COMPOSITIONAL FACTORS AFFECTING THE MAILLARD REACTION IN COMMERCIAL DRIED SWEET WHEY BASED POWDERS DURING STORAGE

by

Patricia Rumrich Pfisterer

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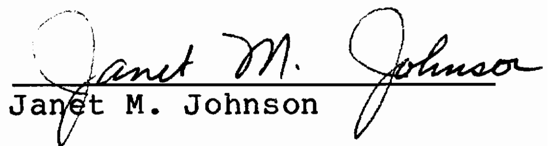
J. Russell Bishop, Chairman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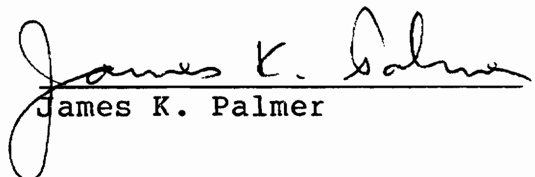
John W. Larkin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Committee Chairman: J. Russell Bishop
Food Science and Technology

(ABSTRACT)

With the increased utilization of whey powders in food products there is a need to investigate the storage stability of whey powders. The objectives were to determine the effects that compositional factors of whey powders had on the Maillard reaction during storage. The Maillard reaction is the major deteriorative reaction limiting the shelf-life of whey powders. Eight commercial whey powders were stored at accelerated storage conditions (35°C) and at five different water activities, a_ws, (0.32, 0.44, 0.52, 0.63, and 0.74) for up to 120 days.

Small quantities of the hydrolyzates of lactose- glucose and galactose (less than 2% dry wt.) caused a sizable increase in the relative rates of the Maillard reaction in whey powders. The relative rate of the Maillard reaction rate was determined by measuring brown pigment formation at 420 nm. Increasing the amount of nonprotein nitrogen (NPN) significantly increased the relative rates of the Maillard reaction in the whey powders. Increasing ash content had a positive but statistically insignificant effect on the Maillard reaction.

The influence of water activity on the storage stability of whey powders was also investigated. Increasing the protein content of the

whey powders increased the a_w where the maximum rate of brown pigment formation was observed. In the highest protein whey powders (46% and 82%) however, the browning rate maxima shifted to lower a_ws.

The loss in protein quality of the whey powders was determined by examining the loss of available lysine using the dye-binding method. Intermediate protein whey powders (31-40% protein) and unprocessed sweet dried wheys lost the greatest relative percentages of available lysine during the storage period.

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This thesis is dedicated to my parents, Peter and Hannelore Pfisterer

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INTRODUCTION

Whey powders are more susceptible to the Maillard reaction than dried milk powders, partially due to the increased amount of lactose in the whey powders (Ferretti and Flanagan, 1971). The Maillard reaction is the major cause of deterioration in the storage of semi-moist protein containing foods. In addition to the development of brown pigments, melanoidins, there is a decrease in the protein quality in foods undergoing the Maillard reaction.

Two related studies have been conducted previously which dealt with Maillard browning in sweet whey powders stored under accelerated storage conditions (Saltmarch, 1980; Soul, 1984). Both used reaction rate kinetics to evaluate the Maillard reaction in sweet whey powders with various compositions, different physical states of lactose, and under different storage temperatures and water activities. Saltmarch (1980) examined the effects of physical conditions (temperature, water activity, and physico-chemical state of lactose) on the Maillard reaction in whey powders. Soul (1984) investigated the effects of compositional factors on storage stability of whey powders, but could only establish general trends. In addition Soul (1984) also studied the effects of temperature and water activity on the Maillard reaction in whey powders. Nonprotein nitrogen levels differed in powders with respect

to processing conditions (i.e. demineralization and ultrafiltration). The effect of NPN on the Maillard reaction was not clear cut. Mineral content also varied in whey powders, with extent of processing, and tended to increase the Maillard reaction as the amount of minerals increased. Increasing protein to lactose ratio in powders (protein contents 21-33%) appeared to decrease the rate of browning. Powders with higher protein to lactose ratios had a shift in their browning maximas to higher water activities when compared to lower protein powders. The relative influence of these various factors can be evaluated and used for storage optimization.

This study is a continuation of the above two studies with the difference being the wide range of protein and lactose contents of the eight powders used and the more varied microcompositions of the powders (i.e. minerals, nonprotein nitrogen, and monosaccharides). Commercial sweet whey powders were selected because there are a wide range of powders on the market and sweet whey powders are the most used source of whey in human foods.

The objectives of this study were 1) to determine which powders developed brown pigments and lost available lysine to the greatest extent, and which powders had the best keeping qualities, 2) to investigate which compositional factors influenced the deterioration of the powders and to what degree, 3) to reaffirm the effects of

storage water activity, a_w, on the reaction rates of brown pigment formation, and 4) to determine if there were any interactions between a_w and the powders.

LITERATURE REVIEW

I. Maillard Reaction in Foods and Model Systems:

Three major nonenzymatic browning reactions can be identified in foods. They are caramelization of sugars, ascorbic acid oxidation, and the Maillard reaction. In the storage of dehydrated and semi-moist protein containing foods, the Maillard reaction is the major cause of product deterioration (Labuza and Saltmarch, 1981a).

A. Positive Effects:

The Maillard reaction is necessary for the development of flavors and odors in foods, such as meats and bakery products (Hurrell and Carpenter, 1977).

Intestinal microflora were found to partly metabolize and liberate amino acids from the Amadori compounds thus, making the amino acids available nutritionally (Finot, 1982).

B. Negative Effects:

Bitter flavors, strong odors, and loss in nutritive value of the protein are disadvantages of the Maillard reaction (Hurrell and Carpenter, 1977). The loss in nutritional value associated with the Maillard reaction is the loss of available amino acids and a decrease in the total nitrogen digestability (Finot, 1982).

There have been studies to investigate the physiological and toxicological effects of the complex

Maillard products (Mauron, 1981; Finot, 1982). After feeding browned egg albumin to rats for 12 months, researchers noticed a strain on the digestive system and organs (Finot, 1982). The rats had enlarged kidneys, liver, and cecum (Finot, 1982). However, complex Maillard products appear not to be mutagenic (Finot, 1982). Large quantities of "premelanoidins" fed to rats showed anti-nutritive properties (Mauron, 1981). There is some concern that the "premelanoidin" products may contribute to nitrosamine formation or that they may be mutagenic (Fennama, 1985). Yen (1985) observed mutagenicity via the Ames test in lysine-glucose systems in the presence of nitrite. It is also well-known that sugar residues bound to proteins increase allergenicity (Finot, 1982).

In the Western world the nutritional losses associated with the Maillard reaction are probably not important, but the deterioration of the product´s quality is vital (Hurrell and Carpenter, 1977). In baby foods, however, the losses are more important since babies are dependent on a few food sources for nourishment (Hurrell and Carpenter, 1977).

C. Brief Review of the Mechanism:

The Maillard reaction occurs between the amino group of either proteins, amino acids, or amines and the carbonyl group of a reducing sugar (Hurrell and Carpenter, 1977). The Maillard reaction is a series of reactions which are

complex and the latter reactions are not fully understood (Hurrell and Carpenter, 1977; Feather, 1985).

The first step in the Maillard reaction is the reversible condensation between the carbonyl group of a reducing sugar with the free amino group of an amino acid or protein (Hodge, 1953; Hurrell and Carpenter, 1977; Finot, 1982; Fennema, 1985). The reaction is a 1:1 ratio between sugar and free amino group (Hodge, 1953). The condensation product immediately loses a molecule of water and is converted to a Schiff's base (Hurrell and Carpenter, 1977). The Schiff's base is converted into a N-substituted glycosylamine which is immediately converted to the 1-amino-1-deoxy-2-ketose, the Amadori compound, by the Amadori rearrangement (Hodge, 1953; Hurrell and Carpenter, 1977; Baltes, 1982). This step in the Maillard reaction is essentially irreversible (Feather, 1985). This initial stage has no visible color (Hodge, 1953). Rearrangement occurs spontaneously in a dry or nearly dry state at 25°C (Hodge, 1953). When the Amadori rearrangement is blocked, there is no browning (Hodge, 1953). After the Amadori rearrangement there are a series of complex reactions. In a basic solution (pH 5 and above) the degradation of an Amadori compound is thought to involve 2,3 enolization, whereas in an acidic solution (pH less than 5) the degradation involves an initial 1,2 enolization (Feather, 1985). This intermediate stage yields colorless or yellow

compounds that have strong absoption in the ultraviolet spectrum, 277-285 nm (Hodge, 1953). The compounds of the final stage are a brown color with a caramel-like aroma. The mechanism of brown pigment formation has not been elucidated (Reynolds, 1969). The brown pigments formed are unsaturated, insoluble, colored polymers of varying composition, termed melanoidins (Hodge, 1953). In these later stages amino acids can be degraded directly by reaction with other carbonyl compounds which are formed during previous reactions (Hurrell and Carpenter, 1977).

Namiki and Hayashi (1983) proposed a new mechanism for the Maillard reaction which involves the formation of free radicals. There is cleavage of the sugar molecule into a highly reactive two carbon fragment in an early stage of the Maillard reaction. Prior to the Amadori rearrangement the cation free radical products were identified as N,N′-dialkylpyrazines, as proof of the new mechanism occurring.

D. Factors Affecting the Maillard Reaction:

1. Compositional Factors Affecting the Maillard Reaction:

a. Sugars Effects:

The amount of pigment formed is directly proportional to the amount of open-chained reducing sugar in the equilibrium solution available for nucleophilic attack by amino nitrogen lone pair electrons (Ellis, 1959; Fennema,

1985). This suggests that the sugar reacts with the amine in the open-chain form.

Reducing sugars, aldoses, uronic acids, and ketoses, are the only carbohydrates that can take part in the Maillard reaction (Reynolds, 1969). Carbonyl groups are necessary for the Maillard reaction because they condense with the free amino groups of the amino acids. The order of reactivity of the sugars at 37°C and 15% moisture, from greatest to least is: D-xylose, D-arabinose, D-glucose, D-lactose, D-maltose, and D-fructose. D-fructose was about one-tenth as reactive as D-glucose (Hurrell and Carpenter, 1977). In another list, hexoses were from greatest to least reactive: D-galactose, D-mannose, and D-glucose (Saltmarch and Labuza, 1982). A model system containing 0.005M of each an amino acid and sugar and adjusted to pH 9.0 was heated to 121°C for 10 min. The order of reactivity from greatest to least in the formation of brown pigments at 420nm was found to be alpha-lactose, D-ribose, D-fructose, and D-glucose (Ashoor and Zent, 1984). From these three lists it is evident that the reactivity of the sugars depends on experimental conditions.

Hodge (1953) used acid hydrolysis on the browning product between glucose and phenylalanine, 50% of the amine source was recovered while no aldose was recovered. Hodge (1953) also recovered amines in browning products, but not the reducing sugar.

b. Protein and Amino Source Effects:

The most important amino acid involved in a Maillard reaction in foods is lysine because it is an essential amino acid (Hurrell and Carpenter, 1977; Dworschak, 1980; Fennema, 1985). Lysine is very accessable to the Maillard reaction because it has a strong, basic epsilon amino group available for condensation with a reducing sugar (Dworschak, 1980). The lysine associated with the Schiff's base appears to be biologically available to rats (Hurrell and Carpenter, 1981). The deoxyketosyl-lysine molecules formed in the "early" stages and under mild conditions (i.e. typical room temperature storage conditions) of the reaction are not available biologically (Hurrell and Carpenter, 1977; Finot, 1982). Under severe heat treatments there is complete destruction of the lysine amino acid (Hurrell and Carpenter, 1977).

In a 0.005M model system of 1:1 molar ratio of sugar and amino acid studied, most amino acids studied had highest browning with alpha-lactose (Ashoor and Zent, 1984). The amino acids which yielded the highest absorbance at 420nm were lysine, glycine, tryptophan, and tyrosine.

After a maximum lysine loss of slightly more than 50% was reached the amount of lysine appeared to increase for a short period of time (Labuza and Saltmarch, 1981a). This was found to be true in a soy model system which was exposed to an extrusion-like process (Labuza and Saltmarch, 1981a).

After this apparent increase, the amount of available lysine levelled off and no further losses were observed (Labuza and Saltmarch, 1981a). The first period is termed the recovery period, while the last period is the no-loss period (Labuza and Saltmarch, 1981a). Many researchers have observed a gradual levelling out of lysine loss after the initial 50% loss. Brown pigment formation however, continues to increase linearly with time (Labuza and Saltmarch, 1981a).

c. Mineral Effects:

Kato et al. (1981) investigated the effects of metals on the Maillard reaction between ovalbumin and glucose at pH 10.0. Four cations, Na^{+}, Cu^{2+}, Fe^{3+}, and Fe^{2+}, which are present in egg white, were added to freeze dried ovalbumin. The sodium cation was the only ion which had no effect on the Maillard reaction. The copper ion had the greatest accelerating effect on the reaction. Fe^{3+} catalyzed the reaction slightly faster than Fe^{2+}. The copper and iron ions seemed to accelerate the destruction of ovalbumin´s secondary structure. The theorized principle binding site seemed to be the imidazole groups of bovine serum albumin above pH 7.0 in an investigation with the metals Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+}.

In an air-stored glucose-glycine system, the addition of 0.003ppm manganese inhibited the rate of the Maillard reaction (Bohart and Carson, 1955).

When potassium citrate was added to a glucose-glycine model system for pH adjustment purposes, the rate was larger than anticipated at such a low pH, suggesting that either the citrate ion or the potassium ion catalyzes the browning reaction (Wolfrom et al., 1974).

Phosphate buffers act to prohibit a pH drop in the medium. A system undergoing the Maillard reaction usually shows a decrease in pH, thus buffers act to enhance the Maillard reaction. Buffers also act as acid-base catalyzers and increase the Maillard reaction in that capacity (Dworschak, 1980). Phosphates may also "increase the polarographic step of sugars and consequently lessen the concentration and the stability of the ring form of the sugar" (Dworschak, 1980).

Koshy et al. (1965) investigated the factors causing browning in spray dried lactose. The mechanism of browning was not clearly established. Spray dried lactose contains slightly higher amounts of other sugars, metals, and ash than U.S.P. conventionally processed lactose. Also, it is exposed to more heat during the spray drying process. Eight percent of the lactose is in the amorphous state. Koshy et al. (1965) found a catalytic effect of phosphate, acetate, citrate and tartrate ions. Borate ions limited the browning of lactose substantially.

Heavy metals have both a positive and negative effect on the Maillard reaction. Generally, copper and iron

promote, while manganese and tin inhibit the reaction (Dworschak, 1980). Upon heating, heavy metal salts hydrolyze and a subsequent pH drop occurs. It is also possible that heavy metals can form complexes with amino acids (Dworschak, 1980).

2. pH Effects:

When the pH of a food is increased above the isoelectric point of the proteins, the protein´s amino groups are exposed and available for reaction (Saltmarch and Labuza, 1982).

Increasing the pH increases the browning rate (Reynolds, 1969). At pH 7 the rate of free amino group loss was ten times faster than at pH 3 (Hurrell and Carpenter, 1977). A pH of 8.0 seemed optimal for the reaction of dipeptides and tripeptiedes with glucose (Saltmarch and Labuza, 1982). At a pH of 3.2 the browning between glycine and glucose was minimized (Saltmarch and Labuza, 1982). After being heated to 121°C for 10 min. an amino acid/sugar solution with each component having a concentration of 0.005M had maximum browning at pH 10. (Ashoor and Zent, 1984). At pH values above 11 there is formation of lactic acid and glycolic acids which increase at the cost of trioses and oxo compounds (Dworschak, 1980). These acids do not participate in the Maillard reaction.

Lea and Hannan (1949) used freeze dried casein-glucose mixtures to determine the effects of pH on

the Maillard reaction. The powders were stored at 37°C and a_ws of 0.55 and 0.70. The reaction rate increases when the pH is increased from 3.0 to 10.0. Lea and Hannan (1949) noted that pH has an ambiguous meaning in a dried system with very little available water.

As the Maillard reaction progresses in a food system the pH of the system decreases (Saltmarch and Labuza, 1982). This is probably due to less basic amino groups in the system (Ellis, 1959). Another explanation proposed is the formation of acidic compounds from the Maillard reaction (Dworschak, 1980).

3. Temperature Effects:

Increasing temperature is the single most important factor affecting the Maillard reaction. Lea and Hannan (1949) increased the temperature in a freeze dried casein glucose system from 0-70°C and found the percent loss of amino nitrogen per hour increased by a factor of 39,000.

At low storage temperatures the initial reactions of the reducing compounds with lysine may occur without any appearance of brown pigment (Saltmarch and Labuza, 1982). Fifty percent of the lysine was lost in a model system after 20 days of storage at 25°C and brown pigment formation did not become visually detectable until after 80 days (Saltmarch and Labuza, 1982).

At high (food processing) temperatures the Maillard reaction in the intermediate phase proceeds by the Strekker

degradation (Saltmarch and Labuza, 1982). "The alpha amino acids degrade to the next lower aldehyde by alpha-dicarbonyls" (Saltmarch and Labuza, 1982). Sugar fragmentation products and pyrazines are also produced (Saltmarch and Labuza, 1982). The aromas of cooked foods such as bread, chocolate, and honey are associated with these compounds (Saltmarch and Labuza, 1982).

Most studies are done under constant, controlled temperature conditions, which are not realistic in commerial food storage (Saltmarch and Labuza, 1982). When storage temperatures were fluctuated, the rate of the Maillard reaction was found to be greater than at the mean temperature of the range studied (Saltmarch and Labuza, 1982). Attempts have been made to predict the changes in a food under fluctuating conditions based on steady-state changes (Labuza and Saltmarch, 1981b; Labuza and Saltmarch, 1982).

4. Moisture Effects:

Water activity, a_w, is related to the moisture content of the food through the moisture sorption isotherm (Saltmarch et al., 1981). Very low and very high moisture contents (water activities) almost halt the Maillard reaction (Hurrell and Carpenter, 1977). At low a_ws, water is chemically adsorbed to polar sites on the surface of a food and is generally unavailable for reaction and solution (Labuza and Saltmarch, 1981a). The BET monolayer is the

upper portion of this region and occurs at a_ws of 0.2-0.3 in foods (Labuza and Saltmarch, 1981a). This is the most stable moisture content for most dehydrated foods (Labuza, 1975; Troller, 1978; Labuza and Saltmarch, 1981a). Water contents above the BET monolayer level are in multilayers, pores, capillaries and possibly entrapped in various structural components (Troller, 1978; Labuza and Saltmarch, 1981a). Many deteriorative reactions increase exponentially in rate as a_w increases above the monolayer (Labuza and Saltmarch, 1981a). But at high a_ws the reaction may level off or even decrease, as is the case with the Maillard reaction (Labuza and Saltmarch, 1981a).

In the Maillard reaction, water can retard the rate of initial glycosylamine reaction in which water is one of the first products. This is termed product inhibition (Loncin _et al_., 1968; Labuza, 1975; Labuza and Saltmarch, 1981a). Three moles of water are produced per mole of carbohydrate reacted (Labuza and Saltmarch, 1981a). Water slows the net Maillard reaction in some important steps of the browning reaction by laws of mass action by diluting the amount of substrate available (Eichner and Karel, 1972; Troller, 1978; Labuza and Saltmarch, 1981a). Conversely, when a_w is low in a semi-moist food an increase in water content acts to dissolve more reactants (Labuza and Saltmarch, 1981a). Increasing water content also acts to decrease the viscosity of the aqueous phase and this can

result in increased mobility of the reactants (Loncin *et al.*, 1968; Labuza and Saltmarch, 1981a). Having water as a product of the Maillard reaction and yielding three moles of water per mole of carbohydrate affects the Maillard reaction to a greater extent than having a decreased viscosity and faster mobility of the reactants (Labuza and Saltmarch, 1981a).

The above phenomena result in the Maillard reaction rate exhibiting a maximum (Loncin *et al.*, 1968; Eichner and Karel, 1972; Labuza, 1975). The browning reaction rate of the Maillard reaction reaches a maximum at a a_w between 0.60-0.75 (Loncin *et al.*, 1968; Labuza and Saltmarch, 1981a). Troller (1978) noted that where a product shows a maximum depends on its sorption isotherm. Humectants, such as glycerol, have been found to shift the maximum rate of the Maillard reaction to a lower a_w, found to be in the range of 0.40-0.55 for a casein-glucose model system (Labuza and Saltmarch, 1981a). The choice of an optimum a_w for storage of dehydrated products is important. The a_w of a dehydrated food is between 0.1-0.5 and intermediate moisture foods exhibit a a_w of 0.6-0.8, where the Maillard reaction often shows a maximum (Troller, 1978; Labuza, 1975; Saltmarch and Labuza, 1982). An optimum a_w exisits where biological and chemical reactions are minimized while oxidation, enzymatic, and microbial reactions also remain sufficiently low (Loncin *et al.*, 1968; Labuza, 1975).

In a study of casein-glucose mixtures the reaction rate for loss of free amino groups was greatest at about 15% moisture (Hurrell and Carpenter, 1977).

Mizrahi et al. (1970) studied the Maillard reaction in dehydrated cabbage. Increasing the moisture content from 2.1% to 7.1% (dry weight basis) caused a decrease in apparent energy of activation for brown pigment formation from 43 to 35 kcal/mole. At low moistures and 30°C the dehydrated cabbage browned linearly except for a brief induction period.

5. Moisture and Temperature Interactions:

Water content seems to decrease the temperature sensitivity of the reaction with increasing levels of water present (Labuza and Saltmarch, 1981a). The activation energy was lowered when the moisture content of dehydrated cabbage was increased (Mizrahi et al., 1970) (See previous paragraph).

Results compiled by Labuza and Saltmarch (1981a) suggested that water content had a smaller effect on the temperature dependence of the lysine loss as compared to the overall browning reaction. The mechanism is different for lysine loss than for brown pigment formation. Lysine participates in the initial as well as the final steps of the Maillard reaction where brown pigments are formed (Labuza and Saltmarch, 1981a).

Kaanane and Labuza (1985) equilibrated fish flour to a_ws of 0.33, 0.44, and 0.65 at 25°C. The flour was then sealed in pouches and stored at three temperatures, 25, 38, and 45°C. Another portion of the fish flour was stored open under specific a_ws. The sealed pouches stored at 38 and 45°C showed shifts to higher apparent water activities whereas the flours sealed and stored at 25°C showed no shift in a_w. The 45°C sealed pouches showed a greater shift to higher apparent a_ws than the 38°C sealed pouches at all a_ws studied. These results suggest that temperature influences a_ws.

6. Oxygen Effects:

The presence or absence of oxygen can have an effect on the browning reaction. Oxidative rancidity leads to the formation of carbonyl reducing compounds capable of producing browning in an intermediate moisture model system of chicken and glycerol (Saltmarch and Labuza, 1982). Adding antioxidants, BHA or EDTA, inhibited the oxidative rancidity and also the brown pigment formation (Saltmarch and Labuza, 1982).

7. Effects of Initial Concentrations of Reactant Species:

The amount and type of reactant species present influence the rate of the Maillard reaction (Saltmarch and Labuza, 1982). In a model food system at a_w 0.52 and a temperature of 45°C, an increase in glucose to lysine ratio

from 0.5 to 3.0 increased the brown pigment formation linearly. The brown pigment formation levelled off when the glucose to lysine ratio was above 3.0 (Saltmarch and Labuza, 1982).

Wolfrom _et_ _al_. (1974) monitored the color of D-glucose-glycine mixtures at 65°C. Water content of the model system was 65%. As Wolfrom _et_ _al_. (1974) increased the amount of reducing sugar in the D-glucose-glycine mixture the browning increased. The greatest browning was at a 1:5 amino acid/reducing sugar content.

II. Using Kinetics to Analyze the Maillard Reaction During the Storage of Food:

A. Previous Kinetic Studies and the Purpose of Using Kinetics:

Jokinen _et_ _al_. (1976) was the first to use kinetics to predict lysine loss as a function of system composition and temperature. Jokinen _et_ _al_. (1976) used temperatures of 80-130°C to study the Maillard reaction. At these higher temperatures Labuza and Saltmarch (1981a) have noted that the Maillard reaction can change pathways.

It is desirable to use kinetics in research because: 1) a specific food system's rate of change in a characteristic can be described by the reaction rate constant, k, 2) it describes the influence of water on Maillard browning in a simple kinetic model, and 3) it

allows comparisons of k's between many products (Labuza and Saltmarch, 1981a).

B. Choosing Experiments to Monitor Deterioration:

For this study, the two experiments utilized to monitor deterioration in the whey powders were brown pigment formation and loss of available lysine.

The variability of experiments determining the chemical changes in food (i.e. the Maillard reaction) is at best ± 10%. Thus, when a product is considered unacceptable after only a 20-30% change in a measurable quality, there are definite limits to the accuracy of predicting shelf-life and measuring rate constants (Fennema, 1985).

The simplest model of browning is a zero order reaction where the rate of pigment formation is a constant, k_b (Labuza and Saltmarch, 1981a). The equation which represents brown pigment formation is:

$$k_b = dB/d(time)$$

where B is the quantity of brown pigment. This equation assumes no induction period which is a fairly good assumption (Labuza and Saltmarch, 1981a). A zero order reaction is valid when reactant concentrations are not limiting for the rate of brown pigment formation (Labuza and Saltmarch, 1981a).

Protein quality loss has been monitored by determining the loss of available lysine. The loss of available lysine has generally been considered a first order

reaction up to at least 75% loss (Labuza and Saltmarch, 1981a). The rate of loss is dependent on the concentration of lysine remaining (Saltmarch and Labuza, 1982). The rate is expressed as:

$$-k_L(L) = dL/d(time)$$

where L is the concentration of lysine and k_L is the rate constant. Upon integration the equation becomes:

$$-k_L(time) = ln(L/L_o)$$

where L_o is the concentration of lysine at time zero (Labuza and Saltmarch, 1981a). Loss of available lysine does not always follow first order kinetics (Labuza and Saltmarch, 1981a). Labuza and Saltmarch (1981a) have noted that lysine may be released after initially being made unavailable from the glycosylamine reaction and after the Amadori rearrangement.

Mauron (1981) reported that the dye binding method using acid orange dye, which was used in this study, may give misleading results. Forty percent of a solution of deoxylactulosyllysine, which is the Amadori compound, became propionylated indicating a free epsilon amino group of lysine (Hurrell and Carpenter, 1981). This would mean that the dye binding method is not valid in the early stages of the Maillard reaction when Amadori compounds are abundant. However, the dye binding method has been previously found to correlate well with the fluorodinitrobenzene (FDNB) method in stored milk powders. The dye binding procedure was

recommended because of its rapid and relatively simple procedure. Hurrell and Carpenter (1981) previously found values of available lysine in skim milk powder to be 60.9 mmol/16g N using the dye binding method and 57.3 mmol/16g N using the FDNB reactive lysine method. However, the powders had not been subjected to any adverse conditions.

Friedman (1982) also noted a number of difficulties with the dye binding method for determining available lysine. There is a need to establish optimum dye to protein ratio in each case. If this is not accomplished there is the possibility of incomplete propionylation of epsilon amino groups. There is a difference in the extent of noncovalent hydrophobic-type interactions between the dye molecule, the starting material, and its propionylated derivative. Finally, Friedman (1982) expressed apparent difficulties with this procedure when using severely browned products, but they were not enumerated. Hurrell et al. (1979) urged the use of a standard each time the dye binding procedure was performed to check on equipment and solutions.

C. Accelerated Storage Studies:

The Arrhenius equation can be used to relate experiments carried out at higher temperatures for shorter periods of time with standard storage conditions (i.e. lower temperatures) (Fennema, 1985). Generally, rate constants from at least three temperature conditions are needed to determine a reasonably accurate and statistically valid

activation energy (Fennema, 1985; Kaanane and Labuza, 1985). At temperatures higher than 80°C, the Maillard reaction proceeds by a different mechanism. This puts a temperature limitation when using the Arrhenius equation for storage studies. Excessively high temperatures will probably produce a nonlinear plot. Activation energies in literature estimate that brown pigment formation in foods ranges from 30-34 kcal/mole. The E_a for lysine loss in food ranges from 10-38 kcal/mole, with the range of 20-25 kcal/mole being cited most often (Labuza and Saltmarch, 1981b). Saltmarch and Labuza (1981b) noted that a_w has little effect on E_a at 95% statistical confidence limit. In whey powders, Labuza and Saltmarch (1981b) found E_a values for relative rate of brown pigment formation and loss of available lysine to be, respectively, 29-34 kcal/mole and 20-25 kcal/mole. Soul (1984) reported the ranges in E_a for the rate of brown pigment formation in whey powders (21-33% protein) to be 29.6-46.6 kcal/mole. The range in E_a for the rate loss in available lysine in whey powders (21-33% protein) was 16.5-27 kcal/mole (Soul, 1984).

III. Dried Sweet Whey and Milk Powders-An Overview of Involvement in the Maillard Reaction:

A. Brief Definition of Whey:

Sweet whey is the major by-product of rennet cheese production and the most utilized source of whey (Evans and Gordon, 1980). In 1984, of 466.2 million pounds of dried whey used in human foods in the U.S., 462.1 million pounds were sweet type wheys (Whey Products Institute, 1984). Eight pounds of liquid whey are produced for every one pound of cheese, so obviously whey utilzation is an important field economically, considering cheese consumption has been increasing yearly (Webb, 1970). Liquid whey contains about 6% total solids, this is half the nonfat solids content of milk (Robinson, 1986).

B. New Technology in Whey Processing:

Since whey proteins are economically, nutritionally, and functionally the most important component of liquid whey, research emphasis has been in the production of high quality protein whey powders via membrane processing (Robinson, 1986). Whey protein concentrates (WPC) were introduced to the food industry in 1977 with limited success because marketing had not defined the product to the buyers (Hood, 1985). WPCs do not offer a functional or economic advantage over other protein ingredients at this time due to 1) inconsistencies in WPC production, 2) a lack of

standardized procedures defining and measuring functionality of WPC in foods and simple aqueous systems, and 3) a lack of understanding and knowledge of the effects that certain processing conditions (i.e. ultrafiltration, pasteurization, and equipment utilization) and compositional components have on the functionality of whey proteins (Melachoris, 1984).

Membrane processing is usually more economical than traditional methods of evaporation and concentration (Hayes, 1985). At the same time membrane processing reduces the heat damage to sensitive whey proteins, maintaining whey protein functionality (Hayes, 1985).

Liquid whey contains almost all the soluble minerals of milk. This has precipitated a considerable amount of research towards reducing the minerals in whey powders (Mavropoulou and Kosikowski, 1973b). Reduced minerals whey powders, as they are commonly known, are especially important in the baby food industry (Houldsworth, 1980).

1. Ultrafiltration:

In ultrafiltration (UF), whey passes under pressure across a semi-permeable membrane film, such as cellulose acetate, polyvinylchloride, or fiberglass. Proteins and insoluble salts are collected while water, lactose, soluble salts, lactic acid, and nonprotein nitrogen are transferred to the film's outer surface (Kosikowski, 1977). Processing via UF can concentrate whey to about 25% total solids and of that 25%, 60-65% is protein (Hayes, 1985).

2. Reverse Osmosis:

The purpose of reverse osmosis (RO) is to concentrate liquid whey and reduce its volume, by the removal of water, to allow further processing (Robinson, 1986). The membranes in RO are similar to those of UF, but the pore sizes are smaller requiring greater pressures than UF and allowing only water and other trace components to pass across the membranes. A major challenge to the dairy industry is keeping the RO membrane sanitary. When liquid whey is processed by RO, the biological oxygen demand in the eluent is 95-99% less than the liquid whey (Kosikowski, 1977).

3. Gel Filtration:

The objectives of column gel filtration are to separate the components of whey by the mechanism of permeation. The column is a crosslinked polysaccharide matrix (Zall, 1979). Smaller molecules are trapped in the matrix while large molecules, such as proteins, flow through rapidly. Preconcentration of the whey is necessary before passing the solution through the column because there is dilution of the concentrate during processing (Zall, 1979). The process is not mechanically complex and operates at cold temperatures which minimizes whey protein denaturation (Zall, 1979). Gel filtration techniques have high initial costs and costs of operation which have caused an apparent abandonment of gel filtration research (Coton, 1985).

4. Demineralization:

There are two direct demineralization methods, ion exchange and electrodialysis. In ion exchange (IE), hydrogen and hydroxyl ions are exchanged with cations and anions, respectively (Hayes, 1985). The IE resin consists of uniform beads, diameters of 0.4-0.8mm, usually made of polystyrene or divinylbenzene (Houldsworth, 1980). By varying the degree of crosslinking, different characteristics of the resin are achieved. In IE, the OH^- or the H^+ are attached to the resin and can be used either together for total demineralization or separately for ion selective deionization (Houldsworth, 1980).

In electrodialysis, an ion selective membrane allows the passage of only one type of charged ion (i.e. sodium, but not chloride) (Hayes, 1985). The complete membrane cell consists of a cation and an anion membrane on opposite sides of the cell; the design is analogous to the plate heat exchanger (Houldsworth, 1980). A current is passed through the whey and cations and anions migrate and pass through to their respective membranes. The voltage is increased during the process to compensate for the decreased ionic concentration of the whey. To obtain significant demineralization, the whey must be recycled until the conductivity drops to a desired level (Houldsworth, 1980).

C. Composition of Whey and Related Milk Powders and their Effects on the Maillard Reaction:

Whey powders are more prone to the Maillard reaction than nonfat dry milk (Ferretti and Flanagan, 1971). Milk and its products are one of the few foods to naturally contain substantial quantities of both proteins and reducing sugars (Hurrell and Carpenter, 1977).

1. Effect of Reducing Sugars:

a. Lactose:

The content of lactose in dairy products and whey powders varies depending on the extent of processing. Spray dried sweet whey, which has not been subjected to further processing has about 70% lactose. Lactose is not the rate limiting factor in the Maillard reaction in spray dried whey powders (70% lactose) (Labuza and Saltmarch, 1981b). This may not be the case in other foods where diffusion and concentration limitations require higher a_ws to solublize reactant species and increase browning rates (i.e. WPC) (Labuza and Saltmarch, 1981b). In WPC (35% protein) stored at 37°C and 75% R.H. the free lactose decreased by 72% after 42 days of storage (Li-Chan, 1983).

A solution of whey containing 5% lactose underwent 30% hydrolysis of the lactose at pH 4.7 and 90°C when heated for up to 50 hours (Ney and Wirotama, 1970). A 5% solution of pure lactose did not undergo hydrolysis under the same

conditions. Ney and Wirotama (1970) contributed the lactose loss to a catalytic effect of the whey proteins.

b. Glucose and Galactose:

The galactose content in mozzarella cheese is higher than other cheeses because the bacterial cultures used in the manufacture of the cheese ferment lactose into glucose and galactose. Most cultures used for the manufacturing of mozzarella cheese do not metabolize galactose, thus it accumulates in the cheese and also apparently in the whey (Johnson and Olson, 1985). Mozzarella cheese contains little lactose. Upon heating the mozzarella cheese, there is a correlation ($r = 0.90$) between galactose content in the cheese and brown color formed (Johnson and Olson, 1985).

Hydrolyzed lactose milk products showed a higher protein quality loss than unhydrolyzed milk products due to the presence of twice as many reducing sugars in the system and more reactive reducing sugars (Labuza and Saltmarch, 1981a). Rawson and Mahoney (1983) reported that the spray drying process decreased the FDNB-reactive lysine by 18% in a lactose hydrolyzed milk powder. Total lysine, determined by amino acid hydrolysis, fell 19%, and net protein ratio (NPR), determined using a feeding study on rats, decreased 16%. Normal spray drying conditions caused 35-40% loss in biologically available lysine in hydrolyzed milk. The loss was not significant in unhydrolyzed milk. The hydrolyzed milk powder after spray drying showed no obvious brown

color, even after 40% loss in available lysine. This indicates that the Maillard compounds formed at this stage are precursors to the melanoidins.

2. Protein Effects:

The two major, globular whey proteins are alpha-lactalbumin and beta-lactoglobulin. They make up 25% and 50%, respectively, of the total amount of whey proteins (McKenzie, 1971; deWit and Klarenbeek, 1984; Robinson, 1986). The remaining 25% of the whey proteins are bovine serum albumin, immunoglobulins, and other minor proteins (McKenzie, 1971; Robinson, 1986). Burvall et al. (1977) reported that lysine is not a limiting amino acid in milk protein and that a 30% loss of this amino acid would not be expected to give any decrease in the nutritive value of pure milk protein. The mode of the Maillard reaction in milk is between the lactose and the k-casein protein fraction (Labuza and Saltmarch, 1981a). This may not be true in milk powders at low a_ws (Labuza and Saltmarch, 1981a).

Milk powders containing 2.5% moisture and stored for several weeks at 60 and 70°C were evaluated by Hurrell and Carpenter (1981). The powder stored at 60°C for 9 weeks had high contents of lactulosy-lysine and about 40% of the lysine was in the deoxyketose form. The powder retained its natural color. At 70°C, however, the powder had undergone advanced Maillard browning. After storage, 83% of the original lysine was destroyed and was not recovered by acid

hydrolysis. Biologically available lysine was 16% of its original value by 4 weeks into storage and remained at this level after the 9 week study. Using the FDNB method to measure available lysine, the amount decreased from 8.4g/100g protein to 3.6g/100g protein after 42 days of storage.

Soul (1984) investigated the relative rates of brown pigment formation and loss of available lysine in commercial WPC at 25 and 35°C. The percentage protein in the powders seemed to influence where the powders exhibited a maximum browning and loss of lysine rate. At 25°C the maximum relative rate of browning in whey powders with less than 25% protein was at 0.52 a_w. In whey powders with greater than 25% protein the maximum relative rate of browning was at 0.75 a_w. At 35°C the maximum relative rate of browning was at 0.44 a_w for powders with less than 25% protein and 0.75 for powders with greater than 25% protein. For rate losses in available lysine, Soul (1984) reported that at 25°C three of four powders lost the greatest amount of lysine at 0.75 a_w while the other lost the greatest amount at 0.65. At 35°C, the powders with less than 25% protein had a maximum relative rate of lysine loss at a a_w of 0.75. The powder with 27.9% protein had a maximum at 0.65, while the powder with 33.4% protein had a maximum loss of lysine at a a_w of 0.45. In a WPC (35% protein) the grams lysine/100g protein,

determined by acid hydrolysis, decreased from 8.7 to 6.0 after 42 days of storage at 37°C (Li-Chan, 1983).

Holsinger et al. (1973) measured the total and available lysine in a spray dried commercial sweet whey powder (12.9% crude protein) after 11 months of storage at room temperature. The powder lost 6.8% total lysine while the available lysine decreased by 18.5%.

3. Nonprotein Nitrogen Effects:

The nonprotein nitrogen (NPN) components of cow's milk include: ammonia, urea, creatinine, creatine, uric acid, and alpha amino acids (Shahani and Sommer, 1951; Webb et al., 1978). Also present in trace amounts are such components as: hippuric acid, orotic acid, indican, phosphoglyceroethanolamine, and phenylacetylglutamine (Schwartz and Pallansch, 1962; Webb et al., 1978). Orotic acid is present in dried whey in concentrations of 64-146mg/100g and concentrations of 7-124mg/100g in modified whey (Empie and Melachouris, 1978). Primary and secondary amines were present in whey and whey powders in concentrations of less than 1mg/liter (Weihrauch and Schwartz, 1974). Free amino acid content of sweet and acid whey powders range from 1.0-11.0g/kg (Mavropoulou and Kosikowski, 1973b). Soluble peptides and free amino acids of whey powders were found to range from 20.3-34.3g/kg (Mavropoulou and Kosikowski, 1973b). Sweet wheys do not contain as much soluble peptides as acid wheys because of

the disruption of the whey proteins and caseins during acid precipitation (Mavropoulou and Kosikowski, 1973b).

Cerbulis et al. (1972) found that when whey powders were dialyzed four days with water, 25% of the nitrogen was dialyzable. NPN is slightly higher in acid whey than sweet whey (Glass and Hedrick, 1977a). When NPN was determined in cheese wheys and laboratory wheys (milk adjusted to pH 4.6) the cheese wheys contained more NPN; probably due to microbial proteolysis and secondary rennin activity (Josephson et al., 1975). The presence of NPN allows the Maillard reaction to proceed more quickly and effectively (Mavropoulou and Kosikowski, 1973b).

Soul (1984) reported that whey powders with the highest amount of free ammonia had the highest rate of browning. Soul (1984) believed that NPN supplied more amino groups to the Maillard reaction, however, the effects of NPN on the Maillard reaction were not as clear cut as the effects of free ammonia. Ultrafiltration and demineralization processes reduce NPN levels in the whey powders.

4. Effects of Minerals:

Whey contains almost all the soluble minerals of the original milk (Robinson, 1986) and greater than 50% of the total minerals of milk (Verma and Sommer, 1957). Many studies have quantified and identified the total mineral contents in whey (Glass and Hedrick, 1977b). In commercial

dried sweet wheys the major minerals included: calcium (0.39, 0.88, and 0.47%), phosphorus (0.68, 1.1, and .58%), sodium (0.91, 1.3, and 0.75%), potassium (2.3, 1.9, and 2.3%), and magnesium (0.1, 0.18, and 0.1%) (Mavropoulou and Kosikowski, 1973a; Glass and Hedrick, 1977b; and Wong et al., 1978, respectively). The trace minerals found in sweet dried whey were aluminum, boron, manganese, zinc, iron, copper, iodine lead, mercury, selenium, cadmium, and arsenic (Mavropoulou and Kosikowski, 1973a; Glass and Hedrick, 1977b; Wong et al., 1978). Josephson et al. (1975) analyzed the composition of fresh wheys and ultrafiltered whey powders. In their analysis they found that the liquid whey with the highest pH had the lowest levels of calcium, magnesium, phosphorus, and ash. Delaveau and Jelen (1979) found that wheys with higher pHs had less free calcium. For the UF sweet whey powders having an average protein content of 61.4%, the mineral distribution was calcium (0.95%), magnesium (0.08%), and phosphorus (0.39%).

Sodium metabisulfite decreased browning in a concentrated lactose hydrolyzed whey (72% solids) stored at 20°C for up to 98 days. Potassium citrate was not effective in controlling the Maillard reaction (Harfouch et al., 1985).

Soul (1984) noted a positive correlation between mineral content and browning rate in commercial sweet whey powders.

5. Effects of Other Constituents:

Lactic acid accumulates in liquid whey if it is held too long unpasteurized or stored at high temperatures (Robinson, 1986). High concentrations of lactic acid make it difficult to dry whey because lactic acid is a liquid at room temperature (Robinson, 1986). The resulting powder is highly sticky and hygroscopic. Hygroscopic powders are less stable and deteriorate via the Maillard reaction more rapidly that nonhygroscopic powders.

Fat globules occur in the free form in milk powders and can be oxidized to form carbonyls which are reactive in the Maillard reaction (King, 1965).

D. Physical Conditions Affecting the Maillard Reaction in Dairy Products and Whey Powders:

1. pH Effects:

Sweet wheys have a pH ranging from 5.2 to 6.4 (Glass and Hedrick, 1977a). A low initial pH in liquid whey is due to lactic acid formation.

Kehrberg and Johnson (1975) stored sweet dried whey at room temperature. During the storage period the pH decreased.

Delaveau and Jelen (1979) noted discoloration of liquid wheys at high pH´s. Soul (1984) observed that the whey powders (21-33% protein) with the lowest initial pH values had the highest and lowest rates of brown pigment formation.

2. Temperature Effects:

The effect of temperature in accelerating the deterioration in dried dairy products is well documented (Soul, 1984). Increasing temperature of storage increased the rate of deterioration of commercial sweet whey powders (25-30% protein) (Soul, 1984). Whey powders stored under fluctuating storage temperatures (25-45°C) with the mean at 35°C had a greater loss of lysine and brown pigment formation than the same powders stored at a constant 35°C (Saltmarch *et al*., 1981).

3. Moisture Effects:

In nonfat dried milk, moisture contents of greater than 5% (wet basis) are necessary for a significant rate of lysine damage during normal storage (Hurrell and Carpenter, 1977; Labuza and Saltmarch, 1981a). In nonfat dry milk powders stored at 40°C for 10 days, the a_w maximum (0.68) for lysine loss paralleled the maximum for the browning reaction (Loncin *et al*., 1968). Henry *et al*. (1948) found a greater decrease in protein quality in a dried milk powder at a a_w of 0.55 than at 0.29. In lactose hydrolyzed milk powders, the greatest loss of biologically available lysine was at a a_w of 0.62 (Kim *et al*., 1981). However, powders were not stored below a_ws of 0.62.

The a_ws of commercial whey powders was found to be between 0.20-0.25 (Sharp and Doob, 1941). This corresponds to the monolayer of water for whey powders (0.2-0.3 a_w) and

is where the reaction rate would be at a minimum (Labuza and Saltmarch, 1981a).

A WPC with 35% protein stored at 37°C had a browning maximum at a a_w between 0.6-0.7 (Li-Chan, 1983). Li-Chan (1983) contributed this higher a_w to the similarity in lactose contents between WPC and milk powder (Li-Chan, 1983). Whey powders with about 25-30% protein had a browning rate maximum between 0.44-0.75 for storage temperatures of 25, 35, and 45°C (Soul, 1984). Hygroscopic whey powders had considerable losses of lysine at low moisture contents (1.5-4.0% wet wt. basis) (Saltmarch et al, 1981). Huss (1974b) noted that whey powders were subject to lysine losses at lower relative humidities of storage than delactosed whey powders (40-50% lactose) and dried milk powders.

At 45°C, a hygroscopic whey powder demonstrated a relative browning rate maximum at a a_w of 0.44-0.52 (Saltmarch _et al_., 1981). The highest rate of browning and protein quality loss in nonhygroscopic and hygroscopic whey powders occurred at a_w 0.44 at all temperatures studied, 25, 35, and 45°C (Kim _et al_., 1981; Labuza and Saltmarch, 1981b). However, only three a_w conditions were used, 0.33, 0.44, and 0.65 (Labuza and Saltmarch, 1981b).

Kim _et al_. (1981) compared the extent of browning in whey stored open in controlled a_w atmospheres and in hermetically sealed packets, previously equilibrated to

specific a_ws. The sealed packet, previously equilibrated to 0.33 a_w, produced the whey powder with the fastest overall browning rate, while the maximum rate of browning for the controlled atmosphere-stored whey powders was at a a_w of 0.44. The whey powders in the hermetically sealed packets browned maximally at a lower a_w than the whey powders stored open at a controlled a_w because of the overall increase in a_w of the sealed system (Kim et al., 1981). The whey stored open at a controlled a_w lost moisture during the study while the closed system's moisture content remained constant.

Berlin et al. (1968) studied water adsorption isotherms for sweet whey powders. At a a_w of 0.4-0.5 at 25°C the stored whey powders exhibited a discontinuity in the isotherms associated with lactose crytallization. A discontinutiy is a drop in moisture content at an increased a_w (Kim et al., 1981).

Saltmarch and Labuza (1980) reported moisture losses in nonhygroscopic and hygroscopic whey powders during 6 weeks storage at 25°C in the a_w range of 0.22-0.75. This indicates a physico-chemical change occurring in the powder. Sharp and Doob (1941) also found moisture losses in precrystallized whey powders at 25°C after 68 days. Moisture losses were more rapid at higher temperatures and were attributed to the initiation of lactose crystallization at the lower a_ws and the continued crystal growth at higher a_ws. Scanning electron microscopy confimed that lactose

crystal initiation and growth increases with increasing a_w in both nonhygroscopic and hygroscopic whey powders (Saltmarch and Labuza, 1980). The subsequent loss of water from the collapsed structure by diffusion or evaporation must be slow enough so as not to slow the Maillard reaction (Saltmarch et al., 1981).

Huss (1970, 1974a, 1974b) related the effect of water on the Maillard reaction in milk powders through a shift of lactose from its amorphous to crystalline form. Lactose in amorphous form in skim milk powders began to crystallize in the 40-42% R.H. range. Huss (1970, 1974a, 1974b) found a greater damage to lysine in powders which had gone through crystallization. At moisture contents of 4.9-6.8%, lactose was in the amorphous form and lysine losses were negligible. But, as lactose crystallization increased moisture levels rose to 5.1-7.0% and lysine loss increased. Hygroscopic milk powders absorbed water on polar sites of the amorphous lactose and proteins at a_ws below 0.40.

4. Effect of the Physical State of Lactose:

Due to the large content of lactose in whey powders a two stage drying process is utilized to convert a majority of the lactose to it´s crystalline (alpha monohydrate) form (Webb and Whittier, 1970). The pasteurized and concentrated whey solution is first dried to 12% moisture content. The product falls onto a conveyor belt and while travelling down the belt to a roller the lactose crystallizes. This process

is termed postcrystallization. At the rollers the powder lumps are ground to fine particles with a final moisture of about 4% (Webb and Whittier, 1970). Hynd (1980) describes another method to prepare nonhygroscopic whey powders by precrystallizting the concentrated whey solution prior to drying. Eighty-five percent crystalline lactose is possible. A nonhygroscopic powder is a product which will not absorb and hold moisture when exposed to 26-49°C and 80-95% relative humidity (Kosikowski, 1977).

In dairy products lactose can be present in three physical forms, alpha-monohydrate, anhydrous beta lactose, and amorphous lactose (Webb et al., 1978). Alpha-monohydrate is the crystalline form of lactose obtained from super-saturated solutions at temperatures below 93.5^{o}C (Webb et al., 1978). Alpha-monohydrate lactose contains one mole of water per mole of lactose (Webb and Whittier, 1970). Anhydrous beta lactose crystals form when a super-saturated solution of lactose is heated above 93.5^{o}C (Webb and Whittier, 1970; Webb et al., 1978). The beta lactose is highly hygroscopic when exposed to normal atmospheric conditions. Amorphous lactose in powders results when solutions of lactose are rapidly dried without allowing crystallization to occur (Webb and Whittier, 1970). Crystalline lactose should not be reactive in the Maillard reaction since it is not in solution (Kim et al., 1981).

Crystallization of amorphous lactose in dried dairy products causes liberation of moisture which causes a discontinuity in the adsorption isotherm (Kim *et al*., 1981). The molecules of amorphous lactose require sufficient mobility to rearrange into a crystalline lattice (Herrington, 1934; Kim *et al*., 1981). Once crystallization is initiated, the bound water is released. Various dried milk products at 25°C had discontinuities in their adsorption isotherms in the 0.50-0.60 a_w range (Troy and Sharp, 1930). The lactose in hygroscopic dried milk powder began crystallizing in a lower a_w range, 0.47-0.52 (Saltmarch *et al*., 1981). The appearance of a discontinuity in the isotherm was time dependent. The rate of recrystallization increases with increased a_w. The relative rate of diffusion of water out of the sample decreases with increased a_w. The caking phenomenon observed in noncrystalline milk powder has been shown to lead to protein insolubility, rupture of fat globules, and accelerate flavor deterioration (King, 1965; Saltmarch and Labuza, 1980). The water given up after the crystallization of lactose can result in the increase of significant deteriorative reactions.

Saltmarch and Labuza (1980) studied the increase of a_w on the physico-chemical state of lactose in a hygroscopic (95% amorphous state) and nonhygroscopic (95% crystalline) whey powder. Discontinuities in hygroscopic whey powder

isotherms were in the range of 0.35-0.52 a_w at 25°C. According to Kim *et al*. (1981) hygroscopic whey powders showed discontinuity in moisture adsorption isotherms at 35°C in the a_w range of 0.33-0.44. By using the scanning electron microscope Saltmarch and Labuza (1980) saw the first evidence of lactose crystallization in the hygroscopic whey powder at a a_w of 0.40 within 1 week at 25°C. There was a noticeable discontinuity in the 6 week moisture isotherm of the hygroscopic whey powder. As lactose crystallization becomes extensive the crystals "purify" and grow, and begin the expulsion of other milk components into the capillary spaces. This was most evident in the micrographs of the nonhygroscopic whey powder at a a_w of 0.75 and 0.85.

Sharp and Doob (1941) stored two whey powders, each containing a majority of one of the two naturally occurring crystalline forms of lactose, anhydrous beta and alpha-monohyrate. They found at 25°C and at a relative humidity of above 65% the anhydrous beta lactose converts to alpha-monohydrate. The small amount of amorphous lactose in the powders absorbed moisture and crystallized to the alpha-monohydrate form at a_ws between 0.30-0.50.

5. Effect of the Physical Structure of Spray Dried Milk Products:

King (1965) described the physical structure of spray dried milk as spherical particles having internally minute

air bubbles. Particle sizes were from 10-100um (King, 1965). Another report on milk powders estimated particle sizes at 10-250um (Hayashi, 1969). The particles of nonhygroscopic whey powder were 100-200um in diameter (Saltmarch et al., 1981). Increasing total solids of the solution and decreasing pump pressure were two factors which increased particle size. Particles below 75um are undesirable because small particles do not dissolve readily in water (Hayashi, 1969).

Roetman (1979) used scanning electron microscopy (SEM) to examine the surfaces of various dried dairy products. Roetman (1979) found that the processing conditions and state of lactose affected the appearance of the lactose. Lactose in the amorphous state had a smooth surface without cracks and folds. When lactose was precrystallized in a concentrated solution prior to spray drying, the crystals were usually tomahawk shaped. The crystals were often enclosed or covered by milk or whey solids which reduces the improved properties of crystalline lactose (i.e. non-caking and free flowing powders). When lactose in spray dried dairy products was crystallized via postcrystallization techniques, the crystals were needle-like in shape. The surface of the postcrystallized product had a more porous structure than any of the other products examined. As lactose content increased the appearance of pores were more apparent (i.e. dried lactose

and dried whey). King (1965) stated, "postcrystallization provokes development of a network of fine interstices and cracks along the sides and edges of tiny crystals." Palmer and Dahle (1922) described the spray dried structure of dried whole milk as containing air pockets which could allow oxidative deterioration to occur in the powder.

MATERIALS AND METHODS

I. Obtaining Whey Powders for this Study:

Eight commercial powders were obtained from four companies, Table 1. The powders were obtained fresh from the production line and sent through the mail. The powders were frozen at -20°C until used. The powders were double bagged in plastic or their lids were double sealed with parafilm. Approximately one pound of each powder was received from each company.

Whey powders were analyzed for moisture, protein, nonprotein nitrogen, lactose, glucose, galactose, ash, and phosphorus to determine initial composition.

II. Compositional Analysis of Commercial Whey Powders:

A. Moisture Determination:

The moisture content of all whey powders was determined using the Karl Fischer procedure. Both the commercial and stored whey powders were analyzed for moisture so that compositional factors could be reported on a dry weight basis. The moisture procedure is a modification of the procedure outlined by Glass and Hedrick (1977a), and Della Monica and Holden (1968).

1.3000-2.0000 grams of whey powder was added to an exact volume (between 40.00-50.00ml) of dry Karl Fischer grade methanol. The samples were shaken well and allowed to

Table 1. Commercial Powders Used in This Study

Product and its Identification No.	Company	Product No.
1. Nutritek 250 Reduced Minerals Whey	Formost Whey Products Baraboo, WI	381
2. Daritek 50 Whey Protein Concentrate	Formost Whey Products Baraboo, WI	357
3. LE-PRO WPC 470	Leprino Foods Denver, CO	470
4. Extra Grade Dry Sweet Whey	Land O´ Lakes, Inc. Minneapolis, MN	27231
5. LE-PRO SW 420	Leprino Foods Denver, CO	420
6. Nutritek 900 Reduced Minerals Whey	Formost Whey Products Baraboo, WI	383
7. Daritek DM Demineralized Whey Protein Concentrate	Formost Whey Products Baraboo, WI	359
8. Savorpro 75 Whey Protein Concentrate	Express Foods Co., Inc. Louisville, KY	2701

stand overnight under refrigeration. The samples were allowed to reach room temperature before analysis and again shaken well. About five minutes before analysis, each sample was shaken well and the whey was allowed to settle. Five milliliter aliquots of sample were added to the titrimeter (Fisher KF Accessory Model 392 and Fisher Burette/Dispenser Model 395, Springfield, NJ) and titrated with Karl Fischer reagent (Fisher no. CC34806-212, Riedel-deHaeen Hydranal Composite/2, Hauppauge, NY,). Blanks were run using the dry methanol solvent. Sample injections were made until samples were reproducible to within 3%. Titers were calculated by injecting 15 or 20ul of distilled deionized water and titrating with the Karl Fischer reagent. The formula for the titer is:

Titer = mg H_2O injected/ml KF reagent used

The formula used to calculate moisture was:

% Moisture = R x T x 100/S

R = net ml of KF reagent to titrate sample
T = KF reagent titer
S = weight of sample injected in mg

B. <u>Protein Analysis by the Macro-Kjeldahl Method</u>:

The procedure according to Richardson (1985) was modified for whey powders as follows.

1. The weighed sample (0.7000-2.2000g) was placed in a 500ml digestion flask. Sample should contain 0.2-0.4g protein. As a standard control, 0.5000g dried glycine (18.66% nitrogen) was weighed into another flask. Samples

were weighed on glassine weigh paper (No. 3 NDC 2-X86-4, Eli Lilly and Co., Indianapolis, IN) and both were placed in the flask. A blank was prepared by placing one sheet of glassine paper into a flask.

2. 3 Kjeltabs (Fisher no. TC-1527-0018) and 25-30ml of sulfuric acid were added to each flask. Each tablet contains 0.4g copper sulfate and 3.5g potassium sulfate.

3. The flasks were placed on the digestion apparatus (cat. no. 55110, Precision Scientific Company Model N-6, Chicago, IL). The exhaust system was turned on for fume removal.

4. The mixture was heated until it was colorless. The mixture was then heated an additional 30 min.

5. The heaters were turned off and the flasks were allowed to cool on the rack until fuming stopped.

6. While the flasks were cooling, the receiving flasks were prepared. Fifty to 75ml of saturated boric acid solution was added to each 300ml receiving flask. 15 drops Tashiro indicator solution was added to each flask. Boric acid solution was prepared by dissolving 1 gram of boric acid (cat. no. B-0252, Sigma Chemical Co., St. Louis, MO) in 18ml of distilled deionized water. The solution was heated to dissolve the boric acid. The Tashiro indicator was prepared by dissolving 0.25g methylene blue (Fisher no. M-225) and 0.375g methyl red (Fisher no. M-219) in 300ml of 95% ethanol.

7. The receiving flasks were placed on the distilling rack so the tip of condenser extended below the surface of the boric acid in the flask. This prevents the escape of ammonia fumes during distillation.

8. After fuming stopped the flasks were removed from the digestion rack and cooled thoroughly by immersing the flasks in cold water. Samples solidified with cooling.

9. One hundred sixty milliliters distilled deionized water was added and the flask was swirled until the sample was completely in solution. The flask was immersed in cold water and cooled again. If necessary, the flasks were capped and held overnight at this stage. Boiling chips (about 6-10 chips) (Fisher no. B-365) and zinc metal (about one half teaspoon) (Fisher no. Z-5) were added to each flask. Twenty-five milliliters potassium sulfide solution was added to each flask while the flask was swirled. The potassium sulfide solution was prepared by dissolving 85g potassium sulfide (Fisher no. P-307) in hot distilled deionized water, cooling and bringing to a final volume of 2000ml in a volumetric flask. Next, the solution was filtered through a Whatman #1 filter under vacuum.

10. The water valve was turned on so cold water would flow around the condensers.

11. The flask was held at an angle and placed on the heater of the distilling unit. Rapidly, but CAREFULLY, 90ml of 40% sodium hydroxide (Fisher no. SO-S-411) was poured down the

side of the flask so that it did NOT mix with the acid solution.

12. IMMEDIATELY the stopper was firmly inserted in the flask to prevent escape of ammonia.

13. The contents of the flask were mixed by shaking it vigorously. It was important to mix the flasks well to minimize superheating and bumping.

14. The heater was turned on and about 125ml of distillate was distilled into the receiving flask. When the volume in the distilling flask was reduced too low, bumping occurred.

15. As the receiving flask was removed, the tip of the condenser was allowed to drain into the flask momentarily. The tip was then rinsed with distilled water, and the rinsings were caught in the receiving flask.

16. The receiving flask was titrated with 0.200N HCl. The endpoint was reached when the solution changed from an initial green through gray tones to a final purple or violet red color.

17. Calculations:

Total nitrogen was calculated as follows:
%N = (ml HCl X N HCl X 1.4)/grams sample
For sweet whey proteins (Karman and van Boekel, 1986): 1 part nitrogen = 6.45 parts crude protein

For the calculation in the browning and available lysine experiments, the universal conversion factor for dairy products, 6.38, was used. Percentage of true protein was calculated for each powder from the difference of total

protein and the nonprotein nitrogen values obtained in the next section. Duplicates of each powder were run and percentage protein values varied less than 2%.

C. Nonprotein Nitrogen Determination Using the Macro-Kjeldahl Method:

A modification of the undenatured whey protein nitrogen procedure for Nonfat Dry Milk in Standards for Grades of Dry Milks -- American Dry Milk Institute, 1971 Ed. was used to determine nonprotein nitrogen.

5.00 grams of whey powder was reconstituted in 45.00g of deionized distilled water. The sample was stirred on a stir plate until dissolved. 10.00g of reconstituted sample was placed into a 50ml polypropylene bottle (UDY Corporation, Boulder, CO) and 40.00g of 15% trichloroacetic acid (TCA) solution was added to the bottle. A blank was prepared with 10.00g deionized distilled water and adding 40.00g of 15% TCA. The protein was allowed to precipitate for at least 60 min. at room temperature. The sample was filtered using 50ml polypropylene filter bottles, filter pulp, filter discs, and filter caps. Centrifugation was also used to settle the precipitated protein when filtration was difficult (15,000xg for 10 min. at 25°C). Nonprotein nitrogen (NPN) was determined by the Kjeldahl method indicated above (as modified from Richardson, 1985) on 20.00g of filtrate.

NPN was represented as a percent of nitrogen. This allowed for the calculation of true protein. The conversion factor used to convert NPN from nitrogen to NPN components was 3.60 (Karman and van Boekel, 1986).

% Crude protein N - NPN as % nitrogen = % True protein N

Duplicates were reproducible to within 5%.

D. Lactose, Glucose and Galactose Determination in Whey Powders:

The procedure according to Jeon *et al*. (1984) was modified to include whey powders of varying protein contents. Also, a neutralization step was added after a pH adjustment to 5.2 so that a calcium-based ionic column could be used.

1. 1.0000-1.2000 grams powder was weighed into a 100ml beaker.
2. Next, 25.0000-26.0000 grams deionized distilled water was weighed into the beaker.
3. The powder was mixed very thoroughly on a Fisher Thermix Stirrer Model 220-T until no lumps remained in the solution.
4. Using 0.25N HCl (Fisher no. A-144) the pH of the solution was adjusted to pH 5.2, the isoelectric point of whey proteins. The pH meter was Fisher Accumet model no. 825MP. The electrode was a Fisher pencil combination polymer body gel filled electrode (model no. 13-639-252).
5. Twenty milliliters of the solution was pipetted into a Kimax test tube and sealed tightly.

6. The test tubes were boiled gently for 10 min. After boiling the tubes were cooled to room temperature under cold water.

7. The solutions were centrifuged using a Sorvall RC-58 centrifuge (DuPont Instruments, Newtown, CT) for 10 min. at 20,000xg and 25°C to sediment the precipitated proteins.

8. The supernatant was decanted into a 30ml polyethylene test tube and the pH of the supernatant was raised to 7.0 using 0.25N KOH (Fisher no. P-250).

9. The supernatant was eluted through a Sep-Pak C_{18} cartridge (cat. no. 51910, Waters Associates, Inc., Milford, MA) according to Waters' directions. The Sep-Pak cartridges removed residual proteins, lipids, and chromophores.

10. At this point the solutions were frozen at -20°C, if necessary. The frozen solutions were thawed in warm water when needed.

11. The solutions were deionized by placing 2.0ml of solution and 0.4ml of resin in a vial and stirring for 10 minutes on a stirplate. The resin was prepared by mixing 63 grams of 100-200 mesh Ag1x8 (Cl^-) converted to the OH^- form and 39.7 grams 100-200 mesh Ag50Wx8 (H^+) (cat. no. 140-1441 and 142-1442, Bio-rad Laboratories, respectively, Richmond, CA). The Ag1x8 (Cl^-) resin was converted to the OH^- form by washing the resin with 1.0N sodium hydroxide until the eluent was chloride free. The solutions were then filtered through a 0.45um filter (cat. no. 4184, Gelman Sciences, Ann

Arbor, MI). Solutions were also frozen at this stage, if necessary. Twenty to thirty-five microliters of solution was injected into the chromatograph after filtering.

12. The standards were prepared from sugars which were previously dried in a vacuum oven for 4 hours at 70°C. The standard sugar solutions were prepared using deionized distilled water and in concentrations to simulate the whey solutions. The sugar standards were deionized and eluted through the 0.45um filters as described above. Twenty to thirty-five microliters of the standard solutions were injected into the chromatograph. Each powder sample was prepared in duplicate when enough powder was available. Lactose values were reproducible to within 3%, generally. Since glucose and galactose concentrations were usually less than 1% and approaching the limits of detection of this method, reproducibility was at worst, 10% for galactose and 20% for glucose.

13. The HPLC System:

The column was a RCM-BR Monosaccharide column from Phenominex Corporation (cat. no. OOH-00130-KO, Rancho Palos Verdes, CA). The column was heated to 80°C during the analyses using a Haake model F423 and F4391 pump and heater assembly (Saddle Brook, NJ). A Carbo-C guard column (cat. no. 125-0128, Bio-Rad Laboratories, Richmond, CA) was used to help extend the life of the column. A Waters Associates Liquid Chromatograph model ALC/GPC 501 and a Waters

Associates Chromatography Pump model M-6000A were used. A Hewlett Packard model 3393A Integrator (Avondale, PA) calculated the areas of each of the lactose peaks. Glucose and galactose values were hand calculated by area measurements.

14. Water (Fisher no. W-5) was used as the mobile phase and was filtered and degassed daily. The water was then heated and held at 60°C. The water was stirred on a stir plate while pumping. The pump's flow rate was 0.6 ml/min. An attenuation factor of 32X was used.

E. Ash Determination:

Percentage ash on a dry weight basis was determined using the procedure outlined in the A.O.A.C. (1975). This method is applied specifically to dry milk products.

1. 2.0000 grams of powder was weighed into a previously tarred and dried porcelain crucible.

2. The samples were ignited in a 550°C furnace until C-free. Overnight (approximately 18 hours) was assumed a sufficient amount of time.

3. Samples were placed in a desiccator and allowed to reach room temperature before being weighed to the nearest 0.0002g. Duplicates had a coefficient of variation of less than 5%.

F. Inorganic Phosphorus Analysis:

The sample preparation for the determination of phosphorus was the same as the sample preparation for the

nonprotein nitrogen analysis, Sec. II C. Ten milliliters of filtrate was analyzed by the Soil Testing Laboratory and Plant Analysis Lab (Smyth 145) at Virginia Tech using an ICAP (Inductively Coupled Argar Plasma) Spectrophotometer (Jarrell Ash Atom Scan 2400). Standards were prepared using dried potassium phosphate (monobasic) in 12% TCA and using the concentrations of 0, 2, 100 and 200ppm. The wavelength for phosphorus was 214.914nm. The duplicates were reproducible to within 5%. One sample, Formost Nutritek 900 Reduced Minerals Whey, was reproducible to only 8%.

III. Preparation for the Storage Study:

A temperature controlled upright box (Vimco warmer) was used to store the powders during the storage study. The box was manufactured by Victory Metal Manufacturing Corporation. A temperature controller was installed in the box by the Virginia Tech Laboratory Support Services. The temperature controller (model no. CN-2010) was from Omega Engineering Inc. (Stamford, CT).

The humidity chambers were 10 gallon aquariums fitted with 1/4" glass lids. The seal between the aquarium was made airtight using petrol gel (USP Mineral Oil, McGlaughlin Oil Co., Columbus, OH).

Saturated salt solutions were used to control the water activity in the aquariums (Greenspan, 1976). The salt solutions were made from the desired salt and distilled

deionized water. Enough water was added to the salt to give a thick slush with about 1/8-1/4" of water above the saturated salt layer. The saturated salt solution covered the bottom of the 10 gallon aquarium and was about 1/2-3/4" deep. The amount of salt needed to make the slurry ranged from 3-5kg per aquarium. The salts used to give the desired water activities were as follows: 0.33- Magnesium Chloride (Fisher no. M-33), 0.44- Potassium Carbonate (Fisher no. P-179), 0.52- Magnesium Nitrate (Fisher no. M-46), 0.65- Sodium Nitrite (Fisher no. S-347) and 0.75- Sodium Chloride (Fisher no. S-271). All salts were ACS certified grade obtained through Fisher Scientific (Springfield, NJ). Since water activity changes with temperature, the regression lines in Rao and Rizvi (1986) were used to calculate the water activity of the individual saturated salt solutions at 35°C. The water activities at 35°C were, respectively, 0.32, 0.44, 0.52, 0.63, and 0.74.

Whey powders were stored in packets made from film permeable to air and water vapor. The packets were made to hold up to 20 grams of whey powder. The film used to make the packets was Tyvek, a spunbound olefin, which is manufactured by DuPont (cat. no. 1059-B, Wilmington, DE). Since the Tyvek was heat sealable the packets, 4 1/2" X 6", were formed using a sealer from Packaging Aids Corporation (San Francisco, CA).

Using packets made the sampling much easier. The thin packets also allowed whey powder to equilibrate much quicker (about 72 hours) in the humidity chambers. Equilibration was done at refrigerator temperatures (4-7°C). Since the packets contained no more than 20 grams of whey powder it was assumed that there were no moisture gradients and thus the powders were not stirred during the storage study. This was confirmed by observing the powders during the study.

The packets were hung from metal rod supports placed in each aquarium. The rods were 1/4" in diameter. The packets were hung from the metal rods using paper clips.

Samples were taken ten times over 120 days. The days sampled were: 0, 1, 2, 4, 8, 16, 30, 59, 85, and 120. Samples were stored at -20°C in 38ml polypropylene test tubes (cat. nos. 55.517 and 65.791, Sarstedt, Princeton, NJ) until analyzed. The caps were double sealed with parafilm.

IV. <u>Analysis for Deterioration of Whey Powders During Storage</u>:

In addition to analyzing the stored whey powders for deterioration by brown pigment formation and loss of available lysine, the powders were also monitored for moisture content and pH. The moisture content was performed as outlined in Sec II A. The pH was measured using the solution prepared in step 3 of Sec. IV A. The pH was an

indication of the formation of acids and of the disappearance of basic amino groups.

Also, for one stored whey powder (no. 5) the lactose, glucose, and galactose levels were measured according to the procedure outlined in Sec. II D. This was done to measure the disappearance of reducing sugars over the storage period.

A. Spectrophotometric Determination of Browning in Whey Powders:

Saltmarch (1980) adapted her procedure from Choi *et al*. (1949), who used trypsin for 1hr. at 45°C to solubilize the brown pigments. The procedure used in this research project is the same as that used by Soul (1984), which is a modification of the three enzyme procedure used by Saltmarch (1980). A protease is used to solubilize the brown pigments of the Maillard reaction in the whey powders. Duplicate analyses were evaluated on the following days: 0, 1, 4, 16, 59, and 120.

1. Each sample was weighed out to contain 62.5mg ± 0.02mg protein on a dry weight basis and placed into a 60ml polyethylene bottle.
2. Ten milliliters of distilled deionized water was added to each bottle and capped. A blank was prepared using 10.0ml of distilled deionized water and continuing with the rest of the procedure.

3. The bottles were placed into a 37°C shaking water bath (Fisher model no. 127).

4. The samples were shaken for approximately 30 minutes to disperse the sample.

5. Each sample was brought to pH 7.5 using 0.1N NaOH and 0.1N HCl.

6. 1.0ml of enzyme solution was added to each sample with a Pipetman P-1000 (Rainen Corporation, Emeryville, CA). The enzyme solution was prepared by dissolving 6 mg/ml protease (cat. no. 4630, Sigma Chemical Company, St. Louis, MO) in deionized distilled water. The solution was adjusted to pH 7.5 with 0.1N NaOH. The enzyme solution was stored on ice and made fresh daily.

7. The bottles were resealed and shaken for <u>exactly</u> 30 minutes in the 37°C water bath.

8. After the 30 minutes of enzyme activity, 2.0ml of 50% wt/wt trichloroacetic acid (TCA) was added to each sample to stop the enzyme's activity and precipitate the protein. Each bottle was swirled to disperse the TCA. The samples were allowed to sit for 10 minutes.

9. The samples were filtered using the filter caps (cat. no. 3500-0534, UDY Corporation, Fort Collins, CO) with fiber filter discs (cat. no. 3000-0520, UDY Corporation, Fort Collins, CO). About 0.2g diatomaceous earth (infusorial earth, Fisher no. I-22) and filter aid (Fisher no. 9-947) were used to aid the filtering process. The filter pulp was

used to plug the top of the filter bottle after the diatomaceous earth was added. Then the filter cap with the fiber filter disc was placed on the bottle. The samples were squeezed into clear, glass test tubes. If the sample was cloudy it was refiltered until it appeared clear.

10. The samples were read on a Perkin-Elmer Lambda 3 System Spectrophotometer (Oak Brook, IL) at 420nm. Early in the shelf-life study absorbance readings were so low that duplicates were approaching the detection limits of the spectrophotometer. As absorbances increased during the storage study reproduciblity between the duplicates was generally less than 10%.

11. The spectrophotometer was zeroed with the blank.

B. Estimation of Available Lysine Using the Udy-Carpenter Dye Method:

Reactive Lysine content of proteins in a given commodity is obtained by measuring the decrease in moles of dye bound when the available lysine epsilon amino groups are blocked prior to reacting with Acid Orange 12 dye (1-phenylazo-2-sulfonic acid). One mole dye is bound to one mole lysine. In theory, the attraction is between the negatively charged azo dye and the positively charged basic amino groups of lysine, histidine, arginine, and terminal amino groups in the proteins of foods suspended an acid medium (Hurrell and Carpenter, 1981). Thus, a difference between the prepared "A" and "B" samples allows

determination of available lysine. The method is applicable to proteins in feed and foods. This method is modified for whey protein powders from the procedure of Udy Corporation (Fort Collins, CO). Duplicate analyses were evaluated on the following days: 0, 4, 16, 59, and 120.

1. Whey powders should be ground to or able to pass through a 0.5mm screen. Appropriate amounts of whey powder were weighed into 60ml polyethylene bottles marked "A" and "B". See step number 10 for an explanation on estimating sample weights. Each sample was prepared in duplicate. 1.0ml isopropanol (Fisher no. A432) was added to each sample bottle and swirled gently to wet all particles.

2. To all sample bottles marked "B", 0.2ml of propionic anhydride (Fisher no. 1126515) was added using a P-1000 Pipetman (Rainen Corporation, Emeryville, CA). The bottles were swirled to mix, then 2.0ml of 5.0% sodium acetate trihydrate solution (Fisher no. S209) using a P-5000 Pipetman (Rainen Corporation, Emeryville, CA) was added. The bottles were quickly capped and shaken for 15 minutes.

3. 20.00ml of Reagent Dye Solution (1.320g/L) (or some other calibrated amount kept constant for all samples) (cat. no. 3000-0602, UDY Corporation, Fort Collins, CO) was added to both "A" and "B" bottles. Then 2.2ml of 5% sodium acetate trihydrate solution was added to all bottles marked "A".

4. The bottles were shaken until equilibrium was reached. For whey powders 40 minutes was usually adequate since the proteins were readily solubilized unlike grain products.

5. The samples were removed from the water bath and filtered. To filter, about 1/4g of Filter Aid (Fisher no. 9-947) was used to plug the top of the 60ml polyethylene bottle (cat. no. 3500-0503, UDY Corporation, Fort Collins, CO). Next, the bottle was capped with a filter cap fitted with a 19mm fiber filter (cat. no. 3000-0520, UDY Corporation, Fort Collins, CO). The bottle was squeezed gently and about 1.0 to 2.0ml was filtered out.

6. The samples were diluted as follows: 4.95ml of distilled water was pipetted into glass test tubes using a P-5000 Pipetman (Rainen Corporation, Emeryville, CA). Next, 0.05ml of sample was added using a Drummond Model 350 pipette (Broomal, PA). The tubes were mixed using a Vortex-Genie model no. K-550-G.

7. Standards were prepared from both the reference (0.600g/L) and the reagent dye (1.320g/L) to generate a standard curve. The standards were diluted 1:100 as was done for the samples. The standards were prepared by weighing the following amounts of dye into a 25ml volumetric flask:

mmoles/L	g/25 ml
1.0	14.59g reference dye
1.2	17.51g reference dye
1.3	18.97g reference dye
1.4	20.43g reference dye
1.5	21.89g reference dye
1.513	10.038g reagent dye
1.6	23.35g reference dye
1.7	24.81g reference dye
2.084	13.826g reagent dye

The dye was next diluted to the mark with deionized distilled water. At least five standards were read with the samples each day.

8. The samples and standards were read on a Perkin-Elmer Lambda 3 Spectrophotometer (Oak Brook, IL) at 480nm. The spectrophotometer was blanked with water. The duplicate readings for this procedure were poor. Coefficient of variations below 10% were usually achieved, but some duplicates varied as much as 20%. Much of the within sample variation can be attributed to the small sample sizes. The sample weights used were 1/2 the recommended level because not enough powder was stored before the study. In addition, the values of available lysine fluctuated tremendously between the days analyzed.

9. The slope, intercept, and correlation were calculated from the standards.

$$(y-b)/m = x \text{ mmoles/L free dye remaining}$$

b = Intercept
m = Slope
y = absorbance at 480nm

10. Most of the previous work using this method has been with grains. Estimating the sample weight so it falls within the desired range (1.515-2.084 mmoles/L or 0.53-0.73g/L) of free dye for whey powders was hindered by the fact that the recommendations for grains were not appropriate due to the significantly different amino acid contents of grains and whey powders. For optimum binding conditions, the dye concentration should be within 1.5 to 2.0 mmoles/L. Although the dye is linear in concentrations from at least 1.0 to 2.0 mmoles/L there is a mass action effect at lower mmole/L values according to Dr. Art King of UDY Corporation (1987). Using the commercial wheys available, it was found that the "A" tubes should contain approximately 35mg of protein to fall within the desired range and the "B" tubes should contain about 82mg.

11. Calculations: Values were expressed as percent available lysine (grams available lysine per 100g powder).

% Available Lysine (for 20.08ml of reagent dye added to each sample) =

$$(1.102561 - 0.3394*C_a)/W_a - (1.102561 - 0.3394*C_b)/W_b$$

Where: C_a = free dye concentration of "A" in mmoles/L
C_b = free dye concentration of "B" in mmoles/L
W_a = weight of sample "A" in grams
W_b = weight of sample "B" in grams

12. Preparation of reagent and reference dyes:

Reagent and reference dye concentrates (cat. no. 3000-0602 and cat. no. 3000-0605, respectively, UDY Corporation, Fort Collins, CO) were prepared according to company directions. To verify that the reagent dye (1.320g/L) was prepared correctly, 40.00ml was weighed on an analytical balance. A correctly prepared solution has a weight of 40.37g $\pm$ 0.05g at 25°C.

RESULTS AND DISCUSSION

I. Treatment of Data from Storage Studies:

The two deterioration tests measured during this investigation of the Maillard reaction in commercial whey powders were brown pigment formation (BPF) and loss of available lysine (LAL). Rate constants were to be calculated for each method according to accepted techniques. However, only the BPF study resulted in an acceptable slope or rate constant for the dependent variable versus day.

A. Brown Pigment Formation:

The browning rate (O.D./100g powder/day) was calculated for each powder and water activity combination (40 rate constants, 8 powders at 5 a_ws) by plotting BPF for each a_w-powder combination versus day of storage (Figure 1 and Figure 2). The slope of the line was taken as the zeroth order reaction rate. A two-way ANOVA was run on the calculated browning rate constants across two classification variables, powder and water activity.

Next, the square root of each relative rate for BPF was regressed against its initial composition and its water activity of storage. The relative rate for BPF was transformed by taking the square root because it significantly increased the adjusted correlation coefficient from about 0.75 to 0.93. All compositional variables were standardized between 0-1 using the following formula:

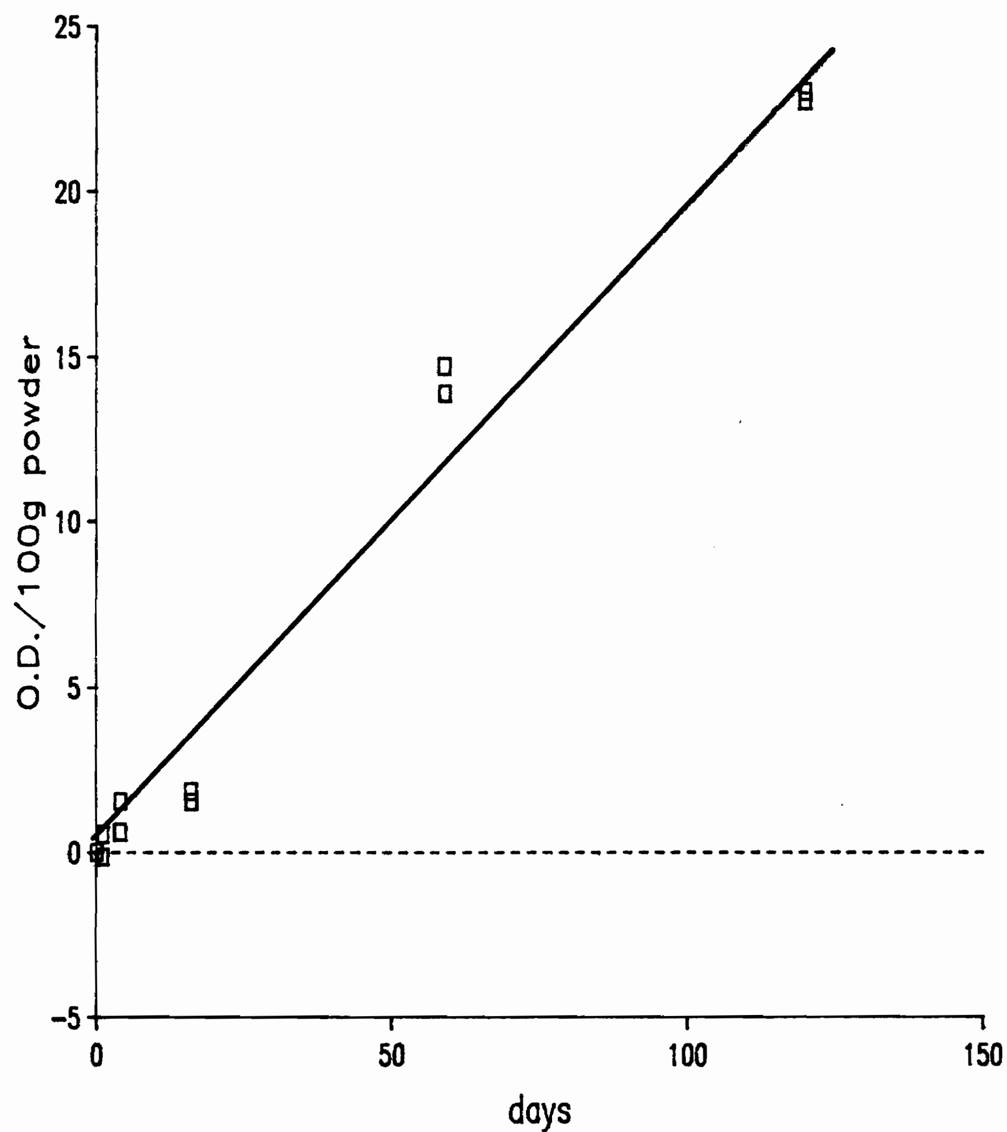

Figure 1 . Formation of brown pigments during storage at 0.74 a_w for powder 4, a SDW, compared to day zero, (B_o-B_d), where B_o is O.D./100g powder at day zero; $y = (0.199)x + 0.075$; $R^2 = 0.98$.

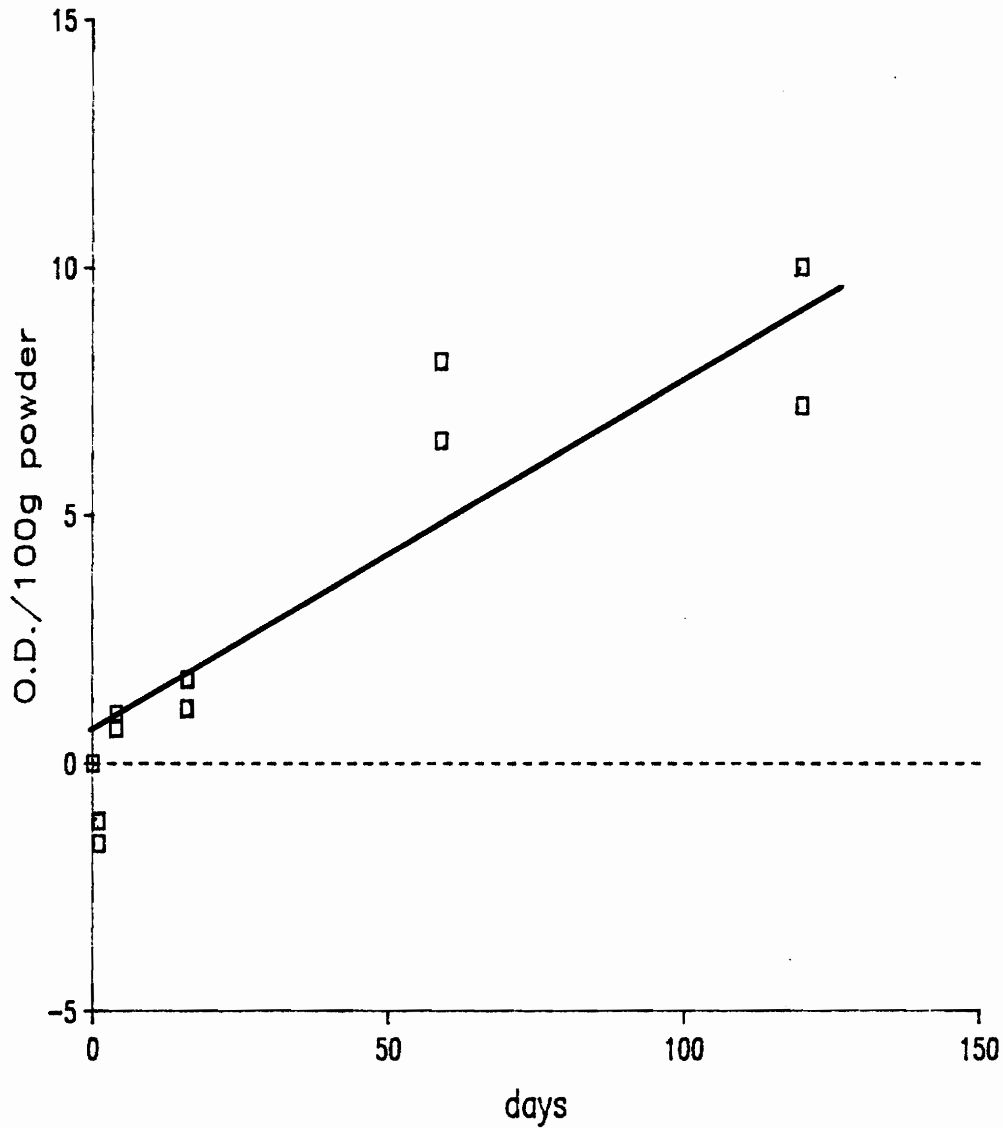

Figure 2 . Formation of brown pigments during storage at 0.32 a_w for powder 4, a SDW, compared to day zero, (B_d-B_o), where B_o is O.D./100g powder at day zero; $y = (0.081)x + 0.117$; $R^2 = 0.85$.

Standardized x = (X-low value in range)/range

where X is the percentage of a compositional variable in a particular powder. Standardization was done to determine the relative importance of each independent variable (X) and lessen multicollinearity (Myers, 1986). Insignificant variables were eliminated from the model by comparing mean square error (MSE) and predicted residual sum of square (PRESS) values between models. The Student's t-statistic for the questionable parameter was not an adequate diagnostic, since there was multicollinearity in the model which leads to unreliable coeffiecients and variances (Myers, 1986). Multicollinearity was further curtailled by combining similar variables (Myers, 1986).

No shelf-life predictions were calculated using the rate constants for brown pigment formation. However, in order to make a statement concerning shelf-life predictions a 10% increase in optical density would be considered a critical point for shelf-life determination. This was the critical point utilized by Soul (1984) when estimating the shelf-life of whey powders (21-33% protein) during storage.

B. <u>Loss of Available Lysine</u>:

No relative rates were calculated due to varied correlation coefficients caused by large within sample variances. The correlation coefficients were calculated from the natural logarithm of the available lysine plots (first order). In addition, many of the LAL plots seemed

biphasic, but not enough days were analyzed to draw two lines confidently. Soul (1984) had used two slopes to characterize the losses in available lysine in whey powders. Sample plots of the % available lysine lost versus day are given in Figure 3 and Figure 4. The Figures show an acceptable and an unacceptable regression of the % available lysine data. Since the data was not consistently unacceptable or acceptable in the first order plots, other transformations on y (i.e. 2nd order plots) were tried and they also could not successfully describe the data for all powder-a_w combinations.

Workers have used 1/4 scale in determining percent available lysine by the dye-binding method for reasons of economy, as this study did. They encountered no special problems, but emphasized the need for careful sample preparation (Hurrell and Carpenter, 1981). However, this researcher believes that larger sample weights should have been used in this study because whey powders absorbed water from the atmosphere during weighing. Larger sample weights would have eliminated much of the within sample variances. In addition there is the possibility that the dye-binding method gives misleading results under certain conditions as was discussed in section II.B. of the literature review.

Two-way ANOVAs were run on the available lysine data. Since day cannot be incorporated into an ANOVA as a classification variable because of severe dependence, four

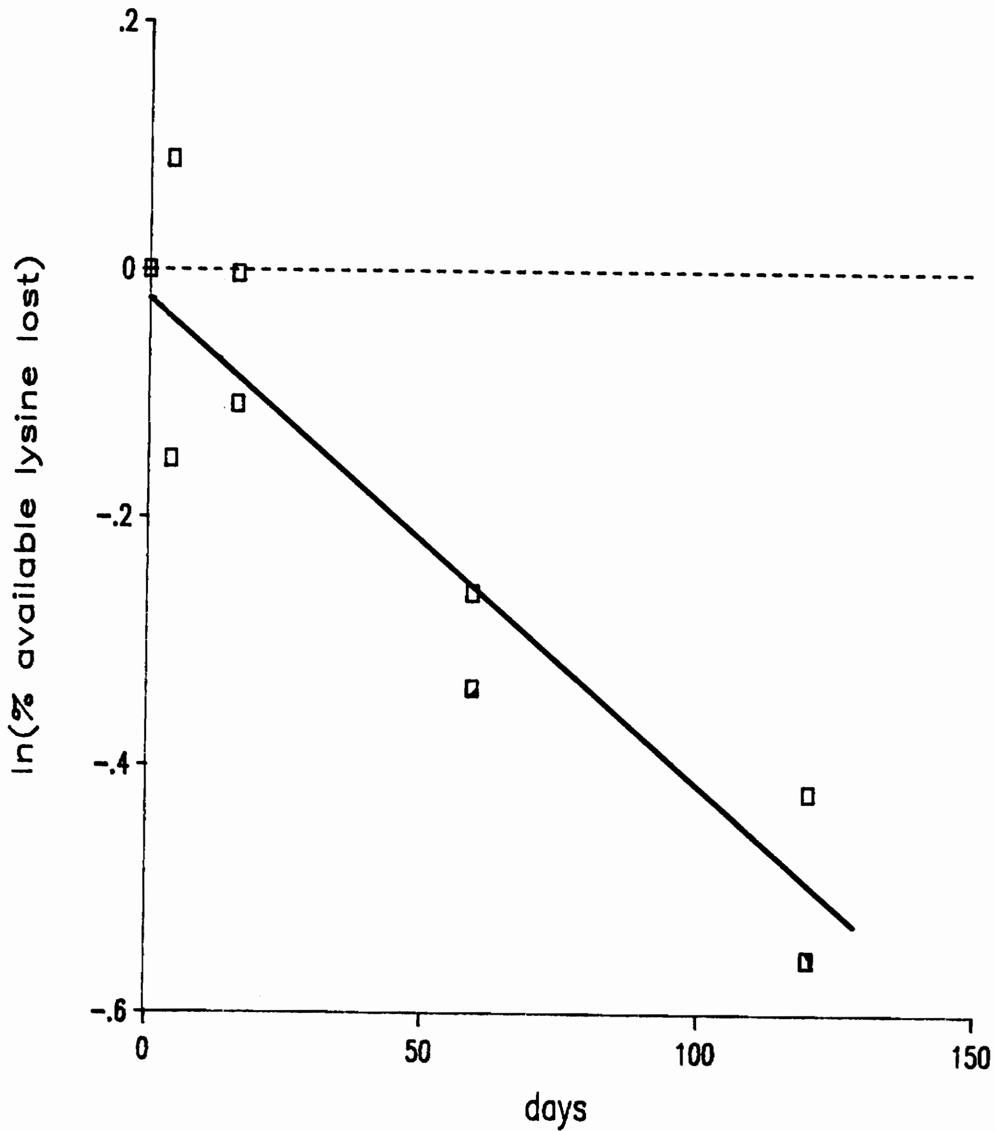

Figure 3 . Natural log of percent available lysine lost during storage compared to day zero, $\ln(L_d/L_o)$, where L_o is the % available lysine at day zero. For powder 3, a mozzarella WPC (31% protein), at 0.74 a_w; $y = (-0.0041)x - 0.0123$; $R^2 = 0.86$.

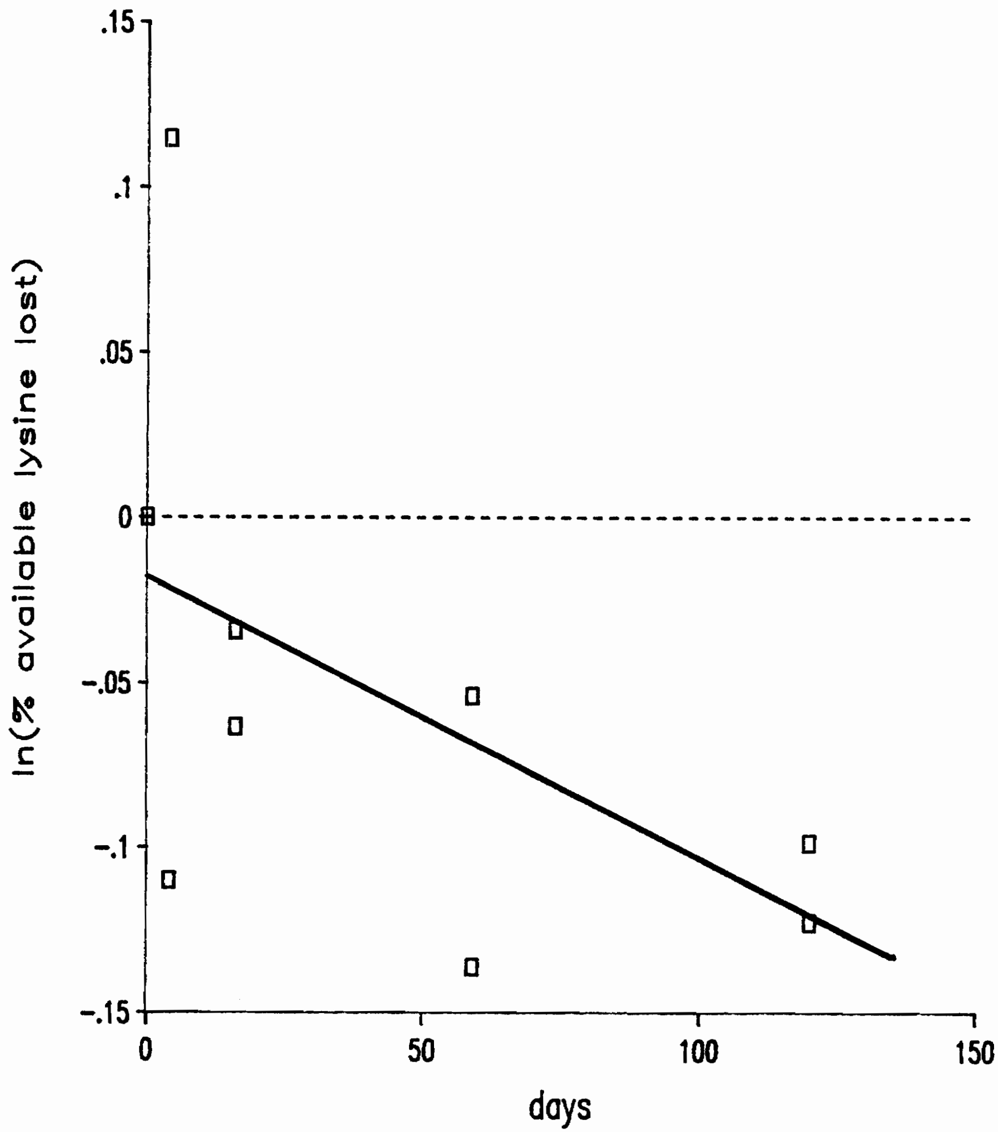

Figure 4. Natural log of percent available lysine lost during storage compared to day zero, $\ln(L_d/L_o)$, where L_o is the % available lysine at day zero. For powder 3, a mozzarella WPC (31% protein), at 0.32 a_w; $y = (-.0009)x - 0.0158$; $R^2 = 0.32$.

separate AVOVAs were performed (Ott, 1984). Day is dependent on other days as stated before and thus cannot be incorporated into multiple regression analysis either, so multiple regresssion was not utilized.

The duplicates for day zero values for a particular a_w and powder combination were averaged. The day 4, 16, 59, and 120 values were subtracted from the average day zero value of percent available lysine. The value calculated was percent of available lysine lost at a particular day compared to the average day zero value. This value was used for the first set of ANOVAs run. The second set of ANOVAs was calculated by taking the first value, percent of available lysine lost, and dividing this by the average value of lysine at day zero; the number was then multiplied by 100 to obtain loss of available lysine as a percentage of day zero. ANOVAs were performed across two classification variables, powder and a_w.

II. Composition of Powders and Processing of the Commercial Whey Based Powders used in this Study:

Table 2 shows the initial compositions of the commercial whey powders used in this study. It was desired to get powders that varied widely in protein, lactose, phosphorus, and NPN content. Obtaining whey powders commercially which varied widely in protein and lactose concentrations was not as difficult as acquiring powders

Table 2. Composition of Commercial Powders Used in This Study

Product and Identification No.	% Protein[a]	% NPN[b]	% Lactose[c]	% Glucose[c]	% Galactose[c]	% Ash[c]	Phos.[d] mg/100g	pH[e]	% Moisture
1. Nutritek 250 Reduced Minerals Whey	9.29	1.81	75.10	-[f]	0.81	5.65	605	6.22	2.49
2. Daritek 50 WPC	46.15	2.59	34.79	-[f]	-[f]	5.45	333	6.60	4.20
3. LE-PRO WPC 470	31.45	2.02	45.61	0.33	0.88	6.36	479	6.47	5.13
4. Extra Grade Dry Sweet Whey	9.67	1.92	74.03	-[f]	0.40	8.55	710	5.90	3.25
5. LE-PRO SW 420	9.40	1.82	71.82	0.38	1.73	8.74	748	5.69	4.42
6. Nutritek 900 Reduced Min. Whey	11.21	1.52	83.09	-[f]	0.15	1.15	157	6.08	5.16
7. Daritek DM Demineralized WPC	40.48	2.14	48.27	0.40	0.46	3.40	253	6.72	3.37
8. Savorpro 75 WPC	82.47	2.70	4.10	-[f]	-[f]	2.93	54	6.27	5.37

[a] % protein is expressed as (%crude nitrogen - %NPN nitrogen) x 6.45; dry weight basis

[b] % nonprotein nitrogen is expressed as %nitrogen x 3.60; dry weight basis

[c] values are on a dry weight basis

[d] inorganic phosphorus; dry weight basis

[e] pH of a solution of whey powder containing 62.5mg protein in 10.0ml distilled deionized water

[f] glucose or galactose not detectable by method used

which had a wide range in phosphorus, ash, and NPN levels. Previous research had shown these factors to be important in controlling the relative rate of the Maillard reaction in whey powders (Soul, 1984).

The powders were assigned random numbers from 1 to 8. A brief description of the powder will be given in an abbreviation after its identification number. The abbreviation will state the percentage of protein in the powder if it is a whey protein concentrate (designated as WPC) and the percentage protein will be after the WPC initials in parentheses. Powders which are sweet dried wheys will have the abbreviation, SDW, and no protein concentrations will be given since all the SDWs had a protein concentration of about 10%. The demineralized (designated henceforth as DM) whey powders will have a percentage value preceding the DM initials which approximates the percent of demineralization. For example, powder 1, a 25% DM SDW, is a 25% demineralized sweet dried whey.

Table 3 lists the conditions under which each whey powder was processed as reported by the whey suppliers. The extent of processing affects the composition of the whey powders as well as the physical properties of the powders. Processing effects no doubt have a very important role in the storage stability of whey powders; however, the emphasis

Table 3. Further Processing of the Spray Dried Sweet Whey Powders

Product and its Identification No.	Processing Treatment(s)
1. Nutritek 250 Reduced Minerals Whey	Electrodialysis to remove sodium chloride ions; calcium and phosphorus only slightly reduced;
2. Daritek 50 Whey Protein Concentrate	Ultrafiltration to remove small molecular weight milk minerals and lactose;
3. LE-PRO WPC 470	100% mozzarella cheese whey; Ultrafiltration;
4. Extra Grade Dry Sweet Whey	Spray dried sweet dairy whey;
5. LE-PRO SW 420	100% mozzarella cheese whey;
6. Nutritek 900 Reduced Minerals Whey	90% of minerals removed by electrodialysis;
7. Daritek DM Demineralized Whey Protein Concentrate	Ultrafiltration to remove portion of small molecular weight minerals and lactose; Electrodialysis to remove additional minerals;
8. Savorpro 75 Whey Protein Concentrate	Ultrafiltration;

of this study has been on compositional effects and their influence on the storage stability of whey powders.

III. Brown Pigment Formation Rate Constant Results:

A. Two-way ANOVA Results:

The two-way ANOVA performed across classification variables, powder and water activity, statisically analyzed the BPF rate constants to determine which powders were significantly different from each other and which a_ws were significantly different from each other. The ANOVA model was significant at an alpha level of 0.001 as were both classification variables.

a. Classification Variables:

1. Powder:

After averaging the browning rates across all a_ws studied for each powder a mean was obtained. As is indicated in Table 4, powder 5, a mozzarella SDW, had the highest browning rate constants of any of the powders stored. The rest of the powders, according to Tukey's studentized range test were not significantly different from each other. However, Tukey's test has a higher probability of accepting the null hypothesis (means are equal) than Waller-Duncan. The Waller-Duncan t-test results found that powder 3, a mozzarella WPC (31% protein) and powder 6, a 90% DM SDW, were significantly different from each other. The importance of this data confirms Soul's (1984) statement

Table 4. Two-way ANOVA across class variable, POWDER, using rate constants for brown pigment formation. Means tested using both Tukey's studentized range test and Waller-Duncan k-ratio t test. Means that are significantly different have different letters (alpha=0.05).

Powder[1]	Means[2]	Waller-Duncan	Tukey
5	1.197	a	a
3	0.544	b	b
1	0.383	b,c	b
2	0.366	b,c	b
8	0.322	b,c	b
7	0.301	b,c	b
4	0.253	b,c	b
6	0.131	c	b

[1] Powder numbers correspond to the powders identified in Table 1 and Table 2

[2] Means for each powder were averaged across a_ws (N=5)

that lowering minerals content and NPN content (powder 6 was lowest in ash and NPN) tended to decrease the browning rate in whey powders. The other five powders (1, 2, 8, 7, and 4) were only significantly different from powder 5, the mozzarella SDW.

2. Water Activity:

In the statistical comparison of the means of a_w, the browning rate constants were averaged across all eight powders at each a_w. As expected, the 0.32 a_w, had a significantly lower browning rate than the other 4 a_ws used in this study (Table 5).

From Table 6, it can be noted that powder 5, a mozzarella SDW, had browning rates considerably larger than the other powders for the a_ws of 0.44, 0.52, and 0.63. These large rate constants caused the results of the ANOVA to be biased. Thus, forcing the ANOVA to show no significant difference between a_ws 0.44, 0.52, 0.63, and 0.74.

3. Elimination of Interaction Test:

No test for interaction could be run because not enough degrees of freedom were available. However, just by looking at Figure 5, the plot of the browning rate constants for all powders studied with respect to a_w, it is obvious that since the curves are not parallel to each other, interaction is present (Ott, 1984). Interaction is evident also by observing that each of the eight powders did not

Table 5 . Two-way ANOVA across class variable, WATER ACTIVITY using rate constants for brown pigment formation. Means tested using Waller-Duncan k-ratio t test and Tukey's studentized range test. Means that are significantly different have different letters (alpha=0.05)

a_w[1]	Mean[2]	Waller-Duncan k ratio	Tukey's Studentized test
0.52	0.589	a	a
0.63	0.559	a	a
0.74	0.510	a	a
0.44	0.417	a	a,b
0.32	0.110	b	b

[1] Water activity

[2] Means for each water activity averaged across powders (N=8)

Table 6. Rate constants for brown pigment formation in powders 1 through 8 as related to water activity. Units are expressed as: (O.D./100g powder) per day x 100.

		a_w				
Powder[a]		0.32	0.44	0.52	0.63	0.74
1	k[b]	17.44	41.63	50.59	48.10	33.62
	SD[c]	0.84	8.20	3.10	3.31	1.61
2	k[b]	19.06	22.34	48.11	62.33	30.92
	SD[c]	5.86	7.15	7.63	6.21	4.78
3	k[b]	1.63	27.66	56.80	75.63	110.44
	SD[c]	0.74	3.26	2.57	4.94	7.24
4	k[b]	8.07	34.14	42.87	21.47	19.86
	SD[c]	1.14	4.16	2.53	1.12	1.03
5	k[b]	33.60	157.81	187.13	137.89	81.99
	SD[c]	2.17	10.16	7.01	5.47	2.61
6	k[b]	3.75	8.34	9.33	21.39	22.64
	SD[c]	0.53	0.90	1.30	2.01	1.20
7	k[b]	0.00	5.75	24.15	48.75	71.58
	SD[c]	2.24	3.11	3.71	3.36	7.14
8	k[b]	4.61	35.83	52.32	31.40	36.70
	SD[c]	3.40	3.55	6.18	11.34	7.48

[a] Powder numbers correspond to the powders identified in Table 1 and Table 2

[b] Rate constant

[c] Standard deviation expressed as S.D. x 100

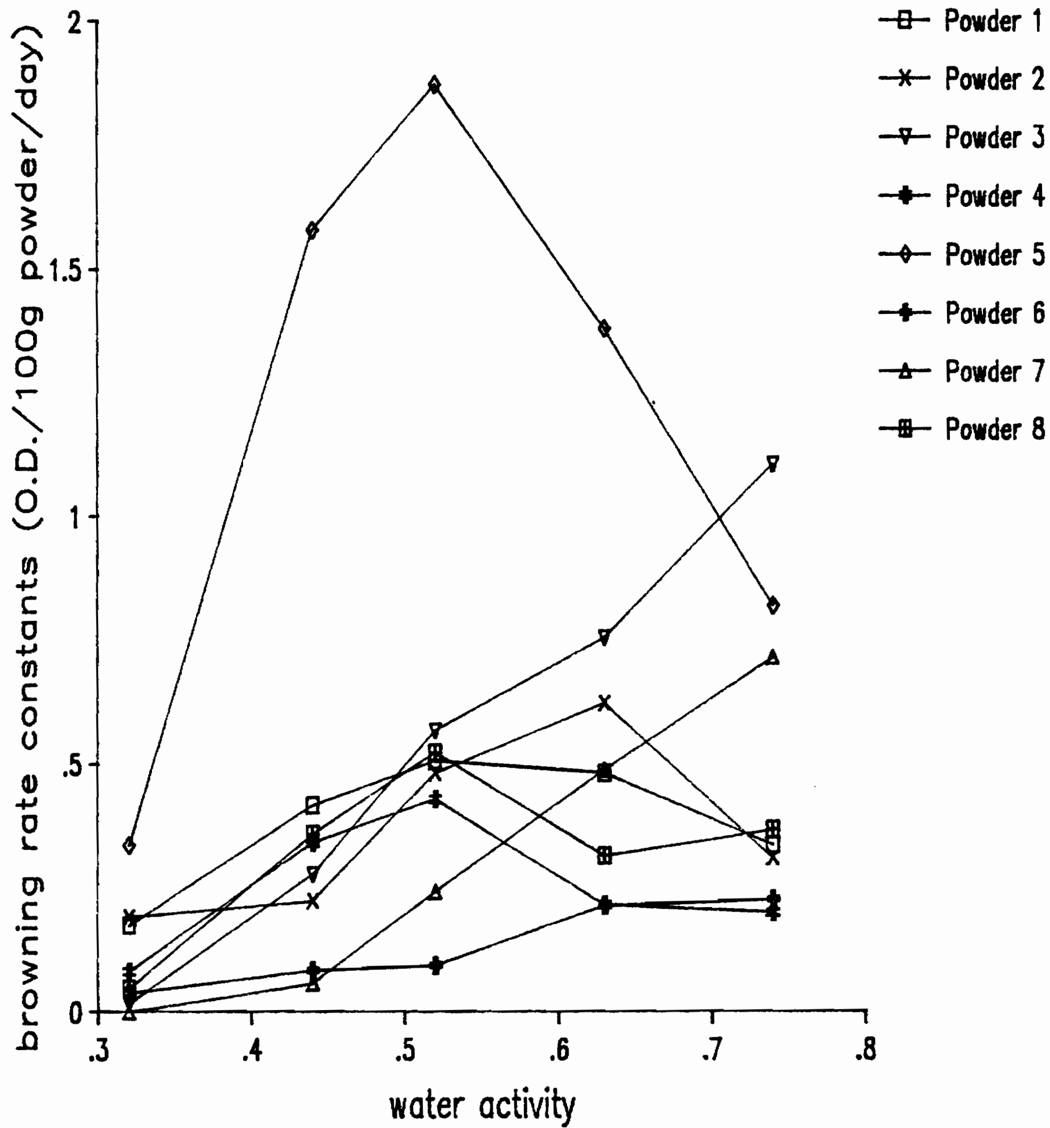

Figure 5. Rate constants for brown pigment formation for the 8 powders studied versus water activity of storage.

exhibit a browning rate maximum at the same a_w of storage (Table 6). The wide range of compositions in the commercial whey powders caused the powders to have different responses with respect to a_w.

B. Multiple Regression Results:

Two separate regression models for rate constants for BPF were developed for this data. The first model was developed to examine the relative importance of compositional variables by examining the coefficient's magnitude and sign. The second is a simple model which can be used to predict the square root of the rate of BPF with a given composition and storage a_w. The second model was a reduction in three variables (protein to lactose ratio, ash, and NPN) from the first model by comparing mean square errors. The compositions were on a wet weight basis and the variables were not standardized in the prediction model in order to keep the model as simple as possible. From an F-test between the complete (unstandardized and on a wet weight basis, first model) and reduced models it was found that the two models were not significantly different from each other.

No intercept was used in either the first model or the second model. This is because a mixture model is not valid when a major component is not present; the model would not be describing a whey powder if the composition of

lactose was zero, for example. An intercept has no meaning when describing a mixture model.

a. Variables Regressed:

The following variables were regressed against the square root of the rate constant for BPF in both models: 1) protein, 2) lactose, 3) glucose, 4) galactose, 5) pH, 6) NPN, 7) ash, 8) phosphorus, and 9) water activity. Also, interactions between each of the above eight compositional factors and water activity were tested for significance in the model. Because none of the interactions showed significance they were dropped from the model.

b. Prediction Model:

Table 7 shows the regression variables significant in this study for predicting the square root of the rate constant for BPF. Curiously, neither protein nor lactose contents were important in predicting the rate constants even though their presence is vital in the Maillard reaction and the formation of melanoidins.

1. Glucose and Galactose:

An important finding in this study was that even small amounts of the monosaccharide reducing sugars present in whey, glucose and galactose, caused a large increase in the browning rate. This is evident in the large positive coefficient for the regression variable of the sum of the glucose and galactose concentrations (Table 7). The whey powder with the highest concentration of glucose and

Table 7. Multiple regression model used to predict the square root of the rate constant for brown pigment formation. Compositional variables are on a wet weight basis (total of one).

Variable	Coefficient	Standard Deviation	Prob > T
glu+gal[a]	22.040	4.753	0.0001
a_w	5.302	1.396	0.0005
a_w^2	-4.146	1.319	0.0033
pH	-0.177	0.055	0.0028

$$(\text{Brown rate})^{0.5} = 5.302a_w - 4.146a_w^2 + 0.177\text{pH} + 22.040(\text{glu+gal})$$

adjusted $R^2=0.93$[b]

[a] Sum of glucose and galactose concentrations

[b] R^2 was adjusted since no intercept was used in the model

galactose was powder 5, the mozzarella SDW, which had a glucose concentration of 0.38% (dry weight) and galactose concentration of 1.73% (dry weight), making a total monosaccharide concentration of 2.11% (Table 2). Since galactose has been found by many researchers to react more readily in the Maillard reaction than glucose in model systems, a higher level of galactose in the whey powders would seem to be even more significant than high levels of glucose (Saltmarch and Labuza, 1982). All whey powders in this study contained more galactose than glucose (Table 2). This fact cannot be proven from the results of this regression due to collinearity between galactose and glucose.

2. Water Activity and Water Activity Squared

The second and third largest coefficients in the prediction equation were a_w and its squared term, a_w^2 (Table 7). As has been stated numerous times before, increasing a_w increases the reaction rate in the Maillard reaction until a maximum is reached, then the reaction rate decreases. For the square root of the rate constants for BPF a a_w^2 is added to describe the decrease in reaction rate after the rate has reached a maximum. This a_w^2 coefficient is negative in the regression for this reason.

3. pH:

pH was found to have a negative influence on predicting the square root of the rate constant for BPF

(Table 7). The importance of pH may be misleading and a consequence of other compositional and processing factors. Refer to the next section (III.B.c.4) on pH which describes specific powders in this study.

c. Model Showing Relative Importance of Variables:

The variables investigated here are not all inclusive. As a result, other whey powders may not be described sufficiently using this model. It is difficult to generalize the results of a single experiment.

1. Water Activity and Water Activity Squared:

As had been presented in the previous section which described the effects of a_w on predicting the square root of the rate constants for BPF, a_w had a tremendous effect on he reaction rate (Table 8). In this model all the variables were standardized on a scale from zero to one. From the standardized variables, one can determine which variables are most important in the regression. These results showed a_w and its squared term described a majority of the change in the square root of the rate constant for BPF. Figure 6 and Figure 7 represent a SDW and a WPC and how each changes with respect to a_w. Table 9 lists the a_ws at which the browning rate maxima occurred for all powders studied. The SDWs tended to have a browning rate maxima at a a_w of 0.52 and the WPCs tended to have a browning rate maxima at the

Table 8 . Multitple regression model estimating magnitude of compostional factors and water activity; the dependent variable is square root of brown pigment formation rate constant. Independent variables are standardized (from 0-1) and the compositional factors are on a dry wt. basis.

Variable	Coefficient	Standard Deviation	Prob. > T
a_w	1.375	0.240	0.0001
a_w^2	-0.960	0.234	0.0003
glu+gal[a]	0.576	0.119	0.0001
NPN	0.478	0.216	0.0339
pH	-0.260	0.127	0.0495
P/L[b]	-0.124	0.167	0.4616
ash	-0.0052	0.131	0.9688

Model M.S.E.=0.0271; Prob>F=0.0001; Adjusted R^2=0.94
Predicted residual sum of squares=1.33

[a] Sum of glucose and galactose concentrations

[b] Protein to lactose ratio in the whey powders

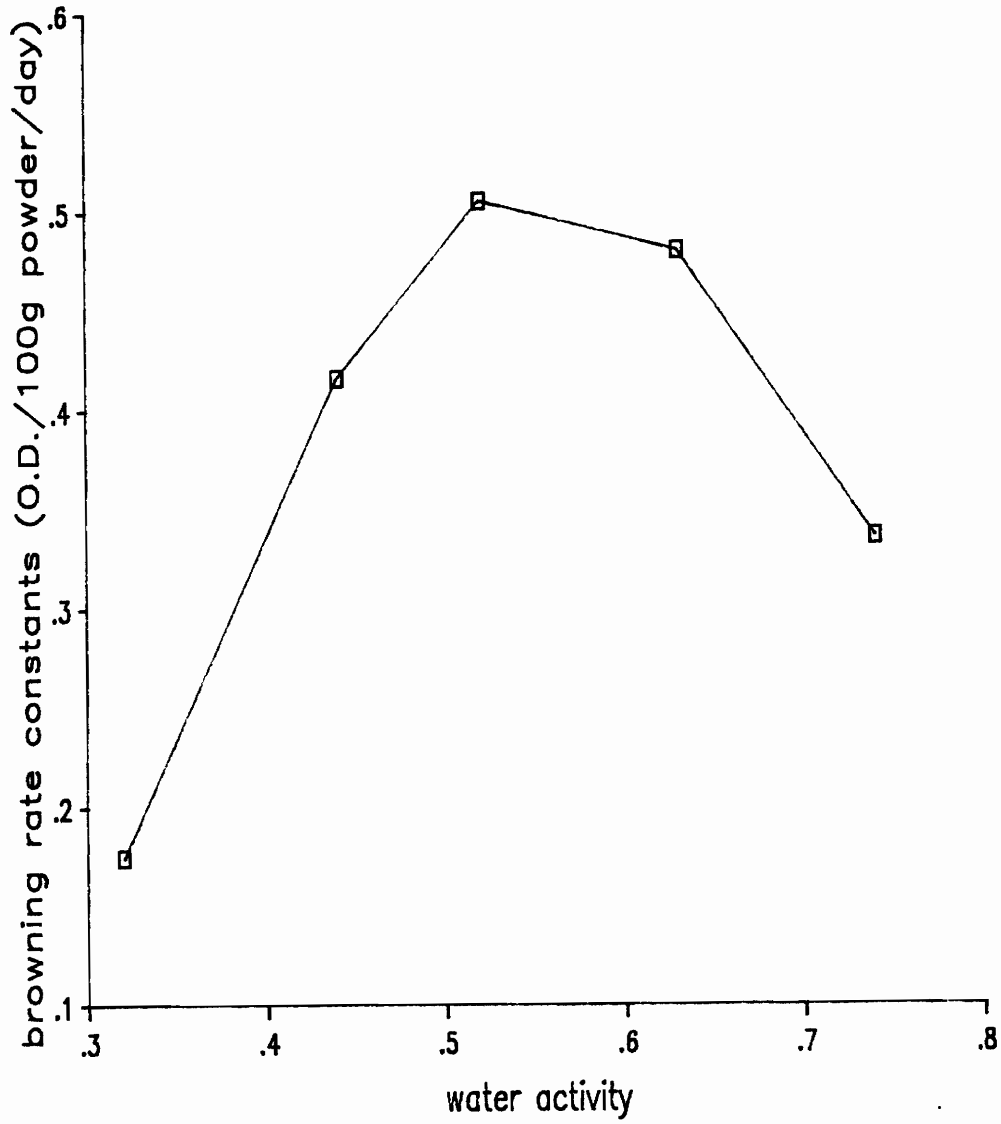

Figure 6. Rate constants for brown pigment formation versus water activity of storage for powder 1, a 25% DM SDW.

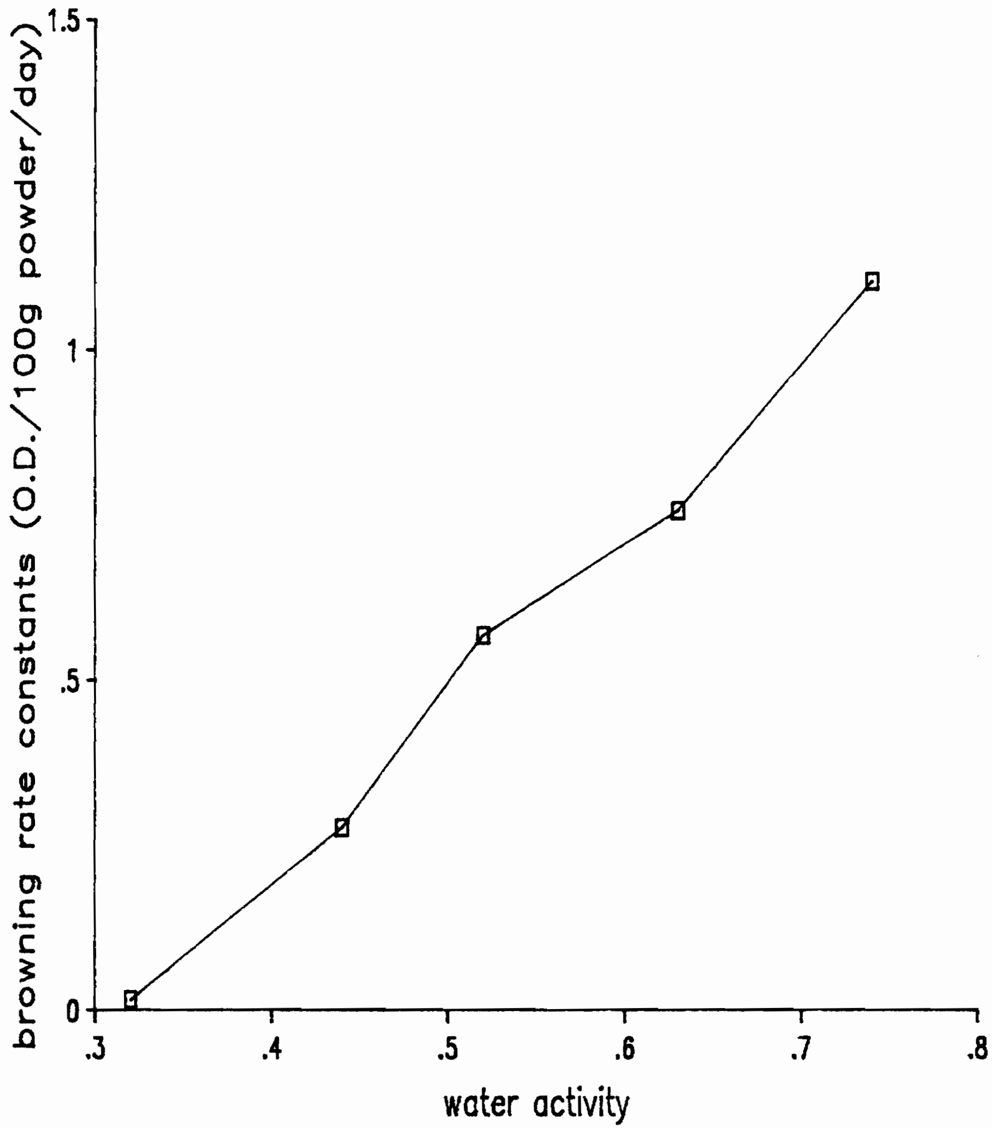

Figure 7. Rate constants for brown pigment formation versus water activity of storage for powder 3, a mozzarella WPC (31% protein).

Table 9 . Comparisons between browning rate maxima, a_w at which the maxima were observed, and % glucose plus % galactose concentration (dry weight basis.)

Powder[a]	Classification	k^b_{max}	a_w	glucose+galactose
5	SDW	187.13	0.52	2.11
3	WPC	110.44	0.74	1.21
7	WPC	71.58	0.74	0.86
2	WPC	62.33	0.63	-
8	WPC	52.32	0.52	-
1	SDW	50.59	0.52	0.81
4	SDW	42.87	0.52	0.40
6	SDW	22.64	0.74	0.15

[a] Powder numbers correspond to the powders listed in Table 1 and Table 2

[b] Rate constant expressed as (O.D./100 g powder/day)x100

highest a_w studied, 0.74. The two exceptions are powder 2 and 8, the WPCs with 46% and 82% protein, which had browning rate maxima at lower a_ws (Table 6). This shift in a_w maxima may have occurred in these high protein whey powders because lactose is limiting and thus high a_ws cause dilution of the lactose component. In addition, since water is a product of the Maillard reaction, product inhibition may have occurred at the higher water activity storage conditions. Another possible reason why a a_w shift in the browning rate maxima was observed in higher protein powders is discussed in section III.B.7. Soul (1984) reported that increasing the protein content of the whey powders tended to shift the browning rate maxima to higher a_ws. However, the highest protein content in that study was 33%.

2. Glucose and Galactose:

The coefficient for the sum of glucose and galactose concentrations had a large positive effect on the square root of the rate constant for BPF (Table 8). Table 9 shows the relationship between the glucose and galactose content of a whey powder and their rate constant for maximum brown pigment formation. Table 9 demonstrates the positive correlation between the rate constants for brown pigment formation and the levels of monosaccharides, glucose and galactose in the whey powders. The effects of the reducing sugars on the square root of the rate of BPF overshadowed, to some degree, the effects of the other compositional

factors which were originally of interest in this study; NPN, phosphorus, and protein to lactose ratio.

The results are important because previous research investigating the compositional effects of the Maillard reaction on whey powders failed to mention the effect of the hydrolyzates of lactose. Villars (1985) suggested that glucose and galactose could also be responsible for the scorching of particles during evaporation and spray drying of the whey powders. Villars (1985) proposed that heat sensitive lactase (beta-D-galactosidase) was present in the whey prior to processing causing an increase in glucose and galactose. Considering the significance of glucose and galactose on the rate of browning it is surprising that these sugars have not been investigated before.

3. Nonprotein Nitrogen:

The coefficient for NPN was positive and significant, but because of the wide range of powders studied (10-82% protein), the coefficient may be lower than expected for a SDW containing a substantial amount of NPN (Table 8). This is because in a high protein powder with an abundance of amino groups, which are not limiting, the level of NPN is less important. The ratio of NPN to protein in a low protein powder is more important than in a high protein powder. The powder with the most NPN, powder 8, had the most protein, 82% (Table 2). The lactose level was very low

in powder 8, 4.1%. The level of lactose in this case would be limiting.

4. pH:

The coefficient for pH was negative and significant, which means increasing the pH had a negative effect on the square root of the rate constant for BPF (Table 8). This is the opposite of what has been reported in the literature for foods (Saltmarch and Labuza, 1982); decreasing pH has a negative effect on the rate of the Maillard reaction and the formation of brown pigments. The importance of the effect of pH on the rate of BPF may be an anomaly. First, the pH in the powders ranged between 5.69 and 6.72, a very narrow range. Second, the powder with the highest rate of BPF, powder 5, a mozzarella SDW, had the lowest pH, 5.69. This could have "forced" the negative value for the coefficient. Perhaps Lea and Hannan (1949) were correct in stating that the use of pH in describing a dry system is ambiguous at best.

One would expect the inital pH to increase in a highly processed whey powder because of 1) the elimination of salts and lactic acid by ultrafiltration and 2) the concentration of whey proteins. For the powders used in this study, the higher protein powders (# 2, 3, 7, and 8) had higher initial pHs than the lower protein powders (# 1, 4, 5, and 6) (Table 2). The demineralized whey powders (#1,

6, and 7) had higher initial pHs than undemineralized powders (Table 2).

Perhaps the holding time of a liquid whey prior to processing can illustrate the misleading concept of pH. If a whey is stored for extended periods of time or stored at high temperatures before processing there is an increase in lactic acid which decreases the pH in a powder. At the same time, there may be residual lactase (beta-D-galactosidase) from the culture used to make the cheese in the whey which hydrolyzes the lactose into glucose and galactose (Villars, 1985). Upon storage, this may cause the powder to brown at a faster rate since monosaccharides react faster than disaccharides in the Maillard reaction and there are twice as many reducing groups than the original lactose molecule. The pH in these powders is low, in spite of this, the powders brown at a faster rate.

5. Protein to Lactose Ratio:

The protein and lactose variables were combined because when in the regression equation separately, the level of multicollinearity was unacceptable. Previous investigations have shown the protein to lactose ratio to be a good indicator of each's respective influence on the Maillard reaction (Warmbier *et al.*, 1976; Soul, 1984). Soul (1984) noted that in whey powders as here, increasing protein to lactose ratio tended to decrease the degree of BPF in the whey powders, making lactose the limiting factor

in the Maillard reaction. The coefficient for the protein to lactose ratio in the regression is negative, supporting Soul´s (1984) finding (Table 8). However, the t-value is not significant which means that the protein to lactose ratio had very little effect on determining the square root of the BPF rate constant in this investigation.

The reason for this insignificant t-value for the P/L ratio is because the intermediate protein powders (#2, 3, and 7) had higher BPF rate constants than some lower protein SDWs (#1, 4, and 6) (Table 6 and Table 9). This researcher believes this occurred for two reasons:

1) the majority of the lactose in the WPCs was not in the crystalline form early in the study and therefore the lactose was more soluble and reactive than the crystallized lactose in the SDWs.

2) The reactant species in a dried powder are not as mobile as in a solution so a ratio of about 1:1, sugar to amino group, is probably a more optimal ratio for maximum rate of browning to occur. Research on solutions of model systems reported that a sugar to amino ratio of 3:1 had the highest degree of brown pigment formation (Saltmarch and Labuza, 1982). Intermediate protein powders have a P/L or L/P ratio close to one. Above or below this ratio, the rate of BPF tended to decrease (Table 6 and Table 2). Neither the protein nor the lactose content seemed to be limiting in the intermediate protein powders because no dilution effects

occurred when storing these powders at high a_ws. These powders browned maximally at the highest water activity studied, 0.74.

6. Ash:

Ash was found to have a small positive effect on the square root of the rate constant for BPF (Table 8). The coefficient was not large and was not significant according to its t-value and therefore not a determining factor in estimating the square root of the rate constant for BPF.

It must be stressed that although a variable is found to be insignificant, it may be because other factors (i.e. NPN, glucose and galactose, or a_w) described the same variablity in the system that the insignificant variable was attempting to describe. This is especially true in a mixture model such as this since the variables being regressed are compositional factors and are not as independent as desired. So, it may be that ash is important in the Maillard reaction, but that ash's variability has already been accounted for in other variables used in the regression.

7. Other Components not Included in this Regression:

Determining the effects of phosphorus on the Maillard reaction was one of the objectives of this study. This study could only conclude that since phosphorus is incorporated in the ash component, it is likely that

phosphorus had a positive effect on the square root of the rate constant for BPF and therefore the Maillard reaction. Phosphorus could not be incorporated into the regression because phosphorus was highly collinear with ash.

Powder 5, a mozzarella SDW, the powder which had the highest browning rates, did have the highest level of inorganic phosphorus (748 mg/100g powder) (Table 2). Powder 6, a 90% DM SDW, had the second lowest inorganic phosphorus (157 mg/100g powder) and overall the lowest rate of browning (Table 4 and Table 6).

To contradict the above statement that phosphorus had a positive effect on the browning rate one could also compare the phosphorus values and the browning rates of powders 4 and 8. Powder 4, a SDW, with the second highest level of phosphorus (710 mg/100g powder) had the second lowest overall browning rate (Table 4). And powder 8, a WPC (82% protein), had the lowest level of inorganic phosphorus, but a higher browning rate than powder 4, a SDW.

Fat may be an important factor in the deterioration of high protein whey powders. The fat content of the whey powders was not determined experimentally. The specifications given by the suppliers was used as a guideline as to the levels in the powders. The level of fat reported by the suppliers for each powder was: 1) 1.1%, 2) 5.9%, 3) 2.25%, 4) 1.2%, 5) 0.75%, 6) 1.2%, 7) 3.2%, and 8) 7.5-8.0%.

There is a possibility of an accelerating effect of fat on the Maillard reaction. After the oxidation of the fat to carbonyl containing compounds, the fat component can partake in the Maillard reaction (Labuza, 1975b).

In one powder particularly, this may have been a factor in its increased browning rate. Powder 8, a WPC (82% protein), had 4.1% lactose and no detectable levels of glucose or galactose. However, powder 8 had a browning rate maximum similar to lower protein powders (Table 6). Powder 8, did have 7.5-8.0% fat. There could be autooxiation of the fat in the WPC which could supply more carbonyls to the system and thus produce a higher rate of browning than would be expected in a powder with such a low level of reducing sugars.

Another interesting point to support the autooxidation of fat in powder 8 would be to note that autooxidation of fat is minimized at a a_w of 0.3 (Fennema, 1985). From Table 6 it can be seen that powder 8 showed very little browning at 0.32 a_w. Autooxidation of fats proceeds faster at a_ws of about 0.1 and in the range of 0.55-0.85 (Fennema, 1985). Unfortunately no a_ws below 0.32 were studied. Autooxidation of the fat could partially explain why powder 8 had a browning rate maximum in the range of 0.52-0.63, whereas the other WPCs had maximas at a_ws at 0.74. Labuza (1975b) reported that in an intermediate moisture food, increasing the a_w from 0.68 to

0.85 decreased the amount of oxygen uptake (i.e. lipid oxidation) in a model system of meat and raisins. Unfortunately, no a_ws below 0.68 were studied. Labuza's (1975b) reasoned that there was a dilution of metal catalysts at high a_ws which decreased fat oxidation. At higher temperatures lipid oxidation occurred at a greater rate in the intermediate moisture food.

The second highest protein powder, powder 2, a WPC (46% protein), also exhibited a browning rate maximum shift to a lower a_w (0.63). Powder 2 also had a high fat content according to its producers, 5.9%. The rest of the whey powders had a fat content of less than 3.2%. The rate of browning of powder 2 at 0.32 a_w was not as low as powder 8, WPC (82% protein), but powder 2 had a greater percentage of lactose (35%) than powder 8. Powder 2 had the poorest reproducibility (S.D.) of any powder in this study because it formed clumps more than any other powder (Table 6).

It should be noted here that this researcher attempted to use the whey processors' values for fat content and regress fat with the other compositional factors against the brown pigment formation rate constants (section III.B.a.). No significant influence of fat was obtained. The fat variable was insignificant in the multiple regression model because this researcher believes fat is only important in accelerating the brown pigment formation rate constants for high protein powders (46-82% protein).

In section III.B.c.1. a dilution effect is discussed which would occur in the high as well as the low protein powders. This would also cause a shift in the maximum rate of brown pigment formation to lower a_ws.

IV. Lysine Results:

The results for the loss of available lysine (LAL) study were less impressive and cannot be correlated firmly to the compositions of the powders. An ANOVA allows only comparisons among powders (a_ws) and differences among powders (a_ws) to be detected. However, after this point, inferences or subjective conclusions must be made as to why the powders were different. The lysine results are divided into two sections, the first looks at the total loss of available lysine in each powder and the second examines the relative loss of available lysine.

A. Loss of Percent Available Lysine in a Two-way ANOVA:

Table 10 shows the statistics obtained from the two-way ANOVA model evaluating the percent loss of available lysine at days 4, 16, 59, and 120. The models were significant for each of the days analyzed. Each classification variable (a_w and powder) was significant at an alpha level of 0.001.

Table 10. Two-way ANOVA for each day. Data is expressed as percent loss of available lysine compared to day zero, (L_o-L_d), where L_o is the % available lysine at day zero.

	DAY			
	4	16	59	120
Prob > F Model[a]	0.0037	0.0001	0.0001	0.0001
Model Mean Sq.	0.501	0.698	0.523	0.994
Mean Sq. Error	0.210	0.072	0.115	0.112
R^2	0.70	0.90	0.82	0.90
Prob > F[b] :				
Powder	0.0003	0.0001	0.0001	0.0001
a_w	0.0026	0.0001	0.0007	0.0001
Powder*a_w[c]	0.2211	0.0001	0.0919	0.0001

[a] Significance of the model

[b] Significance of each of the variables tested

[c] Test for interaction between two class variables

a. Separation by Day:

As was stated previously, the percent loss of available lysine was separated by day. Trends through the storage of the powders and a_ws can be obtained by comparing the ANOVA results at each day.

b. Classification Variables:

1. Powder:

Powder 7, a WPC (40% protein) and powder 3, a WPC (31% protein) lost the greatest percent available lysine throughout all days tested and were not significantly different from each other (Table 11). On day 59 and 120, powder 2, (46% protein) was not significantly different from powders 3 or 7 (Table 11). As would be expected from the nature of the calculations for this statistic, the higher protein powders lost the most lysine except for powder 8, a WPC (82% protein). Powder 8 lost a significantly lower percent of available lysine than powder 3 and 7 from day 16 through day 120. This suggests that when the protein content reaches a certain percentage, available lysine cannot be made unavailable because not enough reducing sugar is available in the system. At some point the protein concentration increases to such a level that available lysine loss is hindered. At what protein level this occurs cannot be determined from this study; it does however, seem to be above 46% protein. Protein contents of 31-46% seemed to show the largest levels of lysine loss (Table 11).

Table 11 . Two-way ANOVA at each day analyzed for class variable POWDER. Data is expressed as percent of available lysine lost in powders compared to day zero, (Lo-Ld), where Lo is % available lysine at day zero. Differences in means calculated using the Waller-Duncan k-ratio t test.

Percent Available Lysine Lost											
DAY 4			DAY 16			DAY 59			DAY 120		
P[1]	mean[2]	sig.[3]	P[1]	mean[2]	sig.[3]	P[1]	mean[2]	sig.[3]	P[1]	mean[2]	sig.[3]
7	0.579	a	7	0.654	a	7	1.117	a	3	1.400	a
3	0.428	a,b	3	0.618	a	3	1.053	a	7	1.330	a
2	0.160	b,c	8	0.224	b	2	0.911	a	2	1.167	a
4	0.053	b,c	4	0.142	b	8	0.613	b	8	0.861	b
1	0.029	b,c	2	0.105	b	4	0.270	c	4	0.332	c
6	0.008	c	5	0.067	b	5	0.196	c	5	0.285	c
5	-.017	c	6	0.065	b	1	0.159	c	1	0.249	c
8	-.533	d	1	0.055	b	6	0.110	c	6	0.246	c

[1] powder identification numbers correspond to the powders identified in table 1

[2] means of the powders averaged across all a_ws investigated (N=10, except powder 2 at day 16, N=8)

[3] means with different letters on the same day are significantly different at alpha=0.05

Early in the storage study, days 4 and 16, the levels of lysine loss were about the same level as the percent error. These high levels of error make interpretation early in the storage study difficult.

As expected, the SDWs lost the lowest percent of available lysine because their protein contents are less than the WPCs.

2. Water Activity:

With the exception of day 16, a_w 0.63 had the greatest loss of percent available lysine (Table 12). At day 120 a_w 0.63 was significantly different from all a_ws studied. At days 4, 16, and 59 percent available lysine losses at a_w 0.63 were not significantly different from a_w 0.44 at days 4 and 16, and a_w 0.74 at day 59. Soul (1984) reported that maximum rate losses in available lysine were dependent on the percentage protein. When percent protein was less than 25%, the maximum rate of lysine loss was 0.74 a_w. When the percent protein was 27.9% the maximum rate of available lysine loss was a_w 0.65, while the powder with 33.4% protein had a maximum loss at a a_w of 0.74. Soul (1984) did not monitor loss of available lysine in any SDWs or WPCs with greater than 33.4% protein. Soul´s (1984) results cannot be confirmed from these results.

As expected, water activity 0.32 had the lowest loss of percent available lysine at all days studied. On day 4 and day 59, however, percent available lysine loss at a_w

Table 12 . Two-way ANOVA at each day analyzed for class variable WATER ACTIVITY. Data is expressed as percent of available lysine lost in powders compared to day zero (Lo-Ld), where Lo is % available lysine at day zero. Differences in means tested using Waller-Duncan k-ratio t test.

Percent Available Lysine Lost											
DAY 4			DAY 16			DAY 59			DAY 120		
a_w[1]	mean[2]	sig.[3]	a_w[1]	mean[2]	sig.[3]	a_w[1]	mean[2]	sig.[3]	a_w[1]	mean[2]	sig.[3]
0.63	0.389	a	0.44	0.598	a	0.63	0.796	a	0.63	1.148	a
0.44	0.212	a,b	0.63	0.590	a	0.74	0.711	a,b	0.44	0.915	b
0.74	0.137	a,b	0.52	0.155	b	0.44	0.543	b,c	0.52	0.768	b,c
0.52	-.013	b,c	0.74	0.081	b	0.52	0.443	c,d	0.74	0.679	c
0.32	-.284	c	0.32	-.158	c	0.32	0.275	d	0.32	0.158	d

1 water activity

2 means averaged across 8 powders (N=16, except for day 16 at a_w 0.63, N=14)

3 means with different letters on the same day are significantly different at alpha = 0.05

0.32 was not significantly different from a_w 0.52. Why this occurred is not known.

3. Interaction:

The interaction term was significant (alpha 0.05) for days 4 and 59 of the study (Table 10). Interaction masks main effects (i.e. powder and a_w) (Ott, 1984). The powders do not respond identically for all levels of a_w. For that reason one has to look at the powder and a_w variables simultaneously. Day 16 and 120 did not have significant interaction terms (Table 10). Section IV.B.3. discusses the meaning in greater detail.

B. Loss of Percent Available Lysine on a Percentage Basis in a Two-way ANOVA:

a. Separation by Day:

Because of the severe dependence of days with other days the ANOVA data had to be separated according to day. Table 13 lists the data for the ANOVA model performed at each day. The significance of the model and classification variables for each day suggest interpretable results.

b. Classification Variables:

1. Powder:

Table 14 shows the significant difference in the percentage LAL according to day. Powder 5, the mozzarella SDW, had the largest rate constants for BPF, but ANOVA results for percentage loss of available lysine did not correlate with this. The possible reason for this is that

Table 13 . Two-way ANOVA at each day analyzed. Data is expressed as % available lysine lost compared to day zero on a percentage basis, $((L_o-L_d)/L_o)x100$, where L_o is % available lysine at day zero.

	DAY			
	4	16	59	120
Prob > F Model[a]	0.0004	0.0001	0.0001	0.0001
Model Mean Sq.	254.47	215.04	196.71	358.32
Mean Sq. Error	84.65	59.69	53.50	61.81
R^2	0.75	0.78	0.78	0.85
Prob > F[b] :				
Powder	0.0029	0.0023	0.0001	0.0001
a_w	0.0001	0.0001	0.0001	0.0001
Powder*a_w[c]	0.0743	0.0145	0.0769	0.0018

[a] Significance of the model

[b] Significance of the variables tested

[c] Test for interaction between two class variables

Table 14. Two-way ANOVA for each day analyzed for class variable POWDER. Data is expressed as loss of available lysine compared to day zero on a percentage basis, ((Lo-Ld)/Lo)x100, where Lo is the % available lysine at day zero. Differences in means were tested using the Waller-Duncan k-ratio t test.

Loss of % Available Lysine on a Percentage Basis											
DAY 4			DAY 16			DAY 59			DAY 120		
P[1]	mean[2]	sig.[3]	P[1]	mean[2]	sig.[3]	P[1]	mean[2]	sig.[3]	P[1]	mean[2]	sig.[3]
7	10.778	a	3	14.211	a	3	24.488	a	3	32.759	a
3	9.700	a,b	7	12.089	a,b	4	22.511	a,b	4	28.015	a,b
4	3.826	a,b,c	4	11.864	a,b,c	7	21.071	a,b,c	5	26.351	a,b,c
2	2.535	a,b,c	5	6.172	b,c,d	5	18.111	b,c,d	7	25.059	b,c,d
1	1.557	b,c,d	6	4.809	b,c,d	2	15.868	c,d	1	20.138	c,d
6	0.437	c,d	1	4.349	c,d	1	12.904	d,e	2	20.049	c,d
5	-1.953	c,d	8	1.876	d	6	7.978	e	6	18.444	d
8	-6.425	d	2	1.295	d	8	6.756	e	8	9.216	e

[1] powder numbers correspond to those powders listed in Table 1

[2] means are for each powder averaged across a_w (N=10, except powder 2 day 16, N=8)

[3] means with different letters on the same day are significantly different (alpha=0.05)

during spray drying, powder 5 lost a substantial quanity of available lysine. This loss formed an abundance of colorless precursors in the powder which did not produce an absorbance at 420 nm, but did cause a decrease in the available lysine. Therefore, during storage the powder did not lose available lysine to a greater degree than the other powders, but did form brown pigments faster than the other powders. Burvall et al. (1977) reported in hydrolyzed whole milk powder a higher LAL immediately after processing than in unhyrolyzed whole milk powder. The hydrolyzed powder did not have a darker brown color than the unhydrolyzed powder. From looking at the results obtained in the LAL experiments at day zero, powder 5 (0.947% available lysine) had a lower average amount of available lysine than powder 4, a SDW (1.008% available lysine), the only other unprocessed sweet dried whey used in this study (Table 3). In addition, the lower losses of available lysine during storage may be contributed to the NPN content (1.82%) in powder 5. The SDWs have a higher NPN to protein ratio than WPCs (Table 2).

Table 14 also shows that intermediate protein powders (31-40% protein) lost high relative percentages of lysine. This is surprising given the high protein contents of powders 7 and 3 (40% and 31%, respectively). Until day 59, these two powders had the highest levels of available lysine loss and were not significantly different from powder 4, a SDW. By day 120 powder 3 was the only WPC not significantly

different from the SDWs, powder 4 and 5. This researcher believes this occurred because powder 3 had the second highest level of reducing monosaccharides (Table 2 and Table 9).

2. Water Activity:

Table 15 shows the highest to lowest LAL on a percentage basis across all days analyzed. At all days, a_w 0.63 had the largest LAL on a percentage basis including days 4 and 59 because LAL was not significantly different from a_w 0.74.

Soul (1984) reported a_ws at which maximum rates occurred in four WPCs. WPCs with less than 25% protein had a maximum LAL at a_w 0.75 and 35°C. WPCs with 27.9 and 33.4% protein had LAL maximum rates losses at a_ws 0.65 and 0.44, respectively.

The results for LAL on a percentage basis (Table 15) analyzed across a_ws are similar to the results obtained for percent available lysine lost (Table 12). Water activity 0.63 showed the maximum LAL in both ANOVAS.

As in the percent available lysine loss ANOVA (Table 12), a_w 0.32 had the lowest LAL on a percentage basis at all days analyzed. On days 4 and 16, the average LAL on a percentage basis for a_w 0.32 was negative, suggesting an increase in the available lysine in the whey powders. This may be experimental error or there is also a possibility that the reversible precursors formed early in the Maillard

Table 15. Two-way ANOVA for each day analyzed for class variable WATER ACTIVITY. Data is expressed as loss of available lysine on a percentage basis compared to day zero, ((Lo-Ld)/Lo)x100, where Lo is % available lysine at day zero. Differences in means calculated using Waller-Duncan k-ratio t test.

Loss of Available Lysine on a Percentage Basis											
DAY 4			DAY 16			DAY 59			DAY 120		
a_w [1]	mean [2]	sig. [3]	a_w [1]	mean2	sig. [3]	a_w [1]	mean [2]	sig. [3]	a_w [1]	mean [2]	sig. [3]
0.74	9.506	a	0.63	16.918	a	0.74	22.680	a	0.63	32.618	a
0.63	8.415	a	0.44	11.648	b	0.63	21.709	a	0.74	25.561	b
0.44	3.421	a,b	0.52	6.770	b,c	0.44	14.960	b	0.52	24.323	b
0.52	1.139	b	0.74	4.430	c	0.52	12.581	b,c	0.44	22.880	b
0.32	-9.696	c	0.32	-2.397	d	0.32	9.124	c	0.32	7.139	c

[1] water activity

[2] means averaged across powders (N=16, except for day 16 a_w 0.63, N=14)

[3] means with different letters on the same day are significantly different (alpha = 0.05)

reaction were converted back into a reducing sugar and an available lysine molecule (Hurrell and Carpenter, 1981). From the results listed in Table 14 it can be seen that the two powders at day 4 showing a negative value for LAL on a percentage basis were powders 5 (the mozzarella SDW) and 8 (the WPC with 82% protein). One must keep in mind that in Table 14 the a_w values for each powder are averaged together and not looked at separately. As has been suggested previously, powder 5, the mozzarella SDW, most likely had a large amount of colorless precusors because of a high content of monosaccharides. These precursors under favorable conditions could reverse, and this may have occurred at the 0.32 a_w condition. Powder 8, a WPC (82% protein), had the highest quantity of protein and upon its spray drying could have formed a substantial amount of colorless precursors which were converted back to available lysine upon storage at a low a_w.

3. Interaction:

Table 13 lists the probability of significance for interaction between powders used in this study and the a_w at which the powders were stored for all days. For days 4 and 59 the interaction term was significant at an alpha level of 0.05. It is odd that only the models for two days showed interactions. Two factors are said to interact if "the difference in mean response for two levels of one factor is not constant across levels of the second factor" (Ott,

1984). So powder and a_w are interacting if, for example, powder 1 and powder 2 do not have the same difference in loss of lysine from a_w 0.32 to 0.44. Since days 16 and 120 did not have significant levels of interaction one cannot clearly state whether there is interaction present or not. It was suggested early, that because of the wide range of powders (10-82% protein) used in this study there may be interaction in the BPF study (Figure 5). This is also believed to be the case for the loss of available lysine data.

V. Effects of Reducing Monosaccharides in Powder Five:

Figures 8 and 9 are plots of the decrease in glucose and galactose during storage of powder 5, a mozzarella SDW. When comparing the decrease in the percent reducing sugars remaining over the days of storage they correlate with the magnitude of the rate constants for BPF. At a_w 0.52 powder 5, a mozzarella SDW, had the largest browning rate constant (Figure 10). This was also the a_w at which glucose decreased the greatest extent. In the galactose plot at a_w 0.44, galactose was lost at about the same rate as a_w 0.52. When looking at the BPF rate constants for 0.44 and 0.52 a_w they are almost equivalent (158 and 187 {O.D./100g powder/day} x 100, respectively) (Table 6). The degree of correlation between the sugar and the browning rate

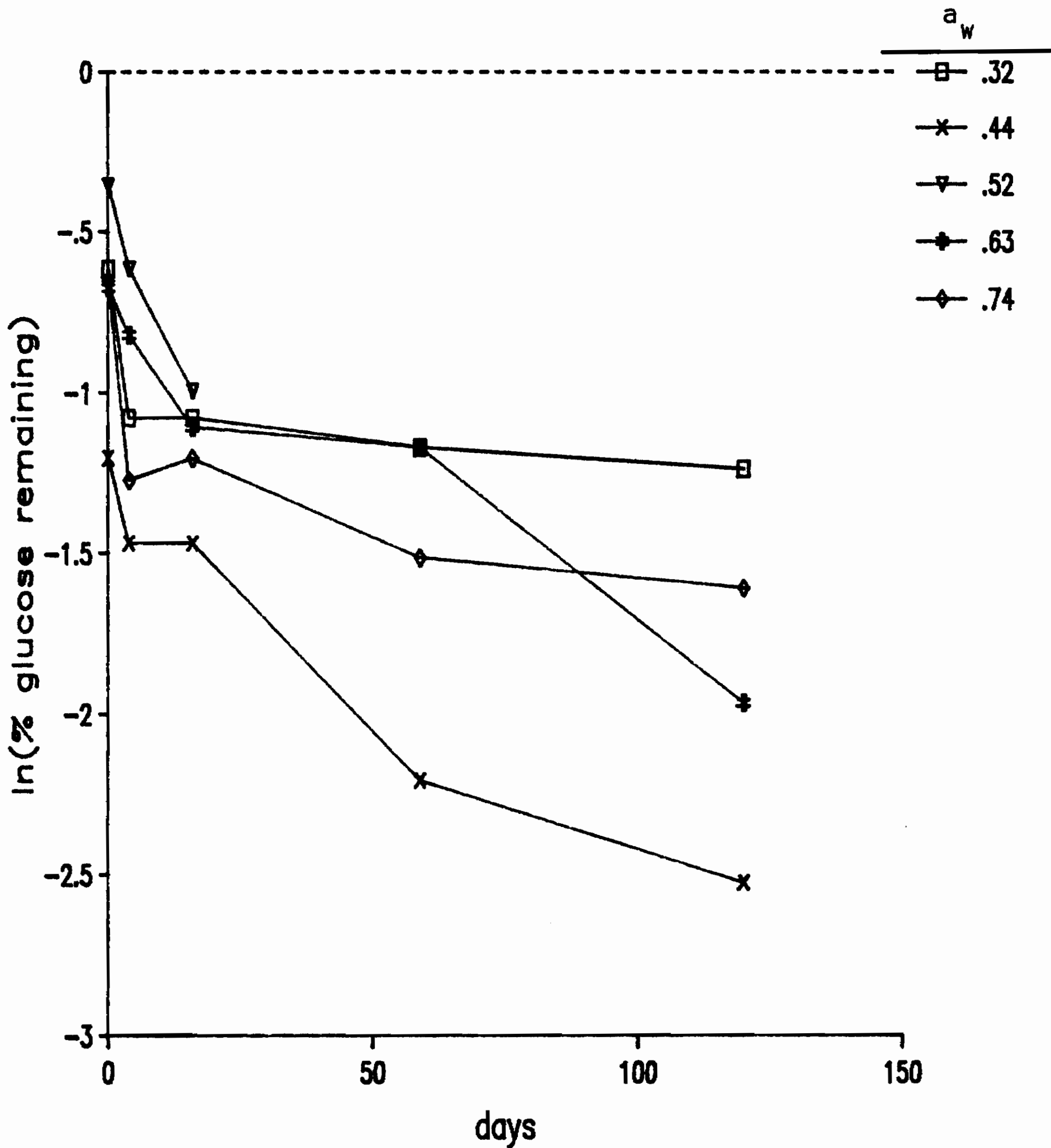

Figure 8. Natural log of percent glucose remaining (dry weight basis) versus days of storage at water activities studied in powder 5, a mozzarella SDW.

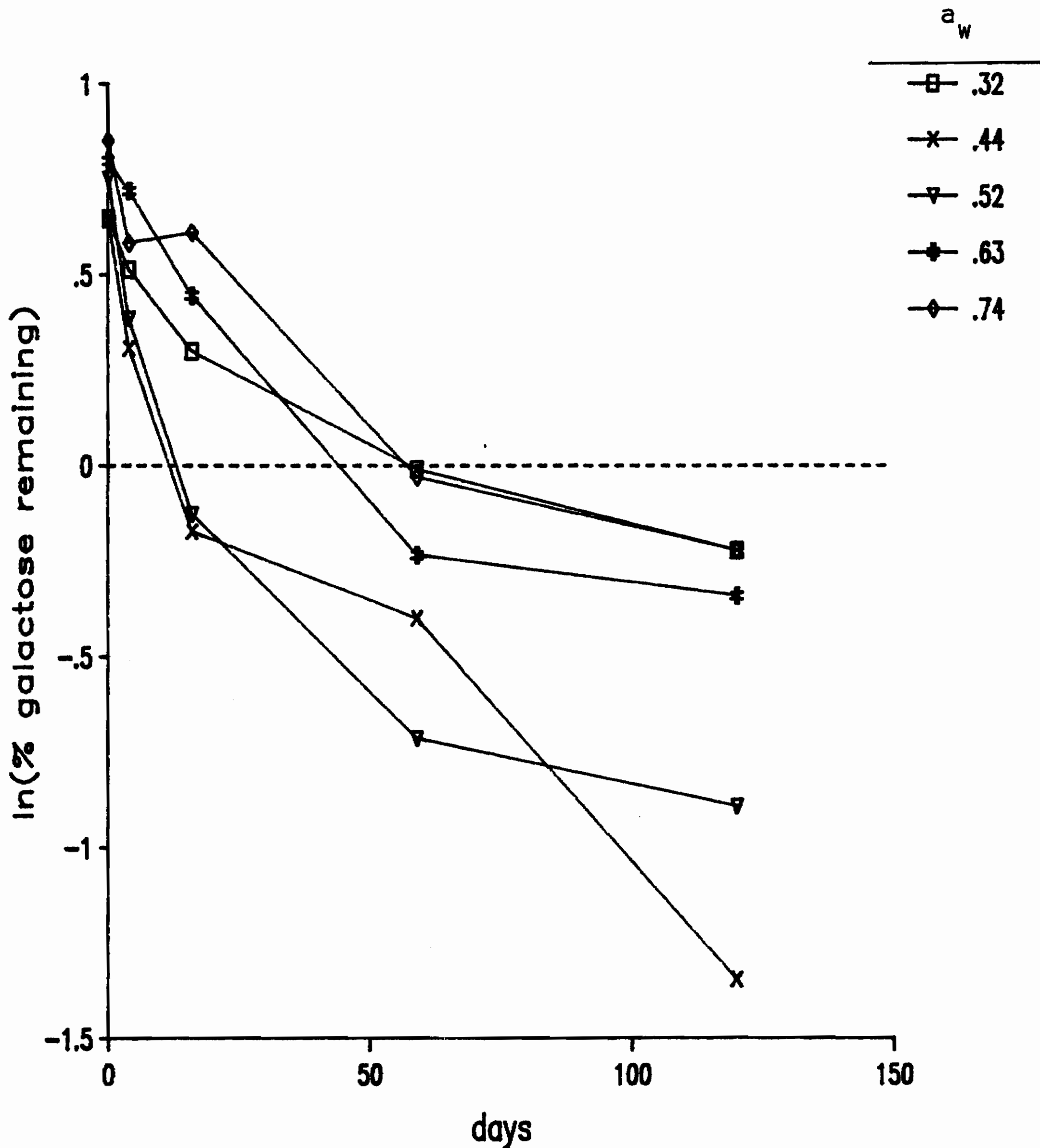

Figure 9. Natural log of percent galactose remaining (dry weight basis) versus days of storage at water activities studied in powder 5, a mozzarella SDW.

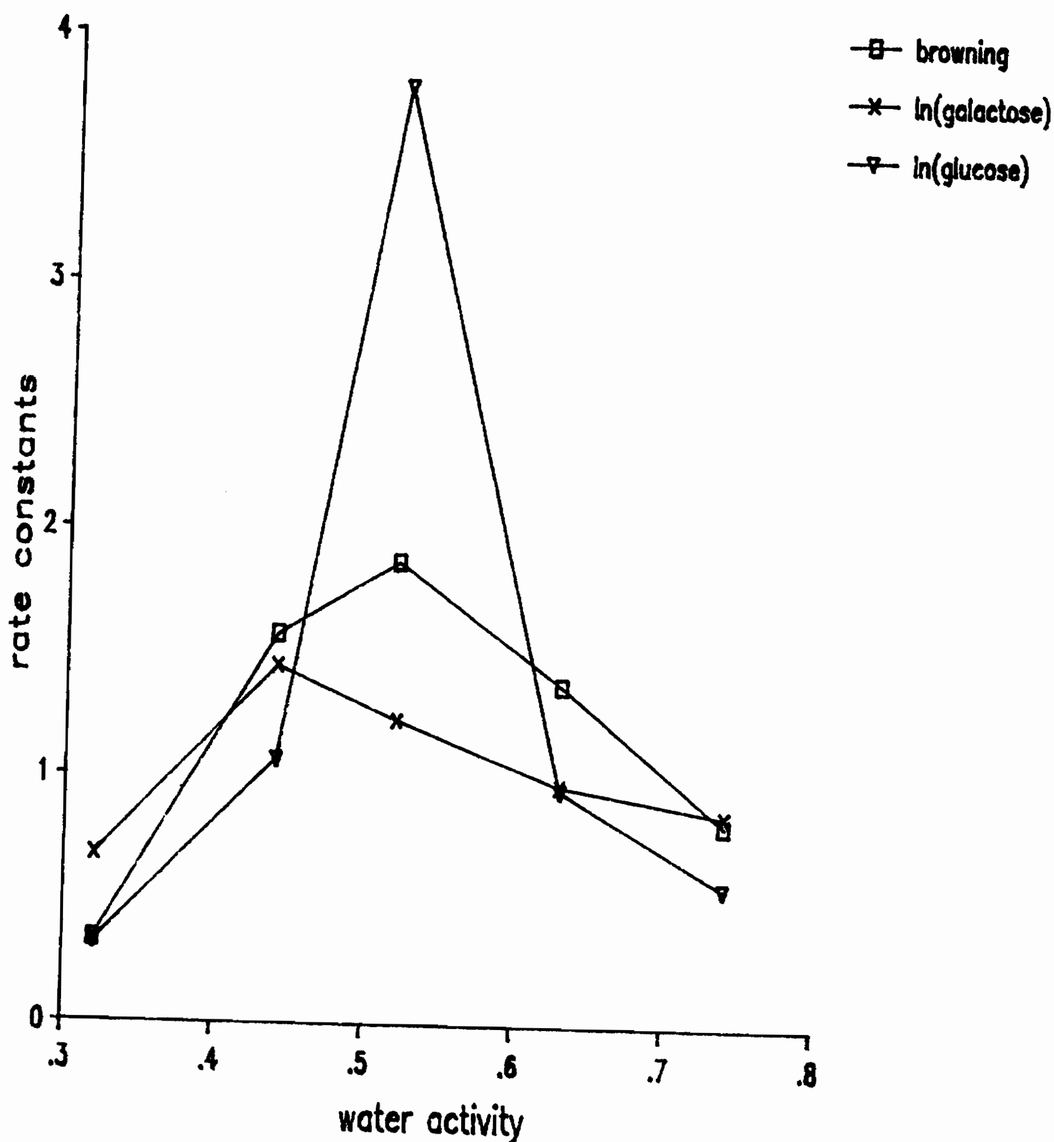

Figure 10. A plot of rate constants for powder 5, a mozzarella SDW, versus water activity of storage.
Browning: Brown pigment formation rate constants, (O.D./100g powder/day).
ln(glucose) and ln(galactose): Rate constants calculated from the natural logarithm of the plots in Figure 8 and Figure 9, respectively. Absolute values of sugar rate constants were taken and multiplied by 100.

constants is impressive given the high percentage error when determining sugar concentration.

The percentage glucose remaining did not decrease faster than the percentage galactose, except at one a_w, 0.52 (Figure 10). This is because glucose was not detectable at day 59 and 120 at 0.52 a_w (Figure 8), resulting in a large rate constant. The natural logarithm of zero is an error so the natural logarithm for day 59 and 120 at a_w 0.52 was not calculated. The same rate loss of reducing sugars in whey powders is important because galactose has been previously found to react at a faster rate than glucose in simple model systems in the Maillard reaction (Saltmarch and Labuza, 1982). Since the glucose and galactose contents were small (2.11% combined dry wt.) and lactose was also present, the availability of the amino groups may have been the component which established the rate of browning.

There were only small losses in lactose levels in these stored powders (less than 4%), and the greatest losses in lactose (a_w 0.74) did not correlate with the a_w at which powder 5 exhibited a maximum, 0.52 (Appendix C). Since most of the lactose in the SDWs in this study was crystallized lactose would be less reactive (Kim _et_ _al_., 1981) than glucose and galactose. The percentage error in measuring lactose was approximately 3%, suggesting that losses in lactose were barely detectable. Reducing sugars are not released in the later stages of the Maillard reaction as is

lysine as indicated in the Literature Review (Section I.D.1.a). The experimental error encountered in quantitating lactose concealed whether actual losses in lactose occurred during the storage study (Appendix C).

Initial glucose and galactose content could be used as an indicator of potential shelf-life in whey powders. The present method to measure sugars was adequate but, quite time consuming considering the levels of detection obtained.

VI. pH Change in Powders During Storage:

The pH change for powder 7, a DM WPC (40% protein), during storage of whey powders follows more closely to a linear loss (Figure 11). There is a strong correlation between magnitude of rate constants for BPF and the rate of decrease in pH of the powders stored at the a_ws studied (Appendix A). This can be observed by comparing the rate constants in Figure 12 and the slopes of the lines in the pH curves in Figure 11. For powder 7 the smallest decrease in pH was observed at a a_w of 0.32 and this is the a_w at which the rate constant for BPF was lowest. For the remaining a_ws, the rate of change of pH correlated with the rate constants for BPF.

Change in pH over storage of powder 6, a 90% DM SDW, is shown in Figure 13. Figure 14 shows the rate constants for powder 6 versus all a_ws studied. The slopes of the lines of the pH values versus a_w correlate with the rate

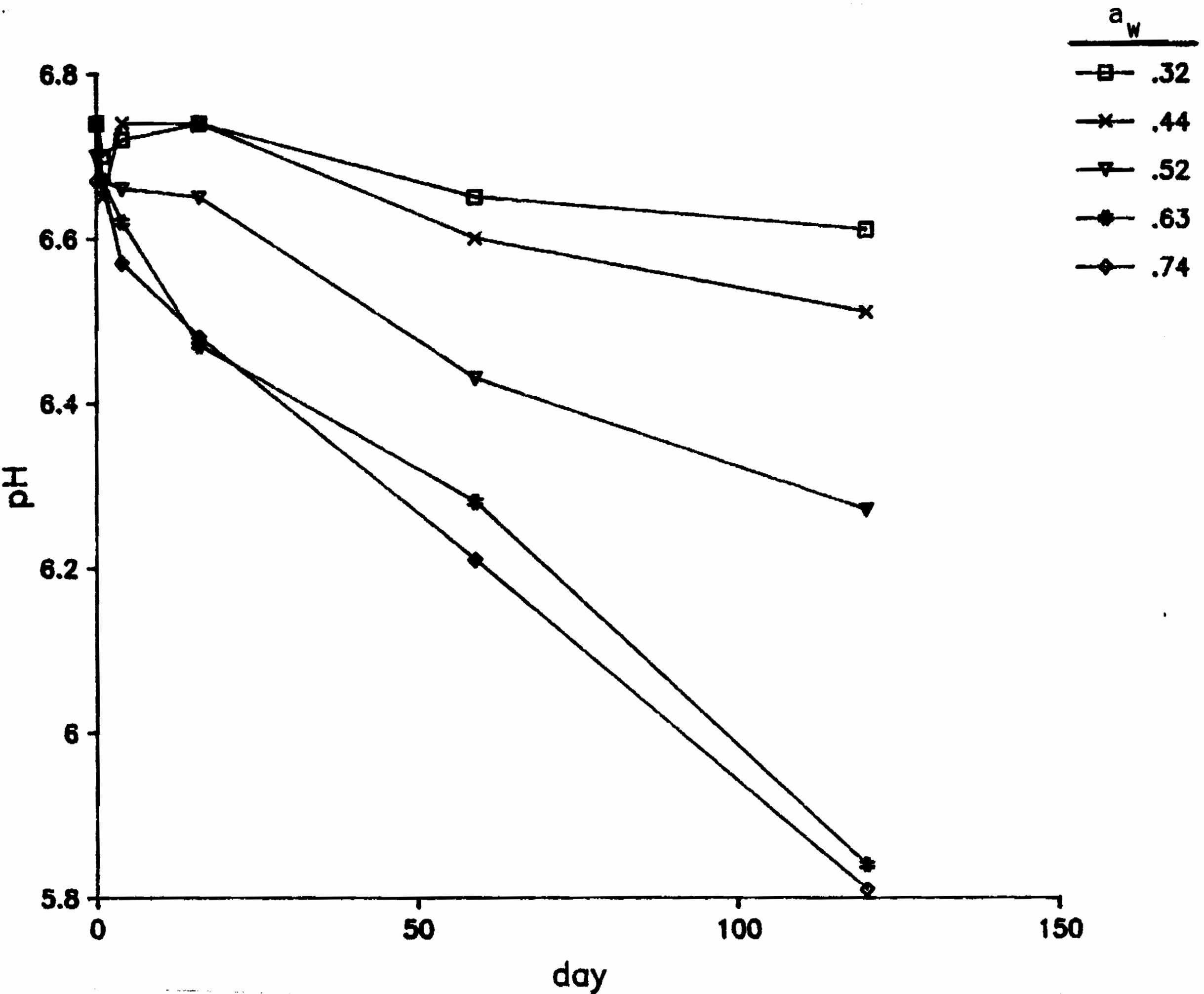

Figure 11. pH change over storage for a solution of powder 7, a DM WPC (40% protein), containing 62.5mg protein in distilled deionized water at water activities studied.

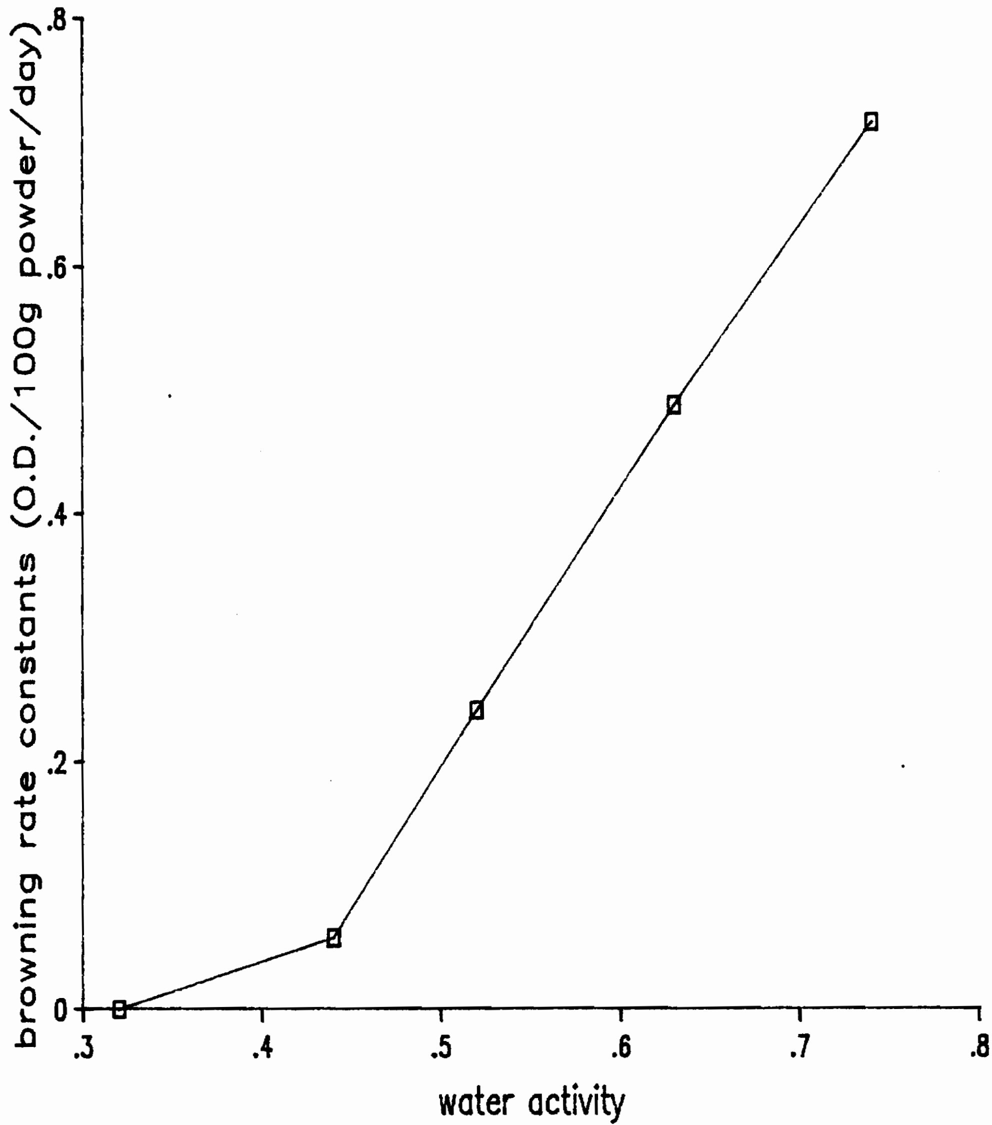

Figure 12. Rate constants for brown pigment formation in powder 7, a DM WPC (40% protein), versus water activity of storage.

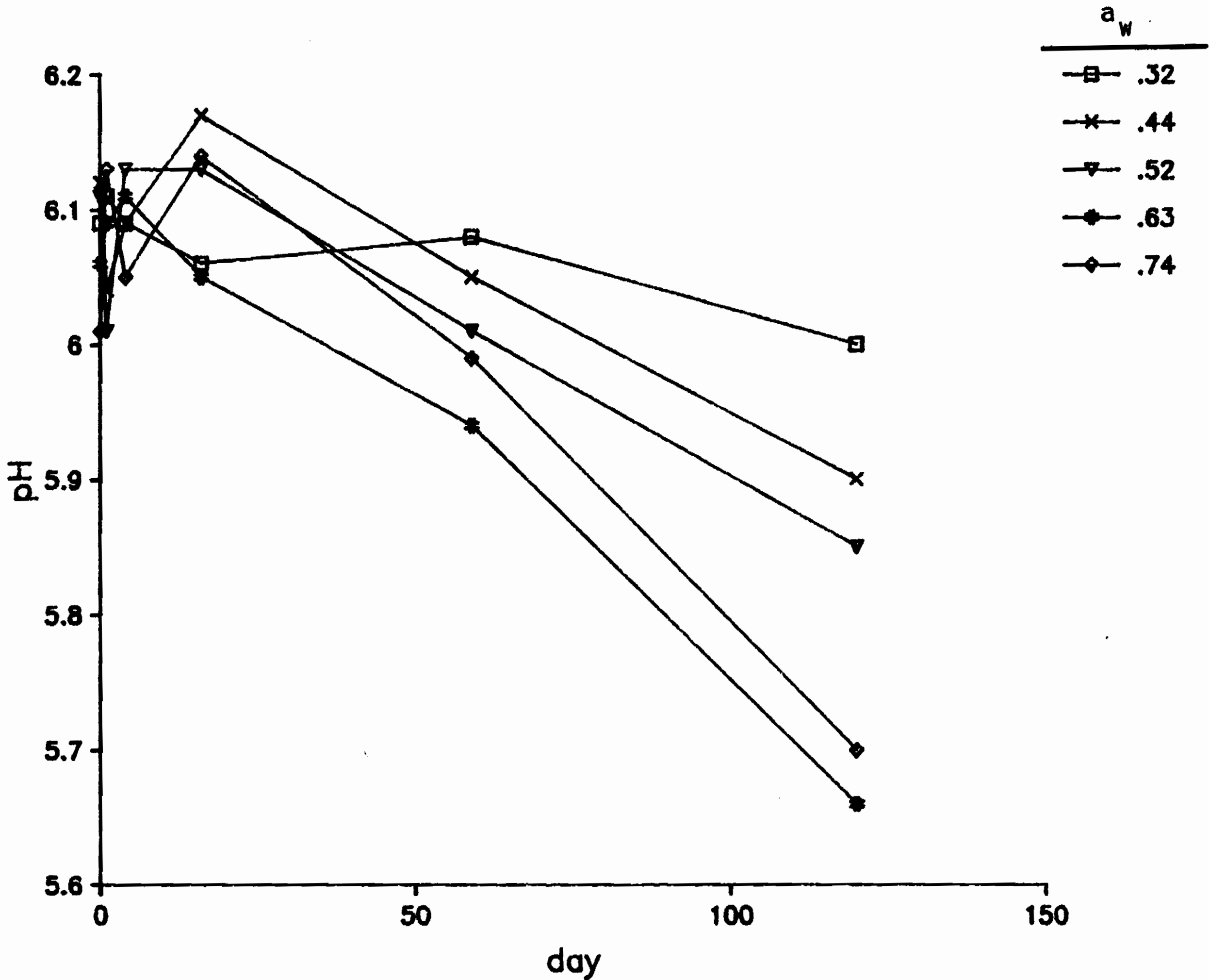

Figure 13 . pH change over storage for a solution of powder 6, a 90% DM SDW, containing 62.5mg protein in distilled deionized water at water activities studied.

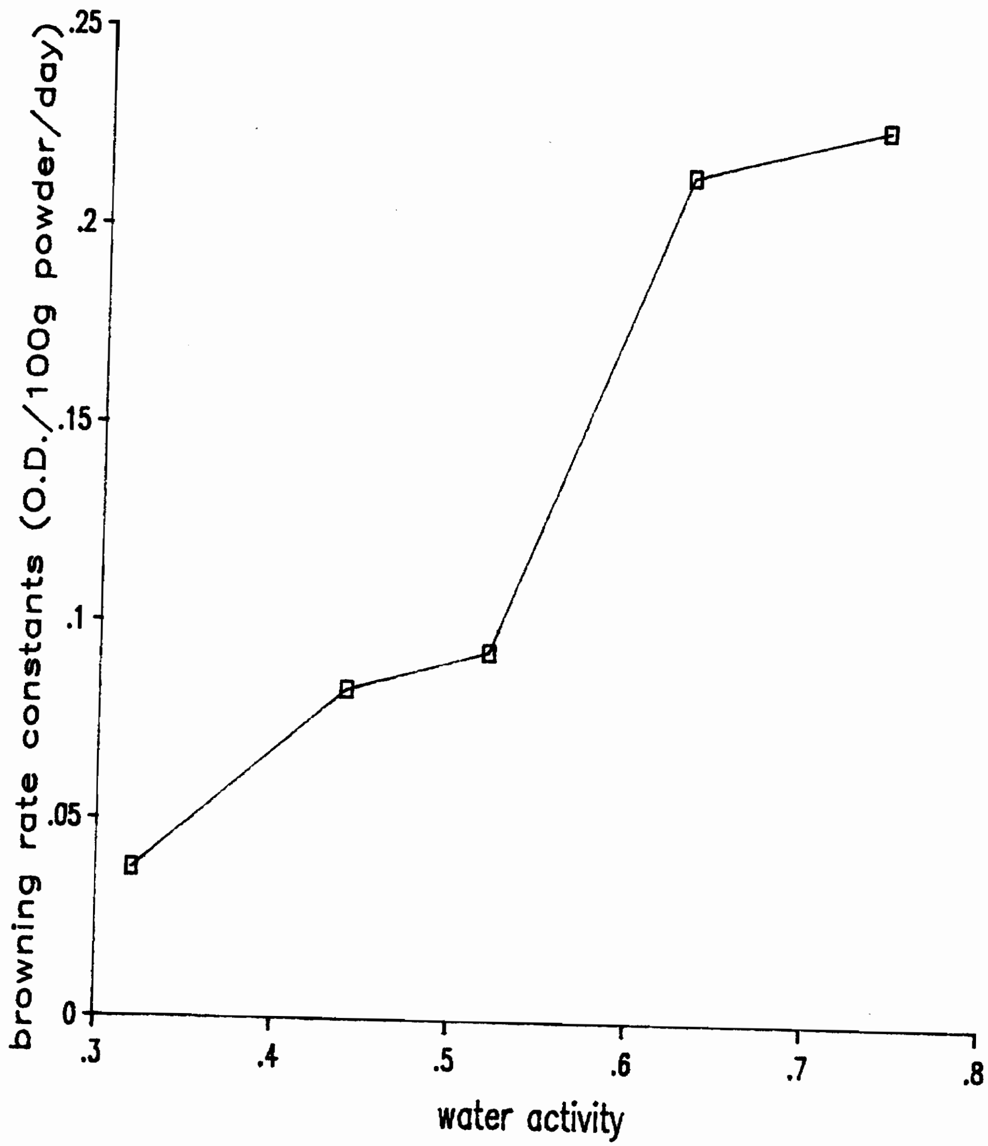

Figure14 . Rate constants for brown pigment formation in powder 6, a 90% DM SDW, versus water activity of storage.

constants for BPF. During the first 30 days of the study, the pH values varied more and overlapped more in powder 6 than powder 7. The total decrease in pH in powder 6 was not as large (about 0.4 pH units at 0.63 a_w) as for powder 7 (about 0.9 pH units at 0.74 a_w) which could account for the overlapping lines early in the study for powder 6. The a_w at which the pH decreased the most during storage was 0.63, but the a_w at which powder 6 had the greatest degree of browning was 0.74. It should be noted however, that the BPF rate constants were not very different at 0.63 and 0.74 a_w (21.4 and 22.6 {O.D./100g powder/day} x 100, respectively).

A decrease in pH occurred in stored whey powders because the basic epsilon amino groups of lysine and other amino sources condensed with reducing sugars and were removed from solution (Dworschak, 1980). Measuring the pH of whey powders in deionized distilled water containing a given amount of protein on a dry weight basis had excellent correlation with rate constants for BPF and deterioration rates of the powders. In addition, pH measurement is a rapid test and could be used as a quick measure of deterioration in a powder. In order for the pH measurement to be accurate, the protein content and the moisture content of the powder must be known.

VII. Moisture Change in Powders During Storage:

The high protein powders, powders 2 (46% protein), 3 (31.5% protein), and 7 (40% protein) did not have a majority of their lactose in the crystalline form. Caking of these powders was observed during storage. Powder 2 did not cake within a week as the other two powders did. The crystallization of powder 2 , a WPC (46% protein), required more time to initiate lactose crystallization because the higher protein content hindered the rearrangement of lactose into a crystalline lattice. By observing the powders during storage, approximations could be made when the caking phenomenon in the whey powders began. Crystallization occurred within the first days (within 2 days) of storage in powders 3, a WPC (31% protein) and 7, a DM WPC (40% protein), at a_ws 0.52-0.74. One would expect a decrease in the bound water of the system and a subsequent decrease in the moisture content of the powders. In Figure 15 the moisture contents of powder 2 over the storage period is shown. Powder 2 did not cake at 0.63 a_w until day 8 of storage and that is when the moisture content decrease is noticed (Figure 15). Conversely, the Maillard reaction yields 3 molecules of water per mole of lactose and this perhaps lessens the water loss evident in the powders. Figure 16 shows the BPF rate constants with respect to a_w for powder 2. WPCs with about 40% lactose tended to lose moisture over the storage period (Appendix B).

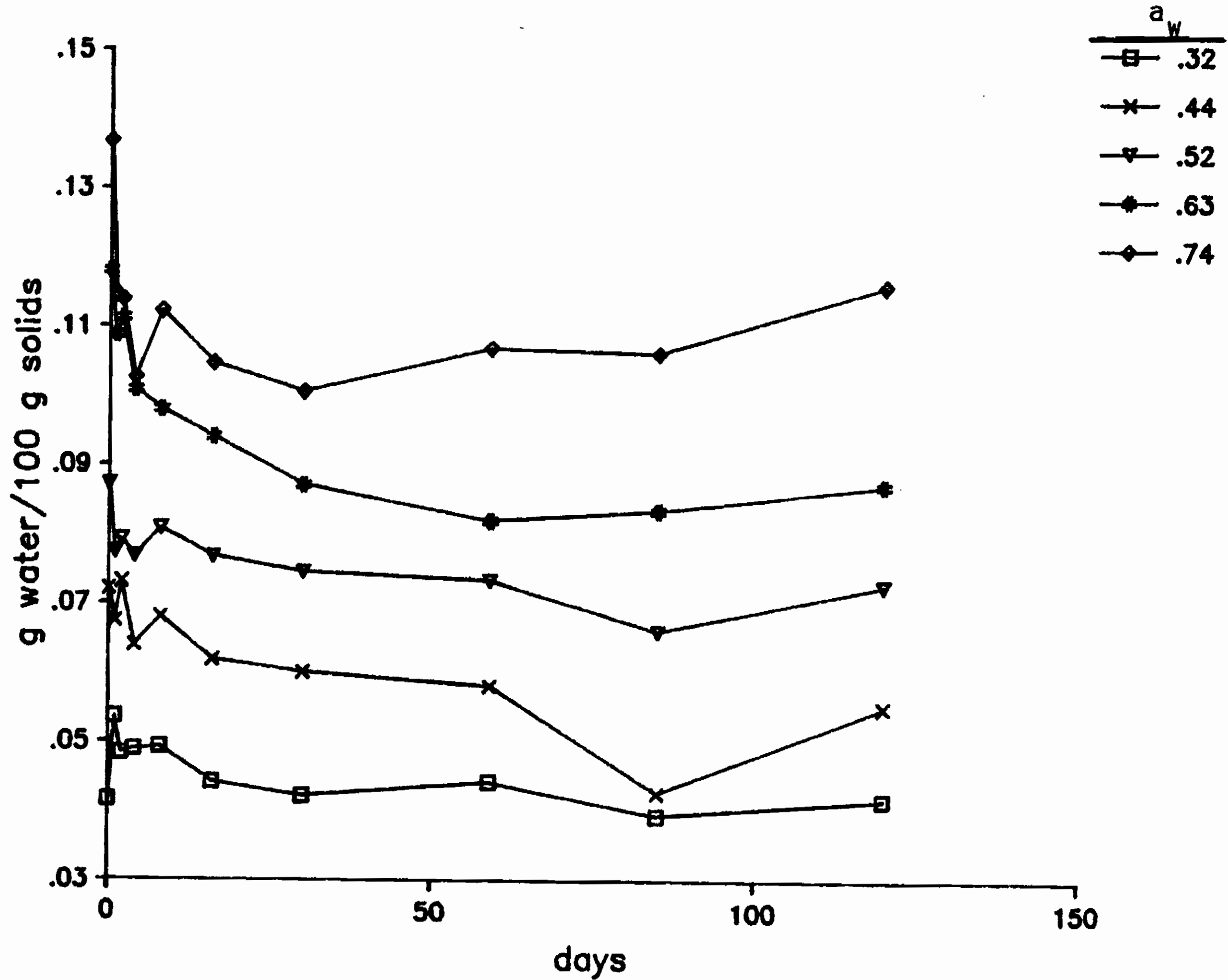

Figure 15. Moisture content (g water/100g solids) versus days of storage at water activities studied for powder 2, a 46% protein WPC.

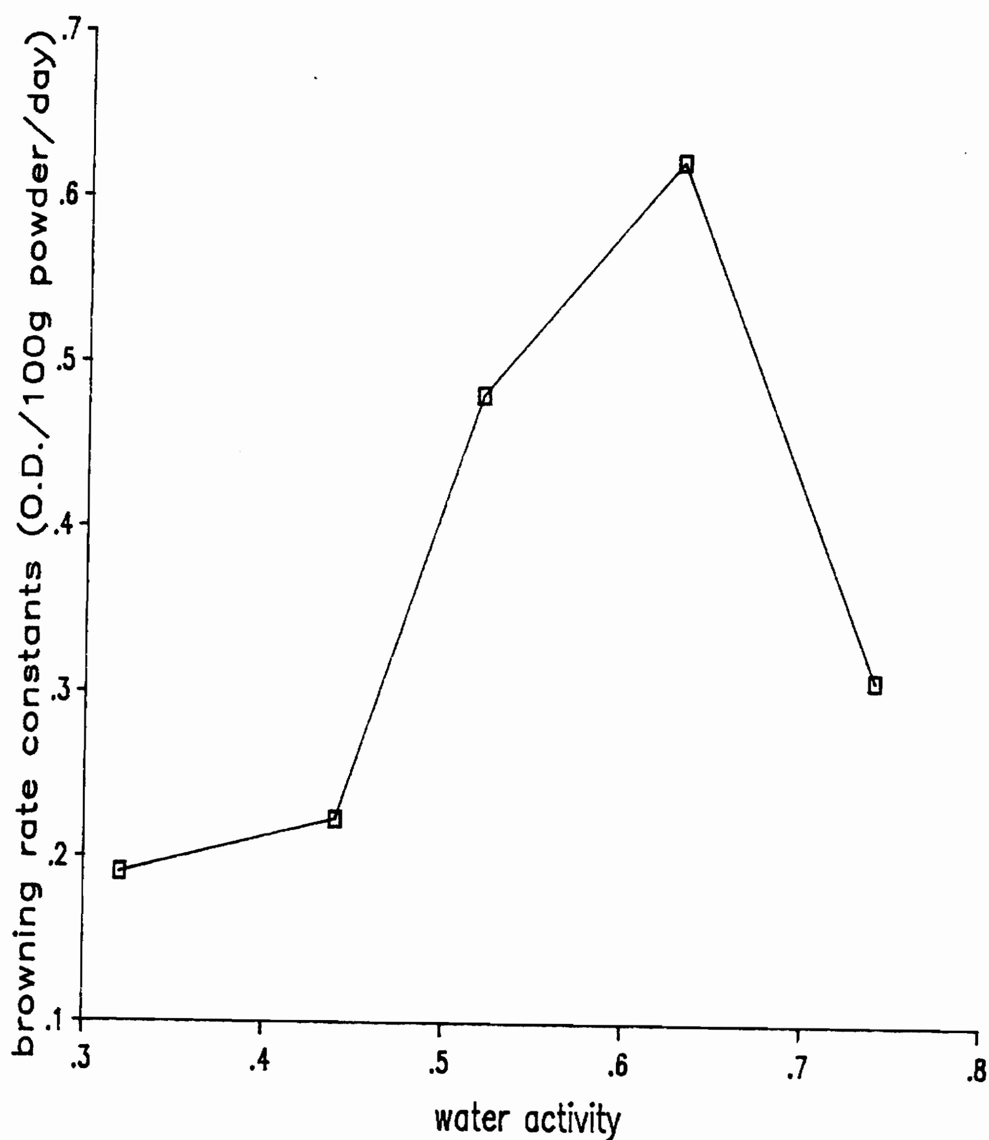

Figure 16. Rate constants for brown pigment formation in powder 2, a WPC (46% protein), versus water activity of storage.

The SDWs tended not to change their moisture content. Figure 17 shows a plot of the change in moisture content in powder 4, a SDW, during storage. In comparison to the WPC, the moisture content for powder 4 seemed to vary about a mean. The exception to this is the day zero results. For all powders studied day zero had a much higher moisture content than any of the other days studied (Appendix B). This may have occurred for two reasons, the powders were not equilibrated to the appropriate a_w at the beginning of the experiment and/or the method used to determine moisture, the Karl Fischer method, may not have been carried out correctly at day zero. The only a_w at which powder 4 appeared to lose moisture over the storage study was 0.63 from day 4 through 30. From observations made during the storage period, at a_ws of 0.63 and 0.74 three of the four SDWs (1, 4, and 5) were semi-caked by day eight. The observations recorded on day 30 and 85 stated that at 0.63 a_w powder 4 was smooth again, but at 0.74 a_w powder 4 was still semi-caked. By day 85, powder 4 had begun to cake at 0.44 a_w, and at 0.52 a_w powder 4 was already semi-caked. Figure 17 does show a drop in moisture content for a_ws 0.44 and 0.52 at day 85. However, the moisture content increases to original levels by day 120 at both a_ws. The slight drop in moisture content in the SDWs could be the rearrangement and growth of the lactose crystals (Saltmarch and Labuza, 1980) into more organized and tightly packed lattices, causing the expulsion

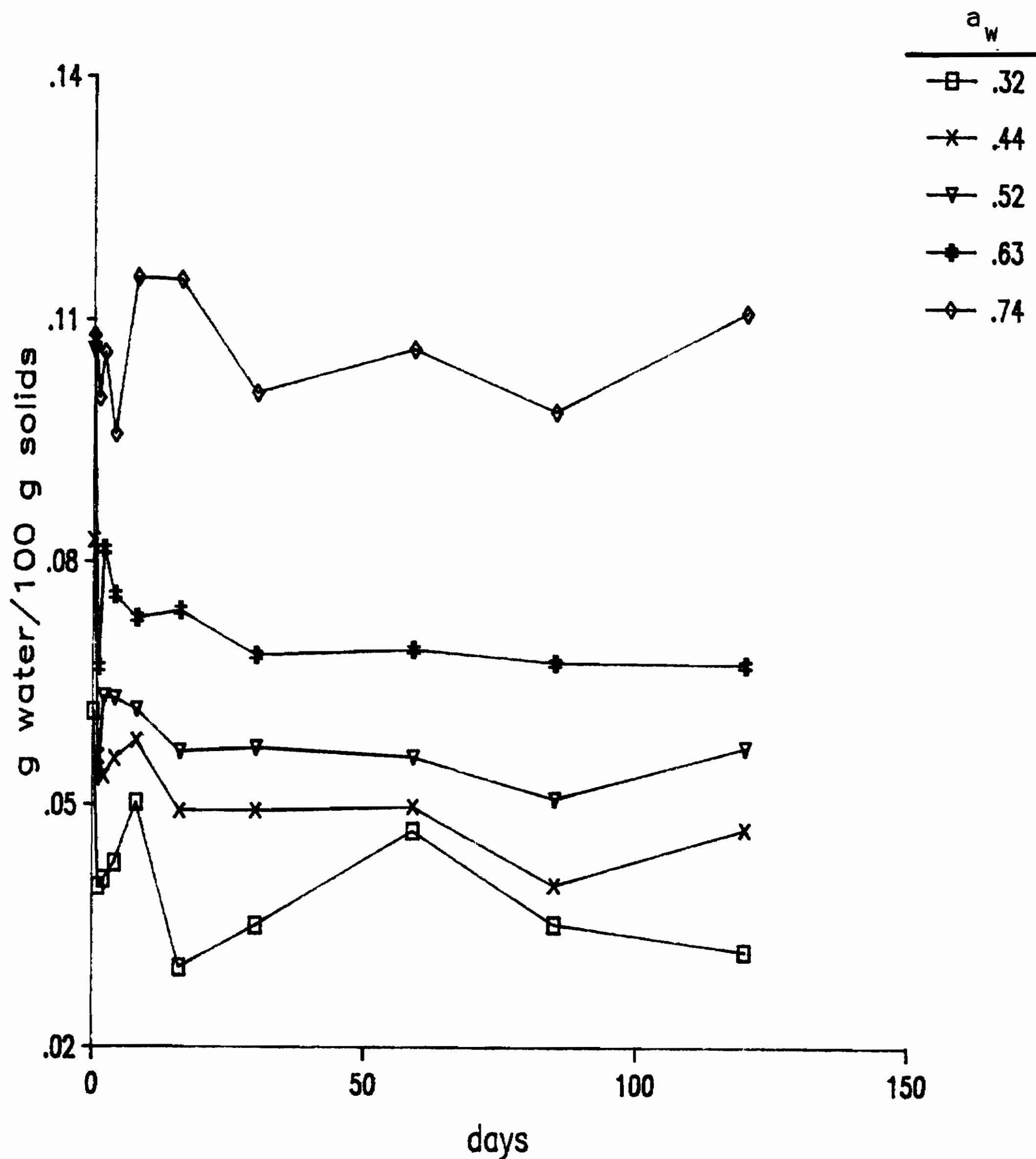

Figure 17. Moisture content (g water/100g solids) versus days of storage at water activities studied for powder 4, a SDW.

of free water. A plot of rate constants for brown pigment formation versus a_w for powder 4 are given in Figure 18. The variation in determining moisture content in this study was too large to be able to make concrete conclusions concerning the decreasing moisture contents of the whey powders during storage.

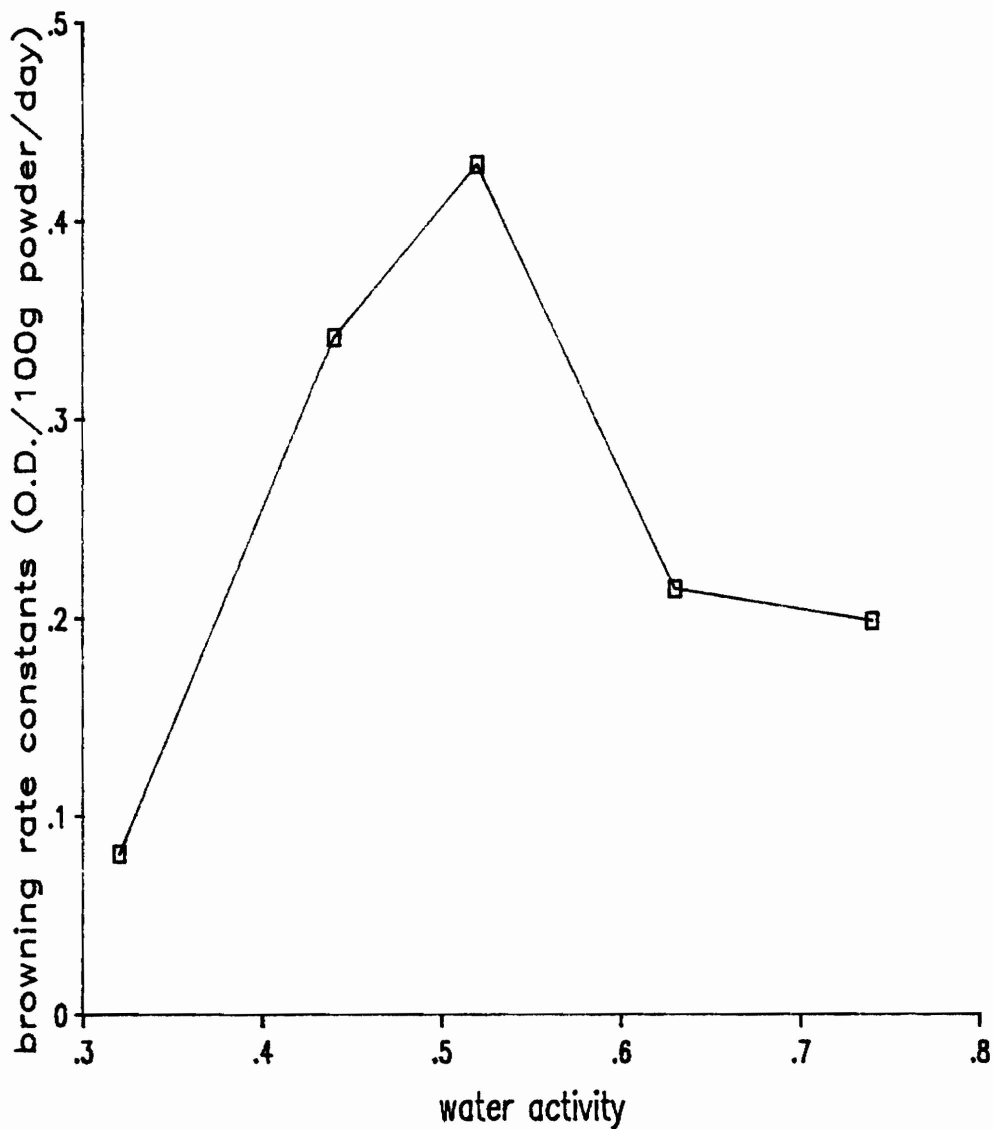

Figure 18. Rate constants for brown pigment formation in powder 4, a SDW, versus water activity of storage.

SUMMARY AND CONCLUSIONS

Eight commercial whey powders with varying compositions (9-82% protein, dry weight) were stored at 35°C and at five water activities (a_ws)(0.32, 0.44, 0.52, 0.63, and 0.74) for up to 120 days. Samples were taken during the accelerated storage study and the deterioration over time was analyzed by brown pigment formation and loss of available lysine.

Prior to storage, the compositions of the powders were determined. The powders were analyzed for protein, lactose, glucose, galactose, nonprotein nitrogen (NPN), ash, phosphorus, initial pH, and initial moisture content. The main objective of this study was to determine which compositional factors and which storage a_ws were important in influencing the Maillard reaction in stored commercial whey powders.

Multiple regression analysis was used to investigate the importance of compositional variables and storage a_ws on the relative rate of the Maillard reaction, evaluated by measuring the rate of brown pigment formation. NPN had a positive influence on the rate of brown pigment formation. Initial pH of a whey powder solution had a negative influence on the relative rate of the Maillard reaction. The reasons for this seemingly contradictory result (Ellis, 1959) may be because of differences in processing for each of the whey powders (i.e. demineralization and

ultrafiltration). Highly processed powders tended to have higher pH values. The powder with the highest rate of brown pigment formation had the lowest pH and was an unprocessed sweet dried whey. Protein and lactose were analyzed as a ratio of %protein to %lactose (P/L). P/L had a negative effect on the rate of brown pigment formation and therefore a negative effect on the relative rate of the Maillard reaction. This means increasing the amount of protein in the powder tended to decrease the relative rate of the Maillard reaction. Although protein and lactose are vital in the Maillard reaction, their coeffiecients were insignificant in the regression analysis. The powders had such varied compositions (because of demineralization and ultrafiltration) that neither protein nor lactose content adequately described the powder. In addition, in a high protein powder (82% protein), the lactose content became limiting and the relative rate of the Maillard reaction decreased to levels below the intermediate (31-40% protein) protein powders. The minerals in the whey powders had a positive but insignificant influence on the rate of brown pigment formation.

This study found that some whey powders contained small amounts of the hydrolyzates of lactose: glucose and galactose. The powders which contained the largest amounts of these reducing monosaccharides were a mozzarella sweet dried whey and a mozzarella whey protein concentrate. All

whey powders contained more galactose than glucose. Johnson and Olson (1985) postulated that large amounts of galactose are present in mozzarella cheese because some cheese cultures used in manufacturing, utilize the glucose but not the galactose. The presence of small quantities of monosaccharides (less than 2% dry wt.) greatly enhanced the Maillard reaction in these powders. The disappearance of glucose and galactose was measured in one commercial whey powder, a mozzarella sweet dried whey, which had the largest concentration of reducing monosaccharides. To use glucose and galactose concentrations as indicators of potential browning, a more sensitive procedure is needed. The 10% relative error encountered in this study was not able to detect low concentrations adequately.

Water activity had dramatic effects on the storage stability of whey powders as has been previously reported (Saltmarch, 1980; Soul, 1984). The powders exhibited various maximas and seemed dependent on the amount of protein in the powders. The lower protein powders (about 10%) had browning rate maximas at a_w 0.52. The intermediate protein powders (31-40% protein) had browning rate maximas at a a_w of 0.74. The highest protein whey powders (46% and 82%) had lower a_ws at which maximum browning rates occurred (0.52 and 0.63, respectively). An explaination why these high protein whey powders exhibited browning rate maxima at lower a_ws may be that these powders also had high levels of

fat which may have undergone oxidation to carbonyls. Fat oxidation is at a maximum at intermediate a_ws and decreases at higher a_ws (Labuza, 1975b). Also, at higher water activities of storage there may have been a dilution effect occurring since the reducing sugars may have been limiting in these powders.

pH decreases of the powders in solution were measured during the storage study. Decreases in pH at a specific a_w, generally correlated with the magnitude of the rate constant for brown pigment formation (i.e. the sharpest decrease in pH generally occurred at a a_w where the whey powder had the highest rate constant for brown pigment formation).

The protein quality loss in powders was measured using the dye binding method which estimates available lysine. These results were evaluated using two-way analysis of variance across classification variables, powder and a_w of storage. No rate constants were calculated because the data did not follow first order (or any other order) rate loss. The data may have been biphasic; however, not enough days were analyzed to draw two lines confidently. For all the powders and at all days, the a_w at which the largest decreases in available lysine were observed was 0.63. The values are averaged over a wide range of powder compositions so the results may be misleading. The powders which lost the greatest percentages of available lysine at all a_ws compared to day zero were the powders containing

intermediate levels of protein (31-40%) and unprocessed sweet whey powders (10% protein). Three of these four powders had the highest levels of reducing monosaccharides (0.88%-2.00%).

During the storage of whey powders a few generalizations can be drawn from this research:

1) Mozzarella wheys seem to have the poorest storage stability due to high initial concentrations of reducing monosaccharides which cause large rates of brown pigment formation. Therefore, storing these powders for long periods requires carefully controlled conditions (low temperature and a_w).

2) For all powders, the initial concentration of monosaccharides was the most important factor in predicting whether the whey powder would have good keeping qualities or not.

3) Obtaining a whey powder with the lowest rate of deterioration requires low concentrations of NPN, ash, and especially monosaccharides. Minimizing water activity of storage will also decrease the deterioration of the powders. Initial pH of the powders may not be an adequate indicator of storage stability.

4) High protein powders (46% and 82% protein) may undergo oxidation of the fat component in the intermediate moisture range (a_w 0.52-0.63) and thus keeping a_ws below 0.40 is important in obtaining storage stability. The

higher protein powders, unfortunately, tend to have high initial moisture contents. Since autooxidation of lipids increases below a_ws of 0.3, it may not be advisable to store high protein powders under this a_w. However, the effects of low a_w lipid oxidation on the Maillard reaction in whey powders are not known since no a_ws below 0.32 were studied.

5) Overall, when disregarding the monosaccharide content of whey powders, the intermediate protein whey powders were more susceptible to deterioration via the Maillard reaction than lower protein sweet dried wheys.

SUGGESTIONS FOR FURTHER RESEARCH

A study should be run to reconfirm the effects of composition on the Maillard reaction. A single type of liquid whey should be used. Ultrafiltration and demineralization could be done by the researcher to vary protein, lactose, minerals, and other minor constituents. It is not clear how to control NPN. The different whey solutions could be spray dried and stored at accelerated storage conditions and a_ws. Processing a single type of liquid whey will eliminate the variation that occurs between different types of wheys and lessen processing effects.

Another study should be done using a liquid whey. The purpose of this study would determine the influence of the individual minerals on the rate of the Maillard reaction in whey powders. The major minerals of whey; potassium, phosphorus, magnesium, sodium, and calcium would be added to the liquid whey in various concentrations. All the powders spray dried should have the same percentage protein and lactose so that direct comparisons could be made between the whey powders.

In addition, a study should be done to investigate the effects of fat on the Maillard reaction in high protein whey powders.

A better method to quantitate lactose, glucose, and galactose in whey powders should be developed. The use of internal standards should be implemented to check for losses of sugars due to the extraction method.

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APPENDIX A. Brown pigment formation, loss of available lysine, and pH data for powders 1 to 8 at all a_ws.

Powder	day	a_w	Brn Pig[a]	% Av Lys[b d]	pH[c]
1	0	.32	6.766377	.9888195	6.19
1	0	.32	7.459326	1.160444	6.17
1	0	.44	6.000223	1.387053	6.22
1	0	.44	6.413653	1.09605	6.26
1	0	.52	9.273908	1.182126	6.25
1	0	.52	7.648706	1.140998	6.21
1	0	.63	6.366838	1.248459	6.24
1	0	.63	6.496143	1.141838	6.25
1	0	.74	5.038117	1.292355	6.21
1	0	.74	5.49701	1.288488	6.21
1	1	.32	8.151008	nd	6.14
1	1	.32	7.833642	nd	6.14
1	1	.44	11.91827	nd	6.17
1	1	.44	11.63616	nd	6.17
1	1	.52	8.919722	nd	6.15
1	1	.52	20.65064	nd	6.17
1	1	.63	8.678611	nd	6.25
1	1	.63	7.675133	nd	6.23
1	1	.74	9.543568	nd	6.23
1	1	.74	10.08845	nd	6.27
1	4	.32	9.120375	1.200437	6.13
1	4	.32	9.97149	1.394744	6.13
1	4	.44	6.706627	1.266212	6.09
1	4	.44	8.359923	1.138277	6.11
1	4	.52	8.441037	1.143439	6.17
1	4	.52	9.960159	1.208779	6.12
1	4	.63	11.24243	1.19669	6.1
1	4	.63	9.979631	1.202808	6.1
1	4	.74	9.648443	1.074162	6.19
1	4	.74	9.864732	.8098037	6.13
1	16	.32	8.969887	.9755935	6.08
1	16	.32	9.38233	1.202468	6.06
1	16	.44	18.65724	1.14972	6.07
1	16	.44	20.37518	1.158537	6.04
1	16	.52	15.61433	1.234046	6.05
1	16	.52	19.34371	1.111526	6.07
1	16	.63	13.09207	1.130286	6.09
1	16	.63	13.34452	1.065617	6.08
1	16	.74	15.13942	1.222913	6.15
1	16	.74	12.81335	1.128835	6.13

APPENDIX A continued

1	59	.32	15.93165	1.067431	6.05
1	59	.32	17.87876	1.007838	6.07
1	59	.44	58.78812	1.137735	5.99
1	59	.44	56.17194	1.057474	6
1	59	.52	44.09405	1.03558	5.98
1	59	.52	45.38051	1.068486	5.95
1	59	.63	40.60942	1.038743	5.92
1	59	.63	47.1607	1.057379	5.92
1	59	.74	31.31397	1.081262	5.93
1	59	.74	32.78466	.783461	5.89
1	120	.32	30.18524	1.164602	6.01
1	120	.32	27.75919	1.199395	6
1	120	.44	43.87388	1.079105	5.92
1	120	.44	61.33931	.7371646	5.93
1	120	.52	67.40726	.9173104	5.66
1	120	.52	71.82052	.6298662	5.65
1	120	.63	67.81768	.9261869	5.95
1	120	.63	57.94702	.9569421	5.98
1	120	.74	46.00249	.8256793	5.71
1	120	.74	49.06704	1.001407	5.69
2	0	.32	9.723573	5.191033	5.58
2	0	.32	10.38062	5.643035	5.42
2	0	.44	8.831522	6.391351	6.6
2	0	.44	8.231582	5.993665	6.58
2	0	.52	10.77296	5.539589	6.55
2	0	.52	4.72526	6.087029	6.62
2	0	.63	9.25681	5.090986	6.67
2	0	.63	1.968117	5.857781	6.65
2	0	.74	3.835336	5.827642	6.62
2	0	.74	3.187962	5.422905	6.66
2	1	.32	10.47486	nd	6.52
2	1	.32	8.333333	nd	6.57
2	1	.44	26.76733	nd	6.61
2	1	.44	32.14774	nd	6.62
2	1	.52	42.95302	nd	6.55
2	1	.52	38.90785	nd	6.56
2	1	.63	4.596192	nd	6.62
2	1	.63	6.6313	nd	6.64
2	1	.74	27.29694	nd	6.65
2	1	.74	21.4704	nd	6.66
2	4	.32	9.023391	6.097292	6.63

APPENDIX A continued

2	4	.32	15.05715	5.948841	6.63
2	4	.44	9.553705	5.500672	6.6
2	4	.44	8.929798	5.484465	6.6
2	4	.52	14.10153	5.972789	6.53
2	4	.52	.672495	5.397928	6.6
2	4	.63	15.2207	5.304682	6.33
2	4	.63	18.05054	5.137028	6.57
2	4	.74	18.75042	5.196698	6.5
2	4	.74	19.16848	5.409414	6.56
2	16	.32	17.45201	5.795171	6.57
2	16	.32	19.14006	6.4737	6.63
2	16	.44	16.2944	5.548158	6.59
2	16	.44	10.221	4.903772	6.67
2	16	.52	6.09096	5.695444	6.56
2	16	.52	11.3424	5.144726	6.64
2	16	.63	lost	lost	lost
2	16	.63	lost	lost	lost
2	16	.74	15.1715	5.898983	6.58
2	16	.74	6.563833	5.795657	6.57
2	59	.32	26.4274	5.87237	6.53
2	59	.32	16.74691	4.494517	6.57
2	59	.44	46.91324	5.00456	6.54
2	59	.44	15.77828	4.946572	6.53
2	59	.52	56.19132	4.988084	6.44
2	59	.52	44.54343	5.032646	6.57
2	59	.63	49.41782	5.002522	6.17
2	59	.63	49.1629	3.127748	6.29
2	59	.74	36.31082	4.349179	6.34
2	59	.74	31.50847	5.121617	6.34
2	120	.32	14.35996	5.993708	6.51
2	120	.32	13.12245	5.136984	6.5
2	120	.44	35.87444	3.805934	6.52
2	120	.44	31.70664	4.212132	6.52
2	120	.52	58.17493	4.840647	6.24
2	120	.52	59.79073	3.922104	6.32
2	120	.63	80.30199	3.453556	5.95
2	120	.63	75.25424	3.453909	5.98
2	120	.74	48.2399	5.521519	5.9
2	120	.74	45.12753	5.033808	5.95
3	0	.32	10.09916	4.528107	6.47
3	0	.32	11.50219	3.679251	6.43

APPENDIX A continued

3	0	.44	12.42579	4.985873	6.51
3	0	.44	5.04194	4.712405	6.49
3	0	.52	12.76615	3.445142	6.45
3	0	.52	13.24987	4.118098	6.46
3	0	.63	7.622293	4.100092	6.47
3	0	.63	6.250837	4.743123	6.48
3	0	.74	7.093142	3.592868	6.47
3	0	.74	4.897596	4.396774	6.45
3	1	.32	6.922012	nd	6.44
3	1	.32	6.132075	nd	6.45
3	1	.44	29.09931	nd	6.43
3	1	.44	21.89101	nd	6.42
3	1	.52	11.97053	nd	6.39
3	1	.52	11.02435	nd	6.39
3	1	.63	7.79459	nd	6.45
3	1	.63	8.249313	nd	6.46
3	1	.74	14.46001	nd	6.49
3	1	.74	9.383378	nd	6.46
3	4	.32	15.12216	3.676077	6.46
3	4	.32	10.8747	4.602679	6.44
3	4	.44	7.405693	4.39155	6.42
3	4	.44	3.709887	3.75232	6.42
3	4	.52	5.50383	3.371106	6.39
3	4	.52	8.241758	3.427467	6.39
3	4	.63	15.70656	3.459059	6.32
3	4	.63	12.25935	3.548221	6.3
3	4	.74	8.26712	4.36493	6.36
3	4	.74	14.08899	3.4271	6.35
3	16	.32	12.50661	3.851657	6.46
3	16	.32	9.576251	3.96602	6.45
3	16	.44	6.061171	3.78736	6.55
3	16	.44	7.791017	3.892393	6.48
3	16	.52	10.24686	3.099279	6.41
3	16	.52	14.80453	3.250198	6.39
3	16	.63	20.73351	3.501551	6.14
3	16	.63	16.95537	3.208036	6.16
3	16	.74	23.05606	3.982464	6.23
3	16	.74	23.33135	3.582419	6.19
3	59	.32	9.937535	3.888392	6.35
3	59	.32	12.22609	3.581196	6.37
3	59	.44	19.56583	3.463988	6.34

APPENDIX A continued

3	59	.44	31.10492	3.586281	6.33
3	59	.52	38.45973	2.987261	6.05
3	59	.52	40.73606	3.100278	6.06
3	59	.63	62.63529	2.808013	5.83
3	59	.63	63.63846	2.420049	5.85
3	59	.74	93.24945	3.079799	5.9
3	59	.74	87.63345	2.851996	5.88
3	120	.32	12.14613	3.718526	6.34
3	120	.32	11.75475	3.630192	6.34
3	120	.44	38.76874	3.11141	6.21
3	120	.44	35.77568	2.993255	6.24
3	120	.52	77.79745	2.787013	5.88
3	120	.52	75.36747	2.351017	5.88
3	120	.63	106.994	2.452143	5.54
3	120	.63	85.41572	2.347569	5.53
3	120	.74	122.549	2.292174	5.58
3	120	.74	148.4929	2.622157	5.59
4	0	.32	7.651662	1.11061	5.85
4	0	.32	6.468491	1.029963	5.87
4	0	.44	8.909757	1.107884	5.92
4	0	.44	7.502194	1.235942	5.96
4	0	.52	8.992308	1.01615	5.9
4	0	.52	7.758597	1.224514	5.91
4	0	.63	5.670502	1.249537	6.94
4	0	.63	7.190564	1.188518	5.9
4	0	.74	7.051894	1.273493	5.86
4	0	.74	7.433614	1.290311	5.87
4	1	.32	5.891884	nd	5.88
4	1	.32	5.439577	nd	5.87
4	1	.44	9.992759	nd	5.83
4	1	.44	10.56746	nd	5.83
4	1	.52	10.17886	nd	5.85
4	1	.52	12.20575	nd	5.85
4	1	.63	9.474591	nd	5.89
4	1	.63	7.443458	nd	5.88
4	1	.74	7.098121	nd	5.97
4	1	.74	7.779939	nd	5.97
4	4	.32	7.773541	1.159768	5.85
4	4	.32	8.060616	1.264772	5.85
4	4	.44	10.43312	1.108689	5.8
4	4	.44	8.539957	.9945609	5.8

APPENDIX A continued

4	4	.52	7.603145	1.078728	5.79
4	4	.52	7.765985	1.264751	5.8
4	4	.63	8.835937	1.073036	5.82
4	4	.63	10.39619	1.228297	5.83
4	4	.74	7.844016	.9312052	6.04
4	4	.74	8.787959	1.091275	6.06
4	16	.32	8.175278	.9572257	5.77
4	16	.32	8.766977	1.134369	5.78
4	16	.44	14.31496	1.06193	5.83
4	16	.44	15.1176	.70347	5.8
4	16	.52	13.49214	1.140552	5.78
4	16	.52	12.56154	1.023477	5.79
4	16	.63	12.39493	1.041044	5.78
4	16	.63	9.972362	.9443292	5.76
4	16	.74	9.085278	1.185393	6.06
4	16	.74	8.765922	1.112627	6.04
4	59	.32	15.17296	.9789184	5.69
4	59	.32	13.55982	.9436816	5.69
4	59	.44	41.52642	.8969963	5.7
4	59	.44	39.09543	.7445737	5.72
4	59	.52	42.60684	1.081108	5.66
4	59	.52	34.23718	.8919141	5.68
4	59	.63	23.74312	.8918911	5.62
4	59	.63	23.54083	.894781	5.63
4	59	.74	21.96648	.8727075	5.71
4	59	.74	21.1186	.8258971	5.76
4	120	.32	17.07726	.8967047	5.64
4	120	.32	14.26555	.7331832	5.69
4	120	.44	40.36504	.8759043	5.62
4	120	.44	53.41662	.9378802	5.63
4	120	.52	59.52209	.8564401	5.67
4	120	.52	56.6294	.8628463	5.64
4	120	.63	32.48891	.7380828	5.42
4	120	.63	33.41425	.7783007	5.41
4	120	.74	29.97086	.9466505	5.55
4	120	.74	30.25719	.7830697	5.47
5	0	.32	8.521868	1.035838	5.7
5	0	.32	11.28513	1.010229	5.72
5	0	.44	10.6267	.9728062	5.73
5	0	.44	10.50793	1.103804	5.73
5	0	.52	9.728708	1.10859	5.68

APPENDIX A continued

5	0	.52	9.701875	1.120366	5.71
5	0	.63	9.640877	1.008147	5.67
5	0	.63	10.56404	1.114689	5.66
5	0	.74	10.23637	1.228643	5.63
5	0	.74	10.0864	1.075679	5.64
5	1	.32	8.765729	nd	5.67
5	1	.32	9.76922	nd	5.67
5	1	.44	18.02431	nd	5.63
5	1	.44	12.03302	nd	5.62
5	1	.52	15.40832	nd	5.66
5	1	.52	18.04953	nd	5.65
5	1	.63	13.19261	nd	5.69
5	1	.63	14.56513	nd	5.68
5	1	.74	10.76861	nd	5.68
5	1	.74	13.36213	nd	5.7
5	4	.32	10.02797	1.24164	5.69
5	4	.32	11.00715	1.078792	5.69
5	4	.44	11.66271	1.123088	5.67
5	4	.44	15.56693	1.077167	5.69
5	4	.52	12.90699	1.13846	5.67
5	4	.52	12.37331	1.050534	5.65
5	4	.63	14.57225	1.124897	5.62
5	4	.63	13.71799	1.04885	5.65
5	4	.74	12.33839	1.078227	5.56
5	4	.74	11.46147	.9909025	5.57
5	16	.32	14.68743	1.200971	5.63
5	16	.32	13.91433	.8822829	5.63
5	16	.44	33.56131	1.090981	5.73
5	16	.44	39.49224	.972076	5.76
5	16	.52	32.76623	1.00508	5.64
5	16	.52	31.35586	.8550881	5.64
5	16	.63	21.70157	.8719268	5.6
5	16	.63	22.63037	.9224231	5.63
5	16	.74	17.97017	1.142875	5.63
5	16	.74	16.18729	1.161353	5.63
5	59	.32	35.04888	.8774464	5.61
5	59	.32	35.85957	.880404	5.62
5	59	.44	132.5873	.8011414	5.64
5	59	.44	137.7196	.9031367	5.65
5	59	.52	139.9391	.9201218	5.49
5	59	.52	136.5308	.9398522	5.5

APPENDIX A continued

5	59	.63	103.6801	.9062343	5.44
5	59	.63	104.171	.7963614	5.42
5	59	.74	63.68916	.9180955	5.59
5	59	.74	61.93785	.874981	5.58
5	120	.32	49.69897	.8843195	5.6
5	120	.32	45.77671	.8567826	5.63
5	120	.44	190.4951	.8077378	5.54
5	120	.44	192.5959	.7976307	5.55
5	120	.52	225.217	.7751939	5.46
5	120	.52	230.6418	.9073344	5.46
5	120	.63	170.9888	.6635447	5.24
5	120	.63	169.4095	.5440359	5.23
5	120	.74	105.6123	.9373673	5.34
5	120	.74	105.8824	.7556418	5.32
6	0	.32	7.282839	1.289836	6.08
6	0	.32	4.455372	1.458828	6.1
6	0	.44	5.188458	1.245401	6.13
6	0	.44	5.637166	1.194497	6.11
6	0	.52	5.57441	1.217909	6.12
6	0	.52	5.394184	1.193577	6.09
6	0	.63	6.705263	1.369587	6.06
6	0	.63	6.216057	1.273646	6.05
6	0	.74	5.75062	1.414452	6
6	0	.74	4.900519	1.404408	6.01
6	1	.32	5.627558	nd	6.11
6	1	.32	5.941266	nd	6.1
6	1	.44	15.24648	nd	6.05
6	1	.44	16.18068	nd	6.02
6	1	.52	11.34824	nd	5.99
6	1	.52	13.54784	nd	6.03
6	1	.63	11.48843	nd	6.12
6	1	.63	7.924465	nd	6.06
6	1	.74	8.215962	nd	6.12
6	1	.74	6.023089	nd	6.13
6	4	.32	7.783945	1.547014	6.07
6	4	.32	6.087456	1.394921	6.1
6	4	.44	4.219124	1.453231	6.03
6	4	.44	6.064384	1.277746	6.15
6	4	.52	8.625793	1.119951	6.12
6	4	.52	8.905468	1.223683	6.13
6	4	.63	9.086473	1.215564	6.13

APPENDIX A continued

6	4	.63	8.557334	1.07466	6.09
6	4	.74	8.865397	1.383317	6.03
6	4	.74	7.649837	1.295319	6.06
6	16	.32	7.673288	1.461993	6.05
6	16	.32	6.943856	1.320663	6.07
6	16	.44	6.768533	1.254434	6.13
6	16	.44	7.779469	1.28186	6.2
6	16	.52	7.293451	1.1656	6.12
6	16	.52	7.764761	1.106024	6.13
6	16	.63	7.083706	1.108669	6.03
6	16	.63	11.62556	1.184518	6.06
6	16	.74	9.902818	1.294045	6.11
6	16	.74	8.018308	1.237633	6.16
6	59	.32	8.33929	1.246337	6.04
6	59	.32	9.317138	1.164192	6.12
6	59	.44	10.17173	1.257536	6.03
6	59	.44	8.796711	1.250239	6.07
6	59	.52	15.69197	1.238211	6.05
6	59	.52	10.53884	1.161709	5.97
6	59	.63	19.56452	1.207177	5.96
6	59	.63	15.14208	1.059137	5.92
6	59	.74	23.91344	1.275703	5.96
6	59	.74	20.09512	1.10249	6.02
6	120	.32	10.1036	1.304	6
6	120	.32	8.141113	1.142872	5.99
6	120	.44	17.06572	1.260706	5.87
6	120	.44	13.86986	1.084409	5.93
6	120	.52	18.02476	.9343561	5.85
6	120	.52	18.00983	1.015267	5.85
6	120	.63	18.66801	1.017455	5.66
6	120	.63	34.37237	1.044855	5.66
6	120	.74	33.60506	.9146257	5.69
6	120	.74	32.48493	.8830461	5.7
7	0	.32	9.639716	4.732908	6.75
7	0	.32	6.037189	5.085656	6.73
7	0	.44	7.181329	5.180834	6.75
7	0	.44	10.01119	4.426196	6.73
7	0	.52	8.244994	4.65219	6.71
7	0	.52	2.942908	6.080778	6.69
7	0	.63	5.14992	5.164852	6.73
7	0	.63	5.064431	5.907714	6.75

APPENDIX A continued

7	0	.74	9.689922	5.843499	6.68
7	0	.74	4.592423	4.918811	6.65
7	1	.32	10.3912	nd	6.68
7	1	.32	12.33046	nd	6.71
7	1	.44	28.41596	nd	6.64
7	1	.44	31.79364	nd	6.66
7	1	.52	37.38872	nd	6.65
7	1	.52	21.54399	nd	6.68
7	1	.63	10.58201	nd	6.65
7	1	.63	12.03438	nd	6.69
7	1	.74	12.91838	nd	6.68
7	1	.74	10.52632	nd	6.66
7	4	.32	11.54173	4.057428	6.72
7	4	.32	10.89061	5.562797	6.71
7	4	.44	9.671764	4.928454	6.75
7	4	.44	10.80497	4.350068	6.72
7	4	.52	7.038949	4.035614	6.67
7	4	.52	0	5.28497	6.64
7	4	.63	14.95842	4.701581	6.63
7	4	.63	15.37643	4.370285	6.6
7	4	.74	18.11266	4.43104	6.58
7	4	.74	15.73885	4.477862	6.56
7	16	.32	14.02952	5.009263	6.73
7	16	.32	16.46442	4.876477	6.74
7	16	.44	6.006006	4.507425	6.73
7	16	.44	19.70502	4.521614	6.75
7	16	.52	10.1541	4.322335	6.63
7	16	.52	10.58824	4.423991	6.66
7	16	.63	7.908505	3.521586	6.45
7	16	.63	14.92092	3.947662	6.49
7	16	.74	8.942944	5.370455	6.51
7	16	.74	9.431738	4.954621	6.45
7	59	.32	17.95655	4.407194	6.68
7	59	.32	9.778158	3.939641	6.62
7	59	.44	16.8037	4.557454	6.57
7	59	.44	11.46166	4.258422	6.62
7	59	.52	26.58004	3.879615	6.41
7	59	.52	20.9807	3.810719	6.45
7	59	.63	36.34414	4.172749	6.27
7	59	.63	39.90085	4.258722	6.28
7	59	.74	74.98535	3.573868	6.22

APPENDIX A continued

7	59	.74	59.95415	3.967389	6.19
7	120	.32	8.433227	4.620171	6.56
7	120	.32	12.30088	4.406661	6.66
7	120	.44	14.95484	3.840405	6.51
7	120	.44	19.1789	3.970916	6.5
7	120	.52	26.19048	3.958553	6.26
7	120	.52	38.86697	3.828563	6.28
7	120	.63	61.55703	4.004172	5.83
7	120	.63	73.74101	2.967193	5.84
7	120	.74	88.37209	3.92801	5.82
7	120	.74	91.96067	3.169192	5.79
8	0	.32	13.29466	8.853489	6.25
8	0	.32	15.49833	8.457623	6.25
8	0	.44	14.13261	10.2896	6.23
8	0	.44	4.737091	9.273586	6.24
8	0	.52	6.997085	9.433237	6.33
8	0	.52	9.287207	7.818832	6.39
8	0	.63	11.2905	8.883412	6.29
8	0	.63	6.845408	10.08146	6.27
8	0	.74	10.33414	9.372486	6.23
8	0	.74	19.15925	7.384843	6.23
8	1	.32	18.54141	nd	6.28
8	1	.32	15.99016	nd	6.28
8	1	.44	46.04486	nd	6.14
8	1	.44	60.41924	nd	6.18
8	1	.52	49.82206	nd	6.19
8	1	.52	16.99029	nd	6.19
8	1	.63	22.22222	nd	6.29
8	1	.63	54.41354	nd	6.34
8	1	.74	20.93023	nd	6.3
8	1	.74	12.98701	nd	6.26
8	4	.32	31.27737	10.15007	6.24
8	4	.32	17.30532	9.424878	6.23
8	4	.44	7.309942	10.3183	6.18
8	4	.44	0	9.035342	6.21
8	4	.52	0	9.77852	6.14
8	4	.52	10.89061	10.08279	6.16
8	4	.63	39.16914	10.10588	6.13
8	4	.63	15.47435	7.402182	6.17
8	4	.74	21.55689	9.401938	6.3
8	4	.74	18.74854	9.479206	6.27

APPENDIX A continued

8	16	.32	24.32498	9.635332	6.23
8	16	.32	15.12097	9.04155	6.26
8	16	.44	14.60209	7.526731	6.28
8	16	.44	11.201	7.669138	6.33
8	16	.52	14.39539	9.848775	6.27
8	16	.52	11.92464	9.46633	6.4
8	16	.63	21.50281	9.267005	6.16
8	16	.63	8.425614	8.496377	lost
8	16	.74	11.74536	7.58846	6.19
8	16	.74	29.00232	9.073473	6.16
8	59	.32	21.44299	8.248663	6.27
8	59	.32	21.84466	8.251587	6.18
8	59	.44	18.26373	8.865012	6.23
8	59	.44	26.41374	9.174688	6.18
8	59	.52	28.75974	8.585751	6.27
8	59	.52	21.78913	8.568363	6.17
8	59	.63	48.21261	9.228242	6.04
8	59	.63	52.60641	7.817644	6.03
8	59	.74	57.39721	7.418525	6.13
8	59	.74	39.85465	7.558687	6.08
8	120	.32	26.21919	8.520999	6.11
8	120	.32	23.33579	8.517642	6.17
8	120	.44	45.98814	7.982537	6.08
8	120	.44	52.74132	8.453611	6.08
8	120	.52	79.81618	7.271335	6.1
8	120	.52	59.79985	8.238129	6.13
8	120	.63	68.42737	8.332695	5.76
8	120	.63	34.85577	7.378458	5.77
8	120	.74	41.61712	7.49591	5.81
8	120	.74	73.81776	9.049645	5.84

[a] Brown pigment formation expressed as O.D./100g powder

[b] Loss of available lysine expressed as percent available lysine

[c] pH of powder in brown pigment formation solution

[d] available lysine not determined at day 1

APPENDIX B. Moisture contents measured at all days and a_ws for powders 1 to 8.

Powder	a_w	day	% Moist.[a]	Powder	a_w	day	% Moist.
1	.32	0	3.99157	5	.32	0	7.48404
1	.32	1	2.90040	5	.32	1	4.91637
1	.32	2	3.09124	5	.32	2	4.97163
1	.32	4	4.63634	5	.32	4	4.97190
1	.32	8	5.23321	5	.32	8	5.01138
1	.32	16	3.07370	5	.32	16	4.17647
1	.32	30	2.98660	5	.32	30	4.44118
1	.32	59	4.56320	5	.32	59	4.41993
1	.32	85	3.07777	5	.32	85	3.88711
1	.32	120	3.17681	5	.32	120	4.20617
1	.44	0	5.29696	5	.44	0	8.35905
1	.44	1	3.68431	5	.44	1	6.12824
1	.44	2	3.89241	5	.44	2	5.45355
1	.44	4	5.12866	5	.44	4	5.72058
1	.44	8	5.73915	5	.44	8	5.75072
1	.44	16	4.12948	5	.44	16	5.02444
1	.44	30	4.62596	5	.44	30	4.92708
1	.44	59	4.65511	5	.44	59	4.71681
1	.44	85	3.75146	5	.44	85	3.99763
1	.44	120	4.13692	5	.44	120	4.83704
1	.52	0	7.28352	5	.52	0	6.85184
1	.52	1	3.79950	5	.52	1	5.80743
1	.52	2	5.12305	5	.52	2	6.47705
1	.52	4	5.89724	5	.52	4	6.30137
1	.52	8	5.97951	5	.52	8	6.23134
1	.52	16	5.34566	5	.52	16	5.72673
1	.52	30	5.26963	5	.52	30	5.72371
1	.52	59	5.41306	5	.52	59	5.30662
1	.52	85	4.52042	5	.52	85	5.28013
1	.52	120	5.30846	5	.52	120	5.61281
1	.63	0	7.97964	5	.63	0	10.1381
1	.63	1	5.07161	5	.63	1	6.83752
1	.63	2	6.32707	5	.63	2	8.05489
1	.63	4	6.76766	5	.63	4	7.78615
1	.63	8	6.73478	5	.63	8	7.20396
1	.63	16	6.37244	5	.63	16	7.24469
1	.63	30	6.40704	5	.63	30	7.13989
1	.63	59	5.98231	5	.63	59	6.87447
1	.63	85	6.04301	5	.63	85	6.43163
1	.63	120	6.29376	5	.63	120	6.92241
1	.74	0	8.81463	5	.74	0	10.6852
1	.74	1	6.14534	5	.74	1	9.22806
1	.74	2	7.38555	5	.74	2	10.4220
1	.74	4	8.08269	5	.74	4	10.1267
1	.74	8	8.46431	5	.74	8	11.6109

APPENDIX B continued

1	.74	16	7.28841	5	.74	16	10.0538
1	.74	30	7.56618	5	.74	30	9.30218
1	.74	59	7.88237	5	.74	59	10.0473
1	.74	85	6.89359	5	.74	85	9.54273
1	.74	120	6.03388	5	.74	120	10.8089
2	.32	0	3.99157	6	.32	0	6.91385
2	.32	1	5.08448	6	.32	1	4.25629
2	.32	2	4.60897	6	.32	2	4.66116
2	.32	4	4.66103	6	.32	4	4.91357
2	.32	8	4.69340	6	.32	8	4.75345
2	.32	16	4.22820	6	.32	16	4.46979
2	.32	30	4.04636	6	.32	30	4.57243
2	.32	59	4.23554	6	.32	59	4.89772
2	.32	85	3.78803	6	.32	85	4.17875
2	.32	120	4.00685	6	.32	120	4.59702
2	.44	0	6.72378	6	.44	0	6.59903
2	.44	1	6.31663	6	.44	1	4.88952
2	.44	2	6.81147	6	.44	2	4.71257
2	.44	4	6.00779	6	.44	4	4.99985
2	.44	8	6.37007	6	.44	8	4.97156
2	.44	16	5.81823	6	.44	16	4.77747
2	.44	30	5.66051	6	.44	30	4.60042
2	.44	59	5.48729	6	.44	59	4.73868
2	.44	85	4.08847	6	.44	85	4.41109
2	.44	120	5.21939	6	.44	120	4.84424
2	.52	0	8.01983	6	.52	0	8.06241
2	.52	1	7.17770	6	.52	1	4.96942
2	.52	2	7.33862	6	.52	2	5.10061
2	.52	4	7.12355	6	.52	4	5.03066
2	.52	8	7.47540	6	.52	8	5.25300
2	.52	16	7.12310	6	.52	16	4.81532
2	.52	30	6.93043	6	.52	30	4.94587
2	.52	59	6.82814	6	.52	59	4.86341
2	.52	85	6.19178	6	.52	85	4.49584
2	.52	120	6.77508	6	.52	120	4.93192
2	.63	0	10.5540	6	.63	0	8.02869
2	.63	1	9.80350	6	.63	1	5.01685
2	.63	2	10.0083	6	.63	2	5.55679
2	.63	4	9.15206	6	.63	4	5.50376
2	.63	8	8.92040	6	.63	8	5.25088
2	.63	16	lost	6	.63	16	5.10365
2	.63	30	8.00963	6	.63	30	5.10233
2	.63	59	7.57172	6	.63	59	5.15950
2	.63	85	7.70598	6	.63	85	4.84193
2	.63	120	8.01637	6	.63	120	5.26275
2	.74	0	12.0302	6	.74	0	7.65559

APPENDIX B continued

2	.74	1	10.2793	6	.74	1	5.78039
2	.74	2	10.2172	6	.74	2	6.61985
2	.74	4	9.29789	6	.74	4	6.07149
2	.74	8	10.0934	6	.74	8	5.78903
2	.74	16	9.47526	6	.74	16	5.78787
2	.74	30	9.13796	6	.74	30	5.79284
2	.74	59	9.66107	6	.74	59	5.62685
2	.74	85	9.60212	6	.74	85	5.43148
2	.74	120	10.3954	6	.74	120	5.86
3	.32	0	7.29107	7	.32	0	5.70591
3	.32	1	5.38779	7	.32	1	4.48965
3	.32	2	4.90682	7	.32	2	4.73752
3	.32	4	4.68066	7	.32	4	4.51845
3	.32	8	4.52072	7	.32	8	4.66356
3	.32	16	4.15007	7	.32	16	4.21711
3	.32	30	4.17734	7	.32	30	4.52659
3	.32	59	4.69642	7	.32	59	4.28337
3	.32	85	3.88	7	.32	85	3.91208
3	.32	120	4.26729	7	.32	120	4.18111
3	.44	0	7.64231	7	.44	0	7.12432
3	.44	1	6.86100	7	.44	1	6.33079
3	.44	2	6.99555	7	.44	2	6.18742
3	.44	4	6.67997	7	.44	4	5.99615
3	.44	8	6.94365	7	.44	8	6.23759
3	.44	16	6.76054	7	.44	16	5.79174
3	.44	30	6.24625	7	.44	30	5.61587
3	.44	59	6.15006	7	.44	59	5.66822
3	.44	85	4.36	7	.44	85	4.42633
3	.44	120	6.12896	7	.44	120	5.62606
3	.52	0	8.52119	7	.52	0	9.07439
3	.52	1	7.89311	7	.52	1	7.13676
3	.52	2	8.20298	7	.52	2	7.58492
3	.52	4	8.24166	7	.52	4	7.78573
3	.52	8	7.80285	7	.52	8	7.39949
3	.52	16	7.34190	7	.52	16	6.99001
3	.52	30	6.70095	7	.52	30	7.12718
3	.52	59	5.64473	7	.52	59	6.76764
3	.52	85	5.17608	7	.52	85	6.13323
3	.52	120	5.41933	7	.52	120	6.51735
3	.63	0	10.1695	7	.63	0	11.6898
3	.63	1	8.08342	7	.63	1	10.0720
3	.63	2	8.25248	7	.63	2	9.41601
3	.63	4	8.18291	7	.63	4	7.31812
3	.63	8	8.19030	7	.63	8	7.04386
3	.63	16	7.62866	7	.63	16	6.20382
3	.63	30	6.70013	7	.63	30	6.27918

APPENDIX B continued

3	.63	59	7.66969	7	.63	59	6.26135
3	.63	85	7.26659	7	.63	85	5.77405
3	.63	120	7.89249	7	.63	120	6.17088
3	.74	0	11.6443	7	.74	0	10.9973
3	.74	1	9.78177	7	.74	1	9.22473
3	.74	2	9.84111	7	.74	2	8.98174
3	.74	4	8.63569	7	.74	4	7.97891
3	.74	8	9.29103	7	.74	8	7.37108
3	.74	16	8.91733	7	.74	16	7.99521
3	.74	30	8.80627	7	.74	30	7.91875
3	.74	59	10.0996	7	.74	59	8.61586
3	.74	85	9.83997	7	.74	85	8.30659
3	.74	120	10.2712	7	.74	120	9.02539
4	.32	0	5.80034	8	.32	0	10.0910
4	.32	1	3.82845	8	.32	1	5.98720
4	.32	2	3.91006	8	.32	2	5.83357
4	.32	4	4.10081	8	.32	4	5.70829
4	.32	8	4.78609	8	.32	8	5.75159
4	.32	16	2.88943	8	.32	16	5.49721
4	.32	30	3.38493	8	.32	30	5.66427
4	.32	59	4.47275	8	.32	59	5.02350
4	.32	85	3.39950	8	.32	85	5.14920
4	.32	120	3.07936	8	.32	120	5.40079
4	.44	0	7.62690	8	.44	0	9.67756
4	.44	1	5.29672	8	.44	1	6.85343
4	.44	2	5.07681	8	.44	2	6.83683
4	.44	4	5.27310	8	.44	4	6.87972
4	.44	8	5.47742	8	.44	8	6.94892
4	.44	16	4.69010	8	.44	16	6.70284
4	.44	30	4.69565	8	.44	30	6.36236
4	.44	59	4.74192	8	.44	59	6.50533
4	.44	85	3.85033	8	.44	85	5.34979
4	.44	120	4.48655	8	.44	120	6.18843
4	.52	0	9.58609	8	.52	0	11.3602
4	.52	1	5.05936	8	.52	1	7.70447
4	.52	2	5.96389	8	.52	2	7.99262
4	.52	4	5.94374	8	.52	4	7.90271
4	.52	8	5.82232	8	.52	8	7.78709
4	.52	16	5.35876	8	.52	16	7.40652
4	.52	30	5.40269	8	.52	30	7.48409
4	.52	59	5.30167	8	.52	59	7.59955
4	.52	85	4.82954	8	.52	85	6.88297
4	.52	120	5.40446	8	.52	120	7.16738
4	.63	0	9.70947	8	.63	0	13.1158
4	.63	1	6.27692	8	.63	1	9.11787
4	.63	2	7.53519	8	.63	2	9.05437

APPENDIX B continued

4	.63	4	7.04837	8	.63	4	9.25226
4	.63	8	6.80626	8	.63	8	9.26362
4	.63	16	6.88731	8	.63	16	8.72730
4	.63	30	6.41233	8	.63	30	8.69788
4	.63	59	6.47554	8	.63	59	8.22462
4	.63	85	6.33119	8	.63	85	8.47104
4	.63	120	6.31187	8	.63	120	8.74114
4	.74	0	9.75032	8	.74	0	12.5787
4	.74	1	9.10980	8	.74	1	10.2304
4	.74	2	9.57715	8	.74	2	11.3653
4	.74	4	8.73920	8	.74	4	10.8506
4	.74	8	10.3336	8	.74	8	10.3084
4	.74	16	10.3115	8	.74	16	10.6489
4	.74	30	9.16620	8	.74	30	10.0603
4	.74	59	9.62008	8	.74	59	10.2726
4	.74	85	8.97802	8	.74	85	10.1529
4	.74	120	9.98862	8	.74	120	10.7569

[a] Percent moisture in whey powders

APPENDIX C. Percent galactose, glucose, and lactose remaining in powder five after storage at various a_ws.

Powder	a_w	day	% galactose	% glucose	% lactose
5	.32	0	1.91	.54	70.6
5	.32	4	1.67	.34	69.13
5	.32	16	1.35	.34	67.27
5	.32	59	.99	.31	70.92
5	.32	120	.8	.29	70
5	.44	0	1.9	.3	71.98
5	.44	4	1.36	.23	70.49
5	.44	16	.84	.23	69.85
5	.44	59	.67	.11	70.09
5	.44	120	.26	.08	69.73
5	.52	0	2.12	.7	67.78
5	.52	4	1.47	.54	69.71
5	.52	16	.88	.37	66.67
5	.52	59	.49	0	66.41
5	.52	120	.41	0	66.73
5	.63	0	2.22	.51	74.15
5	.63	4	2.05	.44	69.42
5	.63	16	1.56	.33	70.33
5	.63	59	.79	.31	70.5
5	.63	120	.71	.14	70.65
5	.74	0	2.34	.52	73.56
5	.74	4	1.79	.28	71.8
5	.74	16	1.84	.3	68.7
5	.74	59	.97	.22	69.96
5	.74	120	.8	.2	68.97

VITAE

The author, Patricia Rumrich Pfisterer, was born December 5, 1963 in Silver Spring, Maryland. She grew up in Gaithersburg, Maryland and graduated from Gaithersburg High School in June of 1981.

In September 1981 she began attending Virginia Polytechnic Institute and State University, working towards a Bachelor's degree in Food Science and Technology. In June of 1985 she graduated from Virginia Polytechnic Institute and State University with a double major in Food Science and Technology, and Biochemistry and Nutrition.

In July of 1985, Paddy began working towards a master's degree in Food Science and Technology under Dr. John W. Larkin.

She is currently a candidate for the degree of Master of Science in the Department of Food Science and Technology at Virginia Tech. Her emphasis is in the area of dairy chemistry and analysis.

Paddy is a student member of the Institute of Food Technologists, the Carolina-Virginia section of the Institute of Food Technologists, and the American Dairy Science Association.

Patricia Rumrich Pfisterer