoing changes. During storage the cells are sensitive to such external influences as temperature, humidity and oxygen, as well as the interplay of internal factors which are difficult to control. The parameters of water content, equilibrium relative humidity and water activity may be used to monitor the chemical stability. Water is generally the major constituent of foods. Its concentration influences the palatability, digestion, transportation and handling of various foods. However, mobility of the water expressed in term of water activity present in foods is involved in their deteriorative processes and in the stability of dehydrated food products. Since water activity is a useful expression of water requirement for chemical and enzymatic stability, the variables studied here are water activity related.

Model systems have often been used by investigators to determine the effect of water activity on chemical stability (Acker, 1962; Labuza, 1973; Kirk et al., 1977). The primary objective of this study was to determine the effect of water activity and time on a real system - freeze-dried apple slices. After determining the sorption isotherm at 25^{o}C for the freeze-dried apple slices, the changes in ascorbic acid, total phenolics and volatiles were studied. The textural index was used as a measure of the hygroscopicity of the apple slices.

LITERATURE REVIEW

Dehydration of Fruits

Drying of fruits is an age-old practice dating back to biblical times. Sun-drying was the method used from those early times and it is regularly used now for drying raisins, apricots and peaches. In the latter part of the nineteenth century attempts were made to use other sources of heat for dehydration of fruits.

Apparently "evaporation" of apples was developed in the early years of the twentieth century in western New York State, using a one-kiln evaporator, which was eventually followed by other types of evaporators (Van Arsdel and Copley, 1964). Low moisture fruit products like apple nuggets, with less than three per cent moisture, were produced commercially by vacuum-shelf drying with a procedure involving reduced pressure and temperatures ranging from 100 to 140^{o}F.

With the advent of vacuum pumps and refrigeration machines, freeze-drying has been made possible. Up to the time of World War II freeze-drying had been regarded as an occasional scientific tool but the opportunity for large-scale use had not arisen. Flosdorf (1949) envisaged its

use for foodstuffs and other bulky materials but only within the last two or three decades has freeze-drying been developed and used extensively as a dehydration operation in food technology. The process has been applied to fruits like apple and peach slices.

Advantages of Freeze-Drying

Theoretically, freeze-drying could be the best technique for preservation of foods if freezing behavior of the material to be processed is studied carefully in order to prevent drastic alteration during the course of freeze-drying. In other words, every given product has to be processed in its own particular way and therefore the process of freeze-drying has to be optimized. Other critical steps of the process include the technique of freezing the material, as well as close control of the sublimation phase (Rey and Bastian, 1962).

The major advantages of freeze-drying are structural integrity, retention of volatiles, reduced browning and preservation of vitamin activity as well as other properties like good organoleptic qualities and good rehydration.

Shrinkage in freeze-drying is usually much less than in ordinary drying because dehydration is achieved at low temperatures. At these temperatures, the mobility of interstitial concentrate is low, so that little change occurs to the porous structure left by the subliming ice.

Flavor retention is much better in freeze-dried foods than in other dehydrated foods, in spite of the high volatility of the aroma constituents and the fact that processing is done under vacuum. Thijssen and associates (1968, 1979) found that the diffusion coefficients of volatiles and water dropped markedly as dissolved solid contents increase, but that the diffusion coefficients for volatiles drop to a considerably greater extent. Thus above a certain dissolved solids content, water is removed by diffusion at a significant rate, but other volatile compounds are not. Flink, Karel, and associates (Flink and Karel, 1970a,b, 1972; Kayaert et al., 1975) have interpreted volatiles retention through the concept of microregion entrapment, in which volatile compounds are immobilized within cages formed by association of molecules of dissolved solids, such as by hydrogen bonding of carbohydrate molecules. The degree of immobilization would then relate to the water content, freezing conditions and other variables.

The low temperature employed for dehydration in freeze-dried foods helps to reduce browning, to preserve vitamin activity and other nutritional properties of the food due to the presence of given biological active compounds. This low temperature process will prevent non-enzymatic browning which causes loss of protein biological value. It will also prevent formation of peroxides produced during lipid

oxidation with proteins or vitamins. Thus organoleptic quality is not impaired.

Eisenhardt et al. (1968) compared the porosity and rehydration of apples that were freeze-dried, air-dried and explosion-puffed. They found that freeze-dried pieces were most rapid in water uptake and this was related to their highest amount of surface-connected pores. Freeze-dried apple slices could be rapidly rehydrated compared to the extremely slow rehydration rates of conventionally dried pieces. Explosive-puffing greatly improved the rehydration rates of conventionally dried pieces.

Utilization of Dehydrated Fruits

Dehydrated fruits are utilized mainly in bakery products, in cooked "sauces" and eating as a snack. As a snack, apple slices prepared by freeze-drying and explosion-puffed have the advantage of a crisp texture compared to conventional air-dried slices. The crispy texture of the dehydrated apple slices would lend itself readily for use as a snack item as the banana chips in the tropical countries. Sonido et al. (1977) studied the equilibrium relative humidity relationships of banana chips as a systematic approach to the proper selection of packaging material for the shelf-life of banana chips. The shelf-life of apple chips can likewise be determined by knowing the moisture sorption isotherm and the effects of different

water activities on the chemical and physical properties.

Effect on Water Activity on Chemical and Physical Stability

Ascorbic Acid

Most of the work related to the influence of a_w on the nutritional composition of foods has been concerned with ascorbic acid. The results have shown that this vitamin is relatively stable at low a_w levels.

Karel and Nickerson (1964), Jensen (1967), Vojnovich and Pfeifer (1970) and Lee and Labuza (1975) have studied the stability of reduced ascorbic acid in various low and intermediate moisture foods and model systems as a function of moisture content, water activity and storage temperature. Results reported by these investigators showed that the rate of destruction of reduced ascorbic acid increased as the total moisture content and water activity increased. Lee and Labuza (1975) have interpreted the increase in destruction rates to be the result of dilution of the aqueous phase which resulted in a decreased viscosity and thus increased mobility of reactants. But above a certain moisture content, the aqueous phase viscosity would not be expected to change very much so the rate of destruction would approach a constant value. There is a greater loss in the desorption systems than in the adsorption systems.

Kinetics data by Lee and Labuza (1975) and Kirk et al. (1977) reported that the loss of total and reduced ascorbic

acid followed the first order reaction. They found that the activation energies for a_w equal to or above the monolayer moisture content ($a_w \geq 0.24$) were not significantly different for destruction of ascorbic acid. Thus the mechanism of the oxidation of ascorbic acid was not considered to be changed as a_w increased. But Kirk et al. (1977) reported that the activation energies at a_w of 0.10 for total ascorbic acid and reduced ascorbic acid to be significantly different from the activation energies for the other a_w levels. These activation energies were interpreted to describe a possible change in the mechanism of ascorbic acid destruction under these storage conditions. Although the greatest stability for total ascorbic acid was observed on model system at low storage temperature and water activity below $20^{o}C$ and 0.24 respectively, Kirk et al. (1977) implicated from their experimental rate constants that dissolved oxygen content was a primary factor in the storage stability of ascorbic acid in dehydrated low and intermediate moisture foods of neutral pH. Karel and Nickerson (1964) reported that all the absorbed water, however, including that adsorbed in a monolayer, appeared to be available for the reactions resulting in destruction of ascorbic acid in orange crystals. Their results suggested that reduction of moisture content to the lowest possible content was necessary for prevention of ascorbic acid losses and that oxygen had little effect on the destruction

of ascorbic acid in orange crystals. This indicates that ascorbic acid decomposition may proceed at and below the BET monolayer.

The preservation of ascorbic acid in foods for extended periods, therefore, requires that the food be stored at low temperatures and at low a_w levels as are practicable.

Total Phenolics

Enzymatic discoloration of fruits is due to the action of a copper-containing enzyme complex on phenolic substances in the tissues of fruit. Walker (1962) reported that the major browning substrate of apple was chlorogenic acid. Only a few phenolic compounds besides chlorogenic acid and l-epicatechin, have been identified as apple polyphenol oxidase substrates. Shannon and Pratt (1967) found that esculetin and dihydroquercetin to be substrates for apple polyphenol oxidase.

Acker et al. (1967a) studied a polyphenol oxidase preparation from potatoes, using cathechol as the substrate. The oxidation was followed by measuring transmittance-color change of the mixtures. They found that as a_w levels increased there was a corresponding decrease in percent transmittance. They concluded that water serves both as a medium for the enzyme reaction and as a vehicle for the substrate.

Optimal and minimal a_w levels for the activity of polyphenol oxidases have not been determined in model

systems or in foods (Troller and Christian, 1978). Acker (1962) cited a number of findings indicating that this class of enzymes is relatively inactive in dried food products in the 5 to 12% moisture range. Generalizations on the effects of a_w limitation on enzyme-substrate reactions are hazardous because reaction rates also depend on the degree of enzyme binding to the substrate and the nature of the substrate.

Total Volatiles

Many measurements have been made of volatiles retention as a function of time during rehumidification of freeze-dried substances (Flink, 1969; Flink and Karel, 1970a, 1972; Chirife et al., 1973). Results typically show that rehumidification at relative humidities below 30% gives very little volatiles loss, yet volatiles loss is substantially complete over a few hours at relative humidities above 70%. Rehumidification at moderate relative humidities can give substantial but not total volatiles loss, with the rate loss becoming very small and perhaps zero after an initial period of more rapid loss.

Two basic mechanisms have been proposed for the interpretation of volatiles loss and retention. One is based upon selective diffusion analysis (Menting et al., 1970b; Chandrasekaran and King, 1972b). The other is the concept of microregion entrapment in which volatile

compounds are immobilized within cages formed by association of molecules of dissolved solids, such as by hydrogen bonding of carbohydrate molecules (Flink and Karel, 1970a, b, 1972; Chirife and Karel, 1973b, 1974a,b; Chirife et al., 1973; Kayaert et al., 1975). Volatiles loss during rehumidification occurs more slowly than does water uptake. Flink and Karel (1970a,b, 1972) found that the seeming asymptotic retention of flavor volatiles at longer times for moderate relative humidities can be attributed to break-up of some, but not all, of the micro-region cage bonds, releasing some of the molecules of the volatiles material but keeping other molecules of the same compound immobilized. Chirife and Karel (1974a,b) further showed that at a constant moisture content in the freeze-dried solid, the loss of volatiles would not occur unless some critical temperature was exceeded. These authors have implicated "collapse" (i.e. loss of structure) as the determining factor in the release of encapsulated materials during rehumidification or exposure to high temperatures.

The selective diffusion and micro-region entrapment concepts are macro and microscale interpretations of the general phenomenon of volatiles retention in freeze-dried foods (King, 1971; Flink, 1974). The selective diffusion approach is a potentially quantitative mathematically formulated model based on transport concepts, while the micro-region entrapment approach is more qualitative and is based

upon molecular concepts (Omatete and King, 1978). They investigated the relationship of the rate characteristics of "collapse" to the rate characteristics of volatiles loss through the selective diffusion concept in terms of the influences of these variables on the dimensionless Fourier group Dt/L^2. Their results showed that with the resulting increase in volatiles loss, there was an increased value of Fourier group.

To and Flink (1978) through other observations of the state of the freeze-dried materials, studied the state of the freeze-dried materials after "collapse" from a practical standpoint. They concluded that it was probably not "collapse" per se which caused the loss of entrapment ability but rather some additional changes in the structure of the material. They then related the "collapse" and recrystallization behavior to volatiles loss and found that recrystallization of matrix solutes following "collapse" was a major cause of loss of entrapment ability in the dry state during rehumidification or exposure to temperatures above critical levels.

Texture

Texture of food is very important in its acceptance and rejection. Evaluation of the effect of relative humidity or a_w level has met with the difficulties of objectively quantifying texture. In spite of the limitations of

textural measurements, a number of studies have shown convincingly that the moisture condition of dried and semi-dried foods plays an important role in determining texture.

Kapsalis (1967) found that increases in equilibrium relative humidity of intermediate moisture foods above the BET monolayer of water (20% R.H.) to a moisture level of 66% produced increases in both hardness and cohesiveness of most samples.

Heldman et al. (1972) used the Instron Universal Testing Machine to determine hardness and chewiness of pre-cooked and freeze-dried beef. They found that the hardness and chewiness of the samples increased as the relative humidity was increased until a moisture content in the 40 to 50% R.H. range was reached. Further increases in moisture decreased both hardness and chewiness. Results for these parameters were similar at 80% and 0% R.H.

Sonido et al. (1977) related the effect of moisture level to the crispiness of fried banana chips via the use of organoleptical test. For plain banana chips they found that at moisture levels of 0.78 to 1.37% ($a_w \leq 0.25$), the chips were very crispy; at 2.2% ($a_w < 0.38$) - crispy; at 3.1% ($a_w < 0.5$) slightly crispy; at 0.45 to 0.65% ($a_w < 0.7$) - not crispy; and at 9.1% ($a_w \geq 0.7$) - wet.

Rheological scientists and instrument manufacturers have developed machines to provide data on such parameters as toughness, crispiness, bioyield strength, etc. According

to Troller and Christian (1978) the most meaningful data relating moisture to texture have been drawn from studies with dried and semidried meat.

A practical approach is to observe the sample during the deformation test. This direct sample observation can provide much insight into the interpretation of texture measurements. The influence of moisture content or a_w on the perception of food texture is obvious. Chemical reactions, hydrogen bonding, colloidal aggregation and changes in hydration among different sorptive groups are some of the possible mechanisms for structural changes in freeze-dried materials during rehumidification.

EXPERIMENTAL PROCEDURE

Preparation of Freeze-dried Apple Slices

Apples of the variety "Schone van Boskoop" were sliced in 2 mm thichnesses with an electrical cutter. The untreated 2 mm slices were cored, frozen in liquid nitrogen for 15 secs and freeze-dried for 24 hours in a "Secfroid"[a] freeze-dryer under the following conditions.

	Start	Finish
Platen temperature	- 15°C	30°C
Chamber vacuum	0.2 Torr	0.05 Torr
Condenser temperature	- 60°C	- 80°C
Conditioner temperature	- 30°C	20°C
Vacuum pump	0.1 Torr	0.05 Torr

Analytical Procedures

Fresh apples were tested for organic acids, sugars and total volatiles. After freeze-drying, samples were tested for moisture, ascorbic acid, total phenolics and total volatiles. The freeze-dried apple slices after two weeks of equilibration in the different water activities at 25°C were measured for moisture sorption isotherm, ascorbic acid, total phenolics and texture. For extended storage another lot of

[a]Freeze-dryer - Secfroid 1024 CH Ecublens - Lausanne, Suisse.

samples were stored at a_w = 0.22 - 0.24, 0.43 - 0.45, 0.61 - 0.70 at 20 - 25°C and ascorbic acid, total phenolics and total volatiles were measured.

Moisture Sorption Isotherm

The moisture adsorption isotherm at 25°C of freeze-dried apple slices was determined gravimetrically by exposing the samples to atmospheres of known relative humidities.

Saturated salt solutions were used to obtain standards of prescribed water activity levels as reported by Rockland (1960) and Gall (1967). Separate desiccators were used for the different equilibration salt solutions. Freeze-dried apple slices were then allowed to equilibrate in the salt atmospheres for two weeks. The moisture content of the equilibrated samples was determined by Karl Fischer titration. Water activity of the samples was measured by an electrolytic hygrometer.

Moisture

The chemical method of Karl Fischer titration was employed (A.O.A.C., 1975). Material used were:

Kark Fischer Reagent (Merck)

Water-free Methanol (Merck)

Sodium Tartrate Dihydrate (Merck)

The Karl Fischer reagent was standardized against Sodium Tartrate Dihydrate which has 15.66% water. A blank was

determined against the solvent water-free methanol. A 200-mg sample of freeze-dried apple slices was dispersed in 10-ml of water-free methanol and electrometric titration was carried out. The presence of excess iodine, detected potentiometrically indicated the end-point of the reaction. Triplicates were done on each sample. Results were reported as percent of moisture on 100 g dry weight.

Water Activity

In the Sina-scope apparatus[a] the electrolyte is Lithium Chloride solution. The operation of this instrument requires that an alternating current be passed through this saturated Lithium Chloride solution which is suspended onto an inert carrier. The measuring cell is made up of two resistances sensitive to relative humidity. The resistances are connected in a bridge with one resistance as a reference and the other is brought in contact with the relative humidity of the sample to be measured. Heat is applied to the cell and eventually the water vapor pressure (w.v.p.) of the Lithium Chloride solution is equal to the w.v.p. of the sample. The direct reading of water activity can be read from the dial indicator.

Ascorbic Acid

The ascorbic acid level in the freeze-dried apple slices was evaluated according to the A.O.A.C. method (1975).

[a]Sina Instrument, Zurich, Switzerland. AG type: SMT - D, Nr. 7-0113.

Ascorbic acid was extracted from 2-3 g sample with a metaphosphoric acid - acetic acid solution and titrated with 2, 6, dichloroindophenol sodium salt solution till a faint pink persisted for 30 sec. Results were reported as mg ascorbic acid per 100 g of dry weight so that the ascorbic acid levels retained are comparable at the different water activities.

Total Phenolics

The method for extraction of total phenolics in the presence of reducing agents according to Khanna et al. (1968) was used.

a. Acetone Extraction in the Cold

Four to five gram samples of freeze-dried apple slices were extracted in a Sorvall mixer with 25 ml of acetone containing cysteine chloride as a reducing agent. Twenty-five ml of 50% acetone was used for each of the first two extractions. The residual solid was extracted twice with two 25 ml aliquots of anhydrous acetone (cysteine chloride was not added). Finally the residue and container were rinsed with acetone. The total volume of the extract was about 140 ml and contained 4×10^{-6} moles of cysteine chloride.

Total phenolics content in every extract was determined in suitably diluted samples, with the Folin-Denis reagent using tannic acid as standard (Goldstein, 1963).

b. Determination of Total Phenolics as Tannic Acid

Five milliliters of the total extract were diluted to 100 ml with distilled water. To 2 ml of the diluted sample solution 2 ml of 10% Folin-Denis reagent (complex phosphotungstomolybdate) were added followed 3 min later by 2 ml of 1 N sodium carbonate. The absorbance was read 20 min later against a blank at 725 nm wavelength. The concentration of cysteine chloride at this stage was about 4×10^{-8} moles which was sufficient enough to prevent oxidation in the sample solution but not enough to give a color to Folin-Denis reagent. From the standard curve for tannic acid, total phenolics were reported as mg tannic acid per 100 g dry matter.

Total Volatiles

A simple method for the analysis of apple volatiles was developed by Feys et al. (1977). This is a modified vacuum cold trap distillation in which the aqueous solution of volatiles is ready for direct GLC analysis.

a. Preparation of Sample

Thirty grams of freeze-dried slices were homogenized in 270 ml of distilled water and 15 ml of 4% TCA (trichloracetic acid) in a Sorvall mixer. The TCA prevented the formation of volatile aldehydes during homogenization of the apple tissue. Two milliliters of antifoaming agent were added to the mixture after blending.

b. Vacuum Cold Trap Distillation

The jar containing the apple pulp was gently heated to prevent cooling as a result of solvent evaporation under vacuum. A stream of nitrogen (15 ml/min) was passed through the apple pulp. Between the jar with the apple pulp and the cold traps with liquid nitrogen, a cooling spiral was placed, cooled to -17°C by means of a cryostat (Haake, KT 33) with methanol as cooling liquid. This was to trap and condense the water. Finally, the whole system was connected to a vacuum pump (Cenco Hyvac 7). The mean volume of the distillate was 16 ml ± 4 ml and the distillation time was one hour.

c. GLC Analysis

This was carried out using a Hewlett-Packard 5720 A instrument with flame ionization detector, connected to a Hewlett-Packard 3380 A integrator. The column used for the identification of the volatiles was a 20% D.E.G.S. on chromosorb P-AW, 60-80 mesh, 20' x 1/8", SS. Column temperatures were programmed at 90 and 110°C, with a N_2 flow of 9 ml/min.

Texture

The Universal Texture Machine Wolpert Amsler, Machine TZM with a maximum force of 10 KN (kilo-newton) was used to measure the texture of freeze-dried apple slices after their equilibration at the different relative humidities.

The 2 mm thick apple slices were placed over a hollow base with a diameter of 8.5 mm which was centered under a 100 N load consisting of an 8 mm diameter puncher. With a load speed of 25 mm/min the puncher ruptured or deformed the apple slice. Energy required to rupture or deform the slices was recorded on a 10-volt full scale chart with a chart speed of 200 mm/min. Readings for each sample were obtained in triplicate. The area under the curve up to the bioyield point was measured with a planimeter in cm^2. The bioyield point is a point on the force-deformation curve at which there occurs an increase in deformation with a decrease or no change in force. This area (cm^2) is directly related to the energy of force required to rupture or deform the apple slice. The greater the area the greater the energy or force required to rupture or deform the apple slices.

Organic Acids and Sugars

The method of Heatherbell (1974) was used for the separation, identification and quantitative analysis of fruit sugars and non-volatile organic acids.

a. Extraction and Separation of Fruit Sugars and Acids

Twenty five grams of apple were blended for five min in a Sorvall mixer with 150 ml of 80% ethanol. To a 5 ml aliquot in a 50 ml centrifuge tube were added 10 mg of tartaric acid, 1 ml of saturated neutral lead acetate solution and 10 ml of 85% ethanol. After mixing thoroughly

the extract was allowed to stand for 45 min and centrifuged. The supernatant contained the sugars, the remaining precipitate contained the acids as lead salts. The tartaric acid which was added was the internal standard for the organic acids. The precipitate containing the acids was then washed twice with 80% ethanol and once with diethylether. The residual ether was evaporated before oven-drying. The dried precipitate was powdered with a spatula and transferred to a vial for silylation. Inositol (0.1 ml of 0.2% w/v) was added to the supernatant containing the sugars as an internal standard. The supernatant was then partitioned between 120 ml of chloroform - methanol - water, 8:4:3 (v/v). Only trace amounts of sugars were detected in the lower methanol - chloroform layer and this was therefore discarded. The methanol - water layer was made up to 100 ml with 80% ethanol. A 1 ml aliquot was used for silylation.

b. Conversion to Trimethylsilyl Derivatives

One milliliter of pyridine, 0.5 ml M.S.T.F.A. (N-methyl-N-trimethylsilyl-trifluoroacetamide), 0.2 ml T.M.C.S. (chlortrimethylsilan) were used. The total contents were heated at 80^{o}C for 30 min.

c. GLC Analysis

The trimethylsilyl derivatives were analyzed on a Varian 1800 gas chromatograph, with a 1.5% SE-30 + 1.5%

SE - 52 U- AW DMCS column, 80 - 100 mesh, 3 m x 3 mm, with a temperature program from 100 to 230^{o}C (4^{o}C/min).

RESULTS AND DISCUSSION

The Sorption Isotherm of Freeze-dried Apple Slices

The equilibrium moisture content at different relative humidities with their standard deviations are presented in Table 3. These data were used in plotting the moisture sorption curve shown in Figure 1.

The sorption isotherm of a food material is best described as a plot of the amount of water adsorbed as a function of the relative humidity or activity of the vapor space surrounding the material at a constant temperature.

$$\text{Activity} = a_w = \frac{P}{P_o} = \% \frac{\text{Relative Humidity}}{100}$$

where P = water vapor pressure exerted by the food material

P_o = vapor pressure of pure water at temperature T_o

T_o = equilibrium temperature of system

There are five types of adsorption isotherm in the classification of Brunauer, Deming, Deming and Teller (BDDT), also known as the Brunauer, Emmet and Teller (BET) classification (Brunauer et al., 1935). The sorption isotherm of a typical food material is sigmoidal in shape and is classified as type II in the BET classification.

This general sorption isotherm has 3 regions as discussed by Labuza (1968). Thus, in theory, the course of water sorption by a dry material is first by the formation of a monolayer, followed by multilayer adsorption and condensation in pores (capillary effects), followed by dissolution of soluble components. These 3 regions may overlap in the sorption isotherm and will differ in magnitude among foods depending upon chemical composition and structure.

The effect of composition of a food on the shape of the sorption isotherm has been studied by Salwin (1963), who found that for starchy foods like potatoes, beans, corn and flour, the sorption isotherm has a sigmoidal shape. These starchy foods have the greatest water holding capacity in the low relative-humidity region.

Protein foods - meat, fowl, eggs, fish and milk have a lower water-holding capacity. Beef has approximately 75% protein 20% fat and only 1% carbohydrate (on a dry basis) and its moisture sorption properties change with temperature. As the temperature increases from 40 to 100^{o}F, the isotherm becomes progressively less sigmoidal and more convex toward the vapor-pressure axis.

Green peppers or group of foods that have high sugar content as well as high-molecular weight constituents have moisture sorption curves sigmoidal in shape at 40^{o}F. But at higher temperatures the solution effects of the sugars

predominate and the curve becomes more convex toward the vapor-pressure axis, except at very low vapor pressures.

The peach is a typical high-sugar item, with 67% sugar, 25% other carbohydrate and 4% protein and has a sorption curve which is entirely convex toward the vapor-pressure axis, which is typical of high-sugar items. Silvia and Chirife (1979) also came to the same conclusion for freeze-dried apple slices. "Boskoop" apples have about 77% sugar (refer to Table 1) and the relationship between water activity and equilibrium moisture content at 25°C is illustrated in Figure 1. It has the characteristic shape of high-sugar food isotherms. But Karl and Nickerson (1964) found that the water sorption for orange crystals to have a sigmoidal shape and that this corresponded to the data on adsorption of water by sugars and pectin, which are also the major components of the orange crystals. It seems then that not only chemical composition but the structure of the dried materials has a bearing on the sorption isotherm curve.

The convex-shaped sorption isotherm of high-sugar item like freeze-dried apple slices, corresponds to the type III BET classification. The sigmoidal-shaped sorption isotherm which is typical of starchy products is type II in the BET classification. And by applying the BET theory of monolayer adsorption which is the safe moisture limit, Salwin (1963) found the monolayer moisture value for these products to be

Table 1. Sugars and non-volatile acids of 'Schone van Boskoop' Apples

Sugars	g/100 g.d.wt.	Acids	g/100 g.d.wt.
Fructose	36.42	Phosphoric	0.12
Glucose	18.09	Maleic	8.18
Saccharose	23.09	Citric	0.25
		Ascorbic	0.10
		Quinic	0.28

Table 2. Chemical composition of freeze-dried apple slices before equilibration

Components	Amount
A_w	0.22
Moisture	4.30% d.wt.
Ascorbic Acid	33.65 mg/100 d.wt.
Total Phenolics as mg Tannic Acid	1620.90 mg/100 g.d.wt.

Table 3. Sorption curve data

A_w	% Moisture on Dry Weight	Standard Deviation
0.14	0.98	0.013
0.21	2.40	0.680
0.26	5.00	0.035
0.32	7.75	0.084
0.43	10.75	0.007
0.53	14.25	0.148
0.64	22.25	0.145
0.74	34.80	0.205
0.76	42.50	0.705
0.81	49.50	0.401
0.86	70.50	0.030

approximately 6% (approximately 15% RH). For freeze-dried apple slices the moisture content is approximately 1.0% at 15% RH. The moisture content exerts a high vapor pressure, even at very low percentages, and is available for deteriorative reactions. The BET equation is then not applicable for the type III BET classification. Complete dehydration is then essential for good stability in freeze-dried "Boskoop" apple slices.

The Effect of a_w and Time on Ascorbic Acid of Freeze-dried Apple Slices

The percent of ascorbic acid retained in the freeze-dried apple slices after 2 weeks of storage at 25°C for the different a_w is shown in Figure 1. The maximum retention seemed to be at a_w = 0.19 and 0.31 with the minimum retention at a_w = 0.66. The dip in percent of ascorbic acid retention at a_w = 0.23 could be due to other factors besides a_w. The exact route of ascorbic acid degradation is highly variable and dependent upon the particular system. Some factors which can influence the nature of the degradation mechanism besides water activity include sugar concentration, enzymes, initial concentration of ascorbic acid, and the ratio of ascorbic acid to dehydroascorbic acid (Fennema, 1976).

Destruction of ascorbic acid as a function of water activity has been studied based upon both kinetic and

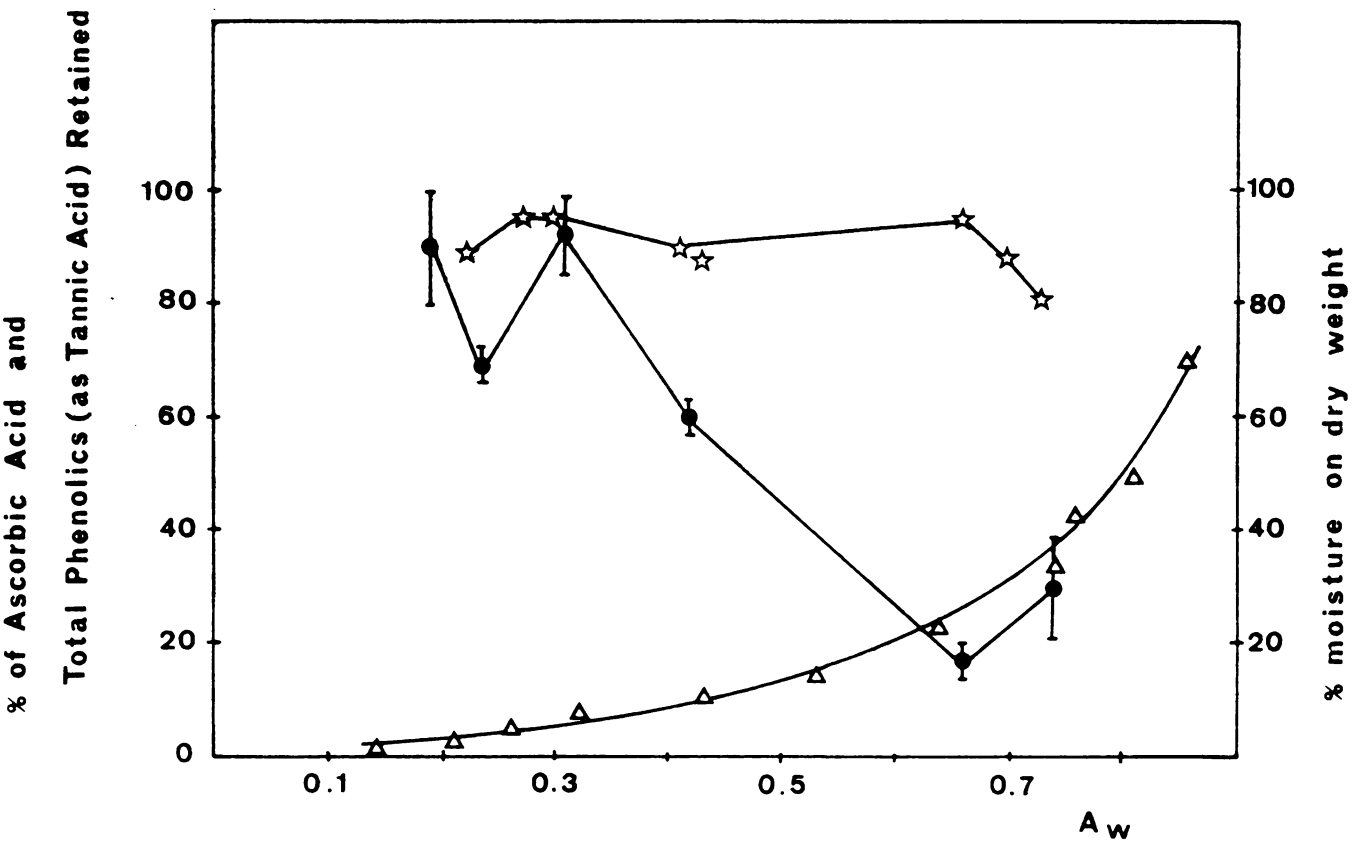

Figure 1. The relationship of moisture sorption isotherm curve of freeze dried apple slices △; with percent retention of Ascorbic Acid ●; and total Phenolics (as tannic acid) ☆ after two weeks equilibration at 25°C.

physicochemical measurements. Many of these studies have been conducted in model systems with a pH less than 2 or in high concentrations of organic acids and therefore may not duplicate the exact pattern in a particular ascorbic acid-containing food product.

It seemed from Figure 1 that at a_w = 0.23 the limiting factor for ascorbic acid destruction is not only a_w and moisture content. It may well be the initial concentration of ascorbic acid or the ratio of ascorbic acid to dehydro-ascorbic acid. Ascorbic acid exists, as the reduced form and oxidized form (dehydro-ascorbic acid). The oxidized form is readily reduced to ascorbic acid and thus has essentially the same activity as ascorbic acid. The analytical procedure used here did not take into account the dehydro-ascorbic acid.

At a_w 0.31 to 0.66 the percent loss of ascorbic acid increases with increasing water activity and moisture content. This effect is due to an increase in water content which increases the mobility of the reaction species in the aqueous phase which also becomes significantly less viscous and diffusion is enhanced (Lee and Labuza, 1975). Also phenolases can oxidize ascorbic acids in the presence of an appropriate substrate (Joslyn & Ponting, 1951).

Above a critical moisture level, as in this case a_w = 0.66, water may act to dilute the concentration of ascorbic acid, thereby reducing the percent loss of ascorbic acid as

can be seen in Figure 1 for a_w = 0.74.

The time effect during storage at 20 - 25°C for the different water activities on the percent of ascorbic acid retained is shown in Figure 2. The best retention is in the a_w = 0.22 - 0.24 where 70% of ascorbic acid is retained after 122 days. For a_w = 0.43 - 0.45 and a_w = 0.61 - 0.70 the loss of ascorbic acid is 50% after 20 days. The destruction is complete at approximately 50 days for a_w = 0.61 - 0.70, whereas the retention at 43 - 45% RH is approximately 30% at 60 days and complete destruction comes at approximately 80 days.

The greatest ascorbic acid losses occur at the highest water activity during storage.

The Effect of a_w and Time on Total Phenolics of Freeze-dried Apple Slices

The percent of total phenolics retained in the freeze-dried apple slices after 2 weeks of storage at 25°C for the different water activities is shown in Figure 1. The percentages lie within the 80% to 95% range. Acker et al. (1967a) showed that in a model system of polyphenol-oxidase-cellulose-catechol, the enzyme activity increased with increasing water activity. In real systems this may not be so simple. In Figure 1 the total phenolics retained are less at a_w = 0.22 than at a_w = 0.27 and 0.3.

Freeze-dried apple slices have a porous structure which leads to a greater susceptibility to oxygen action.

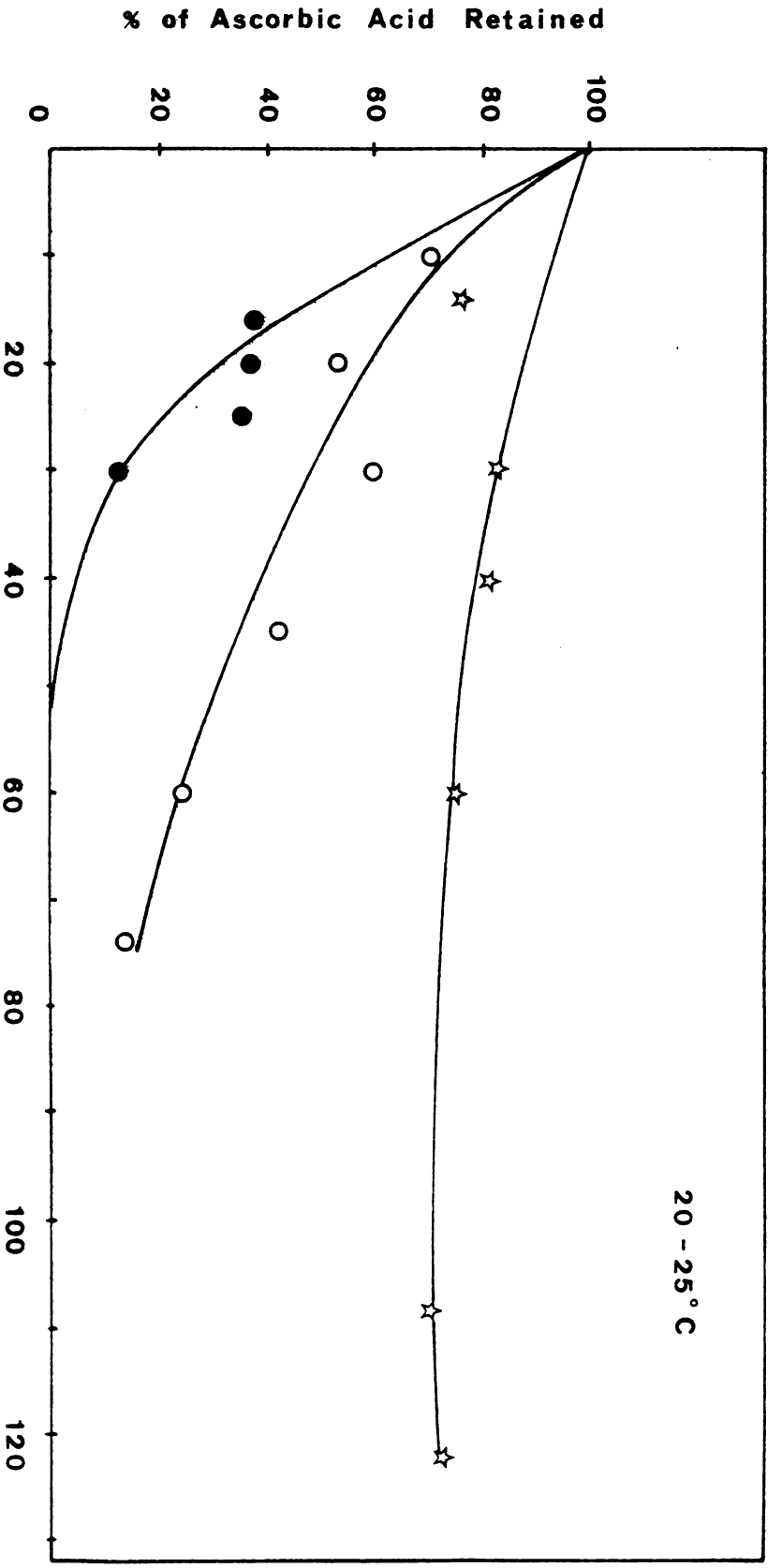

Figure 2. Percent of Ascorbic Acid retained at a_w = 0.22 - 0.24 ☆; a_w = 0.43 - 0.45 o; a_w = 0.61 - 0.70 ●, with storage time.

Enzymatic systems like polyphenol oxidase are oxygen dependent in their reactions. At a_w = 0.22, 88% of total phenolics are retained compared to 95% at a_w = 0.27 and 0.30. This could be due to the higher diffusion of oxygen into the more porous structure at a_w = 0.22, thus resulting in a lower percent of total phenolics. The colorless quinones formed at this stage may then be reduced to the dihydroxyl state, probably by ascorbic acid which is concurrently oxidized to dehydroascorbic acid. This could account for the dip of the curve of ascorbic acid at a_w = 0.23 in Figure 1.

At a_w = 0.3 to 0.66 the percent of total phenolics differ by 5% only. There is not much enzymatic reaction in this range, probably due to some inhibitory action of ascorbic acid. As can be seen ascorbic acid decreases from 92% to 17%. No browning occurs at this stage after a 2-week storage because the colorless quinones formed have not reached the indole, 5, 6 quinone stage.

At a_w = 0.7 and above there is a decrease in percent of total phenolics retained. Here it seems that the ascorbic acid is not effective or has lost its ability to act as an antioxidant due to the dilution effect at this water activity and moisture content. On the other hand, the moisture content at this water activity may serve as a medium for the enzyme reaction and as a vehicle for substrate mobility. There is some slight browning of the apple slices here.

The time effect during storage at 20-25°C for the different water activities on the percent of total phenolics retained is shown in Figure 3. At a_w = 0.61 - 0.70 the enzymatic reaction is greatest. This is expected since water acts as a medium for substrate and enzyme mobility. At a_w = 0.43 - 0.45 lesser enzymatic activity occurs than at the higher relative humidity. A maximum color development occurs at 30 days. The higher relative humidity has a slightly darker reddish-brown hue than the lower relative humidity. The total phenolics levels off after 30 days of storage. This may be due to the fact that the substrates for polyphenol oxidase in the apple slices are exhausted or that the enzyme activity decreases during storage. It may also be due to end product inhibition of the enzymatic reaction which prevents further enzyme activity.

In the lowest water-activity there is less loss in total phenolics during the same period of storage. The moisture content here is low to slow down enzymatic reaction. Nevertheless, minor activity could revive during long-term storage. At 122 days the total phenolics drop to 73%. No browning occurs in these apple slices. This could be due to the 70% retention of ascorbic acid at this stage where the ascorbic acid could act as an antioxidant.

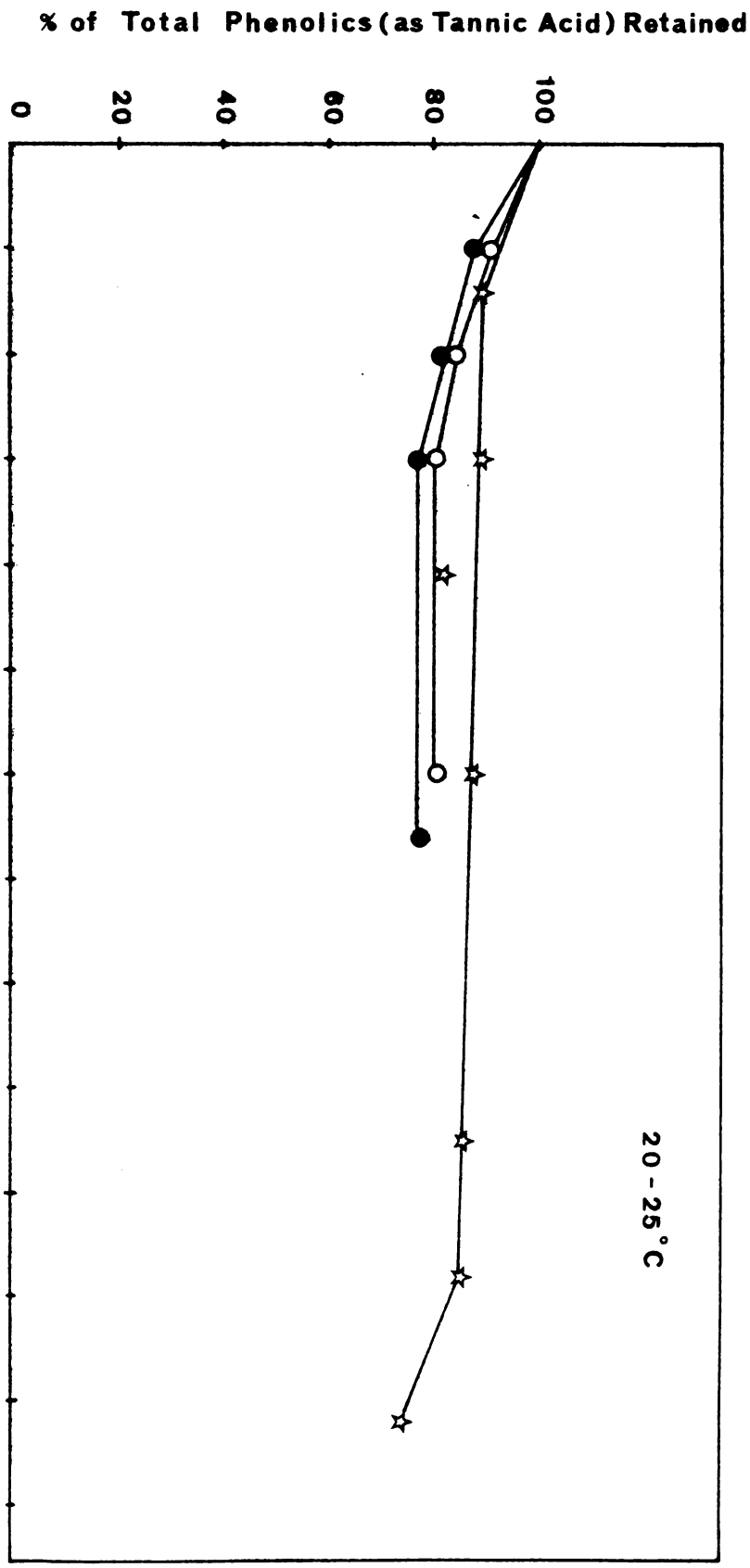

Figure 3. Percent of total phenolics (as tannic acid) retained at a_w = 0.22 - 0.24 ☆; a_w = 0.43 - 0.45 ○; a_w = 0.61 - 0.70● with storage time.

The Effect of a_w and Time on Total Volatiles of Freeze-Dried Apple Slices

Feys et al. (1977) identified the volatiles of "Schone van Boskoop" by using different GLC columns and known compounds as standards. Their results showed that "Boskoop" is a typical alcohol type variety because more than 90% of the total amount of volatiles are alcohols.

There is a considerable loss in volatiles after freeze-drying as shown in Table 4. Acetone seemed to have the highest retention. On the whole flavor volatiles retention in freeze-dried products is much better than other methods of dehydration. Kayaert et al. (1975) and Thijssen et al. (1968, 1969) have studied flavor, retention in freeze-dried substances in detail and have developed the theories of micro-region entrapment and selective diffusion respectively.

Gas-liquid chromatograms of fresh and freeze-dried apples (A_w=0.22-0.24) with respect to their volatile compounds are shown in Fig. 4. It may be seen that there were no new volatiles formed as a result of the freeze drying treatment. The data indicated that freeze-dried apple slices possess an aroma and flavor profile comparable to that of fresh apples.

Flink and Karel (1972) and Chirife, Karel and Flink (1973) found that exposure of freeze-dried substances containing trapped volatiles, to various levels of relative humidity would give decreased volatile content, which was

Table 4. Comparative analysis of total volatiles of fresh and freeze-dried apples.

Components	Fresh µg/100 g D. Wt.	Freeze-dried µg/100 g D. Wt.	% Retention
Diethylether	11.6	-	0
Acetaldehyde	2865.5	310.7	10.83
n-Propanal	107.4	-	0
Acetone	253.7	116.8	45.85
i-Propanol	229.1	52.2	22.71
n-Butanal	122.9	28.0	22.95
Ethanol	26380.0	5626.5	21.33
n-Propanol	443.8	132.4	29.80
Ethylbutrate	659.4	-	0
i-Butanol	723.3	175.7	24.20
n-Butanol	9385.5	2516.7	26.81
n-Pentanol	129.3	-	0
n-Hexanol	1348.0	458.4	33.98

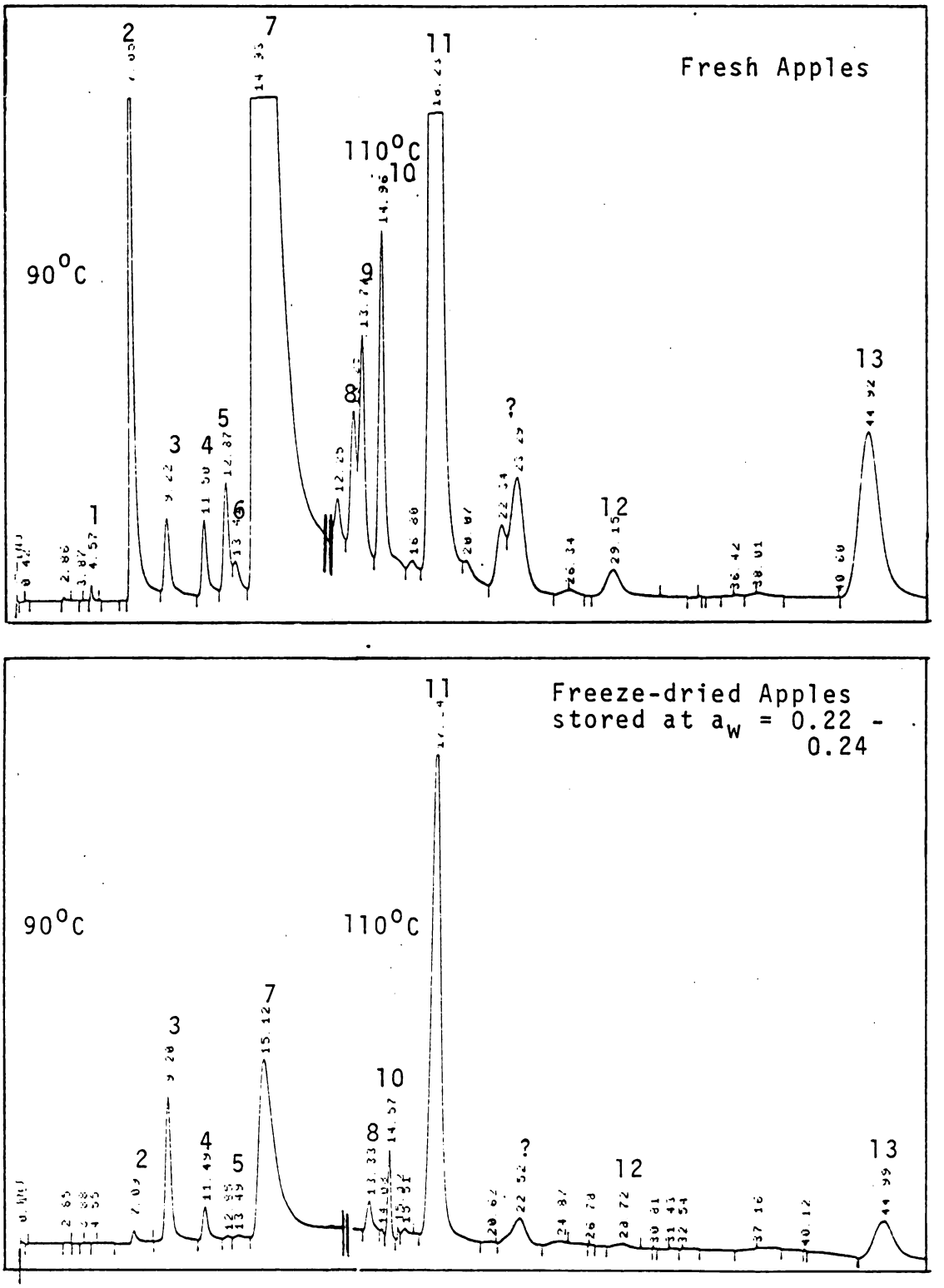

Figure 4. Volatiles of Fresh Apples and Freeze-dried Apples at a_w = 0.22 - 0.24 at 20 - 25°C for 41 days. 1) Diethylether, 2) acetaldehyde, 3) n-propanal, 4) acetone, 5) i-propanol, 6) n-butanal, 7) ethanol, 8) n-propanol, 9) ethyl-butrate, 10) i-butanol, 11) n-butanol, 12) n-pentanol, 13) n-hexanol, ?

lower as the relative humidity was increased. From Table 5, it seemed that the data agreed with their findings. As expected in the lowest a_w = 0.22 - 0.24 storage, the retention of volatiles was the best for the same period of time. The highest a_w = 0.61 - 0.70 had the highest loss in volatiles in terms of concentration (see Table 5).

After 30 days of storage at a_w = 0.43 - 0.45 there were complete loss of i-propanol, n-propanol and i-butanol. After 30 days storage at a_w = 0.61 - 0.70 there were no detectable levels of i-propanol or n-propanol left in the samples. At these two storage water activities the apple slices became sticky and the pieces fused at the surfaces with subsequent "collapse" or loss of structure (Tsourorflis et al., 1976; To and Flink, 1978b). These authors had implicated 'collapse' (i.e. loss of structure) as the determining factor in the release of the encapsulated materials such as volatiles

The Effect of Water Activity on Texture of Freeze-dried Apple Slices

The texture of apples is derived from the pectin, hemicelluloses, cellulose, pentosans and hexosans and their interactions and interconnections (Hulme, 1958). The behavior of these polysaccharides, which attract and retain water, is governed by the sorption isotherms.

Table 5. Comparative analysis of total volatiles of freeze-dried apple slices equilibrated at different water activities

Components µg/100 g D. wt.	A_w									
	0.22 - 0.24				0.43 - 0.45			0.61 - 0.70		
	Days									
	21	30	41	90	10	20	30	10	20	30
Acetaldehyde	147.0	58.1	175.5	97.7	-	-	-	47.9	47.4	44.8
n-Propanal	-	-	-	-	-	-	-	-	-	-
Acetone	202.0	136.4	95.9	204.9	494.7	214.4	410.5	163.3	239.4	99.9
i-Propanol	28.2	-	25.7	16.2	-	-	-	8.1	-	-
n-Butanal	-	-	-	34.2	37.9	-	21.3	-	-	-
Ethanol	2840.0	898.4	4855.5	1898.0	2857.5	5609.0	796.5	1636.0	1262.5	755.4
n-Propanol	98.3	-	123.0	184.0	69.5	40.6	-	-	-	-
i-Butanol	126.5	239.1	235.6	299.8	120.4	79.2	-	26.5	26.2	22.8
n-Butanol	1965.0	539.0	2606.0	1476.5	1497.0	1191.5	486.6	406.6	698.0	357.2
n-Hexanol	314.6	-	386.9	209.5	341.9	259.6	115.1	112.1	81.3	78.5

In this study puncture test were used to give a graphic representation of the texture, which ranged from brittle to rubbery as shown in Figures 5a and 5b. At a_w <0.25, moisture content is less than 5.0% and the apple slices are crispy. The structure of the slices is probably comparable to that of an amphorous dried matrix. The polysaccharide hydroxyl groups can form intermolecular hydrogen bonds in the absence or presence of low polar solutes. This dried matrix does not require much energy to cause a rupture point on the apple slice.

At values of a_w between 0.25 <a_w <0.4, the increased layers of water molecules form hydrogen bonds with the hydroxyl groups of the polysaccharides. These layers of water molecules adjacent to the hydroxyl groups of polysaccharides are therefore partially ordered and immobilized. Formation of this atmosphere of associated water assists in dissolving or dispersing some of the large molecules of polysaccharides and this rehydration gives a viscous texture to the apple slices. This could account for the increased amount of energy required to puncture the surface of the apple slice.

At a_w >0.74 and higher, the moisture content does not necessarily disrupt all the intermolecular hydrogen bonding among the polysaccharide molecules. This fraction then is the insoluble fraction which can be responsible for the rubbery texture. Epstein et al. (1969) referred to the

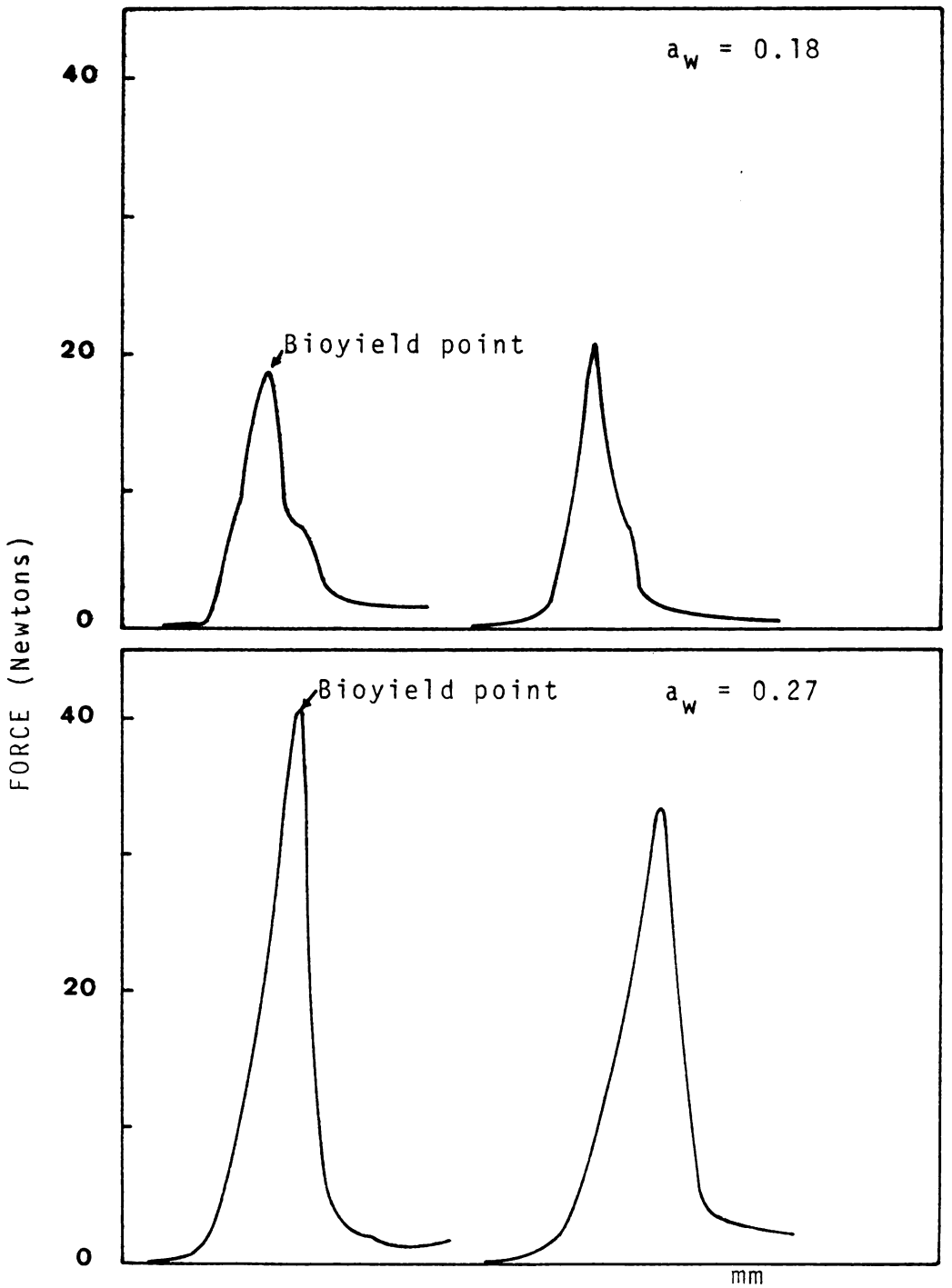

Figure 5a. Textural profile of freeze-dried apple slices at a_w = 0.18 and a_w = 0.27 for two weeks at 25°C.

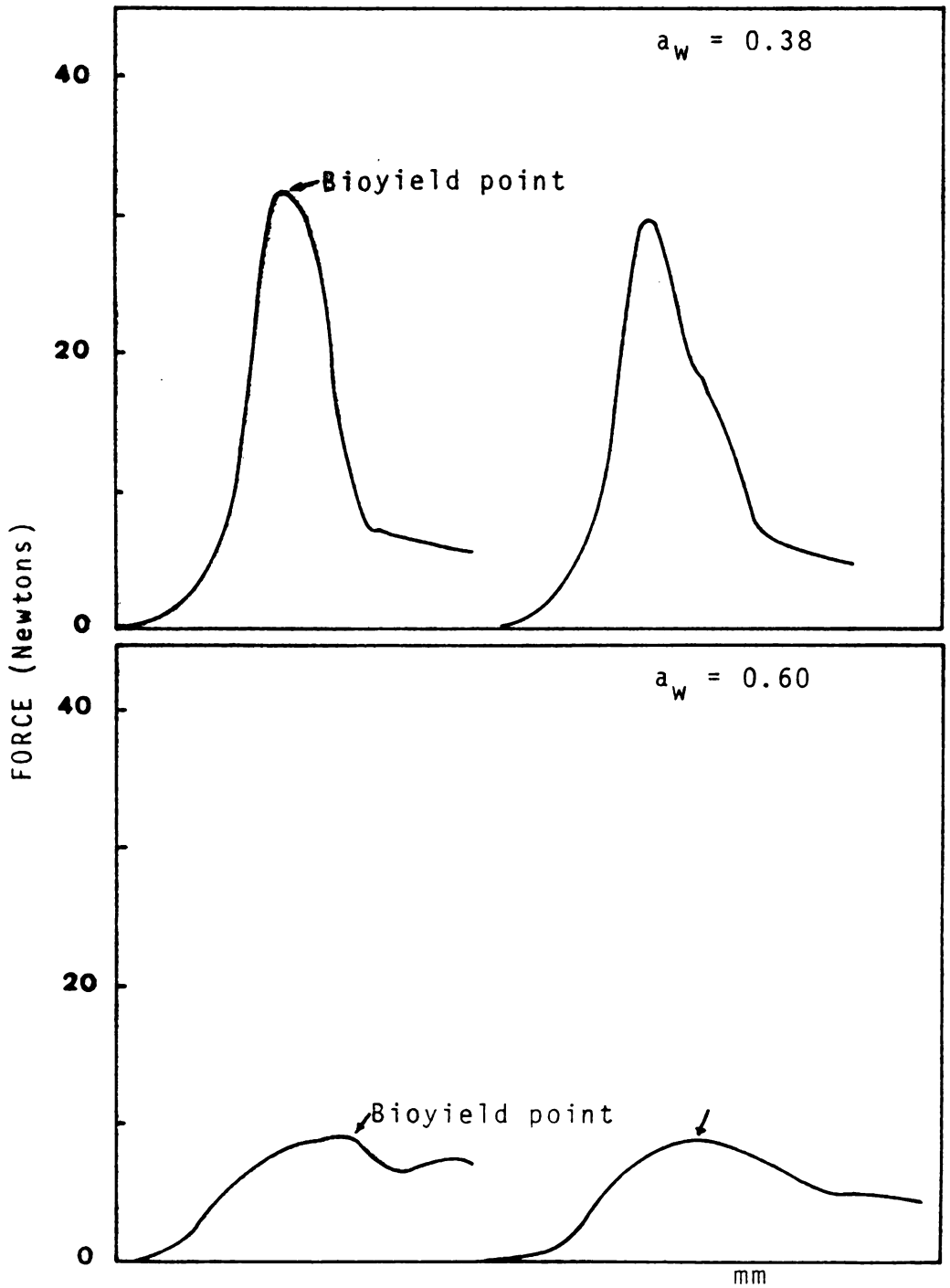

Figure 5b. Textural profile of freeze-dried apple slices at a_w = 0.38 and a_w = 0.60 for two weeks at 25°C.

insoluble fraction of polysaccharide as the cause of adhesion of dried apple slices. At this stage of hydration there is no indication of initial cell rupture in the apple slice. The force-deformation energy required here is the same as for the brittle apple slice (Figure 6) since a considerable plastic flow deformation takes place at a_w = 0.74 after the bioyield point. A bioyield point occurs on a force-deformation curve (Figures 5a and 5b) at which there occurs an increase in deformation with a decrease or no change of force.

For the preservation of textural quality the best storage condition is at a a_w of less than 0.25.

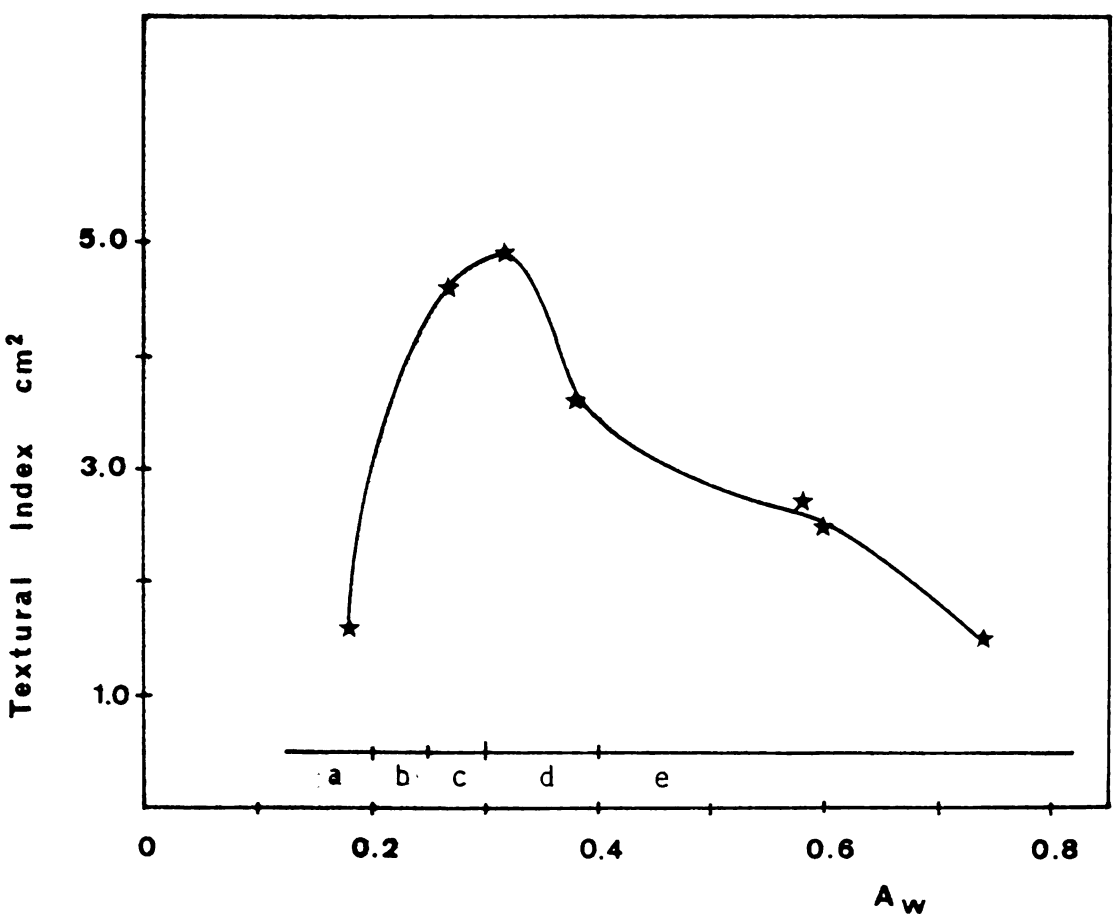

Figure 6. Texture of freeze-dried apple slices after two weeks equilibration at the different water activities at 25°C. Regions a = brittle; b = crispy; c = fairly crispy; d = soft; e = rubbery.

SUMMARY AND CONCLUSIONS

"Schone van Boskoop" apples were freeze-dried for 24 hours and the final moisture content was 4.30% on a dry basis. The freeze-dried apple slices were put in different desiccators with known relative humidities for two weeks for equilibration at 25°C. The moisture sorption isotherm was then determined. The curve was typical of one with high-sugar content because Boskoop apples had about 77% sugars. The BET theory does not apply to this type of sorption isotherm which corresponds to type III in the BET classification. The moisture here exerts a high vapor pressure even at very low percentages and is available for deteriorative reactions.

There was a correlation of ascorbic acid and total phenolics retention with water activities. For the same period of storage ascorbic acid content affected the total phenolics or vice versa. In the low a_w = 0.22 where the porosity of apple slices was conducive to oxygen diffusion, the drop in total phenolics caused a drop in reduced ascorbic acid. No browning occurred here because ascorbic acid was an effective antioxidant. As the ascorbic acid was degraded in the a_w = 0.3 to 0.66, the total phenolics changed very little. When the ascorbic acid was diluted

in the higher a_w = 0.7, the total phenolics decreased and there was some browning because the dilution effect on ascorbic acid made it ineffective in preventing browning. No browning occurred as long as ascorbic acid was effective as an antioxidant. During extended storage periods the greatest ascorbic acid losses occurred for the high a_w = 0.61 to 0.70. Complete destruction occurred at 30 days and reddish-browning occurred then as the loss of total phenolics leveled off. The same was true for a_w = 0.43 to 0.45, though the loss of ascorbic acid was slightly slower and total phenolics retention was slightly greater. Complete ascorbic acid destruction occurred at 80 days but the reddish-brown hue appeared at 30 days. The reddish-brown hue was a little less than in the higher water activity samples. Also the total phenolics levelled off. Once the brown color became apparent the total phenolics levelled off. This could be due to substrates exhaustion for the enzyme polyphenolase or the enzymatic end-products had an inhibitory effect on further enzymatic oxidation during prolonged storage.

The best storage was at $a_w \leq 0.24$ when the ascorbic acid degradation and total phenolics reactions were slow because no browning occurred and the flavor was good.

Freeze-dried apple slices just after freeze-dring lost 50% or more of the volatile components typical of Boskoop apples. Acetone had about 46% retention, n-hexanol

had about 34%, acetaldehyde had 11% and the rest of the alcohol-type volatiles and ethylbutyrate had an average retention of 25%. During storage the best retention of volatiles was in the a_w = 0.22 to 0.24. High losses of volatiles occurred at a_w = 0.61 to 0.70 and at a_w = 0.43 to 0.45 the retention was not much better.

The texture of freeze-dried apple slices at each different water activity were measured. At the low a_w = 0.2 the porosity of the slices caused the texture to be brittle. As water activity increased to about 0.3 there had been some solubilization of the components of the slices to cause them to be less brittle but crispy. As more moisture was absorbed, the slices became sticky, the texture was rubbery and the slices did not break but rather just deformed under the force applied to them. From this observation, it was concluded that the porosity of the cells in the apple slices gave a crispy texture.

For freeze-dried apple slices to maintain their quality and acceptable chemically and physically, they have to be kept at as low a water activity as possible. Complete dryness is essential for good stability of dried apple slices.

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