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**PROTEIN QUALITY AND DIGESTIBILITY
OF WHOLE WHEAT AS AFFECTED BY
DRUM-DRYING AND SINGLE SCREW EXTRUSION PROCESSING**

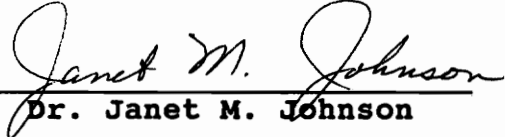
by
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(ABSTRACT)

The objective of this study was to examine the effects of two thermal processes, drum-drying and thermoplastic extrusion, on protein quality and digestibility of whole wheat.

Coker 916 whole wheat flour was made into a simulated whole wheat spaghetti by extrusion cooking (single screw, 50 psi, 93°C) and a flaked product by drum-drying (152°C). Protein Efficiency Ratios (PER) of the original whole wheat kernels and the two processed wheat products were determined. The apparent digestibility of the four diets was determined from Kjeldahl nitrogen analysis of feces. Amino acid composition, available lysine analysis, colorimetry (Hunter L, a, b color values), and Differential Scanning Calorimetry were also conducted to investigate the effects of thermal processing on protein quality.

Both thermal processes significantly increased protein digestibility while PER's of the drum dried flakes (1.66) and unprocessed whole wheat (1.59) were significantly greater than the extruded product (1.42). Thermal processing also resulted in substantial reductions in lysine (>10%) and several other essential

amino acids. Hunter L, a, b values indicated that the drum-dried flakes were lightest in color, followed by the unprocessed whole wheat and the extruded product. The observed decrease in lysine and PER of the extruded product may be due in part to Maillard Browning, as indicated by Hunter color values. It appears that total lysine or Hunter L color values may be reasonable predictors of protein quality of processed whole wheat. DSC results suggest that starch was fully gelatinized during drum-drying of the whole wheat but on partially gelatinized during extrusion cooking.

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Table of Contents

Title.....	i
Abstract.....	ii
Acknowledgements.....	iv
Table of Contents.....	vi
List of Tables.....	ix
List of Illustrations.....	xi
List of Appendices.....	xiii
1.0 Introduction.....	1
2.0 Review of Literature.....	4
2.1 Wheat.....	4
2.2 Milling.....	5
2.3 Food Processing.....	9
2.4 Drum Drying of Foods.....	10
2.5 Extrusion Processing of Foods.....	12
2.6 Lysine: Nutritional Significance.....	15

2.6.1	Maillard (Nonenzymatic) Browning Reaction.....	15
2.6.2	Color Measurements of Food Products.....	17
2.6.3	Available vs. Total Lysine.....	18
2.6.4	Amino Acid Balance in the Diet.....	19
2.7	Effects of Drum-Drying and Extrusion Processing on Food.....	20
2.7.1	Determining Protein Nutritive Value.....	26
2.8	Starch Gelatinization.....	28
3.0	Objectives of the Study.....	31
4.0	Materials and Methods.....	32
4.1	Materials.....	32
4.1.1	Wheat.....	32
4.1.2	Chemicals.....	33
4.2	Milling.....	33
4.3	Preparation of Extruded Product.....	33
4.4	Preparation of Drum-Dried Product.....	34
4.5	Proximate Analysis.....	35
4.5.1	Determination of Crude Protein.....	35
4.5.2	Determination of Moisture.....	36
4.5.3	Determination of Crude Fat.....	36
4.5.4	Determination of Ash.....	36
4.5.4	Determination of Crude Carbohydrate.....	37
4.6	Determination of Hunter L, a, b Color Values.....	37

4.7	Determination of Amino Acid Composition.....	37
4.8	Determination of Available Lysine.....	39
4.9	Determination of Protein Efficiency Ratio.....	43
4.10	Determination of Apparent Digestibility.....	44
4.11	Differential Scanning Calorimetry.....	45
4.12	Statistical Analysis.....	46
5.0	Results and Discussion.....	47
5.1	Milling of Wheat.....	47
5.2	Proximate Analysis.....	47
5.3	Hunter L, a, b Color Values.....	50
5.4	Amino Acid Composition.....	52
5.5	Available Lysine Analysis.....	58
5.6	Protein Efficiency Ratio.....	60
5.7	Apparent Digestibility.....	65
5.8	Starch Gelatinization.....	76
6.0	Summary and Conclusions.....	81
7.0	Suggestions for Further Research.....	84
8.0	References.....	86
9.0	Appendices.....	94
Vita.....		96

List of Tables

Table 1.	Proximate Composition of Wheat and Wheat Fractions.....	7
Table 2.	Mean Percentages of Moisture, Fat, Protein, Ash and Carbohydrate of Unprocessed, Extruded and Drum-Dried Whole Wheat Flour.....	48
Table 3.	Mean Percentages of Fat, Protein, and Ash of Unprocessed, Extruded and Drum-Dried Whole Wheat Flour on a Dry Weight Basis.....	49
Table 4.	Hunter Color Values of Milled, Extruded and Drum-Dried Whole Wheat Flour.....	51
Table 5.	Amino Acid Composition of Unprocessed, Extruded and Drum-Dried Whole Wheat.....	53
Table 6.	Available Lysine Content of Milled, Extruded and Drum-Dried Whole Wheat.....	59
Table 7.	PER of Unprocessed, Extruded, and Drum-Dried Whole Wheat Flour.....	62

Table 8. Apparent Nitrogen Digestibility of Unprocessed,
Extruded, and Drum-Dried Whole Wheat Flour.....67

Table 9. Mean Feed Intake and Weight Gain of Rats Fed
Unprocessed, Extruded and Drum-Dried Whole
Wheat Diets.....69

Table 10. Mean Fecal Weight of Rats Fed Unprocessed,
Extruded and Drum-Dried Whole Wheat Diets.....72

Table 11. Mean Total Fecal Nitrogen of Rats Fed
Wheat Diets.....74

List of Illustrations

Figure 1. A Kernel of Wheat.....	6
Figure 2. A Typical Top Feed Drum-Dryer.....	11
Figure 3. A Cross-Section of a Typical Food Extruder.....	13
Figure 4. Total Lysine and Hunter L Color Values of Unprocessed, Drum-Dried, and Extruded Whole Wheat.....	57
Figure 5. Available Lysine and Hunter L Color Values of Unprocessed, Drum-Dried, and Extruded Whole Wheat.....	61
Figure 6. Average Weight Gain of Rats Fed Unprocessed, Drum-Dried, and Extruded Whole Wheat Diets Over Time.....	64
Figure 7. Comparison of Unprocessed, Drum-Dried, and Extruded Whole Wheat Hunter L Color Values and the PER of Unprocessed, Drum-Dried, and Extruded Whole Wheat Rat Diets.....	66

Figure 8. DSC Thermograms of Wheat Starch, Commercial Whole Wheat Flour, and Whole Wheat Flour Used in this Study.....77

Figure 9. DSC Thermograms of Drum-Dried and Extruded Whole Wheat.....78

List of Appendices

Appendix I. Standard Curve for Available Lysine Analysis..95

1.0 Introduction

Wheat is one of the world's major cereal grains. Based on United Nations data published in 1982, approximately 25% of the total calories consumed in the world came from wheat (Nelson, 1985).

Wheat proteins are a significant part of the world's daily diet (Nelson, 1985). Dietary intake of protein is essential for the nutritional well-being of humans for growth, maintenance, and repair of tissue proteins (Munro and Crim, 1988). The ability of a protein to meet the nitrogen and amino acid requirements of an organism can be defined as 'protein quality'. (Pellett, 1978; Cheftel et al, 1985). Protein quality depends on the amino acid composition of the protein and its digestibility, as well as other factors in the diet (i.e. phytic acid, protease inhibitors and indigestible compounds) and the nutritional status, age and health factors of the organism consuming it (Pellett, 1978; Kies, 1981;

Cheftel et al, 1985).

Thermal processing can, at times, be beneficial to the protein quality of wheat and other foods because heat and shearing forces can denature proteins, gelatinize starch, and solubilize dietary fiber such that the susceptibility of the protein, starch, and fiber to enzymatic hydrolysis is enhanced (Björck et al, 1984; Cheftel, 1986; Cubadda, 1988; Phillips, 1989). However, severe processing conditions may decrease the digestibility, nutritional quality, and safety of the food through Maillard Browning, β -elimination, cross-linking, and racemization reactions as well as reactions of proteins with oxidized lipids (Swaisgood and Catignani, 1982; Cheftel et al, 1985; Cubadda, 1988; O'Brien and Morrissey, 1989; Phillips, 1989).

This study is concerned with measuring protein quality and digestibility of unprocessed whole wheat as well as whole wheat subjected to drum-drying and single screw extrusion thermal processing. Whole wheat was chosen because of its importance to the world's diet and because many food products contain the whole grain. Drum-drying and extrusion processing methods were selected because these methods are widely used in the food industry (Potter, 1986; Davidson et al, 1984b) to make such products as biscuits and breakfast cereals (Björck et al, 1984), instant noodles and pasta (Dexter et al, 1984), instant dried soups or gravy bases, instant beverages, snacks,

and crackers (Linko and Colonna, 1981). The processed and unprocessed wheat was analyzed for total amino acids and available lysine. Wheat is deficient in lysine and this deficiency can be exacerbated from losses due to browning reactions during processing (Dexter et al, 1984). The processed and unprocessed wheat were analyzed by Differential Scanning Calorimetry to examine starch gelatinization. Extrusion and drum-drying are known to cause changes in the physical composition of the starch granule (Davidson et al, 1984a).

2.0 Review of Literature

2.1 *Wheat*

The major cereal grains grown worldwide are wheat, corn, rice, oats, barley, rye, sorghum, and millet. These grains provide the world with most of its calories and about half of its protein (Wu and Inglett, 1988; Potter, 1986). Based on United Nations data published in 1982, approximately 25% of the total calories consumed in the world came from wheat (Nelson, 1985). Wheat is a unique cereal grain due to its ability to form gluten, a requirement for a strong, viscoelastic dough which is necessary for retention of leavening gases in baked products.

The basic composition is similar for all cereal grains. Their structure includes a large, centrally located starch-containing endosperm and an embryo or germ usually located near the bottom of the seed. The bran consists of protective

outer layers such as the epidermis and seed coat layers (Figure 1). The bran contains cellulose material that is indigestible to man and is rich in B vitamins and minerals (Nelson, 1985; Potter, 1986). The germ, also high in B vitamins and minerals, is very rich in oil and enzymes (Nelson, 1985; Potter, 1986), such as lipoxygenases, peroxidase, and β -amylase (Richardson and Hyslop, 1985). The proximate composition of wheat and wheat fractions are presented in Table 1.

Wheat is classified into two types: hard and soft. Hard wheat is generally higher in protein content, yields a stronger flour, which forms a more elastic dough suitable for breadmaking. In contrast, soft wheat has a lower protein content, yields a weaker flour, which forms doughs and batters better suited for cakes and pastries.

2.2 Milling

Conventional milling usually involves the removal of the bran and germ from the endosperm. Milling is necessary due to the indigestibility of the bran and the potential rancidity of the germ. In addition, these components can interfere with the functional properties of the endosperm in some food production processes (Potter, 1986).

In the first steps of conventional milling, the wheat

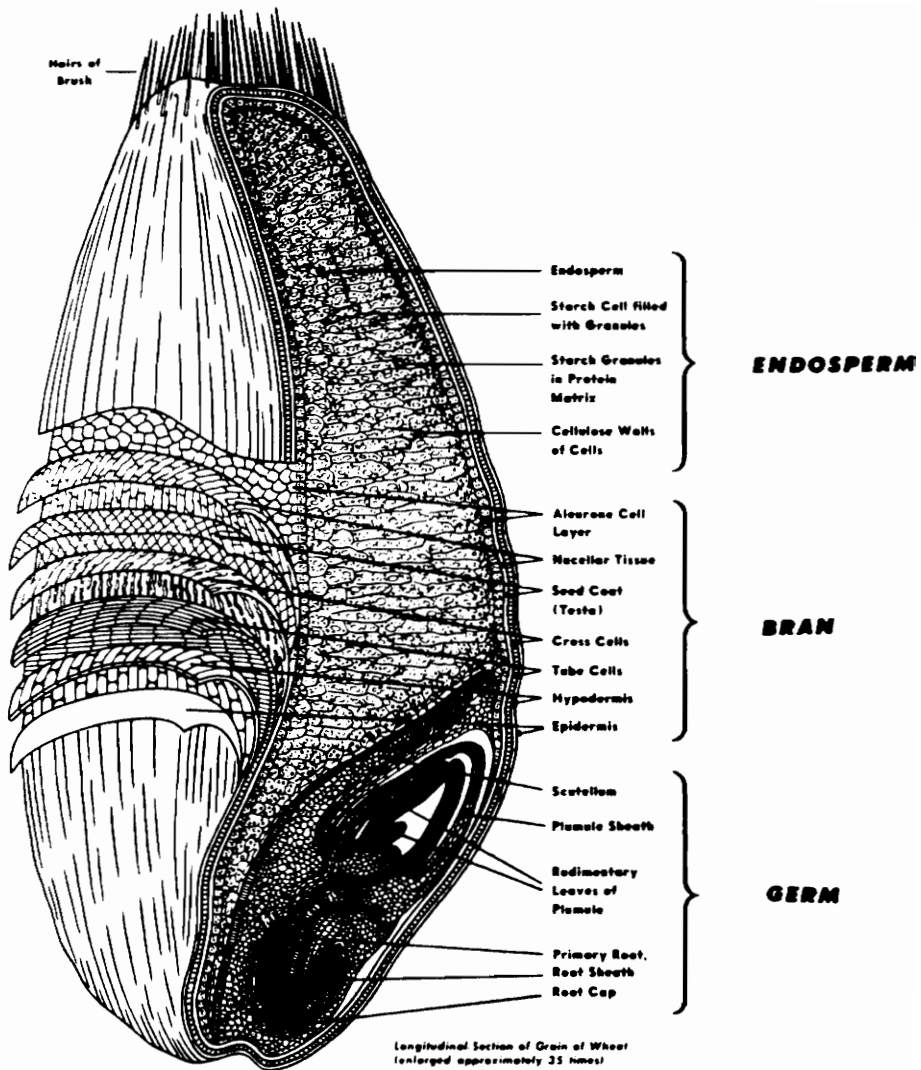


FIGURE 1. A KERNEL OF WHEAT
(Nelson, 1985)

**TABLE 1. PROXIMATE COMPOSITION OF WHEAT
AND WHEAT FRACTIONS**

CONSTITUENT (%)	WHEAT	BRAN	GERM	PATENT FLOUR
Protein	12.0	14.5	30.0	11.0
Fat	2.1	3.3	10.0	0.9
Moisture	12.0	14.1	10.5	13.9
Ash	1.8	6.0	4.0	0.4
Carbohydrate	72.1	62.1	45.5	73.8

Adapted from Wu and Inglett, 1988.

kernels are separated from foreign matter such as other seeds and soil. The kernels are tempered, or soaked in water, to moisten the bran and germ. This allows for better separation from the endosperm and optimizes milling. The milling process involves a progressive series of disintegrations followed by sievings (Potter, 1986). The disintegrations are made by rollers set gradually closer and closer together. The first set of rollers break open the bran and frees the germ from the endosperm. This material is sifted to separate the fractions. The second and third set of rollers further pulverize the endosperm and flatten out the germ (Potter, 1986). The flakes of bran and flattened germ are continually removed by the sieves under these sets of rollers. The pulverized endosperm is run through successive rollers set closer and closer together to grind it into finer and finer flour (Nelson, 1985; Potter, 1986). As it passes through these rollers, the amount of crushed bran and germ progressively decreases. As a result, the resulting flour becomes whiter in color but lower in vitamin and mineral content (Potter, 1986). To replace the lost nutrients, it is common practice to enrich milled flour (Potter, 1986). However, with the recent gains in knowledge of the importance of dietary fiber in the diet, whole wheat flour, which contains the germ and bran, is used in the production of many food products.

2.3 Food Processing

Food is processed for three main reasons: (1) to preserve, package and store food; (2) to produce desirable food products; and (3) to prepare foods for serving (Karmas, 1988).

Raw foods are perishable. The rate at which deterioration occurs depends mainly on the content of biologically available water in the tissues. Raw foods with a high water activity, such as raw meat and leafy vegetables, can be stored for only a few days before they begin to spoil. In contrast, foods such as dry seeds which contain only structurally bound water can be stored for years under proper conditions (Karmas, 1988).

The major causes of food spoilage are microbial growth and enzymatic and chemical changes (Karmas, 1988). These reactions are favored at a high water content, and at specific temperatures and pH. The principles of food processing are based on the manipulation of these environmental conditions. Moisture removal, such as dehydration and concentration, and heat applications, such as pasteurization and extrusion cooking, are two of the basic food processing methods used to preserve foods (Karmas, 1988). Removing water and/or applying heat helps to destroy the microorganisms and enzymes that cause food spoilage.

2.4 Drum Drying of Foods

Drum drying is a dehydration method used to preserve foods. Dehydration removes the biologically active water in the food which prevents growth of microorganisms and reduces the rate of enzymatic and chemical reactions (Karmas, 1988).

The process of drum drying involves applying a viscous slurry, puree, paste, or mash into the trough between two revolving, heated steel drums (Figure 2). The drums are usually heated internally by steam. The slurry is applied continuously and the thin layer loses moisture. After the layer on the drum has revolved almost one full turn, a knife, or doctor blade, is positioned to peel the thin, dried layer of food from the surface of the drum. The drum speed can be regulated so that the product will be dry before reaching the doctor blade (Potter, 1986). The thickness of the product can also be regulated by the width of the trough between the two drums (Potter, 1986). The drum surface temperature may reach well above 100°C, and is often held at 150°C. Rapid drying is achieved by holding the temperature at least above 120°C (Bluestein and Labuza, 1988). This high temperature gives products a more cooked flavor and color (Potter, 1986). Drum drying is one of the least expensive methods of thermal processing (Grieg, 1971) particularly for relatively heat-resistant food products.

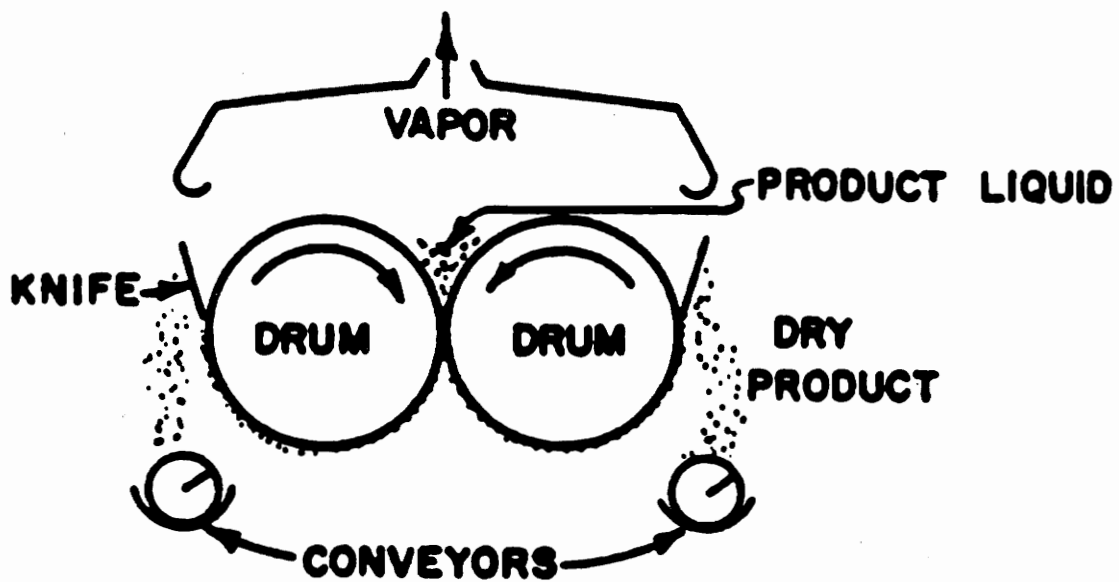


FIGURE 2. A TYPICAL TOP FEED DRUM-DRIER
(Potter, 1986)

2.5 Extrusion Processing of Foods

Extrusion is a heat treatment method that is applied to foods to denature microbial proteins and native food enzymes (Karmas, 1988).

Extrusion technology was first commercially used in the production of pasta and ready-to-eat (RTE) cereals in the 1930's (Harper, 1981). A food extruder consists of a shallow, flighted screw which rotates in a tightly fitting stationary, cylindrical barrel (Figure 3). The raw food ingredients enter through the feed hopper and are conveyed forward by the rotating action of the screw. As the constricted space between the flights and the barrel become completely filled with food material, the food material is compressed and mixed into the form of a dough-like mass (Harper, 1988; Harper, 1981). Heat is added to the food material as it moves through the screw by a combination of mechanisms, including dissipation of the mechanical energy required to turn the screw by fluid friction, heat transfer from electrical heaters or steam contained in jackets surrounding the barrel, and direct steam injection through the barrel wall into the food material (Harper, 1988; Harper, 1981).

As the moist, hot dough moves through the length of the screw, it is subjected to thermal and mechanical shear forces. In addition, the pressure within the barrel increases due to

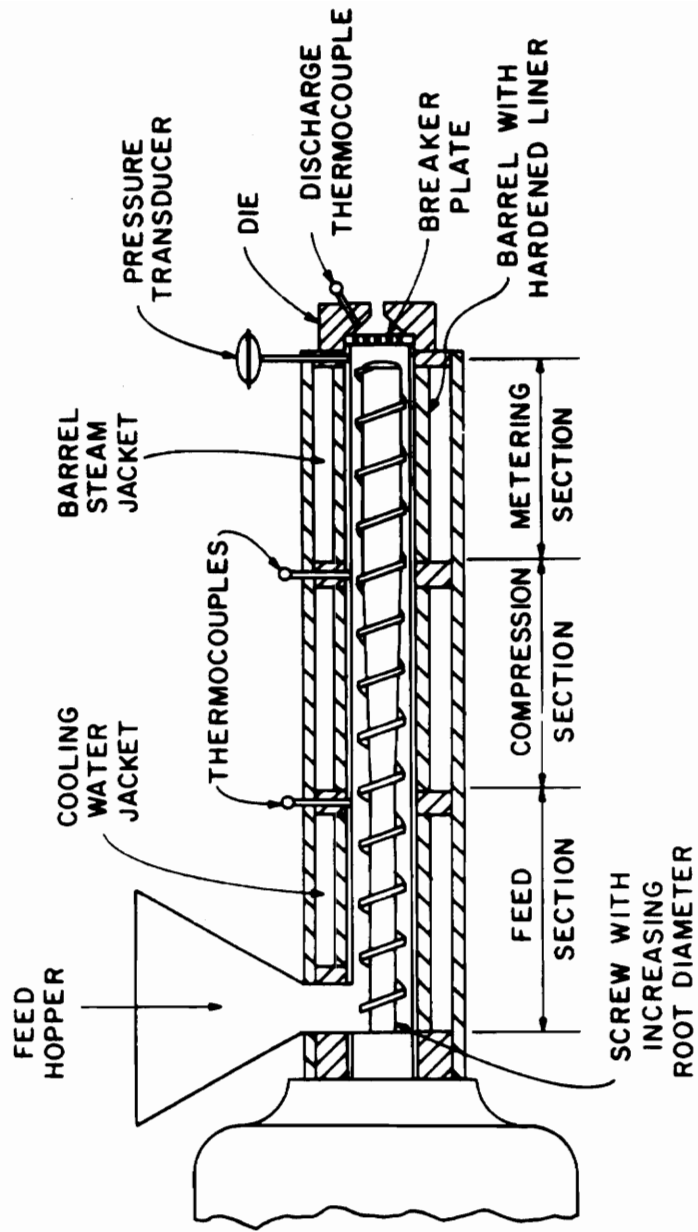


FIGURE 3. CROSS-SECTION OF A TYPICAL FOOD EXTRUDER
(Harper, 1981)

a restricted output at the end of the barrel. This small opening is referred to as a die. Due to the high pressure within the barrel, boiling or flashing of water contained in the dough does not occur. Once the food emerges from the die and the pressure is released, the heated water rapidly boils and the formed piece puffs or expands (Harper, 1981; Potter, 1986). The loss of moisture causes the product to cool nearly instantaneously at the die face (Harper, 1988). The product solidifies and sets in its extruded shape. Additional oven drying of the extruded product may then be necessary (Potter, 1986) to reduce the water activity.

Extrusion may occur over a wide range of temperatures. High temperature - short time (HTST) extrusion occurs at temperatures between 140°C and 190°C with a residence time between 15 and 60 seconds (Harper, 1988). At the other extreme of extrusion processing is the forming extruder, used for macaroni, which operates at temperatures below 60°C and residence times of 120 seconds or greater (Harper, 1988). HTST extrusion processing in the absence of oxygen promotes desirable cooking reactions such as the disruption of starch granules and denaturation of proteins, thus aiding in their digestibility. The inactivation of enzymes and anti-nutritional agents, such as trypsin inhibitors in soy can also be achieved at HTST conditions (Cheftel, 1986; Harper, 1988).

2.6 Lysine: Nutritional Significance

Lysine is one of the nine essential amino acids required by human adults for nutritional well-being. It is well known that lysine is the limiting amino acid in cereal proteins (Cheftel et al, 1985), i.e., lysine is present in the lowest amount with respect to requirements.

The side chain of lysine contains a four-carbon chain with a terminal epsilon amino group. This ionizable epsilon amino group can chemically react with other food components during processing and storage, making the lysine biologically unavailable. This becomes an important consideration in cereal products due to the limiting nature of lysine.

2.6.1 Maillard (Non-enzymatic) Browning Reaction

Perhaps the most studied food reaction involving lysine is the Maillard or Nonenzymatic Browning reaction discovered by the French chemist, Louis Camille Maillard in 1912 (Dworschák, 1980; O'Brien and Morrissey, 1989). The Maillard or Nonenzymatic Browning reaction proceeds via a complex series of chemical reactions (Dworschák, 1980; O'Brien and Morrissey, 1989). The first step of the reaction involves the condensation of a nonionized amino group (such as the terminal epsilon amino group of lysine or the terminal alpha amino

group of a peptide or protein chain) with reducing carbohydrates (such as glucose, fructose, lactose, or maltose) or carbonyl compounds (such as aldehydes and ketones formed from the oxidation of fat) (Cheftel et al, 1985). In this first step, a Schiff base is formed which rapidly isomerizes into an aldosylamine or a ketosylamine, depending on the initial reacting carbohydrate (Cheftel et al, 1985). These N-substituted glycosylamines are unstable compounds and transform, by way of the Amadori or Heyn's rearrangement into stable ketosamines or aldosamines (Cheftel et al, 1985; Dworschák, 1980; O'Brien and Morrissey, 1989).

During the second step of this series of reactions, the ketosamines and aldosamines evolve into numerous carbonyl and polycarbonyl unsaturated derivatives (Cheftel et al, 1985). Some of these resulting derivatives may react with amino acids in the Strecker degradation (Baltes, 1982) resulting in the formation of ammonia and new carbonyl compounds (Cheftel et al, 1985).

During the third step, the polycarbonyl unsaturated derivatives undergo both scission and polymerization reactions, leading to volatile, aromatic substances and/or black or brown pigments (Baltes, 1982; Cheftel et al, 1985; O'Brien and Morrissey, 1989). These pigments, called melanoidins, have high molecular weights and complex structures (Cheftel et al, 1985). They are responsible for

the brown color in bread crust.

2.6.2 Color Measurements of Food Products

Color is one of the most important sensory quality attributes of food (Francis, 1985). A food product can be rejected based only on its color, even if the color does not adversely effect the flavor or nutritional value of the food (Blouin et al, 1981). Maillard browning products can greatly influence the color of foods, such as bread crust, coffee, caramel, chocolate, and syrups (Cheftel et al, 1985; O'Brien and Morrissey, 1989). As explained by Blouin et al (1981), color measurements must specify three fundamental quantities: (1) hue or spectral color, which identifies the object as yellow, blue, red, green, or an intermediate color between these, (2) saturation or purity, which is the strength or intensity of the hue, and (3) luminance or lightness of the object, which is the amount of light reflected or transmitted from the object.

A Hunter Color Meter expresses these quantities as Hunter L, a, and b color values. The L value measures the lightness of the object, or the amount of light reflected or transmitted. The a and b values are color coordinates that provide information about the hue and saturation. The a value measures redness (positive numbers) and greenness (negative

numbers). The b value measures yellowness (positive numbers) and blueness (negative numbers) (Blouin et al, 1981).

2.6.3 Available vs. Total Lysine

Severe heat processing and/or poor storage conditions can reduce the availability of lysine in foods to a greater extent than is measured by simple amino acid analysis (Hurrell and Carpenter, 1981). "Available" lysine refers to the lysine that is biologically available to the organism during digestion; i.e. those lysine units which have free reactive ϵ -amino groups (Hurrell and Carpenter, 1981). The lysine in the early stage of Maillard browning, such as the Schiff base, is biologically available and the lysine can be set free under the acidic conditions within the stomach. However, lysine in Amadori or Heyn's rearrangement reaction is no longer biologically available (Cheftel et al, 1985). The major form of blocked lysine after Maillard reactions is this unavailable deoxyketosyl compound (Hurrell and Carpenter, 1981). Methods used to analyze lysine that begin with acid hydrolysis have been found to overestimate lysine content (Carpenter, 1960). Therefore, it is necessary to determine the amount of lysine that is biologically available by measuring the lysine units that possess a free, unreacted ϵ -amino group. A number of chemical methods have been developed, such as Carpenter's FDNB

(1-fluoro-2,4-dinitrobenzene) method (Carpenter, 1960), Hall et al's TNBS (2,4,6-trinitrobenzenesulphonic acid) method, (Hall et al, 1973), Finot's Furosine method, (Hurrell and Finot, 1985), and Hurrell and Carpenters' dye-binding capacity (DBC) method (Hurrell and Carpenter, 1976).

2.6.4 Amino Acid Balance in the Diet

Food preferences are influenced by the protein or the amino acid balance of the diet (Harper and Peters, 1989). A balanced consumption of amino acids is important for overall health and growth. Characteristic signs of an amino acid imbalance in the diet include depression of food intake and growth (Harper, 1964; Harper and Peters, 1989). An amino acid imbalance occurs when the amino acid pattern of the diet is altered so that the proportions of the essential amino acids vary from the proportions in which they are required, i.e., if there is a deficiency of one or more essential amino acids, or if there is an excess of one or more essential amino acids (Harper and Peters, 1989). The daily lysine requirement for a rat is 600 μ moles/100 grams body weight (Munro and Crim, 1988). Dietary deficiency of lysine has been shown to decrease feed intake, feed efficiency, and weight gain in weanling rats (Bolze et al, 1985; Leung, et al, 1968). Weight gains in mice were found to be proportionally (Finot et al,

1978) or semilogarithmically (Friedman and Gumbmann, 1981) related to the amount of lysine in the diet. Diets that contain less 0.72% lysine do not support weight gain in rats (Cieslak and Benevenga, 1984). While some experiments seem to indicate that an amino acid imbalance decreases voluntary food intake (Bolze et al, 1985; Leung, et al, 1968), Cieslak and Benevenga (1984) found that rats fed suboptimal levels of lysine will consume more energy per unit of body weight compared to rats fed a more adequate lysine level. Brookes et al (1972), also found that body weight gain was depressed approximately 20%, yet the average daily food intake was unchanged or increased. This increased food consumption appears to increase fat deposition (Cieslak and Benevenga, 1984).

2.7 Effects of Drum-Drying and Extrusion Processing on Food

Processing can modify food proteins in ways that change the functionality, nutritional quality, and safety of the food. Food processing has overall beneficial effects since it decreases food spoilage and enables a wide variety of seasonable foods to be available worldwide in shelf-stable forms (Cheftel et al, 1985). In most instances, food processing has little or no adverse effect on the nutritional value of proteins. In fact, processing can be beneficial to

the digestibility of the food because heat and other forces can denature proteins and gelatinize starch such that the susceptibility of the protein and starch to enzymatic hydrolysis is enhanced (Cheftel, 1986; Cubadda 1988; Phillips, 1989). However, severe conditions of processing favor reactions that result in a decrease in the content of essential amino acids, nutritional quality, digestibility, and safety of the food or in the formation of antinutritional and sometimes toxic derivatives (Cheftel et al, 1985). The damage that results can be detrimental when the diet consists of a restricted number of foods or has a minimal protein content, such as in the diets of elderly persons, infants, and poor populations (Cheftel et al, 1985).

Because drum drying and extrusion processing are extremely versatile, it is difficult to generalize their impact on protein quality. Any effect is highly related to the processing condition variables such as temperature, shear forces, pressure, residence time, screw and drum speed, screw geometry, screw compression ratio, moisture content, and the composition of the raw material (Phillips, 1989; Cheftel, 1986; Hurrell and Finot, 1985).

Nutritional modifications do occur as a result of drum-drying and extrusion processing. Several researchers (Aguilera and Kosikowski, 1978; Björck et al, 1983; Björck and Asp, 1984; Cubudda, 1988; Hansen et al, 1975; Maga and Sizer,

1979) have reported changes in amino acid content in extrusion cooked products. Cubudda (1988) found that millet-gram flour lost 24% total lysine, sorghum-gram flour lost 27% total lysine, and millet-fish dry soup lost 11% total lysine when extruded at 150 to 160°C with a residence time of 90 seconds. Decreases in cysteine, methionine, isoleucine, and arginine were also reported.

Björck et al (1983) found an 13% decrease in total lysine in a mildly processed (170°C, 195 psi, 80 rpm, 13% moisture, residence time 42s) protein-enriched biscuit and a 37% decrease in the most severely processed (210°C, 95 psi, 80 rpm, 13% moisture, residence time 44s,) one. Marked losses of methionine (28%) and tryptophan (12%) were also observed under these conditions.

Björck and Asp (1984) found a significant 9% decrease in total lysine of whole grain wheat flours extruded at 166°C (200 rpm, 20% moisture, 200 g/min feed rate). No significant losses occurred in other amino acids.

Hansen et al (1975) found significant losses of total lysine, arginine and cystine in wheat flour heated at 174°C for 10 minutes (33% moisture).

Drum-drying usually results in greater nutrient deterioration than that caused by spray or tunnel drying (Bluestein and Labuza, 1988). For example, MacDonald (1966) has shown that spray-drying destroyed 0 - 4% of the available

lysine in milk and drum-drying destroyed from 3 to 16%. Similarly, Rolls and Porter (1973) reported that available lysine decreased 3 - 10% in spray-dried milk whereas the drum-dried milk lost 5 - 40% of available lysine. High temperature drying (85°C) of spaghetti after extrusion processing, resulted in a 31% decrease in available lysine (Dexter et al, 1984).

Maga and Sizer (1979) examined the combined effects of drum-drying and extrusion processing on free amino acids in potatoes. They reported that high free amino acid losses occurred during commercial drum-drying of raw potatoes. Drum-dried potato flakes lost 68% of free lysine and 86% free methionine. Extrusion of the flakes at 160°C (48% moisture, 100 rpm, 42s residence time) destroyed free amino acids as well with losses of 96% lysine, 88% isoleucine, 78% leucine, 87% phenylalanine, and 57% methionine.

Aguilera and Kosikowski (1978) examined the effects of drum-drying (40 psig, 35% moisture, 4 rpm) or extrusion (90°C, 600 rpm, 230 g/min feed rate, residence time of 30s, 35% moisture) on mixtures of corn, soy, and whey. They found that both processing methods reduced the quantity of essential amino acids (5% total lysine destroyed by drum-drying, 10% destroyed by extrusion). Drum-drying was less damaging than extrusion for all essential amino acids except methionine. P.E.R. values for all mixtures were not significantly

different from casein (2.50). Both processing methods increased the P.E.R. (2.47 - drum-dried; 2.66 - extruded) over that of the unprocessed mixture (2.41).

The most important source of nutritional quality loss is the Maillard browning reaction (Dworschák, 1980; Hurrell and Finot, 1985; Phillips, 1989). This reaction is largely responsible for the loss of lysine in processed foods. Gumbmann et al (1983) found that the Maillard browning reaction decreased protein quality by lowering apparent nitrogen digestibility in casein and casein mixtures of glucose and starch heated at 180 - 240°C. Noguchi et al (1982) found that up to 40% of FDNB-reactive lysine losses in an extruded wheat flour mixture containing 20% sucrose were due to the Maillard browning reactions.

The presence of reducing sugars in the original mix is not required for Maillard browning to occur. Reducing sugars can be formed from starch or oligosaccharides under severe extrusion conditions (Linko et al, 1981; Björck and Asp, 1984) or from the hydrolysis of sucrose (Noguchi et al, 1982).

McAuley et al (1987) reported that Hunter L color values were positively associated with TNBS-available lysine and protein digestibility of wheat-based breakfast cereals. It has been suggested that Hunter L color values may be used as a crude predictor of protein quality, especially lysine availability, in products subjected to Maillard browning

(Bookwalter and Kwolek, 1981).

The mutagenic characteristics of Maillard reaction compounds are amply reviewed in the literature (Dworschák, 1980; Baltes, 1982; O'Brien and Morrissey, 1989). It is known that Maillard compounds can react easily with nitrogen oxides to form nitrosoamines (Baltes, 1982) which were the first thermally induced carcinogens recognized in protein foods (Magee and Barnes, 1956). However, Commoner *et al* (1978) suggested that mutagenic products in heated protein foods result mainly from amino acid pyrolysis, not Maillard browning reactions.

It is known that heat and alkali racemize amino acids in proteins. Amino acid racemization and crosslink formation in proteins decrease nutritional quality both by reducing digestibility and by producing forms of amino acids (i.e. D-isomers or lysinoalanine) which are less available, nonavailable, or even potentially toxic (Friedman *et al*, 1981; Thompson and Erdman, 1981; Friedman and Masters, 1982).

Another major loss of certain amino acids results from β -elimination reactions of cystinyl and substituted threonyl or seryl residues (Swaisgood and Catignani, 1982). This reaction produces a dehydroalanyl residue that is very reactive and will undergo a vinyl-type addition, particularly with cysteine and lysine, forming protein chain crosslinking residues, lanthionine and lysinoalanine (LAL), respectively (Thompson

and Erdman, 1981; Swaisgood and Catignani, 1982). These residues limit protein digestibility and bioavailability of other amino acids (Friedman et al, 1981).

Dworschák (1980) demonstrated that during processing, food lipids can be oxidized or hydrolyzed to species that may react principally with cystine, methionine, lysine and tryptophan. The covalent interactions between oxidized lipids and amino acids that reduce nutritional quality occur slowly at room temperature but are accelerated at the temperature range in which extrusion takes place (Pokorny, 1977). However, Linko et al (1981) found that extrusion cooking usually causes an increase in the oxidative stability of lipids due to inactivation of enzymes such as lipoxigenase and peroxidase.

2.7.1 Determining Protein Nutritive Value

The various methods that exist to determine the nutritive value of a protein source can be divided into two main categories: in vivo and in vitro methods.

Webster's Dictionary (Woolf, 1976) defines in vivo as "in the living body of a plant or animal." Therefore, in vivo assays used to determine protein value involve measurements based on the growth or nitrogen retention in animals, such as rats, or in humans (Cheftel et al, 1985). The most well known

of these methods are the Protein Efficiency Ratio (PER), Net Protein Ratio (NPR), Net Protein Utilization (NPU), Biological Value (BV), and Digestibility (True or Apparent) (Pellett, 1978).

PER relates weight gain of the test animal to protein intake and NPR relates weight gain of the test animal to protein intake including a non-protein diet. PER is the most well known method for evaluating protein quality and is the official procedure within the United States (Pellett, 1978). However, the method has important limitations. It is highly variable due to the strain of rat used and the amount of feed consumed. It is difficult to reproduce and is not proportional (a PER of 2 does not mean twice the nutritional quality of a PER of 1). PER is a measure of the growth requirements of the rat and it does not make allowances for maintenance requirements (Pellett, 1978; Satterlee, 1979; Cheftel et al, 1985). The NPR assay evaluates the ability of proteins to support maintenance as well as growth (Cheftel et al, 1985).

NPU relates the nitrogen retained by the body plus nitrogen lost in the feces and urine to the nitrogen intake (Pellett, 1978; Cheftel et al, 1985). Similarly, BV relates the nitrogen retained by the body to the nitrogen absorbed (Pellett, 1978). BV reflects the balance of essential amino acids in the absorbed protein (Cheftel et al, 1985).

Digestibility assays compare the nitrogen absorbed by the body to the total nitrogen intake (Pellett, 1978).

Methods that are classified as in vitro are done "outside the living body and in an artificial environment" (Woolf, 1976). These include chemical (amino acid analysis), microbiological (*Tetrahymena pyriformis*), and enzymatic (trypsin, chymotrypsin, peptidase) digestion assays.

2.8 Starch Gelatinization

Starch granules contain two different polysaccharides; amylose and amylopectin. These polymers consist of repeating D-glucose units. Amylose is an essentially linear chain in which the D-glucose units are linked by α -1:4 bonds. Amylopectin is a highly branched polysaccharide molecule with α -1:6 and α -1:4 glycosidic linkages (Harper, 1981; Whistler and Daniel, 1985).

The process of gelatinization as it occurs in starch alters the structure of the starch granule, causing it to absorb heat, become less crystalline as determined by X-ray diffraction and birefringence, and become more water soluble (Harper, 1988; Ghiasi et al, 1982). As the starch granules absorb water and expand, the helical structure of amylose is disrupted. The amylose begins to diffuse out of the granules causing the starch granule to collapse. Water molecules bind

to the exposed hydroxyl groups on the starch chains. The granules, which contain mainly amylopectin, are supported in a matrix of amylose and water, forming a colloidal gel (Harper, 1981; Whistler and Daniel).

When starches are heated in the presence of water, phase transitions are induced (Ghiasi et al, 1982). These phase transitions can be studied by Differential Scanning Calorimetry (DSC). DSC can examine the temperature range over which gelatinization occurs as it is affected by various experimental conditions, as well as the enthalpy involved in endothermic transitions.

Extrusion processing is usually conducted on food materials with less than 50% moisture (Harper, 1981). Because of this low moisture content, traditional gelatinization including swelling and loss of birefringence in polarized light does not occur (Harper, 1988). However, starch gelatinization can occur during drum-drying processing because the slurries usually have very high moisture contents (Bluestein and Labuza, 1988).

Researchers investigating the endothermic gelatinization transition of wheat starch by DSC, have reported peak temperatures of 64°C (Ghiasi et al, 1982), 68°C (Wootton and Bamumuarachchi, 1979), 65°C (Kugimiya et al, 1980) and 64°C (Ghiasi et al, 1983). Ghiasi et al (1983) observed a peak temperature of 61°C for the gelatinization endotherm of wheat

flour.

Kugimiya et al (1980) and Ghiasi et al (1983) reported that when wheat starch was heated in an excess of water, two endotherms were obtained. The first endothermic transition representing starch gelatinization occurred in the temperature range of 65 - 75°C and the second transition occurred at a higher temperature near 100°C. Kugimiya et al (1980) proposed that the second endothermic peak was a disordering transition of amylose-lipid complexes. Wheat starch contains significant amounts of lipid (1.12%) (Kugimiya et al, 1980). The authors found that after extraction of the wheat starch with methanol, the endothermic peak previously observed at 100°C was diminished to about two-fifths of its original area. They further demonstrated that amylopectin did not complex with lipid. Ghiasi et al (1983) reported that the second, minor endothermic peak occurring at 100°C in thermograms of wheat starch and wheat flour was from the amylose-lipid complex.

3.0 Objectives of the Study

The goal of this study was to determine the effects of drum-drying and single screw extrusion on the protein quality and digestibility of whole wheat.

The specific objectives of this study were:

1. To determine if drum-drying and single screw extrusion significantly altered the PER and apparent nitrogen digestibility of Coker 916 whole wheat.
2. To determine if drum-drying and single screw extrusion resulted in significant changes in the total and/or available amino acids of Coker 916 whole wheat.
3. To determine if there was any association between protein quality of whole wheat products and the degree of Maillard Browning as determined by Hunter L, a, b color values and total and available lysine.

4.0 Materials and Methods

4.1 Materials

4.1.1 Wheat

Coker 916, a soft, red, winter wheat used in this study, was grown by the Agronomy Department of Virginia Polytechnic Institute and State University in Giles County during the 1989 growing season. Various levels of nitrogen were applied to the wheat at growth stages 25 and 30. Amounts of either 30 or 60 pounds of nitrogen per acre were applied at growth stage 25, which usually occurs during November; and 30, 60, or 120 pounds of nitrogen per acre were applied during growth stage 30, which usually occurs in February. The wheat kernels were stored in paper bags at -20°C until used.

4.1.2 Chemicals

All chemicals used in this study were at least reagent-grade. Distilled and deionized water was used in all experiments, unless otherwise stated.

4.2 Milling

Prior to milling, 16.4 kg of whole wheat kernels were tempered to 14% moisture in a sealed container for 18 hours. This was necessary to enhance the separation of the bran from the endosperm during milling. The wheat was milled into flour over a two day period with a Brabender mill, Model Quad Jr. II. The bran fraction was mixed with the flour fraction to create a whole wheat flour.

4.3 Preparation of Extruded Product

Of the whole wheat flour, 8.2 kg (18 pounds) was made into a dough (45% moisture) and extruded using a single-screw (one inch diameter) Killion extruder at 93°C and 50 psi. The screw speed was approximately 59 r.p.m. and the average feed rate was 62 grams per minute. The extruded product resembled whole wheat spaghetti. The extruded product was dried

overnight in a Precision Scientific Co. Thelco oven, Model No. 28 at 60°C and ground into a powder using a Regal Coffee and Spice Mill, No. V505 (Regal Ware, Inc. Kewaskum, WI) for analysis (Davidson et al, 1984a). The extruded product was stored at -20°C in cotton sacks until used.

4.4 Preparation of Drum-Dried Product

Of the whole wheat flour, 8.1 kg (17.8 pounds) was combined with water to form a slurry (61% moisture) and drum-dried using an American Drum Dryer (Overton Machine Co., Dowagiac, Michigan), Model No. P19, at 152°C and a steam pressure of 55 p.s.i.g. The drum dried product resembled dehydrated, flaked potatoes. This was dried overnight in a Precision Scientific Co. Thelco oven, Model No. 28 at 60°C. The drum dried product was stored at -20°C in double wrapped polyethylene bags until used. The drum-dried product used for analyses was ground into a powder using a Regal Coffee and Spice Mill, No. V505 (Regal Ware, Inc. Kewaskum, WI) (Davidson et al, 1984a).

4.5 Proximate Analysis

The procedures used for determining the proximate analysis were modifications of the methods recommended by the Association of Official Analytical Chemists.

4.5.1 Determination of Crude Protein

The method used to determine the crude protein content in the original whole wheat was a modification of the AOAC Method 920.87 (AOAC, 1990), also known as the Kjeldahl method. The whole wheat sample was first coarsely milled in a Wiley Mill before analysis. The nitrogen content of the samples was multiplied by 5.83 to determine protein content (Jones, 1926; Hansen et al, 1975; Kies et al, 1978).

The method used to determine crude protein content in the rat diet samples and crude nitrogen content of the rat feces was a modification of the automated AOAC Method 976.05 (AOAC, 1991). The block digester unit used was Buchi Model No. 430 (Switzerland). The automatic steam distillation and titration analyzer used was Buchi Model No. 322. The protein content of the diet samples was determined by multiplying the nitrogen content by 5.83 except for the ANRC casein control diet which was multiplied by 6.25 (Chapman et al, 1959).

4.5.2 Determination of Moisture

AOAC Method 925.10 (AOAC, 1991) was used to determine moisture content by drying whole wheat samples that were placed in aluminum pans of known weight in a Brabender oven (Hacksensack N.J.), Type SAS, Model No. 692, at 130°C for 3 hours.

4.5.3 Determination of Crude Fat

The crude fat of the whole wheat samples was determined by a modification of the AOAC method 920.39 (AOAC, 1991), also known as the ether extraction method. A Goldfish extraction apparatus was used. The method is based on the weight lost by a sample after extraction of ether soluble compounds for 8 hours with anhydrous petroleum ether (Fisher Scientific Co., Fair Lawn, New Jersey).

4.5.4 Determination of Ash

The ash content of the whole wheat samples was determined by a modification of the AOAC Method 923.03 (AOAC, 1991). The weighed samples were placed in porcelain crucibles and combusted in a Fisher muffle furnace, Model No. 495, at 575°C for 10 hours.

4.5.5 Determination of Crude Carbohydrate

The crude carbohydrate of the whole wheat was determined by difference. Crude carbohydrate equals 100 minus the percentage of crude protein, moisture, crude fat, and ash.

4.6 Determination of Hunter L,a,b Color Values

The color of the Drum Dried and Extruded products, as well as the whole wheat flour, was determined by the Hunter Color Difference Meter, model Labscan by Hunter Lab (Reston, Virginia). The instrument was zeroed using the black tile, standardized with the white tile (Standard No. LS-12147), and calibrated with the green tile. Hunter L, a, b color measurements were taken in duplicate.

4.7 Determination of Amino Acid Composition

The amino acid composition of the drum-dried and extruded products, as well as the unprocessed whole wheat kernels, was determined by Medallion Laboratories of Minneapolis, Minnesota. The following methodology was used: One ml (2.0 mg) of norleucine internal standard made up in 6 N HCl was added to 40 mg of sample in a hydrolysis flask. Hydrolysis took place under a nitrogen flush for 22 hours after

approximately 15 ml of 6 N HCl was added to the hydrolysis flask. When hydrolysis was complete, the sample was filtered through a GFA microfilter into a 50 ml round-bottom flask. Three drops of octanol was added and the hydrolysate was evaporated to dryness using a rotary evaporator at 40°C. When dry, 2 ml of deionized water was added to the flask and the hydrolysate was evaporated to dryness again. Following the second drying, two additional rinsing/drying steps were done using methanol instead of water. Twenty-five ml of sample dilution buffer was added to the flask to dissolve the residue. The residue was transferred to a microcentrifuge tube and centrifuged for 2 minutes.

The samples were analyzed by a Carlo Erba 3A29 Amino Acid Analyzer. A calibration standard that contained known amounts of each amino acid and the internal standard, norleucine, was run with each set of samples. The relative response of each amino acid compared to norleucine was determined from the calibrated standard.

The disulfide cystine was analyzed by a "protected" method. A 1 ml aliquot of standard cystine and 2.0 ml of the 200 µg/ml norleucine solution was pipetted into a standard taper 24/40 joint 125 ml Erlenmeyer flask. The mixture was evaporated to dryness using a rotary evaporator at 40°C. One ml of norleucine internal standard solution made up in 6 N HCl was pipetted into a standard taper 24/40 joint 125 ml

Erlenmeyer flask. Thirty mg of sample was added to the flask. Under a hood, 10 ml of 0°C performic acid was added to the standard cystine and sample flasks and swirled in an ice bath to wet the particles. While swirling in the ice bath, 2.0 ml of 48% HBr was added to each flask. The solutions were evaporated to dryness at 40°C using a rotary evaporator. After the addition of 6 N HCl, the samples were refluxed for 22 to 24 hours. After hydrolysis was completed, the sample preparation and analyses were conducted as described above.

4.8 Determination of Available Lysine

The method used to determine the amount of available lysine in the processed and unprocessed whole wheat samples was adapted from the dye-binding capacity (DBC) method of Hurrell and Carpenter (1976). The determination of available lysine by this method is thought to be based on an electrostatic interaction between negatively charged azo dyes and the positively charged basic amino groups of lysine, histidine, and arginine as well as the terminal amino groups in the proteins of foods suspended in an acid solution (Hurrell and Carpenter, 1981). The insoluble dye-protein complex precipitates out and, after filtration, the loss of dye from the solution gives an estimate of the protein content of the samples (Hurrell and Carpenter, 1981). However, lysine

alone can be determined by difference because only lysine groups can be propionylated after which they no longer react with the dye (Hurrell and Carpenter, 1976; Hurrell and Carpenter, 1981). Therefore, the determination of available lysine was based on measuring the difference in the dye-binding capacity of a protein before and after the masking of reactive lysine groups by propionylation.

All determinations were performed in duplicate during three different runs.

i) Samples A and B were weighed out into 50 ml polypropylene sample bottles obtained from the Udy Corporation (Fort Collins, Colorado). Sample A contained approximately 52 mg of protein and Sample B contained approximately 2 times the weight of A to compensate for the acylated lysine after addition of propionic anhydride. Two ml of isopropanol (Fisher Scientific Co., Fair Lawn, New Jersey) was added to each bottle and they were swirled gently to wet all particles.

ii) To the bottles representing sample B, 0.2 ml of propionic anhydride (Fisher Scientific Co.) was added. The bottles were swirled to mix, and then 2.0 ml of a 5% sodium acetate (Fisher Scientific Co.) solution was added. The bottles were quickly capped and mixed gently. The 'B' bottles were then placed on a Dubnoff Metabolic Shaking Incubator (Precision Scientific

Co.; Chicago, Illinois) and were shaken at 156 r.p.m. for 15 minutes.

iii) To the bottles representing sample A, 20 ml of Udy Reagent Dye solution (Udy Corp.) was added. The bottles were swirled to mix, and then 2.2 ml of the 5% sodium acetate solution was added. To the 'B' bottles, 20 ml of the Udy Reagent Dye solution was added. All bottles were vortexed and then placed on the shaker at 156 r.p.m. for 60 minutes.

iv) The samples were then filtered using caps fitted with filter discs and ash-free analytical filter pulp (Schleicher and Schuell, Inc.; Keene, New Hampshire).

v) The samples were diluted 1:100 by adding 0.05 ml of the clear supernatant to 4.95 ml of distilled-deionized water. The dilutions were vortexed and the absorbance was read in clean, plastic cuvettes using a Milton Roy Spectronic 601 set at 480 nm against a water blank. The amount of residual dye in millimoles per liter was obtained from a linear regression curve of the working standards (Appendix I).

Standards were prepared as follows: stock solutions of the Standard Reference Dye Solution (Udy Corp.) were prepared at concentrations of 1.2, 1.3, 1.4, 1.5, 1.6, and 1.7 mmol/L

by weighing out the appropriate amount of dye and diluting each of the stock solutions to 25 ml with distilled-deionized water. The working standards were prepared by diluting the stock solutions 1:100 with distilled-deionized water. Their absorbance readings were used to plot a standard linear regression curve (Appendix I).

The available lysine content was calculated as follows: the amount of residual dye obtained from the standard curve was used to calculate the amount of available lysine (grams lysine/ 100 grams protein) in the samples. The following equation was used to convert these numbers into grams of lysine per 100 grams of protein:

$$\text{Lysine(g) per 100 g protein} = [(A-B) \times 146.3/1000] / \% \text{ protein}$$

where A = (3.77 - mmol/L of sample A)/ g sample A = mmol/L of dye bound per gram of sample, and B = (3.77 - mmol/L of sample B)/ g sample B = mmol/L of dye bound per gram of sample, % protein = decimal percent of protein as determined by AOAC Method 920.87 (AOAC, 1990), 3.77 = concentration of Reagent Dye solution in mmol/L, and 146.3 = molecular weight of lysine.

4.9 Determination of Protein Efficiency Ratio

The Protein Efficiency Ratio (PER) of the extruded and drum dried products was determined by the AOAC Method 960.48 (AOAC, 1991). Four isocaloric rat diets were prepared: 1) an Animal Nutrition Research Council (ANRC) approved reference casein (ICN Biochemicals; Cleveland, Ohio) control diet, 2) an unprocessed whole wheat kernel diet, 3) an extruded product diet, and 4) a drum dried product diet. The diets were formulated to contain 10% protein, 13.6% fat ("Mazola" corn oil), 1% vitamin mix (AIN 76, ICN Biochemicals), 4% mineral mix (AIN 76, ICN Biochemicals), and cornstarch (ICN Biochemicals) to make 100%. Forty 21-23 day old weanling, male Sprague-Dawley rats obtained from Dominion Laboratories of Dublin, Virginia, were divided into 4 groups of 10 rats each. The mean initial weights were equal for the 4 groups. The rats were individually housed in wire cages in a controlled environment of relative humidity (30 - 70%) and constant temperature ($22 \pm 0.2^{\circ}\text{C}$) with a 12 hour light and dark cycle. The rats were fed the experimental diets ad libitum for 28 days and were weighed twice per week. The amount of food consumed by each individual rat was determined each day by subtracting the weight of the food remaining in the feed cup from the weight of the food offered on the

previous day.

The PER calculation, an indication of protein quality, is based on the ratio of the weight gained by the animal to the amount of protein consumed over the 28 day period.

The following equation was used to determine PER values:

$$\text{PER (Protein Efficiency Ratio)} = \frac{\text{weight gain (g)}}{\text{protein intake (g)}}$$

The PER values obtained by the above equation were standardized by the following equation as suggested by Chapman *et al* (1959):

$$\text{Corrected PER} = \frac{2.5}{\text{Determined PER for Reference Casein}} \times \text{Determined PER of Test Diet}$$

where the Determined PER for Reference Casein = the mean of the unadjusted or initial PER casein values.

4.10 Determination of Apparent Nitrogen Digestibility

The apparent nitrogen digestibility of the four rat diets was determined by the method of Gumbmann *et al* (1983). Apparent nitrogen digestibility was based on the amount of diet consumed and Kjeldahl nitrogen analysis of fecal collections from the 14th to the 21st day of the PER study.

The following equation was used to calculate the apparent nitrogen digestibility:

$$\text{Apparent Nitrogen Digestibility} = \frac{(\text{N intake} - \text{fecal N})}{\text{N intake}} \times 100$$

where N intake = (diet consumed for 8 days) x (% N in diet)
and fecal N = (% N in feces) x fecal weight (g).

4.11 Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) was conducted with a Perkin-Elmer System 4 Thermal Analysis Microprocessor Controller (Norwalk, Connecticut) and the thermograms were analyzed with a Perkin-Elmer Thermal Analysis Data Station. The samples were weighed with a Perkin-Elmer AD-6 computerized microbalance accurate to 2 mg ± 0.1 µg into stainless steel capsules. Water was added with an automatic pipette and the capsule was reweighed to determine the amount of water added. The sample capsule was hermetically sealed to prevent vaporization and volatiles from entering the system. The reference material was an empty pan. Indium was used as a standard for calibrating temperatures and enthalpy measurements. The sample was heated from 25 to 120°C at a scan rate of 10°C per minute. The sensitivity was set at 5 mcal per second and was rescaled to either 1.0, 1.5 or 2.0

mcals per second.

4.12 Statistical Analysis

Statistical analyses (means, standard deviations, and Fisher's LSD) were conducted using the Number Cruncher Statistical System, Version 5.0 (Hintze, 1987).

5.0 Results and Discussion

5.1 Milling of Wheat

The milled whole wheat yielded an extraction rate of 66%. Total recovery of the bran and endosperm fractions was 99.8%.

5.2 Proximate Analysis

The results of the proximate analysis for the whole wheat flour, drum-dried product after drying, and extruded product after drying are presented in Table 2. The protein content of the extruded product was significantly higher ($p < 0.05$) than the milled whole wheat flour and drum-dried product. This difference is most likely an effect of the decreased water content in the extruded product. In order to eliminate the confounding effect of water, protein and ash were calculated on a dry weight basis and are given in Table 3. However, the

**TABLE 2. MEAN PERCENTAGES OF MOISTURE^a, FAT^b, PROTEIN^c,
ASH^a AND CARBOHYDRATE^d OF UNPROCESSED, EXTRUDED AND
DRUM-DRIED WHOLE WHEAT FLOUR**

CONSTITUENT	MILLED WW FLOUR	EXTRUDED	DRUM-DRIED
Moisture	12.6	5.3	6.2
Fat	2.34	0.86	1.39
Protein	10.97	12.6 ^e	11.37
Ash	1.65 ^f	1.75	1.76
Carbohydrate	72.4	79.5	79.3

^a based on the analysis of 6 samples of whole wheat and 3 of extruded and drum-dried products

^b based on the analysis of 2 samples

^c based on the analysis of 6 samples of whole wheat and 4 of extruded and drum-dried products

^d determined by difference

^e significantly different from milled whole wheat flour and drum-dried product by Fisher's LSD ($p < 0.05$)

^f significantly different from extruded and drum-dried products by Fisher's LSD ($p < 0.05$)

**TABLE 3. MEAN PERCENTAGES OF FAT^a, PROTEIN^b, AND ASH^c
OF UNPROCESSED, EXTRUDED AND DRUM-DRIED
WHOLE WHEAT FLOUR ON A DRY WEIGHT BASIS**

CONSTITUENT	MILLED WW FLOUR	EXTRUDED	DRUM-DRIED
Fat	2.34	0.86	1.39
Protein	12.55	13.3 ^d	12.12
Ash	1.89	1.90	1.88

^a based on the analysis of two samples

^b based on the analysis of 6 samples of whole wheat and 4 of extruded and drum-dried products

^c based on the analysis of 6 samples of whole wheat and 3 of extruded and drum-dried products

^d significantly different from milled whole wheat flour drum-dried product by Fisher's LSD ($p < 0.05$)

protein content of the extruded product remained significantly greater ($p < 0.05$) than the milled whole wheat flour and the drum-dried product. It is unknown why the protein content remained significantly different in the extruded product.

5.3 Hunter L, a, b Color Values

The Hunter color values of the milled whole wheat flour, extruded, and drum-dried products are presented in Table 4. The extruded product was much darker than the drum-dried product and the milled whole wheat flour. The Hunter L value for the milled flour shows that it is darker than the drum-dried product. The Hunter a values are slightly positive which indicate a small red coloration in the samples. This is an expected observation because Coker 916 is a red wheat. All of the samples displayed positive b values, indicating some yellow coloration in these products. The yellow color is caused by naturally occurring carotenoid pigments (Cole et al, 1991) and/or Maillard Browning. These results are in relative agreement with the Hunter color values obtained by Blouin et al (1981) for wheat flour. They reported color values of 89.5, 0.6, and 6.7 for L, a, and b, respectively. It is not known if whole grain flour or white flour was measured, or what type of wheat was used. McAuley et al (1987) reported a Hunter L value of 73.75 for whole wheat; however, the wheat

**TABLE 4. HUNTER COLOR VALUES^a OF MILLED,
EXTRUDED AND DRUM-DRIED WHOLE WHEAT FLOUR**

VALUE	MILLED WW FLOUR	EXTRUDED	DRUM-DRIED
L	59.94	49.19	65.25
a	5.07	5.51	2.35
b	13.32	13.09	14.07

^a based on the analysis of two samples

type evaluated was not reported. The variation in reported data is expected because carotenoids exist in differing concentrations depending on the type of wheat (Coles et al, 1991). For example, bread wheats naturally contain less carotenoid pigments than durum wheats (Lepage and Sims, 1968).

5.4 Amino Acid Composition

The amino acid composition of Coker 916 whole wheat (kernels were supplied to the reference laboratory), extruded and drum-dried products determined on a wet weight basis is given in Table 5.

The observed amino acid composition of the whole wheat was consistent with previously reported results (Wu and Inglett, 1988; Björck and Asp, 1984). In addition, McAuley et al (1987) reported a total lysine value of 2.36 (g/100 g protein) for whole wheat flour and Dexter et al (1984) reported a total lysine value of 2.15 (g/100 g protein) for semolina wheat flour (endosperm only).

The essential amino acid losses of the drum-dried product ranged from 16% (methionine) to 20% (isoleucine), while the extruded product losses ranged from 1% (isoleucine) to 16% (lysine). The nonessential amino acid losses of the drum-dried product ranged from 1% (cystine) to 10% (alanine), and the extruded product losses ranged from 1% (tyrosine) to 20%

**TABLE 5. AMINO ACID COMPOSITION OF UNPROCESSED,
EXTRUDED AND DRUM-DRIED WHOLE WHEAT (g/100 g protein)**

AMINO ACID	WHOLE WHEAT	EXTRUDED	DRUM-DRIED
Essential Amino Acids			
Lysine	3.01	2.54 (16)	3.17
Threonine	3.10	2.78 (10)	3.25
Valine	4.28	4.05 (5)	4.67
Methionine	1.46	1.51	1.23 (16)
Isoleucine	3.19	3.17 (1)	2.55 (20)
Leucine	7.20	6.75 (6)	6.95
Phenylalanine	4.28	4.29	4.75
Tryptophan	1.01	1.08	1.12
Nonessential Amino Acids			
Histidine	2.64	2.62 (1)	2.81
Arginine	5.65	4.52 (20)	5.19 (8)
Aspartic Acid	5.01	4.52 (10)	5.37
Serine	4.83	4.60 (5)	5.19
Glutamic Acid	34.64	30.79 (11)	35.18
Proline	10.48	10.40 (1)	10.64
Glycine	4.74	4.37 (8)	4.40 (7)
Alanine	4.01	3.65 (9)	3.61 (10)
Tyrosine@	2.73	2.70 (1)	3.17
Cystine*@	2.83	2.38 (16)	2.81 (1)
Ammonia	4.19	4.52	4.49

* protected cystine method

@ tyrosine and cystine have a sparing effect on phenylalanine and methionine, respectively, and therefore can be considered semi-essential (Munro *et al*, 1988).

(#) percentage of amino acid loss

(arginine). The 16% decrease of total methionine in only the drum-dried whole wheat is more difficult to understand because the thioether group does not normally react with reducing sugars (Björck et al, 1983). Similar results were reported by Björck et al (1983), who found that extruded cereal-based biscuits (210°C, 13% moisture, and 80 rpm screw speed) lost up to 28% total methionine; and Cubadda (1988), who found that extruded millet-grain flour (150°C, 32% moisture, and 57 rpm screw speed) lost 20% total methionine.

The different temperatures of processing (extrusion at 93°C and drum-drying at 152°C) would appear to prevent any comparison between the two products. However, during drum-drying, the temperature of the whole wheat did not increase above 100°C as long as moisture was evaporating. Immediately after dehydration, the dried layer reached the doctor blade and was scraped from the surface of the drum, preventing any further rise in temperature. Thus, the two products were processed at approximately the same temperature.

The extrusion process caused more destruction of the amino acids present in the original whole wheat than the drum-drying process. This may have been a result of the differences in water content in the first stage of thermal processing. Whole wheat flour was made into a slurry (61% moisture) for drum-drying and a dough (45% moisture) for extrusion. Several studies have reported that available

lysine retention is enhanced by increasing the moisture content during processing (Björck et al, 1983; Noguchi et al, 1982; Cubadda, 1988). As explained by Noguchi et al (1982), this may be attributed to the law of mass action, since water is a product of the condensation reaction in the Maillard browning sequence. Moisture content may have been involved in the loss of the amino acids lysine and cystine by only the extruded product. While lysine retention may have been enhanced by the increased moisture content during processing, the whole wheat remained at a higher moisture content immediately after extrusion (40%) than after drum-drying (15%), prior to oven-drying. Lysine retention could have also been enhanced in the drum-dried product due to immediate dehydration of the whole wheat. This lowered the water activity of the drum-dried product below the optimum water activity required for Maillard Browning reactions.

The loss of cystine in the extruded product could also be due to the high moisture content of the whole wheat after extrusion. Free water is required for the reduction reaction that splits the disulfide bridge of cystine yielding two cysteine residues. The free water necessary for this reaction to occur was present in greater amounts in the extruded product than in the drum-dried product immediately after processing.

The greater loss of amino acids in general, in whole

wheat as a result of extrusion cooking may have also been caused by the shear forces that are inherent in extrusion processing. The process of drum-drying does not subject food materials to mechanical shear forces.

Several amino acids in the drum-dried product increased in content. It is unknown why this increase occurred. Björck et al (1984) reported similar gains of amino acids in extrusion cooked whole grain wheat flours.

In Figure 4, the total lysine content of the three samples was plotted against their Hunter L Color values. There was a significant ($p < 0.05$) correlation between the lightness of the three whole wheat products and their lysine content ($r = 0.997$). This is due to the Maillard browning reaction. As the reaction occurs, the product becomes darker in color and lysine is lost. This correlation was also observed by Björck and Asp (1984), who reported a relationship ($r = 0.95$) between reflectance and total lysine content of extruded wheat flour products.

The correlation between total lysine and Hunter L color values observed in this study ($r = 0.997$) suggests that protein quality, especially total lysine content, can be roughly predicted by the Hunter L color value, as suggested by Bookwalter and Kwolek (1981), who reported a linear relationship between Hunter L values and DNFB-available lysine content in high-protein corn-soy-milk blends.

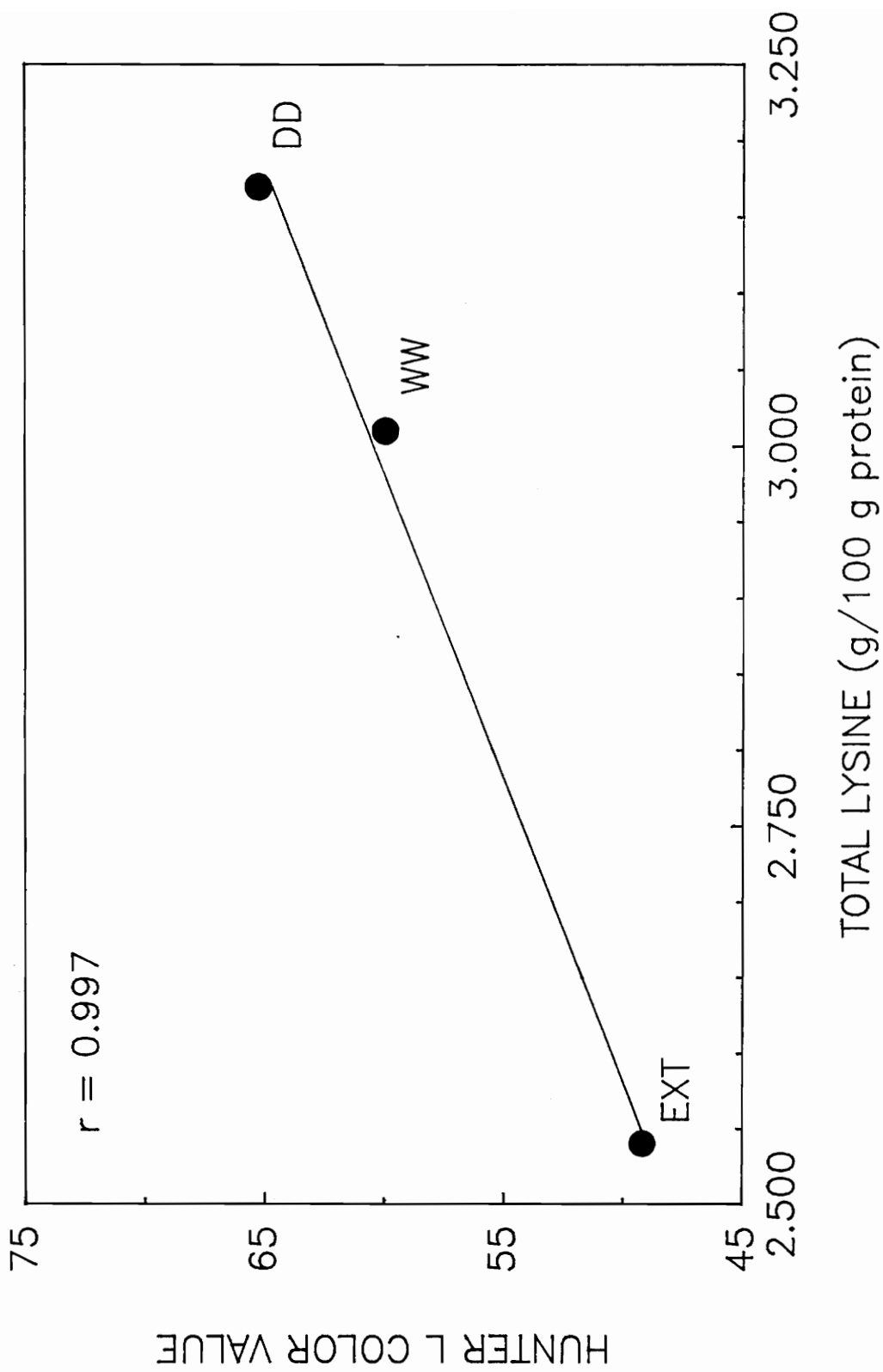


FIGURE 4. TOTAL LYSINE AND HUNTER L COLOR VALUES OF UNPROCESSED, DRUM-DRIED, AND EXTRUDED WHOLE WHEAT

5.5 Available Lysine Analysis

The results of the available lysine analysis are presented in Table 6. The available lysine analysis was performed using the dye-binding capacity (DBC) method of Hurrell and Carpenter (1976). It has been reported (Hurrell and Carpenter, 1981; Hurrell and Finot, 1985) that in foods containing early Maillard browning reaction products, the dye-binding procedure overestimates the reactive lysine, because the dye reacts with the basic deoxyketosyl lysine derivative, which is biologically unavailable. This would not be a problem if the dye still bound with the deoxyketosyl lysine after propionylation. However, this has not been confirmed (Hurrell and Carpenter, 1981). Hurrell and Finot (1985) reported that about 30% of the Amadori compound of lysine was propionylated like reactive lysine.

Taking into consideration the possibility of overestimation of available lysine by the DBC method, the amount of available lysine was less than the total lysine for all three samples. The observed percent of total lysine for whole wheat flour (76.4%) agrees with the result reported by McAuley et al (1987) (77.4%) determined by a modified TNBS method. The drum-dried product had a significantly greater amount of available lysine than the extruded product and the whole wheat flour. These results support the previously

**TABLE 6. AVAILABLE LYSINE CONTENT¹ OF MILLED
EXTRUDED AND DRUM-DRIED WHOLE WHEAT (g/100 g protein)**

SAMPLE	AVAILABLE LYSINE	% TOTAL LYSINE
Milled WW	2.30 ± 0.055 a	76.4
Extruded	2.19 ± 0.101 a	86.2
Drum-dried	2.64 ± 0.134 b	83.3

Mean values followed by the same letter are not significantly different ($\alpha = 0.05$), Fisher's LSD Test.
¹ based on the analysis of 6 samples

stated observation that extrusion processing was more detrimental to lysine than was drum-drying processing. The increase in available lysine in the drum-dried product may mean that the protein was more susceptible to the Udy dye binding.

The available lysine content of the three samples was plotted against their corresponding Hunter L Color values (Figure 5). Although the correlation ($r = 0.887$) is not as strong as between total lysine and Hunter L color values ($r=0.997$), there seems to be some relationship between available lysine content and lightness of the sample. This may be due to the Maillard browning reaction as previously discussed. McAuley et al (1987) observed a relationship ($r=0.55$) between Hunter L values and available lysine of wheat-based ready-to-eat breakfast cereals. Rhee and Rhee (1981) reported a significant ($p<0.01$) correlation between the browning index and available lysine of heated oilseed/glucose solutions.

5.6 Protein Efficiency Ratio

The PER (Protein Efficiency Ratio) results of the rat feeding study are presented in Table 7. The PER values were adjusted, or standardized as suggested by Chapman et al (1979). The use of ANRC reference casein as a standard in the

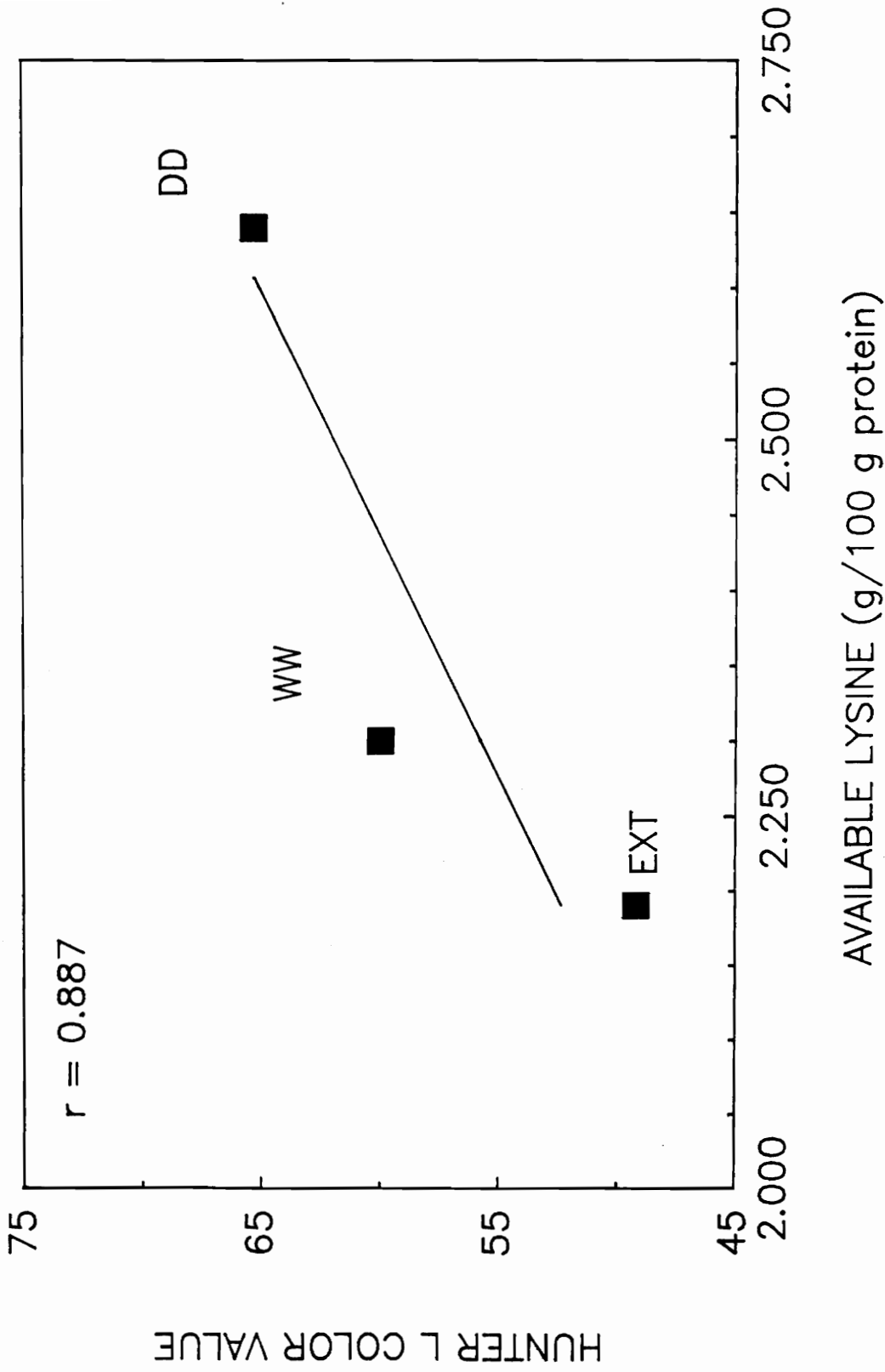


FIGURE 5. AVAILABLE LYSINE AND HUNTER L COLOR VALUES OF UNPROCESSED, DRUM-DRIED, AND EXTRUDED WHOLE WHEAT

**TABLE 7. PER OF UNPROCESSED, EXTRUDED,
AND DRUM-DRIED WHOLE WHEAT FLOUR¹**

RAT DIET	ACTUAL PER	ADJUSTED PER
Casein Control	2.65 ± 0.30 <i>a</i>	2.50 ± 0.28 <i>a</i>
Drum-Dried	1.77 ± 0.23 <i>b</i>	1.66 ± 0.22 <i>b</i>
Unprocessed	1.69 ± 0.08 <i>bc</i>	1.59 ± 0.08 <i>bc</i>
Extruded	1.50 ± 0.13 <i>c</i>	1.42 ± 0.12 <i>c</i>

Mean values in the same column followed by the same letter(s) are not significantly different ($\alpha=0.05$), Fisher's LSD Test.

¹ based on 10 rats per dietary treatment

rat feeding study, reduces the variation in PER values from different assays (Chapman et al, 1979). However, Hegarty (1975) questions the wisdom of the setting the PER value of 2.5 for the reference casein because of higher values reported in the literature. The PER value of the ANRC reference casein control obtained in this study (2.65) was higher than 2.5 before adjustment. The adjusted PER values will be used for discussion.

The PER of the ANRC casein control (2.5) was significantly greater than any of the test diets. The PER of the casein control and the drum-dried product (1.66) was significantly greater than the extruded product (1.42). The process of drum-drying increased the protein quality of the whole wheat by disrupting the protein structure, which increased their susceptibility to enzymatic digestion by the rat. This may have affected release and absorption of amino acids from the small intestine. The PER of the extruded product was not significantly different from the PER of the unprocessed whole wheat kernels. This may be due to the loss of essential amino acids, especially lysine and threonine, that occurred during the extrusion process. A comparison between the average body weight of the rats consuming the different diets over the 28 day study period is given in Figure 6. The rats consuming the casein control diet consistently gained the most weight throughout the study and

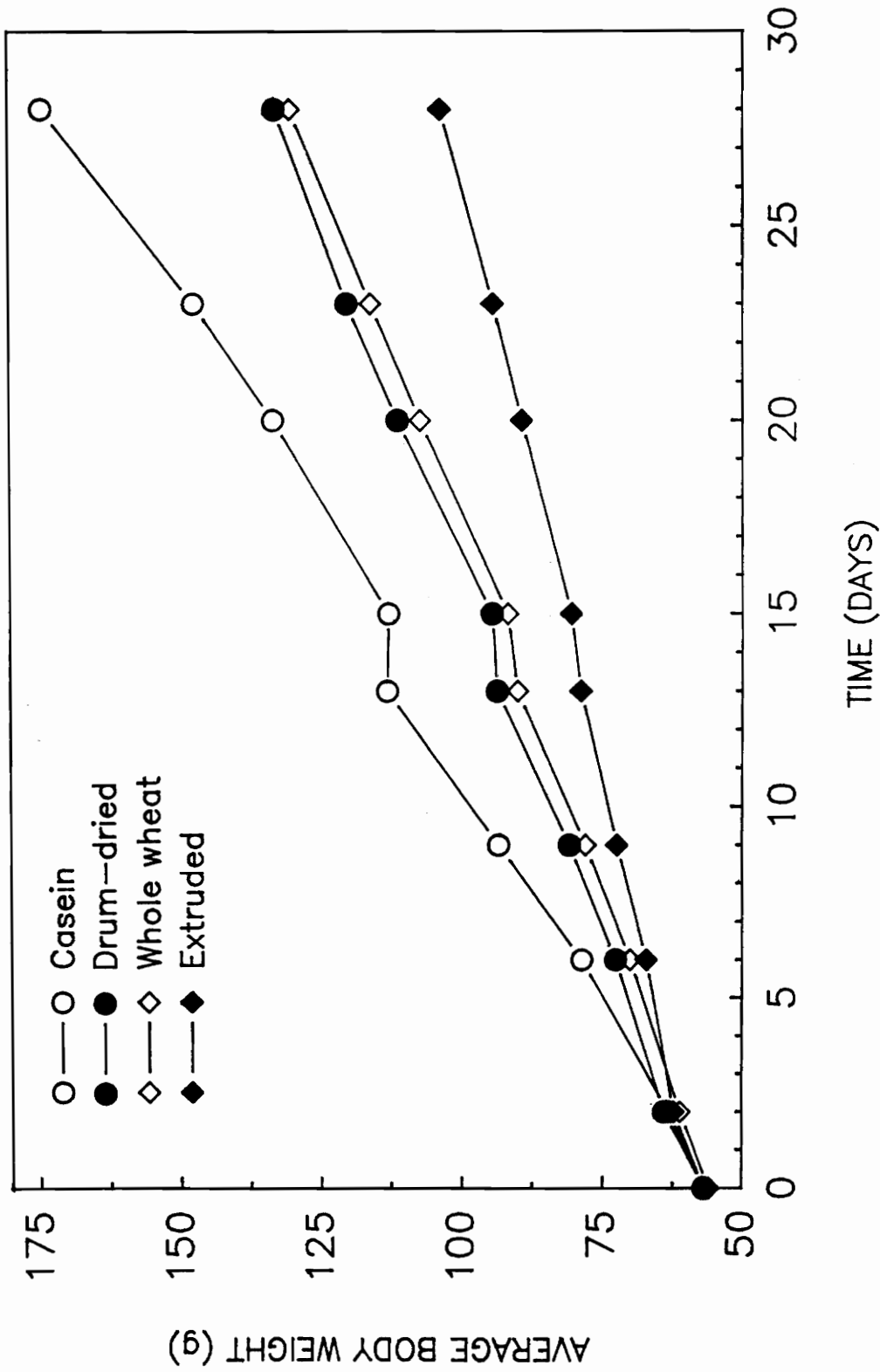


FIGURE 6. AVERAGE WEIGHT GAIN OF RATS FED UNPROCESSED, DRUM-DRIED, AND EXTRUDED WHOLE WHEAT DIETS OVER TIME

had a final average body weight of 174.2 g. The rats consuming the drum-dried and the whole wheat diets were similar in weight gain throughout the study with final average body weights of 132.98 g and 130.26 g, respectively. The rats consuming the extruded diet consistently had the lowest average body weights throughout the study and had a final average body weight of 103.57 g.

The PER of the three rat diets was plotted against their corresponding Hunter "L" Color values (Figure 7). There was a significant correlation ($p < 0.05$) between the lightness of the three whole wheat products and their PER's ($r = 0.999$). This correlation indicates that it is possible to predict protein quality with Hunter L Color values. As the lightness of a product decreases, the amount of Maillard browning increases, which causes a lower protein quality. This relationship was also observed by Bookwalter and Kwolek (1981) who suggested it was possible to predict PER, and thus protein quality, with Hunter "L" Values.

5.7 Apparent Digestibility

The apparent digestibility of the casein and the three whole wheat diets are given in Table 8. Apparent digestibility indicates the relative percentage of nitrogen absorbed by the rat from each diet. Both drum-drying and

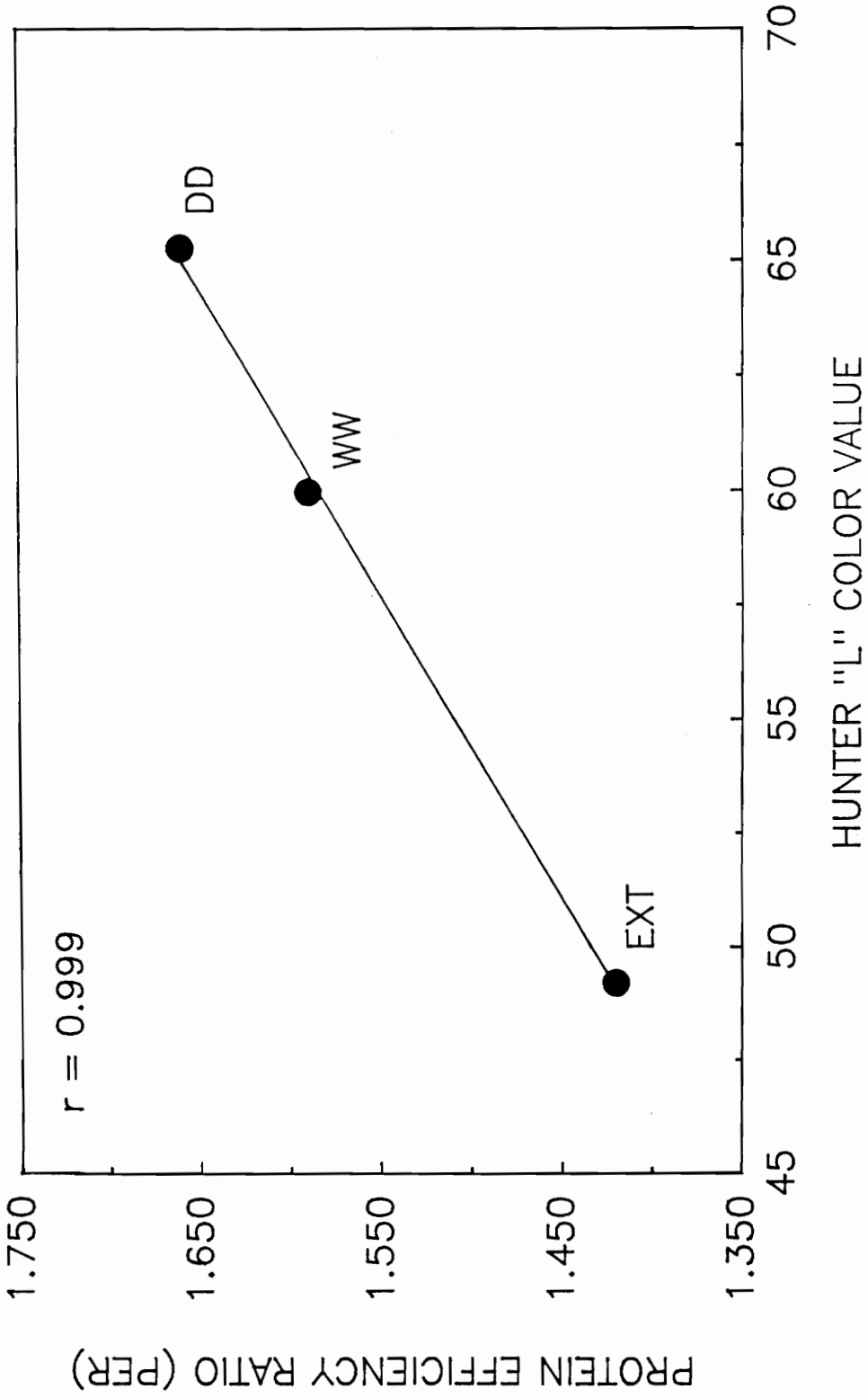


FIGURE 7. COMPARISON OF UNPROCESSED, DRUM-DRIED, AND EXTRUDED WHOLE WHEAT HUNTER L COLOR VALUES AND THE PER OF UNPROCESSED, DRUM-DRIED, AND EXTRUDED WHOLE WHEAT RAT DIETS

**TABLE 8. APPARENT NITROGEN DIGESTIBILITY OF
UNPROCESSED, EXTRUDED, AND DRUM-DRIED
WHOLE WHEAT FLOUR¹**

RAT DIET	APPARENT N DIGESTIBILITY (%)
Casein Control	92.4 ± 1.71 <i>a</i>
Drum-Dried	90.0 ± 1.63 <i>b</i>
Unprocessed	82.5 ± 2.04 <i>c</i>
Extruded	87.2 ± 1.54 <i>d</i>

Mean values in the same column followed by the same letter(s) are not significantly different ($\alpha=0.05$), Fisher's LSD Test

¹ based on 10 rats per dietary treatment during days 14 - 21

extrusion cooking significantly increased the apparent digestibility of Coker 916 whole wheat. Drum-drying was significantly more effective than extrusion, however, in improving nitrogen bioavailability. The unprocessed whole wheat diet had a significantly low digestibility compared to all other diets. Intestinal release and absorption nitrogen from the extruded diet may have been impaired by the formation of undigestible crosslinked and Maillard browned products. Toxic compounds may have also formed under the conditions of extrusion (Gumbmann et al, 1983).

The mean feed intake and weight gain of the rats during the feeding study are noted in Table 9. It is interesting to separately examine the two parameters that compose the PER calculation, i.e. feed intake (protein intake) and weight gain. Mitchell (1927) questioned the common practice of drawing experimental conclusions based on the composition of the diet alone without considering the amount of food consumed by laboratory animals. He recognized that the growth rate of rats was influenced by their food intake. Knipfel (1981) reported that weight gains in rats fed autoclaved casein, soy, egg, or fish protein diets decreased markedly due to depressed caloric intake. It was also reported by Harper and Peters (1989) that food intake of animals depends on the protein content and the amino acid balance of the diet. Characteristic signs of an amino acid imbalance in the diet

**TABLE 9. MEAN FEED INTAKE AND WEIGHT GAIN OF RATS
FED UNPROCESSED, EXTRUDED AND DRUM-DRIED
WHOLE WHEAT DIETS**

RAT DIET	FEED ¹ INTAKE (g)	WEIGHT ¹ GAIN (g)
Casein Ctrl	444.4 ± 32.0 a	117.3 ± 12.4 a
Drum-Dried	432.9 ± 40.6 a	76.2 ± 11.1 b
Unprocessed	440.7 ± 42.2 a	74.3 ± 6.5 b
Extruded	311.8 ± 28.5 b	46.7 ± 4.9 c

Mean values in the same column followed by the same letter(s) are not significantly different ($\alpha=0.05$), Fisher's LSD Test.

¹ based on 10 rats per dietary treatment during a 28 day period.

include depression of food intake and growth (Harper, 1964; Harper and Peters, 1989). In this study, weanling rats fed the extruded product consumed significantly less food and gained significantly less weight than rats in the other diet groups (Table 9). Leung and Rogers (1987) observed that when rats are offered a choice between two diets, they will select a protein-free diet or a relatively balanced amino acid diet over a diet with an imbalance of amino acids. They proposed that specific brain mechanisms exist that provide feedback control for depression of food intake when animals ingest diets that would drastically alter their amino acid pools. In addition, Cieslak and Benevenga (1984) reported that diets containing less than 0.72% lysine do not support weight gain in mice. The unprocessed whole wheat, drum-dried, and extruded whole wheat diets in this study contained 0.23%, 0.26%, and 0.22% available lysine, respectively. Therefore, it is possible that the rats consuming these three diets did not receive adequate amounts of lysine for optimum growth, even though the available lysine content of the whole wheat was enhanced by drum-drying (Table 6). It also seems likely that the rats fed the extruded product diet decreased their food intake to avoid amino acid imbalance. However, palatability of the diets cannot be ignored. Rats fed the extruded product diet may have consumed less feed simply because they disliked the taste.

The mean dry fecal weights of the rats are given in Table 10. It has been reported that fecal weight increases with the addition of dietary fiber to the diet (Nyman and Asp, 1982; Björck et al, 1984; Aoe, 1989). By definition, dietary fiber resists the action of the digestive enzymes in the gastrointestinal tract (Björck et al, 1984). However, fermentation occurs in the large intestine through the action of bacterial enzymes (Nyman and Asp, 1982). The degree to which fermentation occurs depends on factors such as particle size, solubility, and chemical structure of the fiber (Björck et al, 1984). Wheat bran fiber has very good bulking capabilities due to its resistance to intestinal fermentation (Nyman and Asp, 1982). In this study, rats consuming the ANRC reference casein control diet, which contained no dietary fiber (Nyman and Asp, 1982), had a significantly lower average fecal weight than rats in the other diet groups. Rats consuming the unprocessed whole wheat diet had a significantly higher average fecal weight than the other groups. It is assumed that since the rats ate whole wheat kernels, the particle size of the bran reaching the small intestine was rather large. Taking these observations into consideration, along with the low apparent digestibility result (82.5%), the unprocessed whole wheat diet may have contained the greatest amount of dietary fiber. Wyman et al (1976) reported that the bulking effect of bran is less pronounced with cooked bran

**TABLE 10. MEAN FECAL WEIGHT OF RATS FED
UNPROCESSED, EXTRUDED AND DRUM-DRIED
WHOLE WHEAT DIETS**

RAT DIET	FECAL WEIGHT ¹ (g)
Casein Control	3.3 ± 0.68 a
Drum-Dried	10.8 ± 1.41 b
Unprocessed	13.6 ± 2.42 c
Extruded	8.1 ± 0.89 d

Mean values in the same column followed by the same letter are not significantly different ($\alpha=0.05$), Fisher's LSD Test.

¹ based on 10 rats per dietary treatment during days 14 - 21 of the study; calculations are based on dry fecal weight.

than with raw.

The effect of extrusion cooking on the dietary fiber in wheat products was studied by Björck et al (1984). They found that extrusion cooking of white wheat flour caused a redistribution of insoluble dietary fiber to soluble dietary fiber; however, the redistribution in whole grain wheat flour was smaller. Similarly, Aoe et al (1989) reported that extrusion cooking of wheat bran significantly increased the availability of dietary fiber in the rat by solubilization of the dietary fiber. These observations indicate that the fecal bulking capacity of the dietary fiber is influenced (Asp and Björck, 1984) by food processing. Based on the lower average fecal weight of the rats consuming the extruded product in this study, it appears that extrusion cooking increased the soluble dietary fiber content of the whole wheat product.

The mean total fecal nitrogen in the feces of the rats is given in Table 11. Knipfel (1981) found a significantly increased fecal N excretion when egg diets fed to rats had been autoclaved for 60 minutes. The author suggested that the increased fecal N was caused partly by heat damage to the protein and partly by an increased endogenous N from the digestive tract in response to heated proteins. Nyman and Asp (1982) observed that an increase in fecal N excretion in rats fed wheat bran fiber was accompanied by a decrease in apparent digestibility values. They proposed that this was caused by

**TABLE 11. MEAN TOTAL FECAL NITROGEN OF RATS FED
UNPROCESSED, EXTRUDED AND DRUM-DRIED
WHOLE WHEAT DIETS**

RAT DIET	FECAL N ¹ (mg)
Casein Control	155.8 ± 39.0 a
Drum-Dried	213.0 ± 35.3 b
Unprocessed	402.5 ± 70.5 c
Extruded	193.8 ± 19.6 ab

Mean values in the same column followed by the same letter(s) are not significantly different ($\alpha=0.05$), Fisher's LSD Test.

¹ based on 10 rats per dietary treatment during days 14 - 21 of the study; calculations are based on dry fecal weight.

poor digestibility of the fiber-associated protein.

In this study, a significant increase in fecal N was observed in rats consuming the unprocessed whole wheat diet. This is may have been due to the presence of high amounts of undigestible dietary fiber as well as inhibition of protein digestion by this fiber (Nyman and Asp, 1982). The fecal N of the rats fed the drum-dried diet was significantly greater than the fecal N of rats fed the ANRC casein control diet. This may have been the result of slight inhibition of protein digestion inhibition due to the presence of dietary fiber (Nyman and Asp, 1982) as well as the presence of indigestible compounds such as Maillard Browning reaction products. The fecal N of the rats fed the extruded whole wheat diet was not significantly different from the fecal N of the rats fed the ANRC casein control diet. This may have been a result of the the increased solubility of the dietary fiber in the extruded diet which would cause a reduction in the inhibition of protein digestion in the intestine. Although the apparent N digestibility of the rats fed the extruded diet was significantly lower than the rats fed the drum-dried diet, it was significantly improved over the digestibility of the unprocessed whole wheat.

5.8 Starch Gelatinization

The Differential Scanning Calorimetry (DSC) thermograms obtained when various whole wheat products were heated with water are presented in Figures 8 and 9. The thermograms are a record of heat flow as a function of temperature. The thermograms of wheat starch, commercial whole wheat flour (Pillsbury's Best Whole Wheat Flour), and Coker 916 whole wheat flour are shown in Figure 8. The peak temperature of the gelatinization transition endotherm was 67.2°C, 70°C, and 69.8°C for wheat starch, commercial whole wheat flour, and Coker 916 whole wheat flour, respectively. These values agree with those reported for wheat starch by Ghiasi et al (1982) (64°C), Wootton and Bamumuarachchi (1979) (68°C), Kugimiya et al (1980) (65°C), and Ghiasi et al (1983) (64°C). The three thermograms in Figure 8 are essentially identical. Similar results were reported by Ghiasi et al (1983). Although there was a slight shift to the right in the peak gelatinization temperature for the two whole wheat flours, the onset, or initial temperature, of gelatinization was very close for the three thermograms (62.6°C, 63.6°C and 62.8°C, for wheat starch, commercial whole wheat flour, and Coker 916 whole wheat flour, respectively).

All three thermograms in Figure 8 exhibited a second endotherm at maximum temperatures of 104.9°C, 104.1°C, and

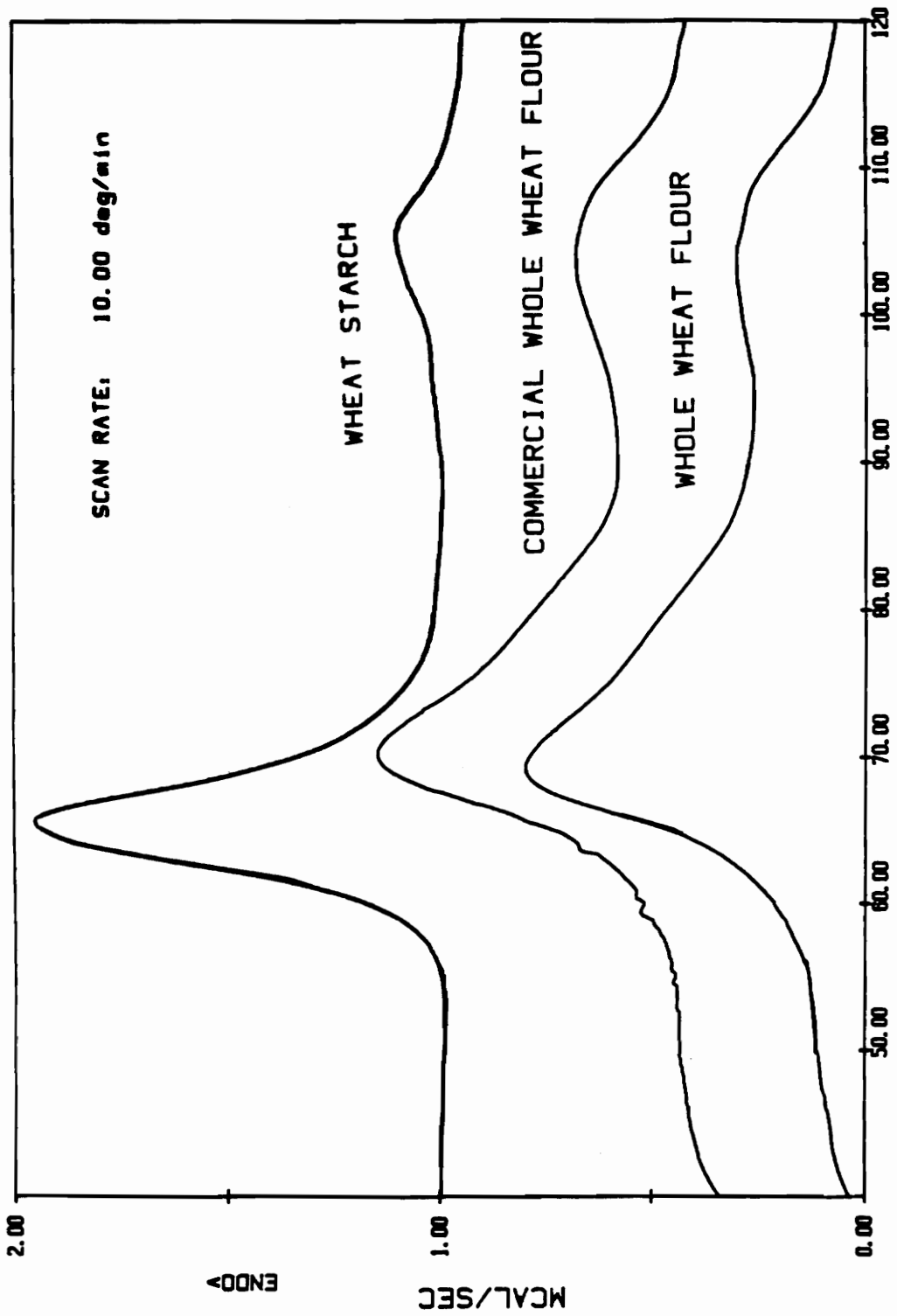


FIGURE 8. DSC THERMOGRAMS OF WHEAT STARCH, COMMERCIAL WHOLE WHEAT FLOUR, AND WHOLE WHEAT FLOUR USED IN THIS STUDY

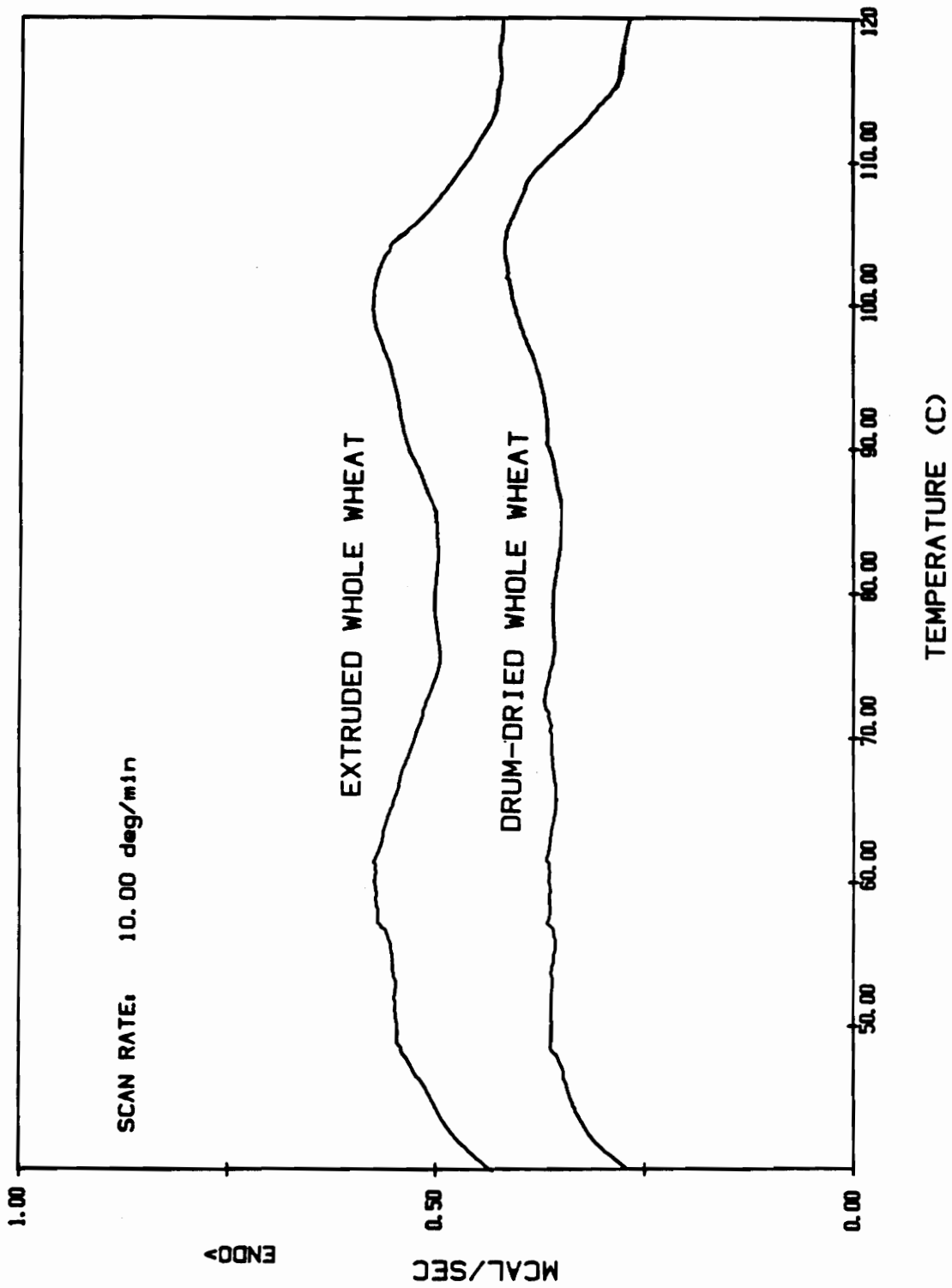


FIGURE 9. DSC THERMOGRAMS OF DRUM-DRIED AND EXTRUDED WHOLE WHEAT

104°C for wheat starch, commercial whole wheat flour, and Coker 916 whole wheat flour, respectively. This endotherm represents a disordering transition of amylose-lipid complexes as reported by Kugimiya et al (1980) and Ghiasi et al (1983).

DSC thermograms for the extruded and drum-dried whole wheat products are presented in Figure 9. There was no discernible endotherm for starch gelatinization present in DSC thermograms of the drum-dried product. A small peak consistent with that previously reported for starch gelatinization did appear, however, at 60°C in the DSC thermograms of the extruded product. Kugimiya et al (1980) reported that the gelatinization endotherm disappeared when previously gelatinized starch samples are cooled and then reheated during DSC. It appears, therefore, that starch was fully gelatinized during the drum-drying of whole wheat, but only partially gelatinized during single screw extrusion. It has been reported by Harper (1981) that wheat flour granules begin to swell at 33% moisture, but complete gelatinization does not occur until the moisture content is > 50%. The moisture content of the extruded wheat flour in this study was 45%. It appears that complete starch gelatinization did not occur during extrusion cooking of whole wheat possibly due to limited moisture content of the extruded product.

The second endothermic peak representing the disordering transition of amylose-lipid complexes occurred at maximum

temperatures of 103.9°C and 100.8°C, for drum-dried and extruded whole wheat, respectively. These temperatures agree with those reported by Kugimiya et al (1980) and Ghiasi et al (1983).

According to Kugimiya et al (1980) it is not possible to determine from calorimetric experiments whether the amylose-lipid complex pre-exists in the native starch granule, or if it is formed upon heating. Björck et al (1984) have reported that these complexes are formed during extrusion-cooking.

According to Larsson and Mieziš (1979), amylose-lipid complexes are resistant to α -amylase in the intestine and may be considered to be dietary fiber. However, more recently, this amylose-lipid complex was reported to be completely digested and absorbed in the rat small intestine (Holm et al, 1983). Thus, physiologically, these complexes should be considered starch rather than dietary fiber (Björck et al, 1984). It is not possible to determine whether these complexes were formed upon heating or were already present in the native starch granule; therefore, it is impossible to speculate about their effects on bioavailability of proteins or other nutrients.

6.0 SUMMARY AND CONCLUSIONS

The results of this study indicate that the net effect(s) of thermal processing on protein quality depends on the type and severity of the thermal process.

PER (Protein Efficiency Ratio) values indicate that drum-drying increased protein digestibility and bioavailability of amino acids in whole wheat, while extrusion cooking decreased amino acid bioavailability below that of unprocessed whole wheat. The observed decrease in PER of the extruded product may be due in part to Maillard Browning, as indicated by Hunter color values. Weanling rats fed the extruded product diet consumed significantly less food and gained significantly less weight than rats in the other diet groups. This may be an indication that the extruded product lacked a proper balance of essential amino acids.

Both drum-drying and extrusion cooking of whole wheat resulted in substantial reductions in essential amino acids.

Lysine losses in the extruded product may be attributed in part to Maillard browning. Lysine losses are an important consideration since wheat is deficient in this essential amino acid. Other amino acids were either directly destroyed as a result of thermal processing and/or rendered perhaps less bioavailable due to participation in protein crosslinking reactions.

Available lysine of whole wheat was not significantly decreased by extrusion. Drum-drying improved the available lysine content of whole wheat presumably by disrupting the protein quaternary structure and increasing their susceptibility to the Udy Reagent Dye. It appears that total lysine or Hunter L color values can predict protein quality of processed whole wheat.

The fecal weights of rats fed the extruded product diet were significantly less than rats in the drum-dried and unprocessed whole wheat diet groups. Extrusion cooking may have lowered the fecal bulking capacity of whole wheat. However, the significantly improved apparent digestibility and the reduction in total fecal N excretion of rats fed the extruded diet over that of rats fed the unprocessed diet indicate that extrusion increased the availability of dietary fiber in the rat by redistributing insoluble dietary fiber to soluble fiber.

DSC (Differential Scanning Calorimetry) results suggest that starch was fully gelatinized during drum-drying of the Coker 916 whole wheat but not during extrusion cooking.

7.0 SUGGESTIONS FOR FURTHER RESEARCH

An area of current interest is the development of wheat strains that have a higher lysine content. This is a worthwhile endeavor that should continue.

There is little research concerning the effect of processing on dietary fiber in grain products. Further studies could investigate the effects of extrusion and other cooking processes on the physiological properties of dietary fiber, such as fecal bulking capacity, accelerated transit time, lowered plasma lipids and cholesterol, and improved glucose tolerance.

It may be possible to make some improvements in the available lysine analysis used in this study. The Furosine method may be more applicable to evaluate heat damaged proteins. This method can only be used when lysine is blocked as Maillard derivatives (Hurrell and Finot, 1985).

The mystery of what exactly happens to proteins during

thermal processing was not completely solved by this study. Further research needs to examine why protein content increases yet, amino acids disappear.

8.0 References

- Aguilera, J.M. and Kosikowski, F.V. 1978. Extrusion and roll-cooking of corn-soy-whey mixtures. *J. Food Sci.* 43:225.
- Aoe, S., Nakaoka, M., Ido, K., Tamai, Y., Ohta, F. and Ayano, Y. 1989. Availability of dietary fiber in extruded wheat bran and apparent digestibility in rats of coexisting nutrients. *Cereal Chem.* 66:252.
- AOAC 1990. "Official Methods of Analysis," 15th ed. Association of Official Analytical Chemists. Arlington, Va.
- Asp, N.-G. and Björck, I. 1984. The effect of extrusion-cooking on nutritional value. In "Thermal Processing and Quality of Foods," Zeuthen, P, Cheftel, J.C., Eriksson, C., Jul, M., Leniger, H., Linko, P., Varela, G. and Vos, G., Eds. Elsevier Applied Science Publishers, Ltd. New York, NY. p.162-167.
- Baltes, W. 1982. Chemical changes in food by the maillard reaction. *Food Chem.* 9:59.
- Björck, I. and Asp, N.-G. 1984. Protein nutritional value of extrusion-cooked wheat flours. *Food Chem.* 15:203.
- Björck, I., Noguchi, A., Asp, N.-G., Cheftel, J.-C. and Dahlqvist, A. 1983. Protein nutritional value of a biscuit processed by extrusion cooking: effects on available lysine. *J. Agric. Food Chem.* 31:488.

- Björck, I., Nyman, M. and Asp, N.-G. 1984. Extrusion cooking and dietary fiber: effects on dietary fiber content and on degradation in the rat intestinal tract. *Cereal Chem.* 61:174.
- Blouin, F.A., Zarins, Z.M. and Cherry, J.P. 1981. Color. In "Protein Functionality in Foods," Cherry, J.P. Ed., ACS Symposium Series 147. American Chemical Society, Washington, D.C. p. 21-39.
- Bluestein, P.M. and Labuza, T.P. 1988. Effects of moisture removal on nutrients. In "Nutritional Evaluation of Food Processing," 3rd ed. Karmas, E. and Harris, R. Ed. Van Nostrand Reinhold Co. Inc., New York. p. 393-422.
- Bookwalter, G.N. and Kwolek, W.F. 1981. Predicting protein quality of corn-soy-milk blends after nonenzymatic browning. *J. Food Sci.* 46:711.
- Brooks, I.M., Owens, F.N. and Garrigus, U.S. 1972. Influence of amino acid level in the diet upon amino acid oxidation by the rat. *J. Nutr.* 102:27.
- Bushuk, W. and Macritchie, F. 1989. Wheat proteins: aspects of structure that determine breadmaking quality. In "Protein Quality and the Effects of Processing," Phillips, D.R. and Finley, J.W. Ed. Marcel Dekker, Inc., New York. p. 345-369.
- Carpenter, K.J. 1960. The estimation of the available lysine in animal-protein foods. *Biochem. J.* 77:604.
- Chapman, D.G., Castillo, R. and Campbell, J.A. 1959. Evaluation of protein in foods. 1. A method for the determination of protein efficiency ratios. *Can. J. Biochem. Physiol.* 37:783.
- Cheftel, J.C. 1986. Nutritional effects of extrusion-cooking. *Food Chem.* 20:263.
- Cheftel, J.C., Cuq, J.-L., and Lorient, D. 1985. Amino acids, peptides, and proteins. In "Food Chemistry," 2nd ed., Fennema, O.R. Ed. Marcel Dekker, Inc. New York. p. 245-369.
- Cieslak, D.G. and Benevenga, N.J. 1984. The effect of amino acid excess on utilization by the rat of the limiting amino acid-lysine. *J. Nutr.* 114:1863.

- Cole, M.E., Johnson, D.E., Cole, R.W. and Stone, M.B. 1991. Color of pregelatinized pasta as influenced by wheat type and selected additives. *J. Food Sci.* 56:488.
- Commoner, B., Vithayathil, A.J., Dolara, P., Nair, S., Madyastha, P. and Cuca, G.C. 1978. Formation of mutagens in beef and beef extract during cooking. *Science* 201:913.
- Cubadda, R. 1988. Nutritional properties and safety of food processed by extrusion cooking. In "Nutritional and Toxicological Aspects of Food Processing," Walker, R. and Quattrucci, E. Ed. Taylor and Francis. New York. p. 157-170.
- Davidson, V.J., Paton, D. Diosady, L.L. and Larocque, G. 1984a. Degradation of wheat starch in a single screw extruder: characteristics of extruded starch polymers. *J. Food Sci.* 49:453.
- Davidson, V.J., Paton, D. Diosady, L.L. and Rubin, L.J. 1984b. A model for mechanical degradation of wheat starch in a single-screw extruder. *J. Food Sci.* 49:1154.
- Dexter, J.E., Tkachuk, R. and Matsuo, R.R. 1984. Amino acid composition of spaghetti: effect of drying conditions on total and available lysine. *J. Food Sci.* 49:225.
- Donovan, J.W. 1979. Phase transitions of the starch-water system. *Biopolymers* 18:263.
- Donovan, J.W. and Mapes, C.J. 1980. Multiple phase transitions of starches and năgeli amyloextrins. *Starch/Stärke* 32:190.
- Dworschák, E. 1980. Nonenzyme browning and its effect on protein nutrition. *CRC Crit. Rev. Food Sci. Nutr.* 13:1.
- Finley, J.W. 1989. Effects of processing on proteins: an overview. In "Protein Quality and the Effects of Processing", Phillips, D.R. and Finley, J.W. Ed. Marcel Dekker, Inc., p. 1-7.
- Finot, P.-A., Mottu, F., Bujard, E. and Mauron, J. 1978. N-substituted lysines as sources of lysine in nutrition. In "Nutritional Improvement of Food and Feed Proteins," Friedman, M. Ed. Plenum Press, Inc. New York. p. 549-670.

- Francis, F.J. 1985. Pigments and other colorants. In "Food Chemistry," 2nd ed., Fennema, O.R. Ed. Marcel Dekker, Inc. New York. p. 545-584.
- Friedman, M. and Gumbmann, M.R. 1981. Bioavailability of some lysine derivatives in mice. J. Nutr. 111:1362.
- Friedman M. and Masters, P.M. 1982. Kinetics of racemization of amino acid residues in casein. J. Food Sci. 47:760.
- Friedman, M., Zahnley, J.C. and Masters, P.M. 1981. Relationship between in vitro digestibility of casein and its content of lysinoalanine and D-amino acids. J. Food Sci. 46:127.
- Ghiasi, K., Hoseney, R.C. and Varriano-Marston, E. 1982. Gelatinization of wheat starch. III. Comparison by differential scanning calorimetry and light microscopy. Cereal Chem. 59:258.
- Ghiasi, K., Hoseney, R.C. and Varriano-Marston, E. 1983. Effects of flour components and dough ingredients on starch gelatinization. Cereal Chem. 60:58.
- Grieg, W.S. 1971. "The Economics of Food Processing," Avi Publishing Co. Inc., Westport, Ct. p.227-295.
- Gumbmann, M.R., Friedman, M. and Smith, G.A. 1983. The nutritional values and digestibilities of heat damaged casein and casein-carbohydrate mixtures. Nutr. Rep. Int. 28:355.
- Hall, R.J., Trinder, N. and Givens, D.I. 1973. Observations on the use of 2,4,6-trinitrobenzenesulphonic acid for the determination of available lysine in animal protein concentrates. Analyst 98:673.
- Hansen, L.P., Johnston, P.H. and Ferrel, R.E. 1975. The assessment of thermal processing on wheat flour proteins by physical, chemical, and enzymatic methods. In "Protein Nutritional Quality of Foods and Feeds," part 2, Friedman, M. Ed. Marcel Dekker, Inc., New York. p. 393-415.
- Harper, A.E. 1964. Amino acid toxicities and imbalances. In "Mammalian Protein Metabolism," vol. 2, Munro, H.N and Allison, J.B. Ed. Academic Press. New York. p. 87-134.

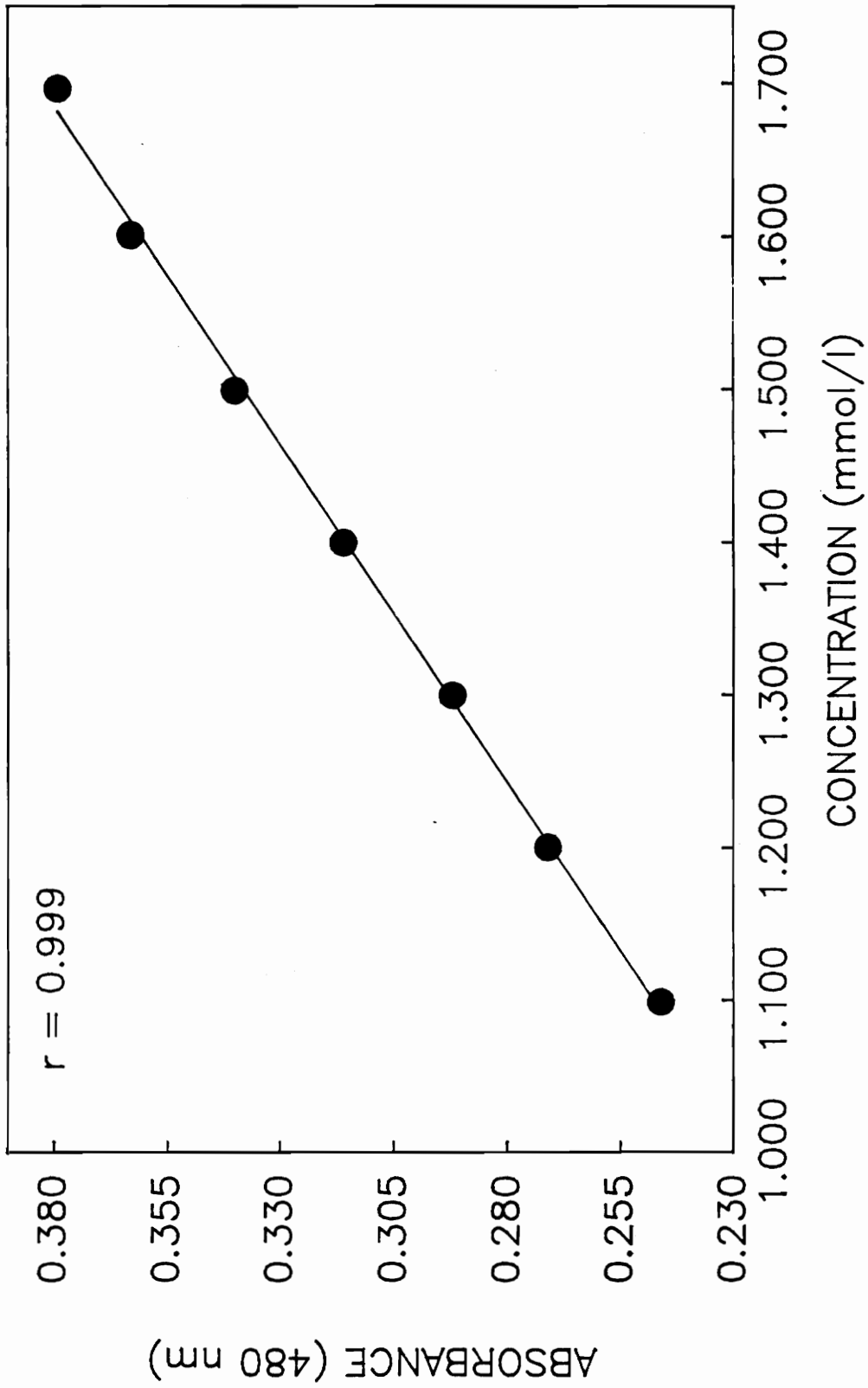
- Harper, A.E. and Peters, J.C. 1989. Protein intake, brain amino acid and serotonin concentrations and protein self-selection. *J. Nutr.* 119:677.
- Harper, J.M. 1981. "Extrusion of Foods," vol. 2. CRC Press, Inc. Boca Raton, Fl. p. 41-60.
- Harper, J.M. 1988. Effects of extrusion processing on nutrients. In "Nutritional Evaluation of Food Processing," 3rd ed. Karmas, E. and Harris, R.S. Ed. Van Nostrand Reinhold Co. Inc., New York. p. 365-392.
- Hegarty, P.V. 1975. Some biological considerations in the nutritional evaluation of foods. *Food Tech.* 29:52.
- Hintze, J.L. 1987. Number cruncher statistical system. Version 5.0. Kaysville, Utah.
- Holm, J., Björck, I., Ostrowska, S., Eliasson, A.-C., Asp, N.-G., Larsson, K. and Lundquist, I. 1983. Digestibility of amylose-lipid complexes in vitro and in vivo. *Starch/Stärke* 35:294.
- Hurrell, R.F. and Carpenter, K.J. 1976. An approach to the rapid measurement of reactive lysine in foods by dye binding. *Proc. Nutr. Soc.* 35:23A.
- Hurrell, R.F. and Carpenter, K.J. 1981. The estimation of available lysine in foodstuffs after Maillard reactions. *Prog. Food Nutr. Soc.* 5:159.
- Hurrell, R.F. and Finot, P.A. 1985. Effects of food processing on protein digestibility and amino acid availability. In "Digestibility and Amino Acid Availability in Cereals and Oilseeds," Finley, J.W. and Hopkins, D.T. Ed. American Association of Cereal Chemists, Inc., St. Paul, Mn. p. 233-246.
- Jones, D.B. 1926. A new factor for converting the percentage of nitrogen in wheat into that of protein. *Cereal Chem.* 3:194.
- Karmas, E. 1988. The major food groups, their nutrient content, and the principles of food processing. In "Nutritional Evaluation of Food Processing," 3rd ed. Karmas, E. and Harris, R. Ed. Van Nostrand Reinhold Co. Inc., New York. p. 7-20.

- Kies, C. 1981. Bioavailability: a factor in protein quality. *J. Agric. Food Chem.* 29:435.
- Kies, C., Fox, H.M., Mattern, P.J., Johnson, V.A. and Schmidt, J.W. 1978. Comparative protein quality as measured by human and small animal bioassays of three lines of winter wheat. In "Nutritional Improvement of Food and Feed Proteins," Friedman, M. Ed. Plenum Press, Inc., New York. p. 91-102.
- Knipfel, J.E. 1981. Nitrogen and energy availabilities in foods and feeds subjected to heating. *Prog. Food Nutr. Sci.* 5:177.
- Kugimiya, M., Donovan, J.W., and Wong, R.Y. 1980. Phase transitions of amylose-lipid complexes in starches: a calorimetric study. *Starch/Stärke* 32:265.
- Larsson, K. and Mieziš, Y. 1979. On the possibility of dietary fiber formation by interactions in the intestine between starch and lipids. *Starch/Stärke* 31:301.
- Lepage, M. and Sims, R.P.A. 1968. Carotenoids of wheat flour: their identification and composition. *Cereal Chem.* 45:600.
- Linko, P., Colonna, P. and Mercier, C. 1981. High-temperature, short-time extrusion cooking. In "Advances in Cereal Science Technology," vol. IV, Pomeranz, Y. Ed. American Association of Cereal Chemists, St. Paul, MN. p. 145-235.
- Leung, P.M.B. and Rogers, Q.R. 1987. The effect of amino acids and protein on dietary choice. In "Umami: A Basic Taste", Kawamura, Y. and Kare, M.R. Eds. Marcel Dekker, Inc. New York, NY. p.565-610.
- MacDonald, F.J. 1966. Available lysine content of dried milk. *Nature.* 209:1134.
- Maga, J.A. and Sizer, C.E. 1979. The fate of free amino acids during the extrusion of potato flakes. *Lebensm. Wiss. Technol.* 12:13.
- Magee, P.N. and Barnes, J.M. 1956. The production of malignant primary hepatic tumours in the rat by feeding dimethylnitrosamine. *British J. Cancer* 10:114.

- McAuley, J.A., Kunkel, M.E. and Acton, J.C. 1987. Relationships of available lysine to lignin, color and protein digestibility of selected wheat-based breakfast cereals. *J. Food Sci.* 52:1580.
- Mitchell, H.H. 1927. Does the amount of food consumed influence the growth of an animal? *Science* 66:596.
- Munro, H.N. and Crim, M.C. 1988. The proteins and amino acids. In "Modern Nutrition in Health and Disease," 7th ed. Shils, M.E. and Young, V.R. Ed. Lea & Febiger, Philadelphia, PA. p. 1-37.
- Nelson, J.H. 1985. Wheat: its processing and utilization. *Am. J. Clin. Nutr.* 41:1070.
- Noguchi, A., Mosso, K., Aymard, C., Jeunink, J. and Cheftel, J.C. 1982. Maillard reactions during extrusion-cooking of protein-enriched biscuits. *Lebensm. Wiss. Technol.* 15:105.
- Nyman, M. and Asp, N.-G. 1982. Fermentation of dietary fibre components in the rat intestinal tract. *Br. J. Nutr.* 47:357.
- O'Brien, J. and Morrissey, P.A. 1989. Nutritional and toxicological aspects of the maillard browning reaction in foods. *CRC Crit. Rev. Food Sci. Nutr.* 28:211.
- Pellett, P.L. 1978. Protein quality evaluation revisited. *Food Tech.* 32:60.
- Phillips, R.D. 1989. Effect of extrusion cooking on the nutritional quality of plant proteins. In "Protein Quality and the Effects of Processing," Phillips, D.R. and Finley, J.W. Ed. Marcel Dekker, Inc., New York. p. 219-246.
- Pokorny, J. 1977. Interactions of oxidized lipids with protein. *Rev. Ital. Sostan. Grasse.* 54:389.
- Potter, N.N. 1986. "Food Science," 4th ed., Avi Publishing Co. Inc., Westport, CT.
- Rhee, K.S. and Rhee, K.C. 1981. Nutritional evaluation of the protein in oilseed products heated with sugars. *J. Food Sci.* 46:164.

- Richardson, T. and Hyslop, D.B. 1985. Enzymes. In "Food Chemistry," 2nd ed., Fennema, O.R. Ed. Marcel Dekker, Inc. New York. p. 371-476.
- Saterlee, L.D., Marshall, H.F. and Tennyson, J.M. 1979. Measuring protein quality. J. Am. Oil Chem. Soc. 56:103.
- Swaisgood, H.E. and Catignani, G.L. 1982. In vitro measurement of effects of processing on protein nutritional quality. J. Food Prot. 45:1248.
- Thompson, D.B. and Erdman, J.W. 1981. Nutrient bio-availability. In "Protein Functionality in Foods," Cherry, J.P. Ed. ACS Symposium Series 147. American Chemical Society, Washington, D.C. p. 243-274.
- Whistler, R.L. and Daniel, J.R. 1985. Carbohydrates. In "Food Chemistry," 2nd ed., Fennema, O.R. Ed. Marcel Dekker, Inc. New York. p. 69-137.
- Wolf, H.B. Ed. 1976, "Webster's New Collegiate Dictionary," G. & C. Merriam Co., Springfield, MA. p. 609.
- Wootton, M. and Bamunuarachchi, A. 1979. Application of differential scanning calorimetry to starch gelatinization. Starch/Stärke 31:201.
- Wu, Y.V. and Inglett, G.E. 1988. Effects of agricultural practices, handling, processing, and storage on cereals. In "Nutritional Evaluation of Food Processing," 3rd ed. Karmas, E. and Harris, R.S. Ed. Van Nostrand Reinhold Co. Inc., New York. p. 101-118.
- Wyman, J.B., Heaton, K.W., Manning, A.P. and Wicks, A.C.B. 1976. The effect on intestinal transit and the feces of raw and cooked bran in different doses. Am. J. Clin. Nutr. 29:1474.

9.0 APPENDICES



APPENDIX I. STANDARD CURVE FOR AVAILABLE LYSINE ANALYSIS

Vita

Jane Elizabeth McMillan was born December 15, 1965 in Radford, Virginia. After graduating from Pulaski County High School in 1984, she attended Virginia Polytechnic Institute and State University and graduated in 1988 with a Bachelor of Science degree in Biology. She attended Roanoke Memorial Hospitals School of Medical Technology during the 1987-88 school year and passed the ASCP Registry exam in August of 1988 to become a certified Medical Technologist. She worked in the Roanoke Memorial Hospital laboratory for one year and then returned to Virginia Polytechnic Institute and State University in the fall of 1989 to pursue a degree in Human Nutrition and Foods. She graduated in 1991 with a Master of Science degree in the Foods option of Human Nutrition and Foods.

A handwritten signature in cursive script that reads "Jane E. McMillan". The signature is written in black ink and is positioned in the lower right area of the page.