

PROTEIN RICH EXTRUDED SNACK FOODS USING
HYDROLYZED PROTEINS

By

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Abstract

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Protein Rich Extruded Snack Foods Using Hydrolyzed Proteins (Title)			
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The effects of the degree of protein hydrolysis and feed concentration of protein were tested on corn meal extrudates using a single screw extrusion system. Four different concentrations of whey protein isolate (0%, 5%, 10%, and 15%) with differing levels of hydrolysis (0%, 4.5%, 10.0%, and 14.9%) were mixed with corn meal and then extruded. Samples of the extrudates were tested for breaking strength, apparent density, true powder density, expansion ratios, and porosity. It was found that extrudates containing the hydrolyzed proteins had physical characteristics similar to those of the 100% corn meal samples. Many of the undesirable effects of adding unhydrolyzed protein to extrusion feed mixtures either did not occur or occurred to a lesser extent when partially hydrolyzed proteins were added. It was also found that as the degree of protein hydrolysis

and as the protein concentration of the extrusion feed mixture increased the breaking strength and the apparent density of the extrudate decreased to values that were lower than the extrudate of the control corn meal. Extrusion runs that utilized hydrolyzed and unhydrolyzed forms of the isolated soy proteins produced similar results which suggests that changes in the physical properties of protein hydrolysate fortified corn meal extrudates are independent of the protein source.

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CHAPTER ONE

Introduction

Extrusion is a means of shaping something by forcing it through an opening. In food processing, extrusion combines heating with the act of extrusion to create a shaped cooked product. Extrusion is accomplished by single screw and twin-screw extruders. The choice of extruders is determined by the moisture content of the extrusion feed mixture. Low moisture extrusion mixtures appropriate for a single screw extruder must have a feed composition of less than 35% moisture. High moisture extrusion mixtures, those with a moisture level of 35% or above, require the use of a twin-screw extruder. The 13.5% (w/w) moisture content of the extrusion mixtures used throughout this study was applicable to a single screw extruder.

There has been extensive ongoing research on the benefits of whey protein in the diet. Some of the findings indicate that whey protein slows the growth of breast cancer, boosts the immune systems of AIDS patients, reduces blood pressure, suppresses appetite, reduces cholesterol, and the inhibits the formation of dental plaque and dental caries. Benefits of whey protein exist for infants, active teens and healthy adults, athletes, and the elderly. The addition of whey protein into a commonly consumed type of snack food would increase the consumption of whey protein as well as possibly benefit the health of many individuals (Pins & Keenan, 2002; Schmidl, Taylor, & Nordlee, 1994; Ziegler, Nitenberg, Coudray-Lucas, Lasser, Giboudeau, & Cynober, 1998; Nelson, Colker, Kalman, & Swain, 2002; Loimaranta, Laine, Soderling, Vasara, Rokka, Marnila, Korhonen, Tossavainen, & Tenovuo, 1999).

Statement of Problem

Increasing numbers of residents in the United States are obese due in part to their consumption of significant amounts of high fat foods. Many common snack foods are fried, making them high in fats whereas extruded snack foods are often not fried. For this reason an extruded snack food with additional protein would not only be more nutrient dense but also lower in fat.

The snack food industry is continually growing with new products becoming available every year. In 2001, the snack food industry produced 332.6 million pounds of product (Table 1) that brought in about 1027.1 million dollars. Of this, 21.8 million dollars were brought in by cheese curls or puffs (Table 1 and Figure 1 & 2).

Table 1

Consumption of Snack Foods over the Past Three Years

Year	Consumption			
	Dollars (millions)	change	Pounds (millions)	change
1999	919.6		310.5	
2000	990.4	+70.8	324.2	+14
2001	1027.1	+36.7	332.6	+8.4

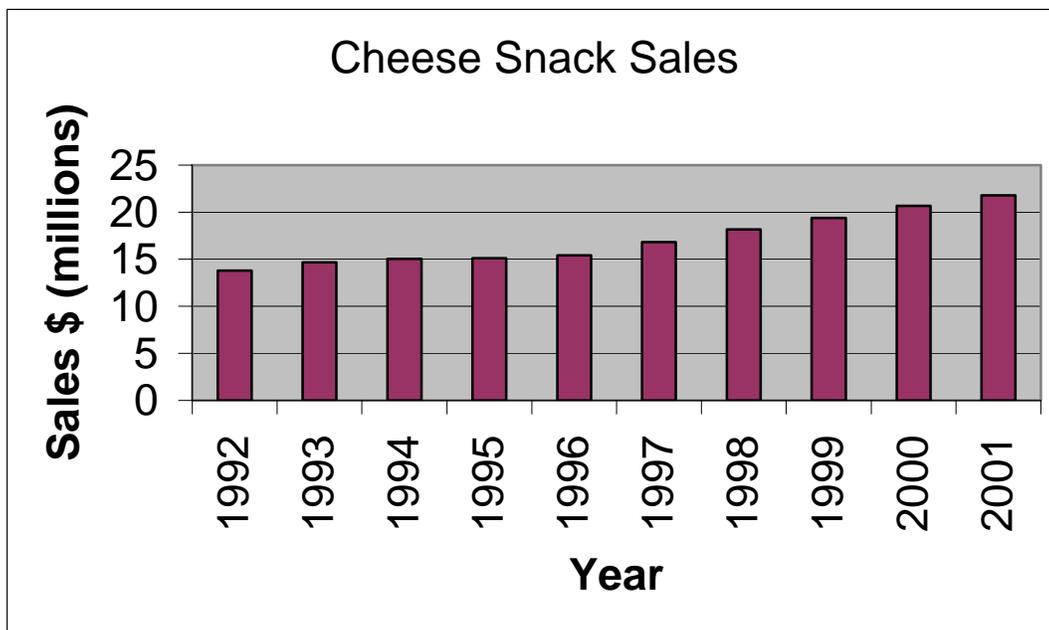
Snack Food Association (1999, 2000, 2001)

Previous studies have shown that when incorporating protein into an extruded snack food there is decreased expansion and increased hardness in the final product, both of which are undesirable effects (Choudhury & Gautam, 2003; Choudhury, Gogoi, & Oswalt, 1998; Onwulata, Konstance, Smith, & Hilsinger, 1998; Onwulata, Smith, & Constance, 2001).

Objectives

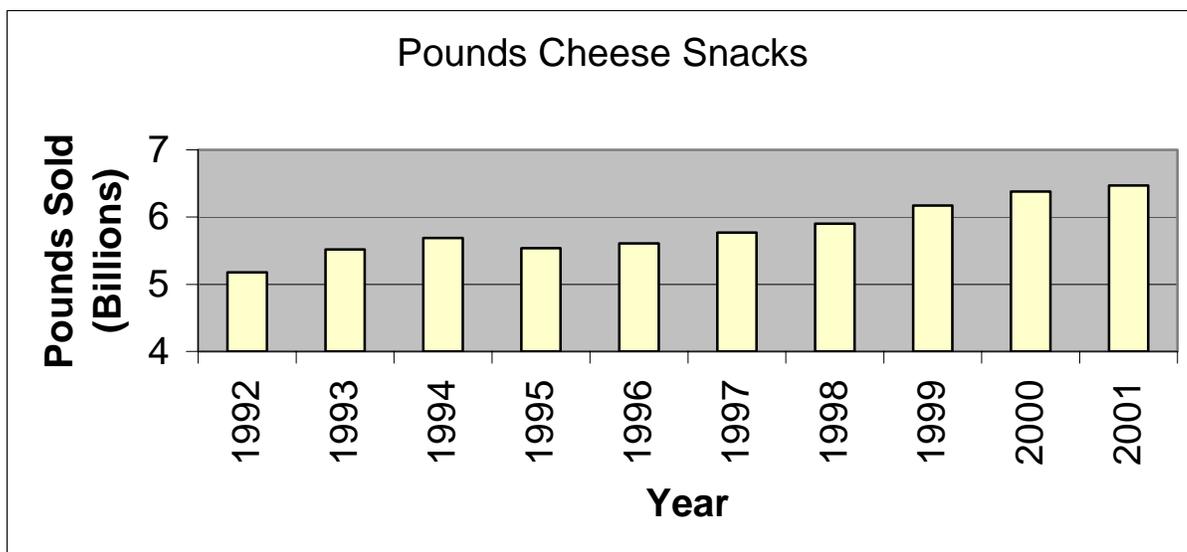
The general objective of this research is to produce quality low moisture high protein snack food using extrusion technology. A single screw extruder, hydrolyzed protein, and corn meal will be used to attempt to make a desirable product. More specifically the research is designed to determine if results with previous research with fish protein can be duplicated with other proteins. Because hydrolyzed protein is a rather expensive food ingredient this experiment is designed to determine the lowest degree of protein hydrolysis that produces the best product as well as the lowest percentage of protein on a dry basis.

Figure 1. Sales of Cheese Snacks



Snack Food Association (1999, 2000, 2001)

Figure 2. Volume of Cheese Snacks Sold



Snack Food Association (1999, 2000, 2001)

CHAPTER TWO

Literature Review

Extrusion

Different extrusion equipment is required for processing high moisture and low moisture feed mixtures. Low moisture extrusion can be performed with single screw or twin-screw extruders but high moisture extrusion must be performed with a twin-screw extruder. Low moisture extrusion is often used to make RTE breakfast cereals and snack foods.

Many modifiable process parameters affect the extrudates of both twin screw and single screw extruders. Variables that can be modified when using a single screw extruder include screw speed, throughput rate, temperature, and most importantly feed composition and moisture composition.

Whey Protein

There has been extensive on benefits of whey protein in the diet. Reported benefits of incorporating whey protein in the diet include decreased growth of breast cancer, enhanced operation of the immune system of AIDS patients, reduced blood pressure, appetite suppression, reduction of serum cholesterol, and inhibition of the formation of dental plaque and dental caries. There have been benefits of whey protein found for infants, active teens and healthy adults, athletes, and the elderly (Pins & Keenan, 2002; Schmidl, Taylor, & Nordlee, 1994; Ziegler, Nitenberg, Coudray-Lucas, Lasser, Giboudeau, & Cynober, 1998; Nelson, Colker, Kalman, & Swain, 2002; Loimaranta, Laine, Soderling, Vasara, Rokka, Marnila, Korhonen, Tossavainen, & Tenovuo, 1999).

Based on preliminary animal research conducted by Joel Pins and others it was found that a hydrolyzed whey protein isolate may be shown to reduce blood pressure. It is thought that this might occur because of it may inhibit angiotensin converting enzyme, causing blood pressure to lower (Pins & Keenan, 2002).

Studies conducted showed that the consumption of whey protein isolate over a 12-week period assists in the decrease of total cholesterol and LDL cholesterol. This study also found whey protein isolates to have an anti-estrogenic effect, which may help in decreasing the estrogen driven adverse health effects. This study was also found to show that whey protein isolate has an immunoenhancing effects that were more pronounced than those reported for ingestion of isolated soy protein (Nelson, Colker, Kalman, & Swain, 2002).

Hydrolyzed Protein

Proteins are often modified in order to improve their functional properties, both for processing and health of consumers. Hydrolysis of protein causes a transient decrease in solubility due to the temporary exposure of previously buried hydrophobic regions. Certain oligopeptides released during protein hydrolysis have been shown to possess physiological activities, such as opioid activity, immunostimulating activity, and the inhibition of angiotension-converting enzyme (Pins & Keenan, 2002; Schmidl, Taylor, & Nordlee, 1994; Ziegler, Nitenberg, Coudray-Lucas, Lasser, Giboudeau, & Cynober, 1998; Nelson, Colker, Kalman, & Swain, 2002).

Hydrolyzed proteins are identified by there degree of hydrolysis which is stated as a percent. Hydrolysis uses enzymes to break the proteins into smaller chains. The degree of hydrolysis measures the percent of the bonds broken by that enzyme in

comparison to the amount that are able to be broken by the given enzyme (Alder-Nissen, 1986).

Previous Studies

In the past few years there have been studies done in attempts to incorporate proteins into a low moisture extruded snack food. Much of this research has shown that with an increase in the protein concentration there is a decrease in product expansion and porosity, as well as an increase in product hardness (breaking strength). These three effects are undesirable and there has been limited research on ways to decrease these effects.

In 2001, Onwulata and others used cheese whey protein concentrate in an attempt to increase the nutrient density of snack foods. Although they did find some instances where expansion and breaking strength were improved the overall conclusion was that the increase in whey protein concentrate caused an increase in breaking strength and a decrease in product expansion. Their mixes tested included 0%, 25%, and 50% whey protein concentration. They tested these with corn meal, potato flour, and rice flour. The corn meal data showed a decrease in expansion ratio for both the 25 and 50% WPC samples. The breaking strength remained similar with the 0 and 25% samples but increased dramatically with the 50% sample. The potato flour showed the same general trend but with much smaller differences while data from the rice flour experiments showed a large decrease in expansion ratio for both the 25 and 50% WPC samples except when a high shear extrusion procedure was used. It was also reported that there was an overall increase in breaking strength for all samples at 25% and with low extrusion a slight decrease from the 25 to 50% sample. The feed moisture used in the low shear

samples ranged from 14.2-14.8%. The high shear moisture ranged from 13.1-17.8% moisture. Changes in the finished products were primarily associated with changes in the moisture content of the feed mixtures. The authors suggested that the addition of the protein to the feed mixture increased moisture retention in the feed mixture by binding water (Onwulata, Smith, & Konstance, 2001).

In 1998, Onwulata and others studied the effects of including milk products in the extrusion feed mixtures. They found that the inclusion of up to 25% (w/w) milk products could be accomplished but was accompanied by a decrease in expansion and an increase in breaking strength. They determined that by changing extrusion parameters, especially moisture, that whey products could be incorporated into extrusion mixtures but that process modification would be needed (Onwulata, Konstance, Smith, & Hilsinger, 1998).

In 2003 Choudhury and others used rice flour and arrowtooth flounder mince to test the effects of the addition of the fish solids and hydrolyzed fish solids. They hydrolyzed the arrowtooth flounder fish solids for 5, 10, and 15 minutes. It was found that with the use of unhydrolyzed fish solids true density, which was measured with a multipycnometer, increased with the increase in percentage of fish solids. The hydrolyzed fish solids showed little difference between times and from the 100% rice flour control (Choudhury & Guatam, 2003).

Additionally, Choudhury and others found that fish solids content and fish solids hydrolysis time affected the apparent density extrudate samples in this series of experiments. Apparent density was calculated from averaged measurements of diameters, lengths and mass of individual extrudate samples. The addition of unhydrolyzed fish

solids caused an increase in the apparent density of extrudates compared to 100% rice flour control extrudates but when the hydrolyzed fish solids were included there was a decrease in apparent density of the extrudates (Choudhury & Guatam, 2003).

Choudhury and others found that the porosity of rice flour extrudates was affected by the inclusion of proteins but not by inclusion of hydrolyzed fish proteins to the extrusion mixture. Within the range of hydrolysis intervals studied (5, 10, 15 minutes), the degree of hydrolysis did not make a significant difference on the porosity of the extrudates. It was apparent that even limited hydrolysis made a difference (Choudhury & Guatam, 2003).

In the studies of experiments conducted by Choudhury and others, the radial expansion of rice flour extrudates decreased with the addition of unhydrolyzed fish proteins to the extrusion mixture but increased as increasing amounts of hydrolyzed proteins were incorporated into the extrusion mixture. The greatest radial expansion was observed in extrudates containing 10% hydrolyzed fish protein for all degrees of hydrolysis studied. In contrast the inclusion of 10% unhydrolyzed fish protein had significantly less radial expansion. This also shows that it was the hydrolysis in general, not the hydrolysis time that affected the expansion. Axial expansion ratios showed similar trends as did overall expansion ratios (Choudhury & Guatam, 2003).

CHAPTER THREE

Materials and Methods

Materials

Whey protein was donated by Davisco Foods International, Inc. (LeSueur, Minnesota). Four different types of whey protein were used, BiPro® isolated whey protein, BioZate® 1, hydrolyzed whey protein isolate, BioZate® 3 hydrolyzed whey protein isolate, and BioZate® 5 hydrolyzed whey protein isolate. Yellow corn meal was donated by Illinois Cereal Mills, Inc. (Paris, Illinois). Samples of Pro-Fam® 781 isolated soy protein and Pro-Fam® H200FG hydrolyzed soy protein were donated by Archer Daniels Midland (Decatur, Illinois). Product specifications for all ingredients are presented in Tables 2 and 3.

Table 2

Approximate Company Specifications

	Moisture %	Protein %	Carbohydrates %	Fat %
Yellow Corn Meal	13.0	7.0	79.0	0.7
BiPro®	4.7	97.8	<1	0.2
Biozate 1®	5.0	97.2	<1	0.2
Biozate 3®	5.0	96.2	<1	0.4
Biozate 5®	4.9	96.1	<1	0.3
Pro-Fam H200 FG®	7.0	82	<1	1.0
Pro-Fam 781®	6.0	90.0	<1	1.0

Table 3

Particle Size Distribution of Corn Meal

Sieve Number	Typical	Range
On US 20	8%	5.0%-20.0%
On US 25	20%	15.0-35.0%
On US 30	35%	25.0%-45.0%
On US 40	35%	20.0%-40.0%
Thru US 40	2%	0.0%-3.0%

Single Screw Extruder

All the experiments used a single screw extruder (Figure 3) (Tiger 20 Bake Type Extruder, American Extrusion International, South Beloit, Illinois, USA). The extruder was equipped with cooling jacket for the throat section of the stator, color touch screen control panel, variable speed feeding hopper, 9" hi pro stator, 9" hi pro feed screw, single hi pro flow plate, standard 12-hole die, 7" heat bands, hi pro stator seals, and standard baked knife blades. The die consisted of a 12-hole die with each hole being 3.57mm in diameter. Eight of the 12 holes were closed with screws and four were allowed to extrude. The screw was 7.62 cm in diameter and 24.48 cm long. There was a 2.54 cm lead and 1.27 cm pitch with double right hand threads. The thread radius was 0.475 cm full and 0.554 cm deep. The nose of the screw was milled at 15° on both sides.

Extrusion Experiments

The degrees of protein hydrolysis and protein concentration were varied. Four different degrees of protein hydrolysis were used as well as four different percentages of whey protein. The percent moisture of each ingredient was determined by drying

Figure 3. Single Screw Extruder



triplicate samples of each in a drying oven (Model MO1450A/SA, Lindberg/Blue, Asheville, North Carolina) for 24 hours at 105°C. The determinations can be found in Table 4. Three blends for each degree of hydrolysis were prepared by mixing (on a dry solids basis) corn meal (95%, 90%, 85%) with hydrolyzed whey protein (5%, 10%, 15%). The dry components were first mixed by hand before being put into a Hobart Mixer (Model A-200, Hobart Corporation, Troy, Ohio). The calculated amount of water to bring the feed to 13.5% moisture was added to the Hobart Mixer and mixed for 10 minutes. The concentrations of protein in each blend are shown in Table 4.

Table 4

Moisture Determinations

Ingredient	Percent Moisture (w/w)
Corn Meal	11.0%
BiPro®	4.9%
Biozate 1®	5.6%
Biozate 3®	5.9%
Biozate 5®	5.8%
Pro-Fam H200 FG®	5.5%
Pro-Fam 781®	5.2%

Table 5

Composition of Extruded Mixes

	Degree of Hydrolysis (%)	% Protein (w/w)	% Corn Meal (w/w)
Control	NA	0	100
BiPro®	0	5, 10, 15	95, 90, 85
Biozate 1®	4.5	5, 10, 15	95, 90, 85
Biozate 3®	10.0	5, 10, 15	95, 90, 85
Biozate 5®	14.9	5, 10, 15	95, 90, 85
Pro-Fam H200 FG®	Hydrolyzed	10	90
Pro-Fam 781®	Unhydrolyzed	10	90

The screw configuration was maintained at 24.48 cm in length and 1.27 cm pitch was constant as was the screw speed at 180 rpm. The temperature was maintained at 175°C by using cooling water. The moisture content of the feed was kept constant at 13.5% (w/w). The auger rate was held constant at 12 rpm. The cutting blade speed was kept at 300 rpm. Once a steady state of product extrusion was reached, samples were taken and evaluated based on the response variables. All results are averages of two replicate runs. Each of the soy protein samples was run just once and only at one level.

Response Variables

Gallon polyethylene storage bags were used to store the corn/protein curls. Five curls from each set were selected so that they would approximate straight cylinders. They were then weighed on a balance (AB204-S, Mettler Toledo, Greifensee, Switzerland). Ten diameter and ten length measurements were taken for each of the five samples using a Vernier Caliper. These length and diameter measurements were then averaged and

used to calculate the apparent density (Equation 1) and specific volume (Equation 2) of the samples.

$$\text{Apparent Density} = \text{mass/volume} \quad (1)$$

$$\text{Apparent Specific Volume} = \text{volume/mass} \quad (2)$$

The true powder densities of the raw ingredient samples as well as of each corn/protein curl were estimated using a Multipycnometer (Quantachrome Instruments, Boynton Beach, Florida). The large reference and sample cell were used. Two samples from each run were weighed and the true powder volume of each was obtained using the multipycnometer. The multipycnometer determines true powder density by the application of Archimedes principle of fluid displacement to determine volume. The fluid used is helium gas, which because of its small atomic dimension assures that atoms of the gas will penetrate the smallest of pores. The multipycnometer measures the pressure difference between the known volume of the reference cell and the cell with the sample. The reference cell pressure is taken followed by the sample cell pressure and these are used to calculate true powder volume (Equation 3). The true powder density is then found by using the mass obtained and the volume found through the previous calculation.

$$V_p = V_c - V_r [(P_1/P_2) - 1] \quad (3)$$

$$V_p = \text{Sample Volume}$$

$$V_c = \text{Cell Volume}$$

$$V_r = \text{Reference Cell Volume}$$

$$P_1 = \text{Reference Cell Pressure}$$

$$P_2 = \text{Sample Cell Pressure}$$

The expansion ratios were then calculated. The first of these is radial expansion (Equation 4), which compares the diameter of the sample to the diameter of the die hole. Overall expansion (Equation 5) is calculated using the apparent specific volume and the true specific volume calculated previously. Axial expansion (Equation 6) is the last of these ratios to be calculated. This calculation uses both radial expansion and axial overall expansion.

$$\text{Radial Expansion} = \text{Area}_{\text{sample}} / \text{Area}_{\text{die}} \quad (4)$$

$$\text{Overall Expansion} = \text{Apparent Specific Volume} / \text{True Specific Volume} \quad (5)$$

$$\text{Axial Expansion} = \text{Overall Expansion} / \text{Radial Expansion} \quad (6)$$

Porosity (Equation 7) is calculated using the apparent volume and the true volume. The porosity value will be a number between zero and one and can be evaluated as a percentage.

$$\text{Porosity} = (\text{Apparent Volume} - \text{True Volume}) / \text{Apparent Volume} \quad (7)$$

Product breaking strength was determined with the use of a Single Column Table Top Load Frame Texture Analyzer (Series 3340, Instron Corporation, Canton, Massachusetts) with a Warner-Bratzler meat shear set in compression mode. The cross-head was run at 500 mm/minute. Twenty-five samples of each product run were used to measure the peak force. The average diameter was used to calculate the cross-sectional area. The peak force was divided by the cross-sectional area to give the value of force per unit area, kPa.

Data Analysis

Data analysis was completed using a program developed with Excel. Data was examined using Analysis of Variance (ANOVA) and Comparison of Means methods.

AXUM was also used in data analysis to obtain regression values.

CHAPTER FOUR

Results and Discussion

Feed Composition

Four different types of whey protein were used in this study. Although the composition of the whey proteins were quite similar (Table 4) the effects of the extrudates were noticeable. The degrees of protein hydrolysis affected the physical appearance and properties of the extrudates as did the amount of the protein added. (Figure 4-8).

The throughputs were calculated for all of the various mixes studied and no matter the mix they were the same. The means were calculated to be 60.18 kg product/hr and to be 65.28 kg feed/hr. This suggests that the feeds all flowed at equal rates and cannot be the cause of the differing extrudates.

Effects of Hydrolyzed Protein on Extrudate Densities

True Density (TD):

Addition of whey proteins to cornmeal had a significant effect on true density of extrudates (Tables 6 & 7). In the samples with hydrolyzed proteins the true densities decrease as the percentage of protein increases. In all cases but the first level of hydrolysis (4.5%) the values are below that of the extruded corn meal samples. In the unhydrolyzed protein samples there is no apparent trend to be observed. It can also be seen that as the level of hydrolysis increases the true density again decreases. The analysis of variance (ANOVA) data suggests that for true density values the degree of hydrolysis does have a highly significant impact but the amount of protein and the interaction between these two variables does not.

Figure 4. Unhydrolyzed Whey Protein Extrudates

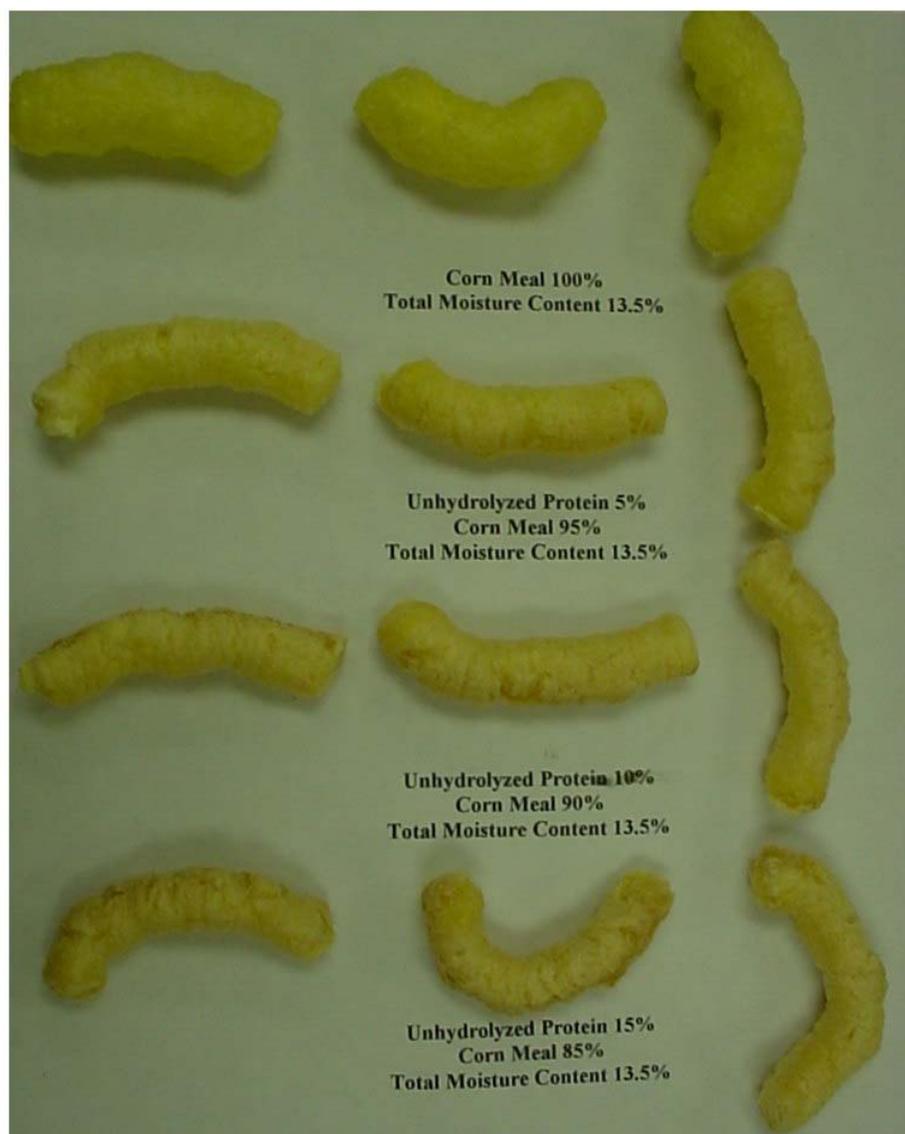


Figure 5. Hydrolyzed (4.5%) Whey Protein Extrudate



Figure 6. Hydrolyzed (10.0%) Whey Protein Extrudate

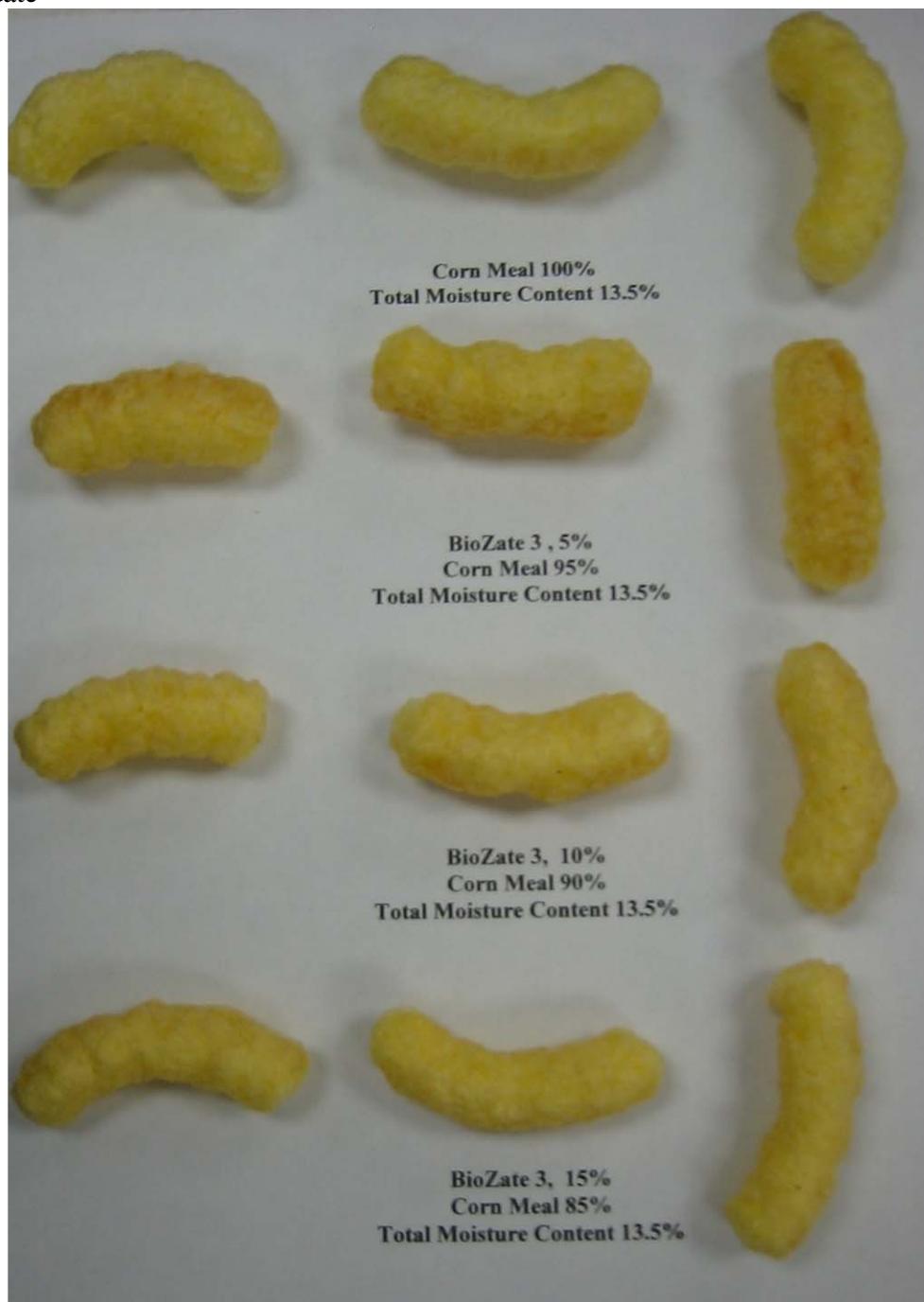


Figure 7. Hydrolyzed (14.9%) Whey Protein Extrudates

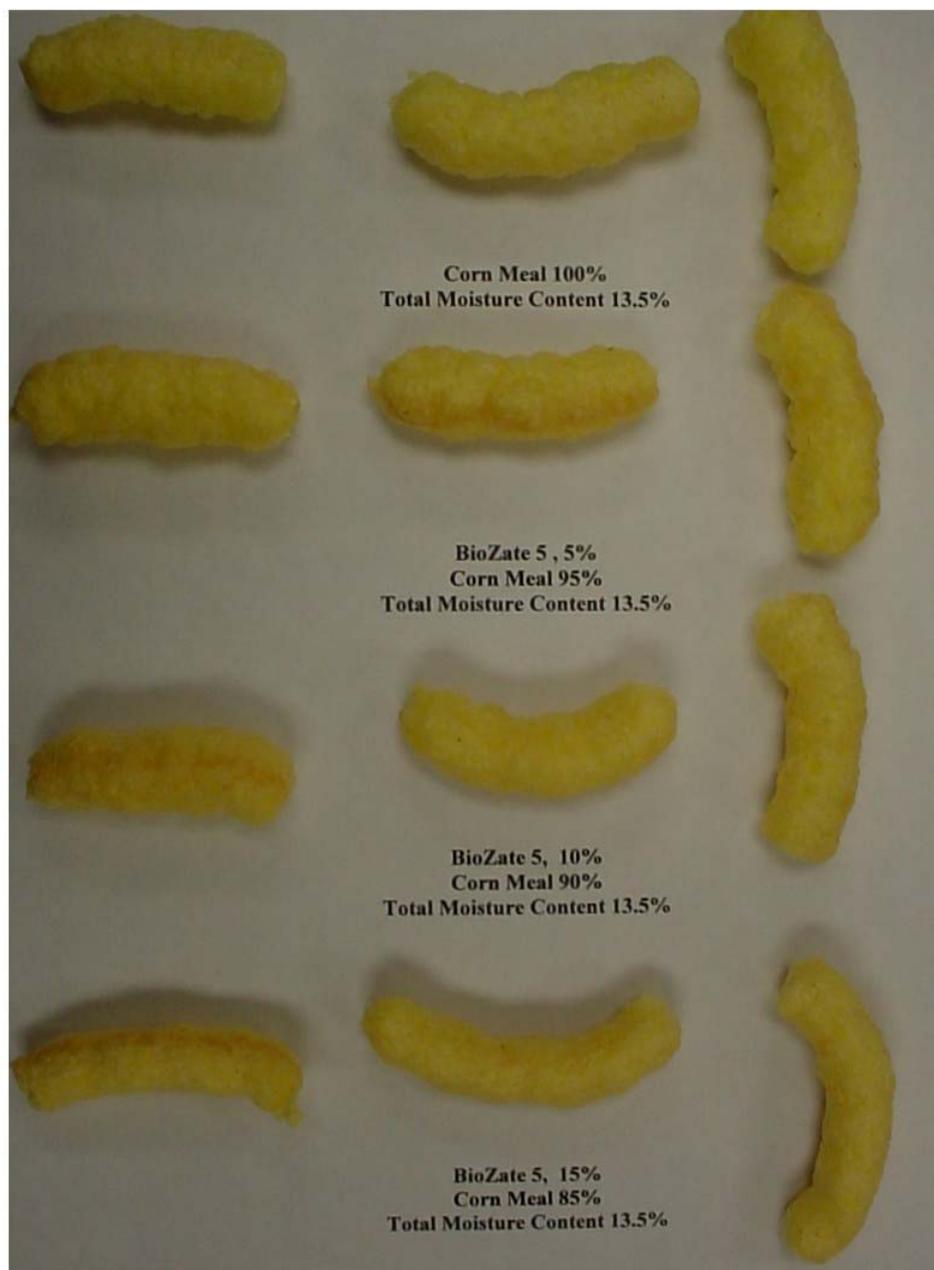


Figure 8. Soy Protein Extrudates

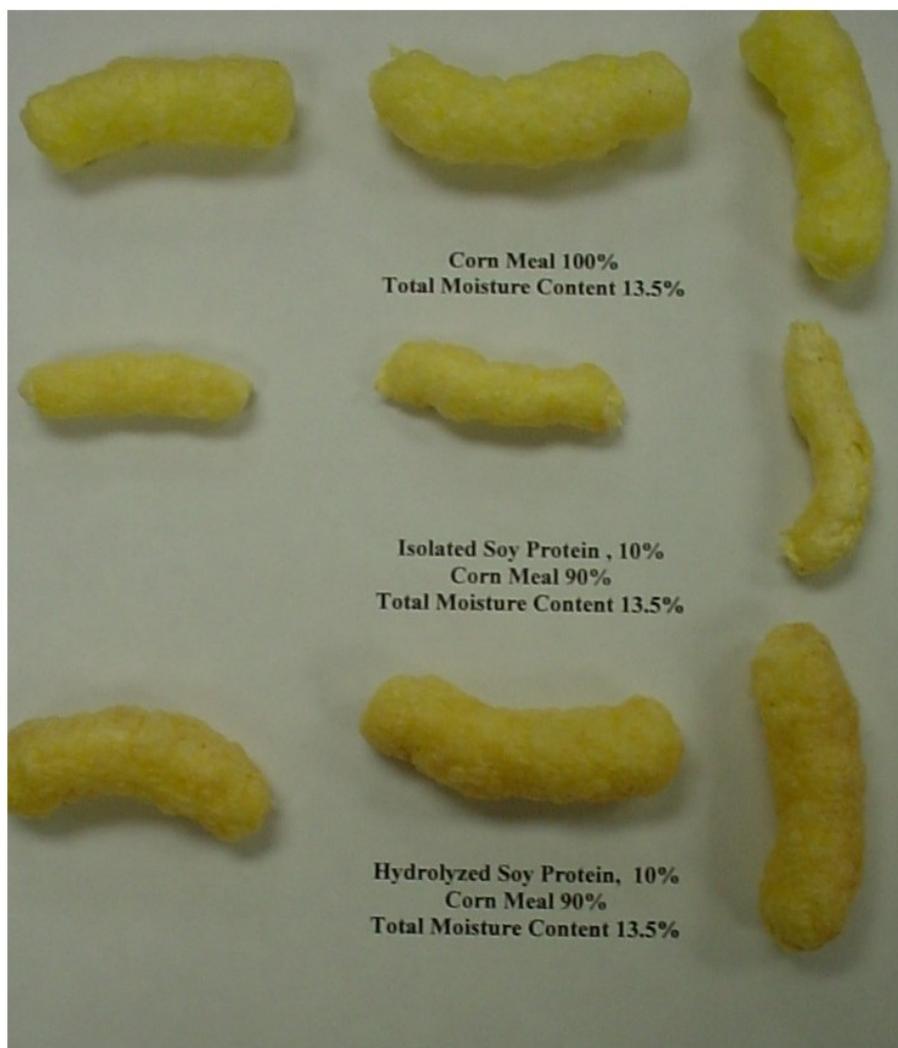


Table 6

Effect of Degree of Hydrolysis and Whey Protein Solids Content on True Density of Extrudates Obtained from Blends of Whey Protein and Corn Meal

Whey Protein Isolate Solids Concentration (%)	True density (kg/m ³) whey protein degree of hydrolysis			
	0%	4.5%	10.0%	14.9%
5	1788.8 ^{AB} ± 69.1	1773.6 ^{ABC} ± 72.65	1593.7 ^{BCDEF} ± 32.2	1471.0 ^{BCDEF} ± 102.0
10	1979.6 ^A ± 56.6	1755.7 ^{ABCD} ± 0.25	1586.0 ^{BCDEF} ± 112.6	1359.5 ^F ± 8.5
15	1574.0 ^{BCDEF} ± 216.0	1743.5 ^{ABCDE} ± 140.5	1552.6 ^{BCDEF} ± 72.35	1304.1 ^F ± 82.9

True density for corn meal extrudate = 1658.45 ± 27.05 kg/m³

Means with same letters are not significantly different

Table 7

Analysis of Variance Data for Densities and Porosity of Extrudates Obtained from Whey Protein and Corn Meal Blends

Source	DF	Mean Sum of Squares (MSS) and F-values for					
		Apparent Density (kg/m ³)		True Density (kg/m ³)		Porosity (%)	
		MSS	F	MSS	F	MSS	F
		Degree of Hydrolysis (DOH)	3	744.95	16.226**	210146.3	9.337**
Whey Protein Isolate Solids (WPI)	2	1892.80	41.229**	38658.2	1.718	0.1152	16.178**
DOH x WPI	6	199.63	4.348*	19848.06	0.882	0.0176	2.465
Error	12	45.91	-	22507.11	-	0.000712	-

**Highly significant at p # 0.01

*Significant at p # 0.05

The true densities were also tested for the soy protein isolate samples run with 10% protein. The hydrolyzed soy protein isolate had a true density of 1327.18 ± 43.3 kg/m³ and the unhydrolyzed was 1392.82 ± 1.15 kg/m³. These were both similar to the whey protein isolate samples with 15% hydrolysis.

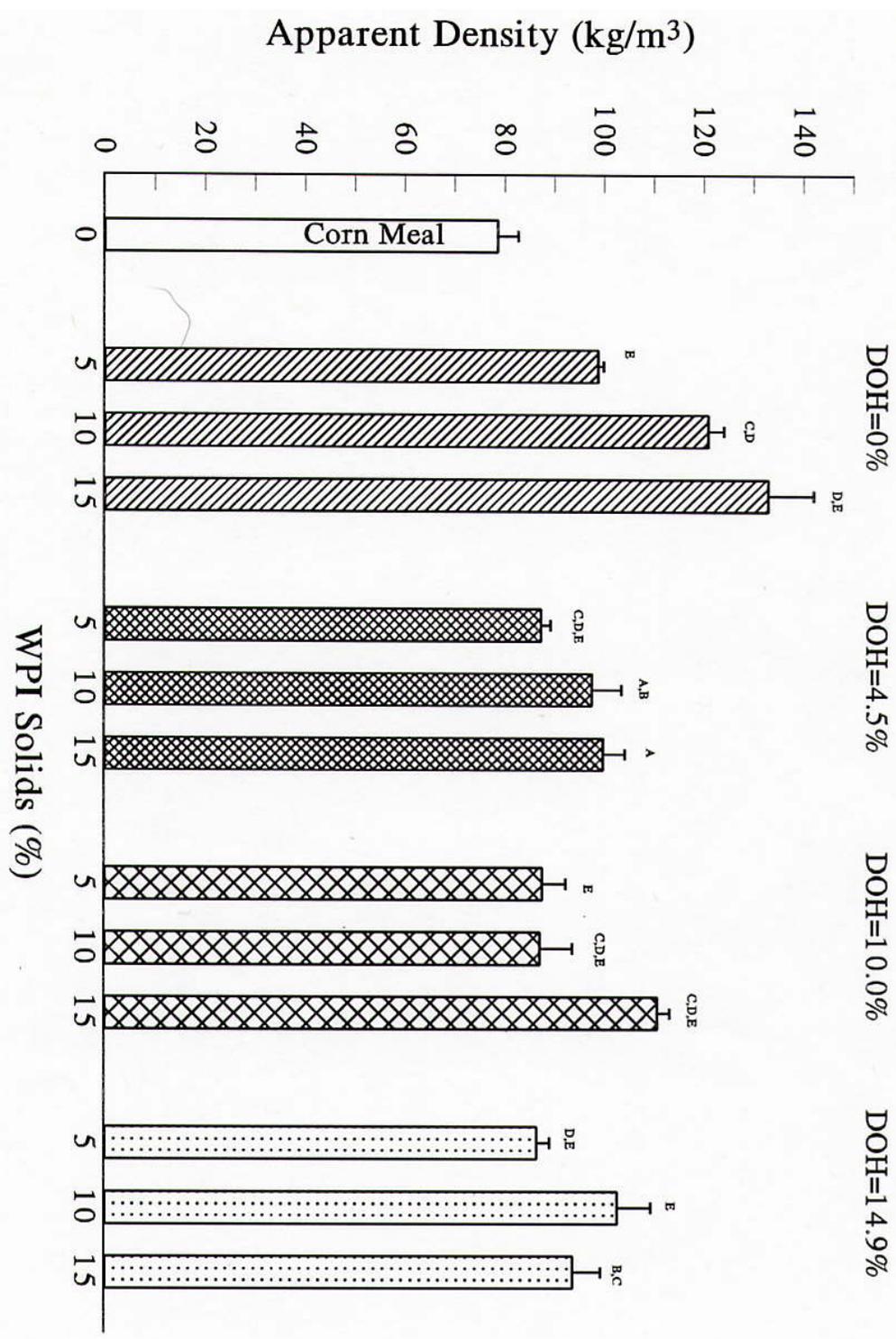
Apparent Density (AD):

The degree of hydrolysis, whey protein content, and their interaction affected the apparent density significantly (Table 7). The effects were different for unhydrolyzed and hydrolyzed whey protein. An apparent density of 78.58 ± 4.16 kg/m³ was obtained for the 100% corn meal extrudate. It increased methodically with addition of unhydrolyzed whey protein to corn meal, from 98.73 ± 1.22 kg/m³ with 5% unhydrolyzed whey protein to

$132.95 \pm 9.05 \text{ kg/m}^3$ with 15% unhydrolyzed whey protein. Similar effect of pink salmon muscle on apparent density has been observed in previous studies. When added to rice flour at high concentrations, pink salmon muscle increased the apparent density of extrudates (Choudhury, Gogoi, & Oswalt, 1998) and with rice flour and arrowtooth flounder (Choudhury & Gautum, 2003).

A similar trend was observed with hydrolyzed whey protein solids. The apparent density value slightly increased to $\sim 87 \text{ kg/m}^3$ upon addition of 5% whey protein solids to corn meal and in general, increased further with increasing level of hydrolyzed whey protein in the blends. When looking at Figure 9 the degree of hydrolysis seemed to have minimal effect on the apparent density, this is indicated by similar apparent density profiles for the three degrees of hydrolysis (Figure 9). The analysis of variance (ANOVA) shows that the percentage hydrolysis has a highly significant effect as does the percentage whey protein solids and the interaction of these two variables (Table 7).

Figure 9. Apparent Density of Whey Protein



The apparent densities for the soy protein isolate samples followed a similar trend as the whey protein isolate samples, with the unhydrolyzed sample having a much higher apparent density than the hydrolyzed and the control. The unhydrolyzed sample had an apparent density about three times higher than both the corn meal and the hydrolyzed (Figure 10).

Effects of Hydrolyzed Protein on Extrudate Porosity

The whey protein solids percentage had a significant effect on the porosity of the samples (Table 7). Figure 11 shows that as the percentage of whey protein solids increase the porosity of the extrudates decrease. This relationship is even more apparent in the unhydrolyzed samples. According to the ANOVA data (Table 7), neither the degree of hydrolysis or the interaction of the change percentage of protein solids and degree of hydrolysis had a significant effect on the extrudates. The porosity of the corn meal extruded sample was 95.5%. The control is held as the most porous sample. All of the samples with the addition of protein had values below the value for corn meal.

Figure 13 shows that the porosities of the unhydrolyzed and hydrolyzed samples were below that of the corn meal. The apparent densities were all above the value of the corn meal as well. Generally the unhydrolyzed samples had a higher apparent density values than did the hydrolyzed samples. This comparison also shows that in general, as the porosity of a sample increases the apparent density decreases.

Figure 10. Apparent Density of Soy Protein Extrudates

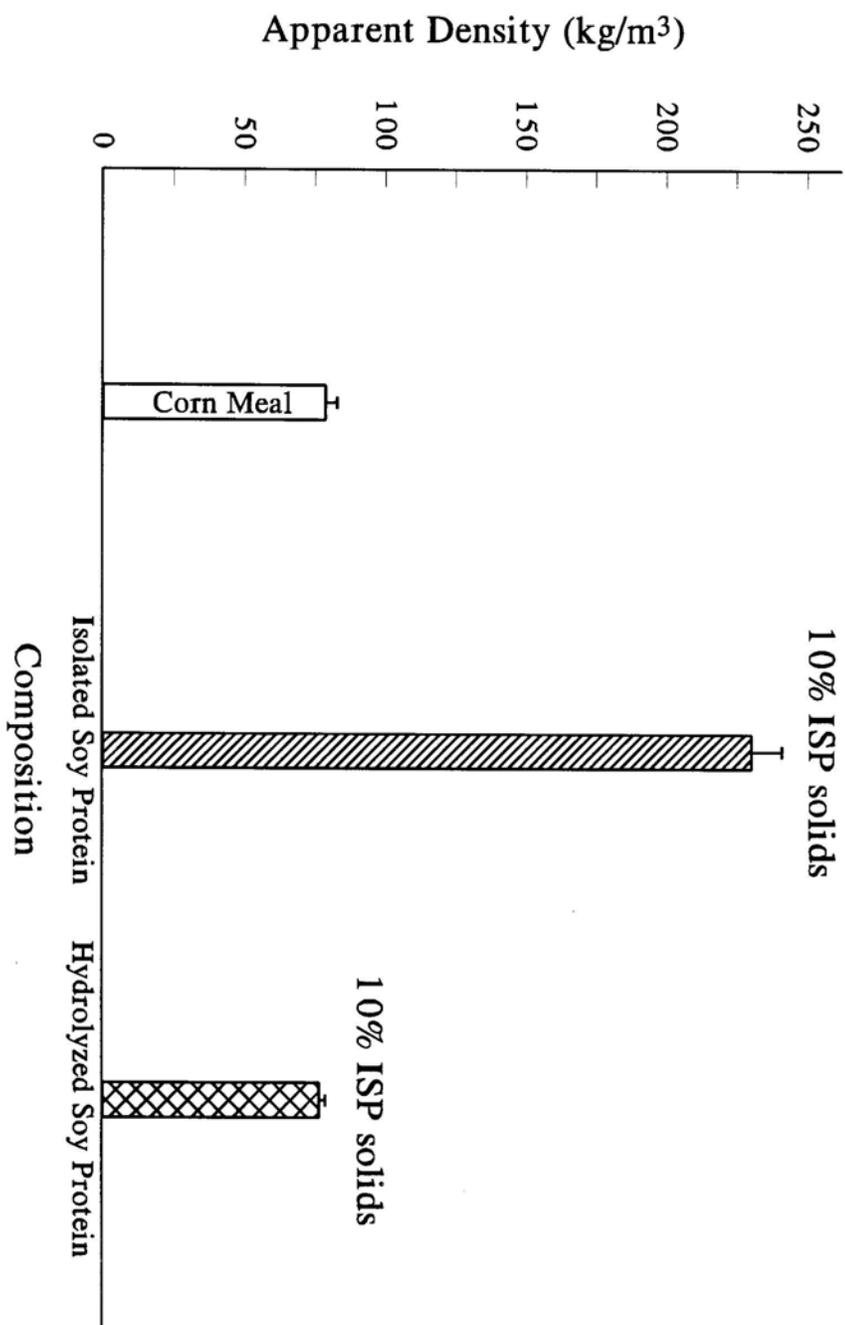


Figure 11. Porosity of Whey Protein Extrudates

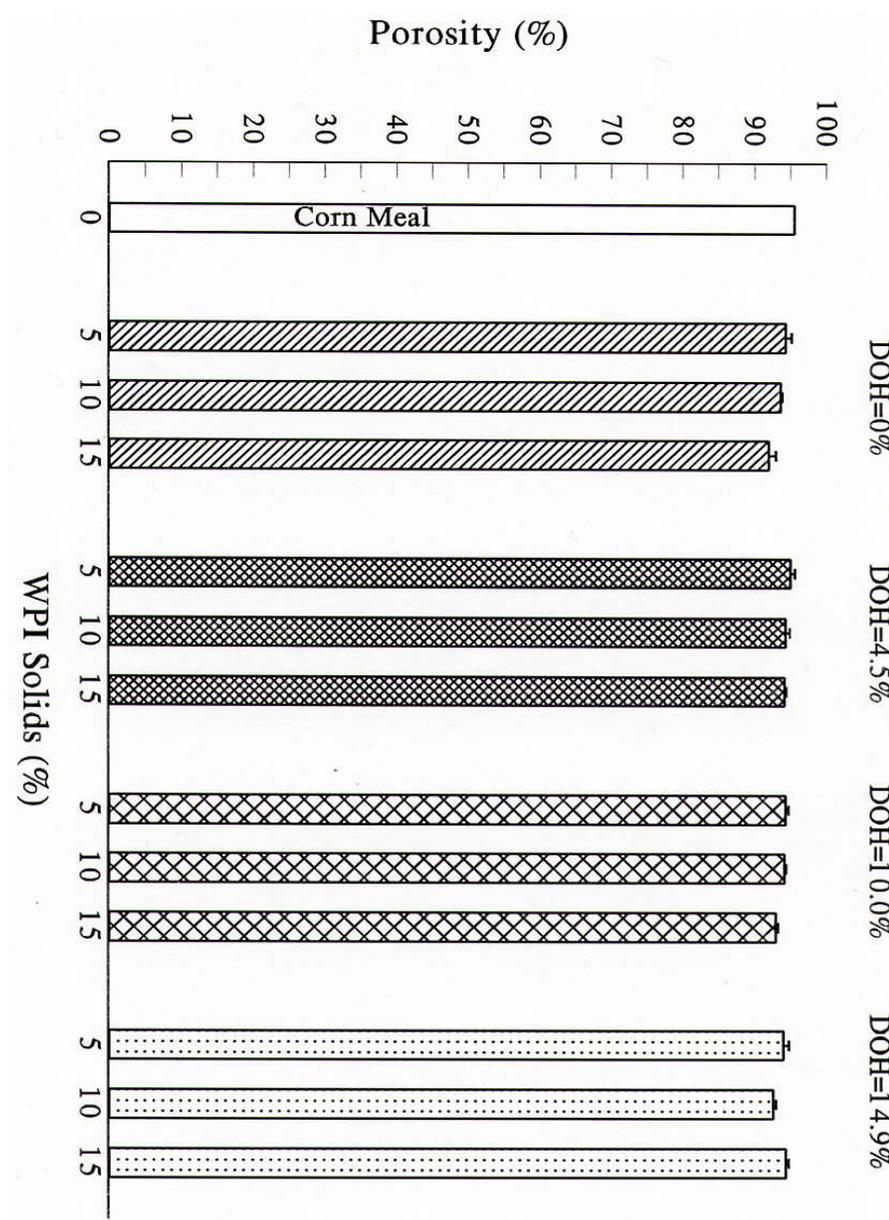


Figure 12. Porosity of Soy Protein Extrudates

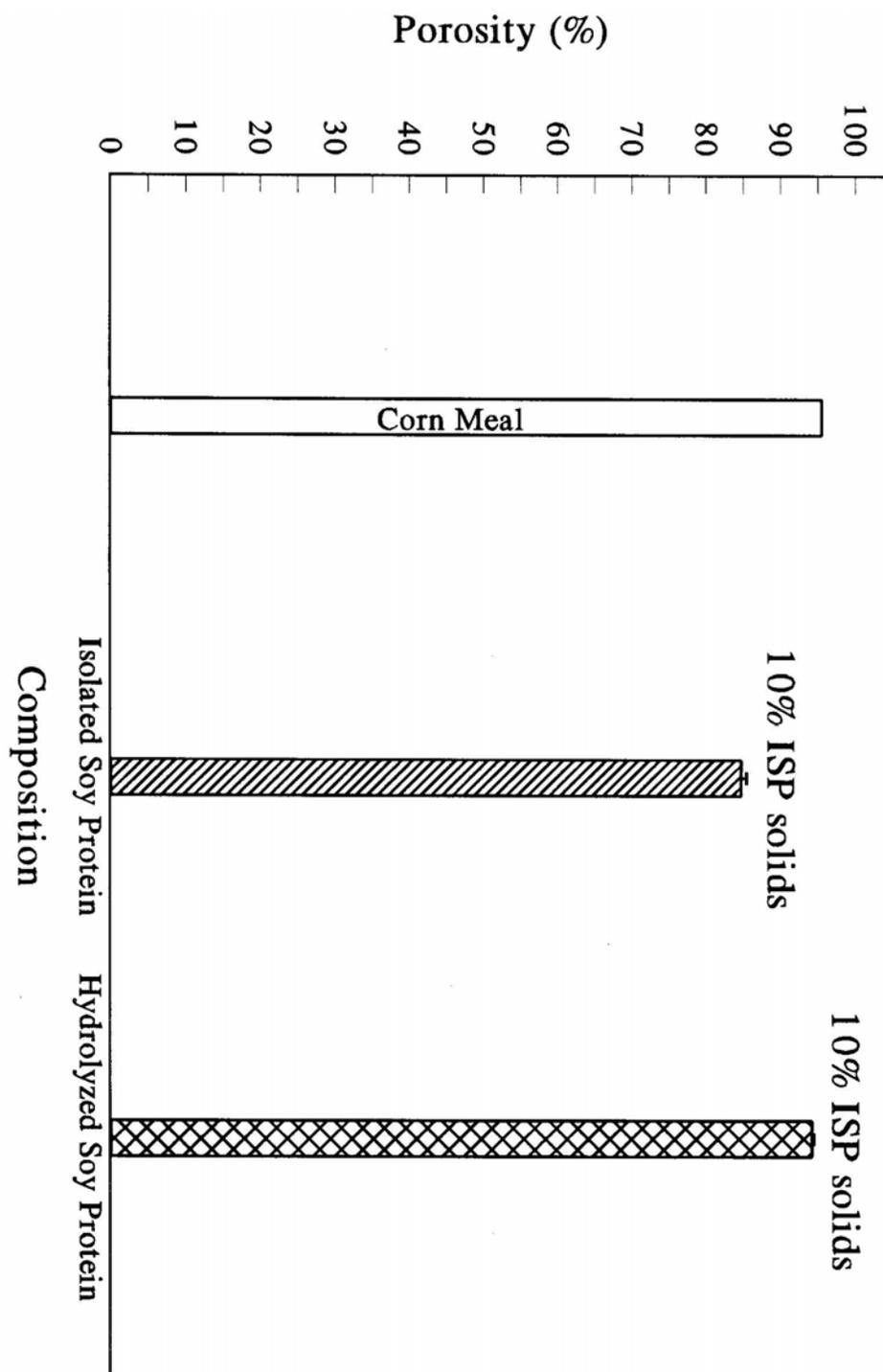
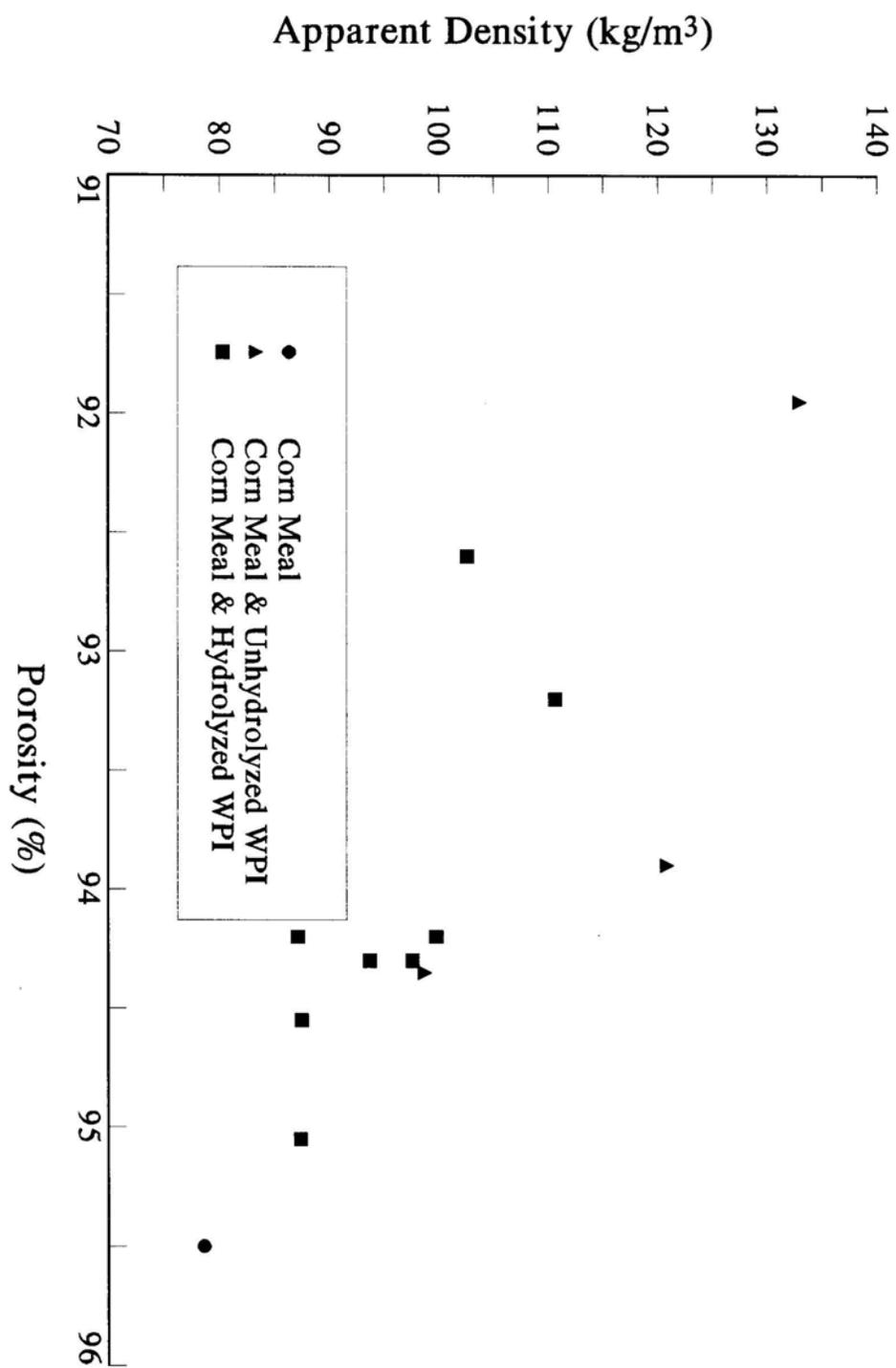


Figure 13. Apparent Density and Porosity of Whey Protein Extrudates



Hydrolysis had a more apparent effect on the porosity of the soy protein samples. The porosity of the unhydrolyzed soy protein was substantially less than that of the corn meal and the hydrolyzed protein sample was very similar to the value of the corn meal (Figure 12).

Effects of Hydrolyzed Protein on Extrudate Expansion

Radial Expansion Ratio (RER):

The statistical significance of feed composition effect on radial expansion of extrudates was due to whey protein solids concentration and degree of hydrolysis but not the interaction of the two (Table 8). The corn meal extrudate had a radial expansion ratio of 20.43. Addition of 5% unhydrolyzed whey protein solids to cornmeal reduced the radial expansion ratio to 14.29. This ratio decreased even further as the percentage of protein solids increased. The radial expansion ratio was 11.34 and 9.97 for the 10% and 15 % feed compositions.

Hydrolyzed whey protein isolate was found to enhance the expansion characteristics of corn meal (Figure 14). The radial expansion ratio of extrudates obtained from blends corn meal and hydrolyzed whey protein isolate were similar to that of the corn meal extrudate but significantly higher than the ratio of the extrudates containing the unhydrolyzed whey protein. As can be seen by (Figure 14) the 5% protein samples always have the highest radial expansion ratios and the ratios decrease as the percentage of whey protein increases, this is true for both hydrolyzed and unhydrolyzed samples.

The soy protein samples again behaved similarly. Because degree of hydrolysis was not tested with these that cannot be speculated on but the absence of hydrolysis

caused the expansion to decrease by more than half. The hydrolyzed sample had radial expansion very similar to that of the corn meal (Figure 15).

Figure 14. Radial Expansion Ratio of Whey Protein Extrudates

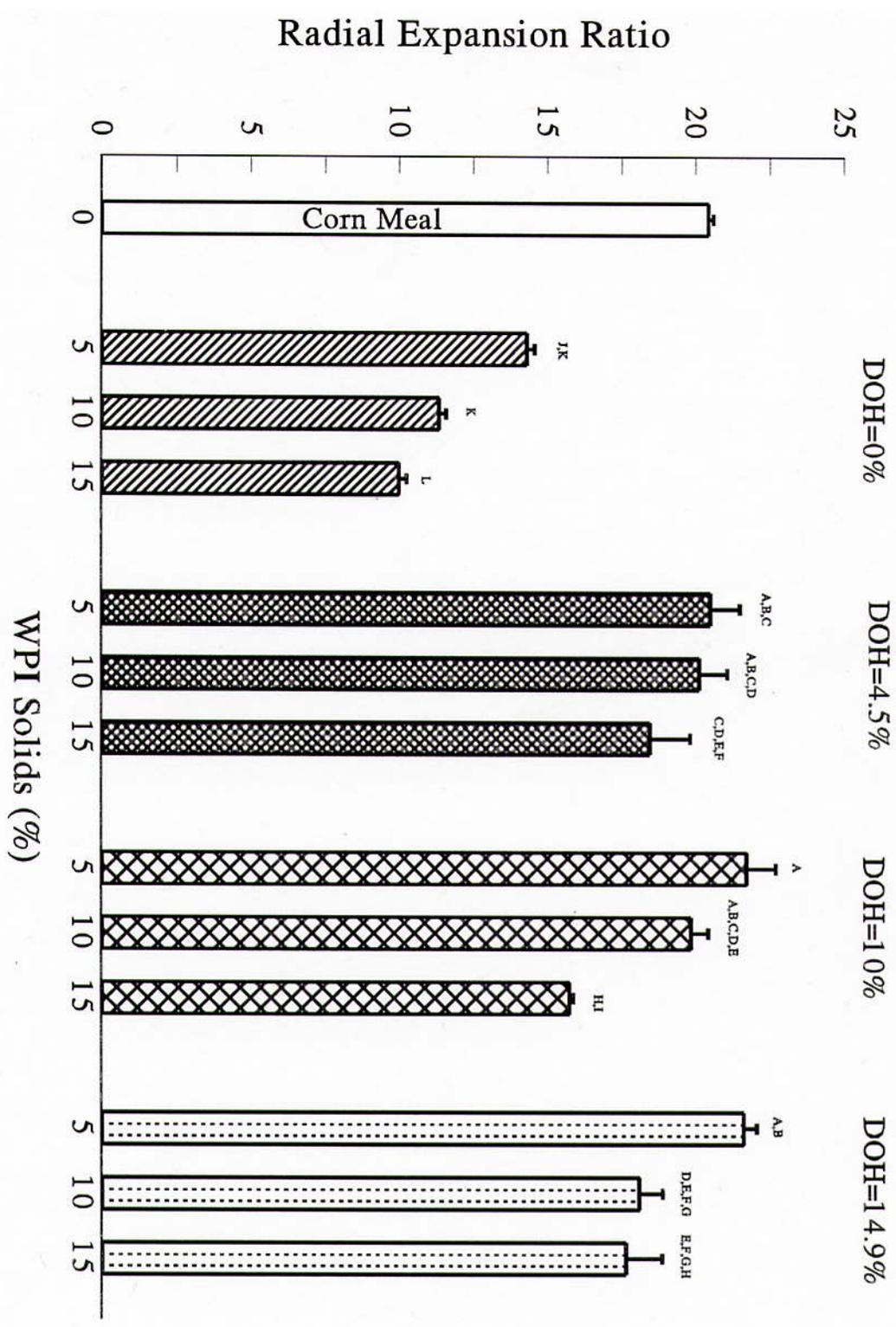
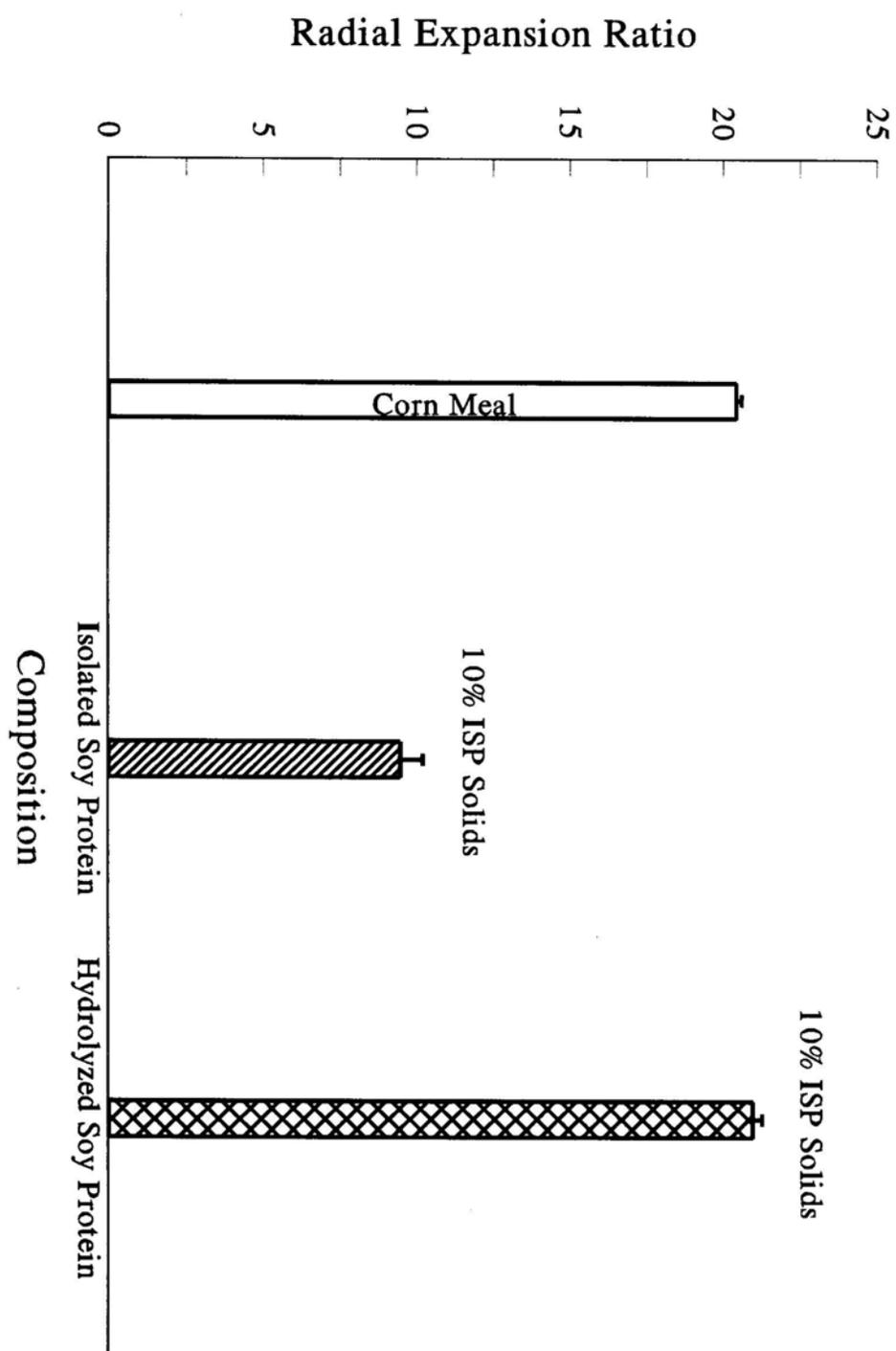


Figure 15. Radial Expansion Ratio of Soy Protein Extrudates



Axial Expansion Ratio (AER):

The effects of the degree of hydrolysis were found to have a significant effect on the axial expansion ratio of the extrudates (Table 8). The value for the corn meal extrudate was an axial expansion ratio of ~1.186. In the samples with unhydrolyzed protein this value increased, the highest of which being the 10% protein sample with a value of ~1.469. As hydrolyzed protein was added, regardless of the degree of hydrolysis, this ratio dropped significantly (Figure 16). The highest ratio for the hydrolyzed protein samples was with the 5% protein sample using the 4.5% hydrolyzed whey protein. The lowest of these values was found to be the 10% protein sample using 14.9% hydrolyzed protein. This suggests that both the percentage of protein and the degree of hydrolysis have a significant effect, although the analysis of variance data shows that the percentage of protein effect was not significant.

The soy protein samples surprisingly behaved in the opposite way here. The unhydrolyzed samples had a significantly lower axial expansion ratio than the corn meal.

Figure 16. Axial Expansion Ratio of Whey Protein Extrudates

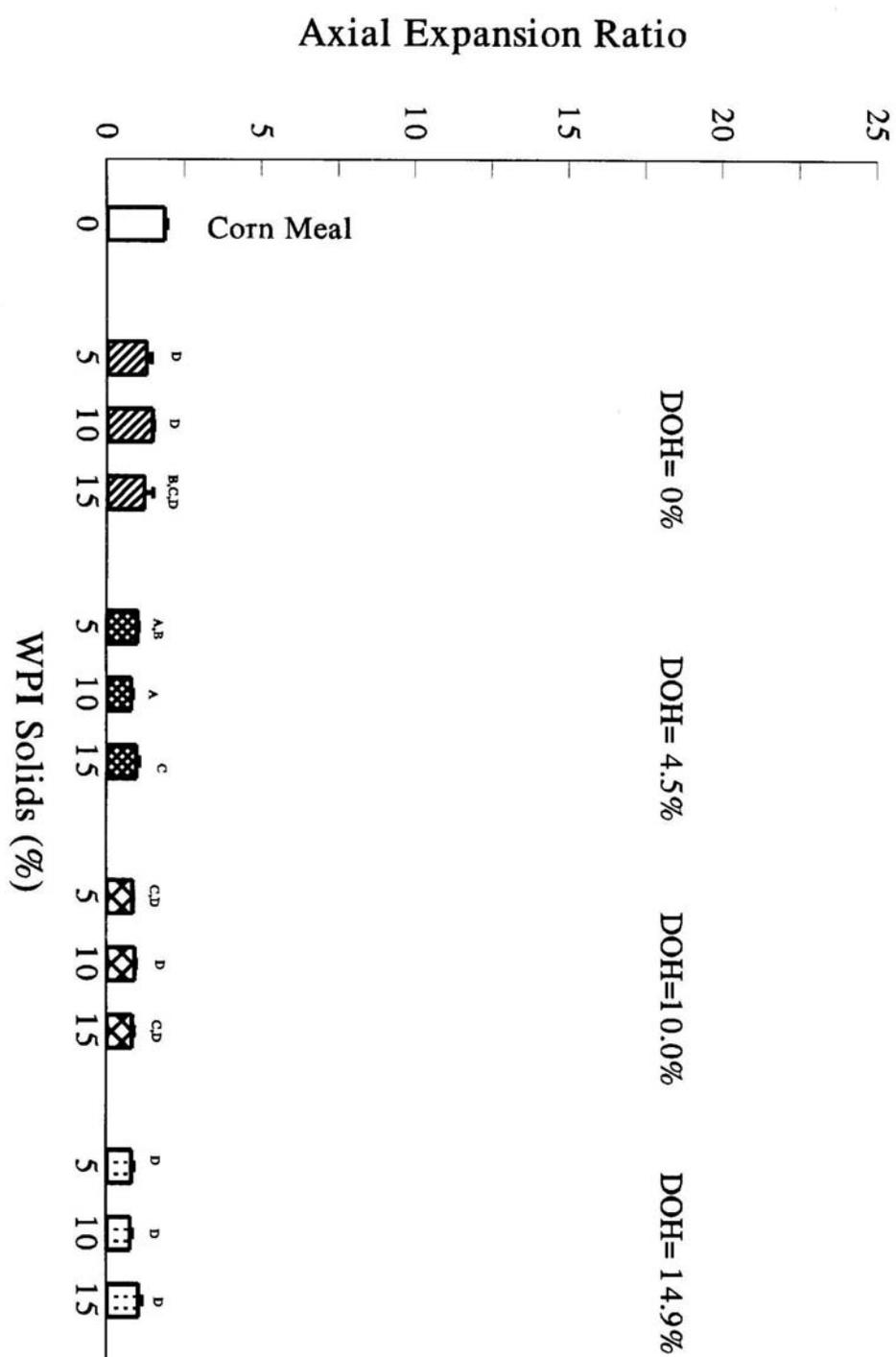


Figure 17. Axial Expansion Ratio of Soy Protein Extrudates

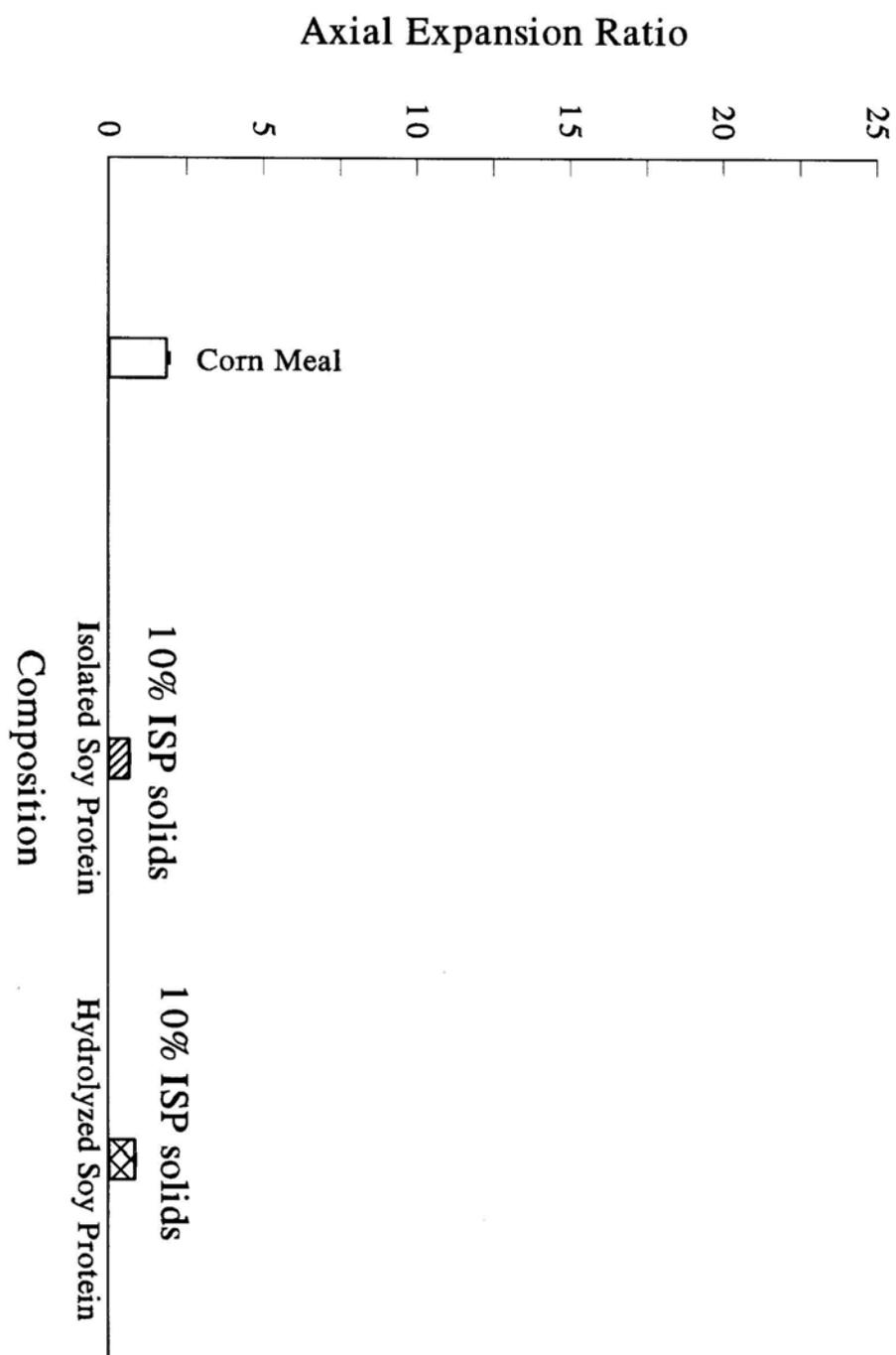


Table 8

Analysis of Variance Data for Expansion Ratios of Extrudates Obtained From Anova

Mean Sum of Squares (MSS) and F-values for Expansion Ratios							
Source	DF	Radial Expansion Ratio		Axial Expansion Ratio		Overall Expansion Ratio	
		MSS	F	MSS	F	MSS	F
Degree of Hydrolysis (DOH)	3	76.42	75.89**	0.3470	21.63**	30.029	6.60**
Whey Protein Isolate Solids (WPI)	2	56.38	55.99**	0.0031	0.1939	58.753	12.92**
DOH x WPI	6	4.54	4.51*	0.02039	1.272	2.867	0.6304
Error	12	1.006	-	0.01604	-	4.548	-

**Highly significant at p # 0.01
blends of whey protein and corn meal.

*Significant at p # 0.05

The hydrolyzed soy also had a lower axial expansion ratio than corn meal, but not by nearly as much (Figure 17).

Overall Expansion Ratio(OER):

The percentage of whey protein solids had a highly significant effect on the overall expansion ratios of the extrudates. The effect of the interaction between the two was not found to be significant nor was the degree of hydrolysis (Table 8). The corn meal extrudate had an overall expansion ratio of 22.9; none of the samples, either hydrolyzed or unhydrolyzed were above this. As the percentage of protein increased in the samples the overall expansion ratio decreased noticeable with the exception of the 14.9% hydrolyzed protein at 15% whey protein solids (Figure 14). The samples with the 4.5 and

10.0% hydrolyzed protein were consistently higher than that of the unhydrolyzed proteins. This did not follow true for the 14.9% hydrolyzed protein samples.

The soy protein behaved similarly in this case as well with neither of the protein samples reaching the higher expansion ratio of corn meal and the unhydrolyzed having a significantly lower value than the hydrolyzed.

There appeared to be a slight indirect relationship between overall expansion and apparent density with the whey protein samples (Figure 19). All values were less than corn meal for overall expansion as well as greater in apparent density. As the apparent density of the extrudates increased the overall expansion decreased.

Porosity had the opposite effect on overall expansion (Figure 20). These values suggest a direct linear relationship between these two response variables. Corn meal alone is the highest in both overall expansion and porosity. As the overall expansion of the extrudates increases so does the porosity. There is linear relationship for both hydrolyzed and unhydrolyzed samples. This was as expected from previous research for the unhydrolyzed proteins but surprising that the overall expansion was not higher for the hydrolyzed proteins. This may be because previous studies were completed with rice flour, which expands less than corn meal.

Figure 18. Overall Expansion Ratio of Whey Protein Extrudates

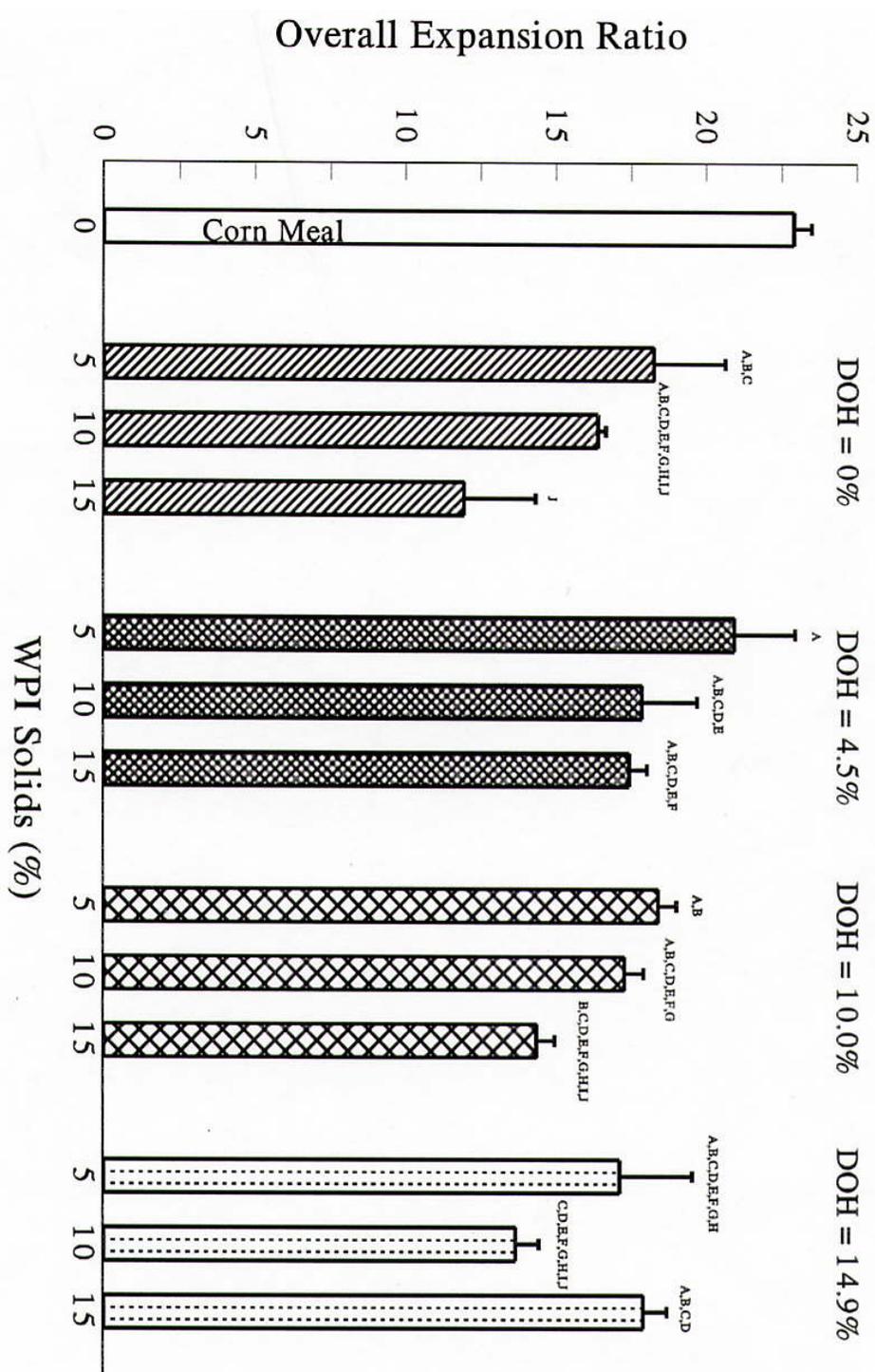


Figure 19. Overall Expansion Ratio of Soy Protein Extrudates

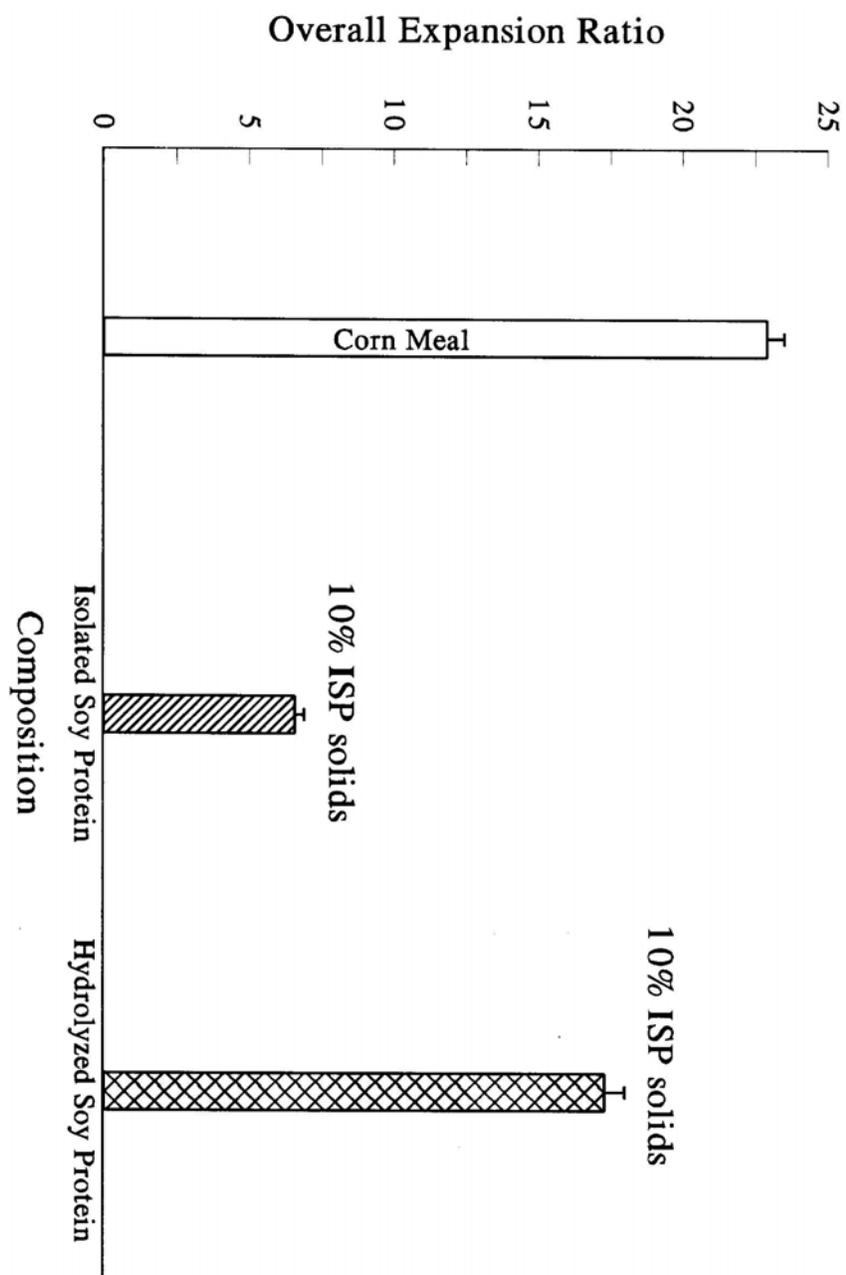


Figure 20. Apparent Density and Overall Expansion Ratio of Whey Protein Extrudates

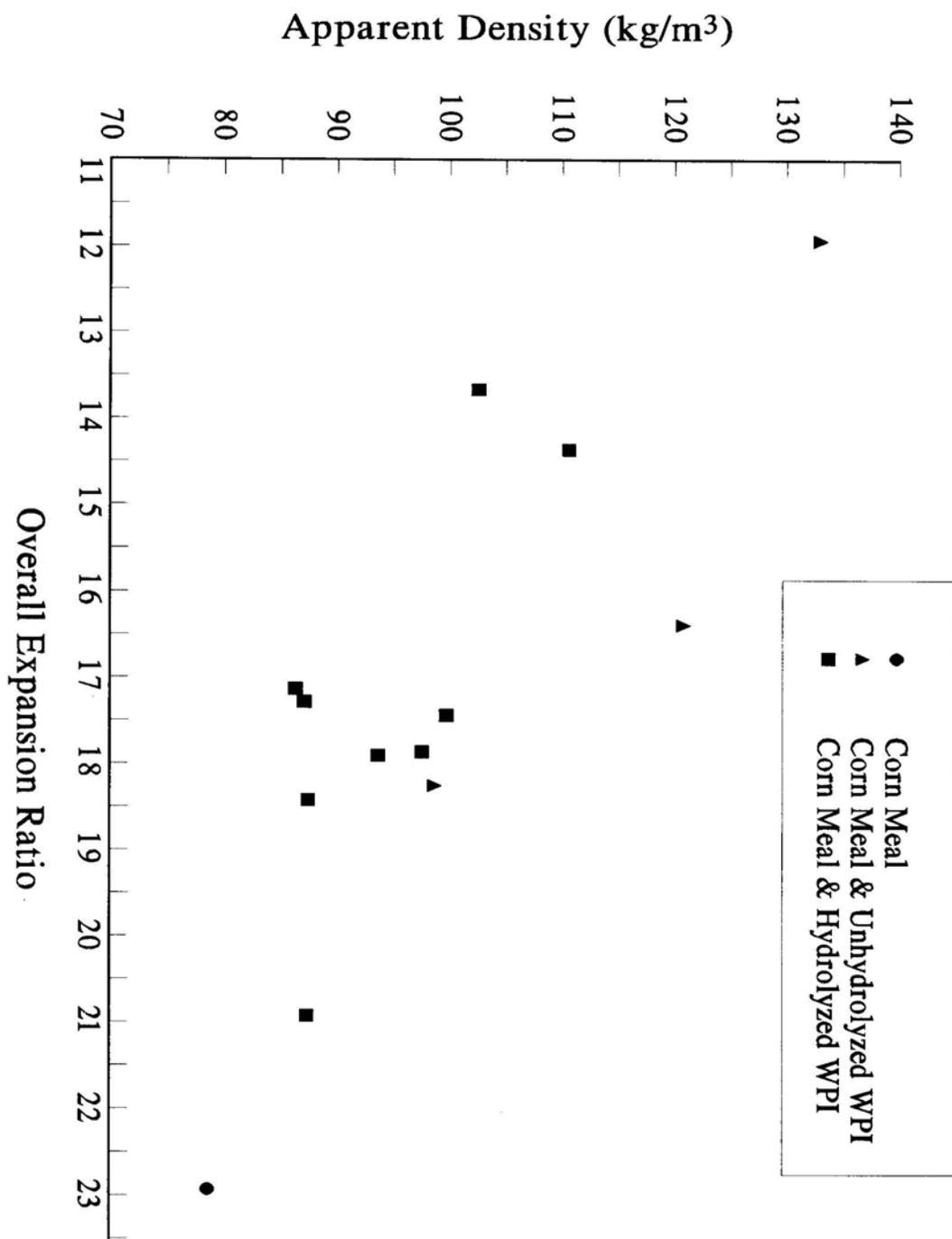
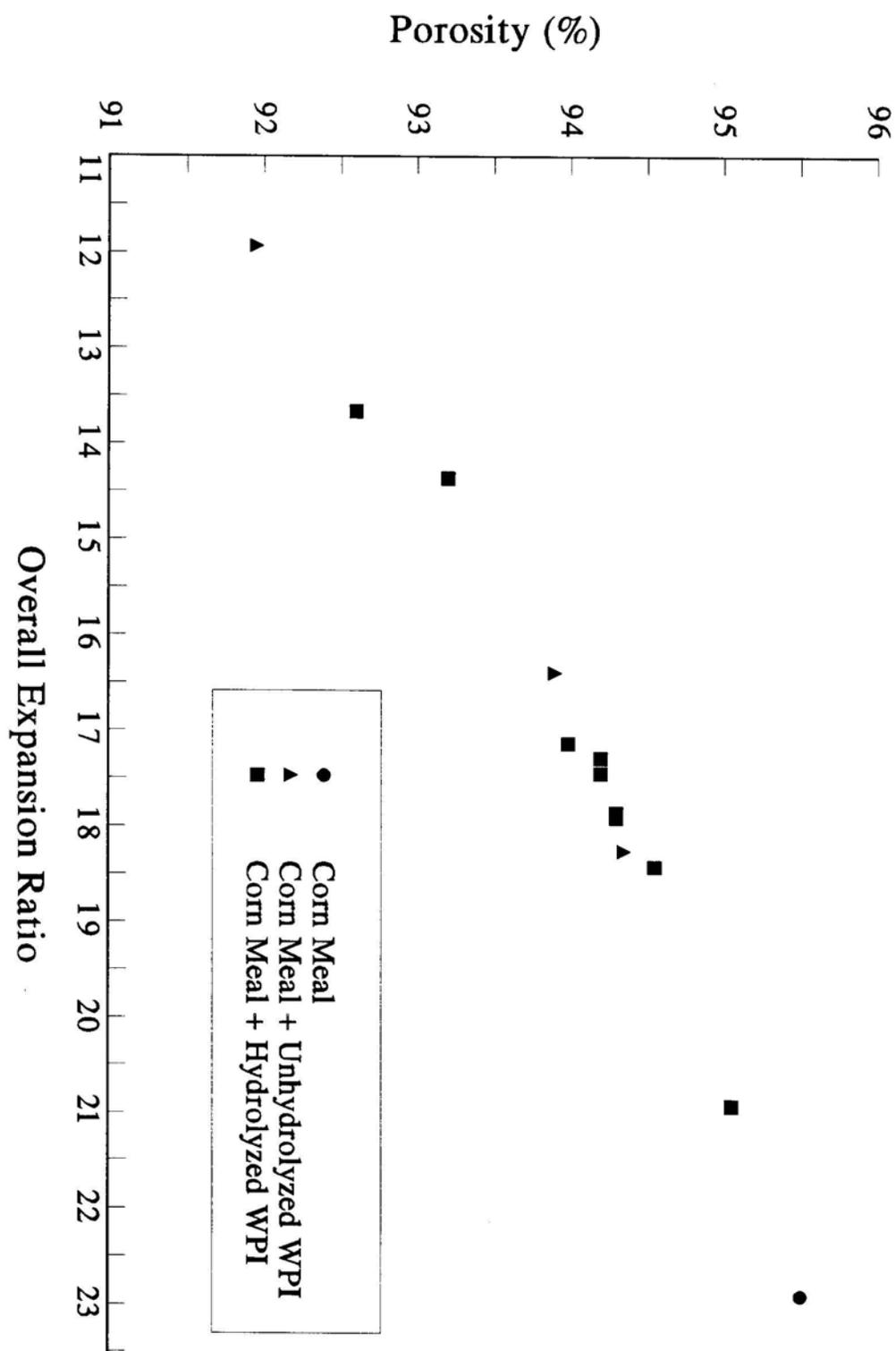


Figure 21. Porosity and Overall Expansion Ratio for Whey Protein Extrudates



Effects of Hydrolyzed Protein on Extrudate Breaking Strength

Among all the macroscopic properties studied, breaking strength was the one affected most by the addition of unhydrolyzed whey protein. In the analysis of variance done on breaking strength values (Table 9) it was found that the degree of hydrolysis, percentage of whey protein isolate solids, and the interaction between these two variables, all had a very highly significant effect on the breaking strength.

Table 9

Analysis of variance data for breaking strength of extrudates obtained from blends of corn meal and hydrolyzed whey protein isolate

Source	DF	Mean Sum of Squares (MSS) and F-values for Breaking Strength (kPa)	
		MSS	F
Degree of Hydrolysis (DOH)	3	141527.8	836.66**
Whey Protein Isolate Solids (WPI)	2	7295.6	43.13**
DOH x WPI	6	5930.6	35.06**
Error	12	169.2	-

**Highly significant at p # 0.01

*Significant at p # 0.05

Figure 22. Breaking Strength of Whey Protein Extrudates

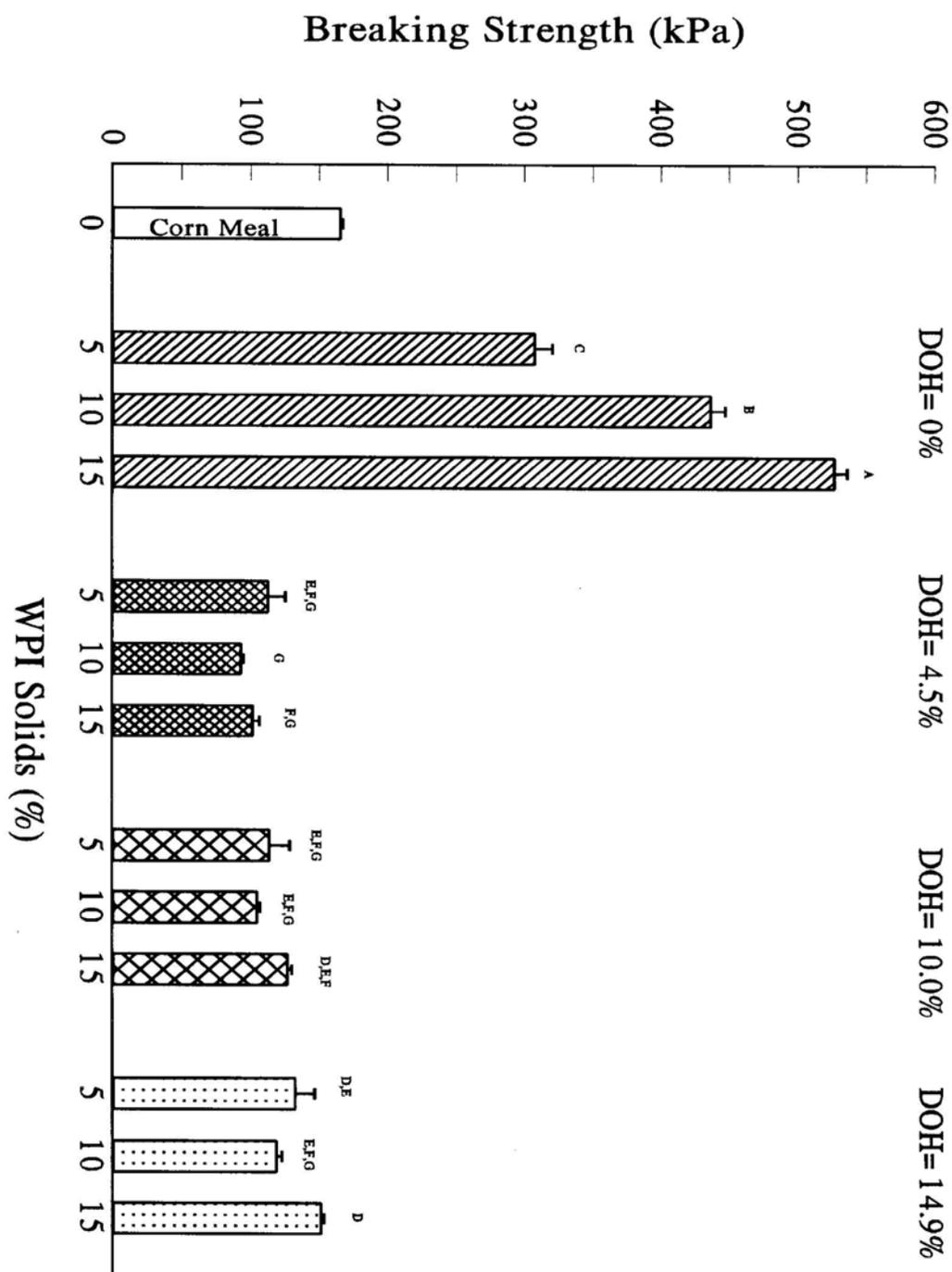
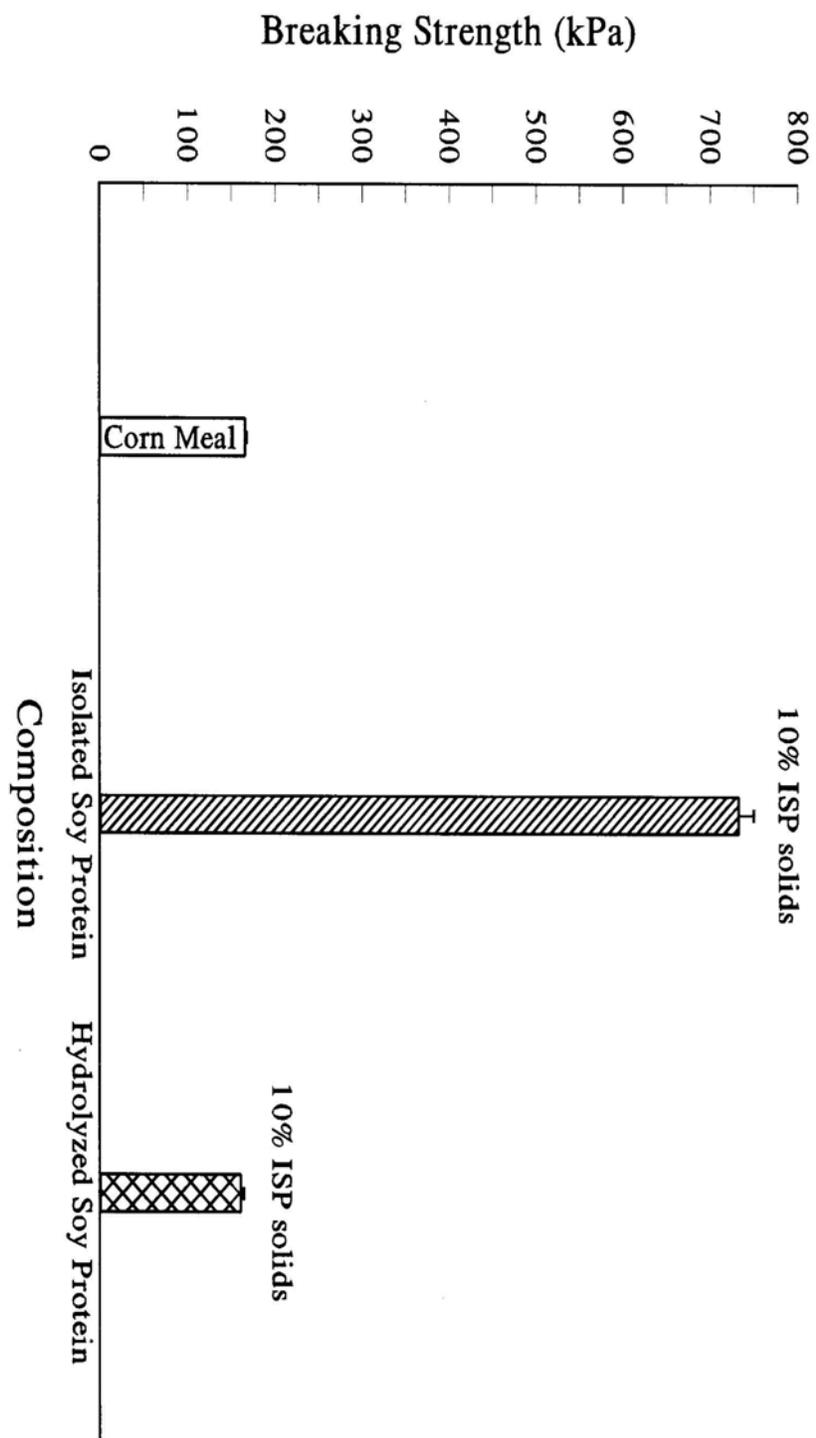


Figure 23. Breaking Strength of Soy Protein Extrudates



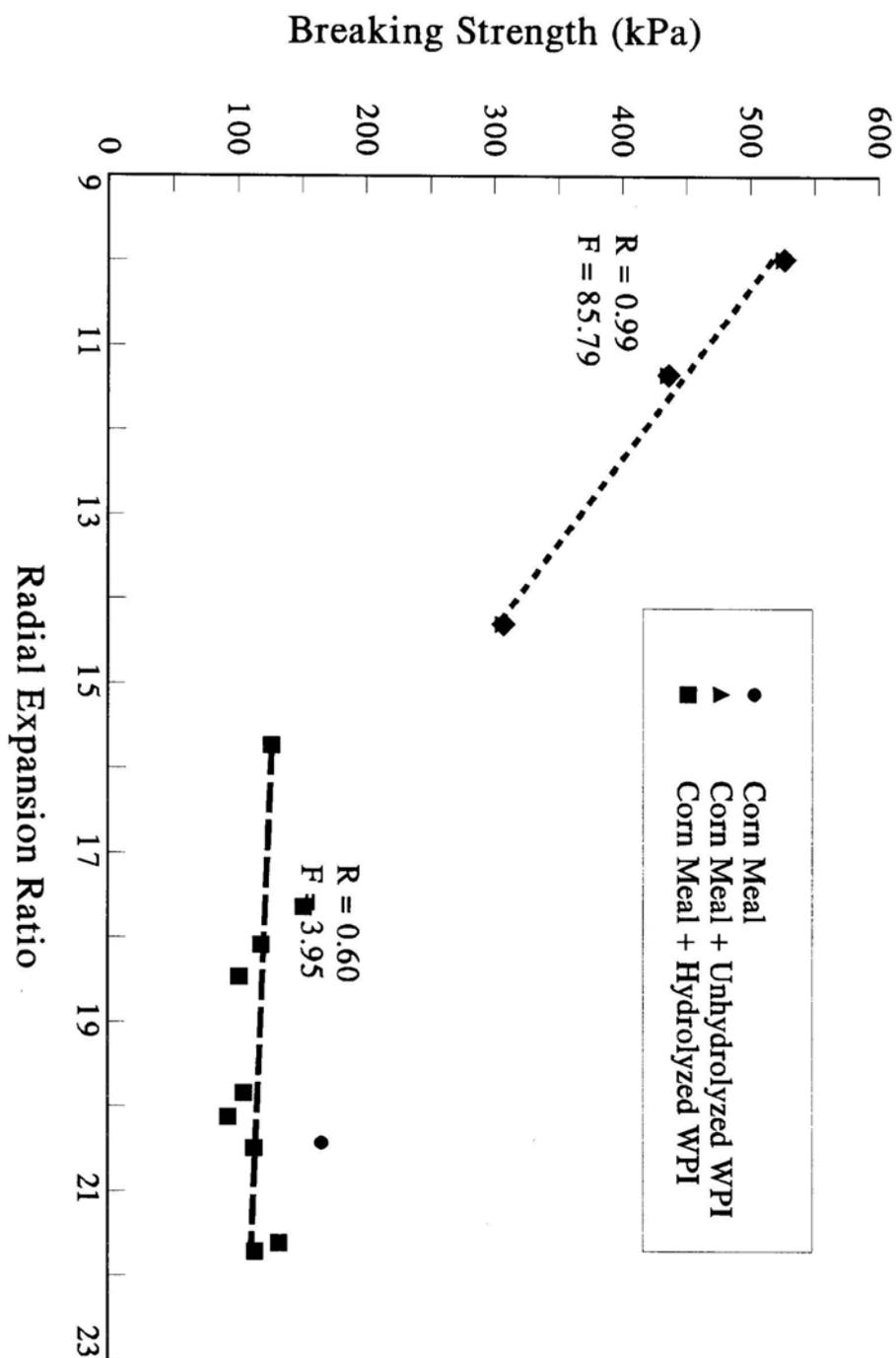


Figure 24. Breaking Strength and Radial Expansion of Whey Protein Extrudates

The addition of unhydrolyzed protein has a substantial effect on the breaking strength of the extrudates, even with the addition of 5% protein (Figure 22). The breaking strength value for corn meal samples was found to be 165.33 ± 1.52 kPa. The lowest percentage protein sample with the unhydrolyzed protein had a breaking strength of 307.3 ± 13.4 kPa and the highest percentage of protein a value of 525.7 ± 9.4 kPa. The addition of hydrolyzed protein had the opposite effect and differs significantly from the unhydrolyzed protein samples. The hydrolyzed samples all had breaking strengths values lower than that of the corn meal extrudate. This suggests a very significant change in the characteristics whey protein after hydrolysis, and suggests that this change depends on the presence of hydrolysis rather than the degree of hydrolysis. Previous studies with pink salmon have demonstrated similar effect on breaking strength at with high fish protein solids (Choudhury, Gogoi, & Oswalt, 1998). And this also agrees with an observation by Areas (1992) that the addition of protein to starch rich flours produces the usual "protein-type" extrudates that are harder and expand less.

Radial expansion of hydrolyzed whey protein had a much less significant effect on the breaking strength (Figure 24). As the radial expansion values decreased the breaking strength increased very minimally. The relationship between radial expansion ratio and breaking strength for the unhydrolyzed samples was a very strong negative linear correlation. As the values for radial expansion decreased the values for breaking strength increased dramatically. This suggests that the less the extrudates expand as they come out of the die the harder they are. But surprisingly this is not as true for the hydrolyzed protein samples. As they expand less they do increase some in breaking strength but not nearly as drastically.

The trend for breaking strength values in soy protein (Figure 23) was just as evident as with the whey protein. The hydrolyzed sample and the corn meal had similar values but the unhydrolyzed had a breaking strength value (~733 kPa) even higher than the highest of the whey protein samples (~525 kPa).

CHAPTER FIVE

Conclusions

Summary

There were many distinguishable differences found in the macroscopic characteristics of the extrudates. The apparent density of the unhydrolyzed proteins was much higher than that of corn meal and the majority of the hydrolyzed samples. The data for the true densities of the products shows that there is a decrease as more protein is added and as the degree of hydrolysis increases for the hydrolyzed proteins. The unhydrolyzed protein data collected was inconclusive and more experiments would need to be run to determine a reason for this finding.

Porosity was also shown to decrease as both the percentage of protein and of hydrolysis increased. However the opposite was true in the case of the isolated soy proteins, in this case the porosity increased with hydrolysis. Porosity and apparent density were found to have a negatively correlated linear relationship with the whey proteins, both hydrolyzed and unhydrolyzed.

Radial expansion ratio decreased drastically with the incorporation of unhydrolyzed whey proteins and increased to the level of corn meal with hydrolyzed proteins. This was also true for the isolated soy proteins. Axial expansion was found to decrease with the use of hydrolyzed whey proteins, and it increased with the use of hydrolyzed soy proteins. Overall expansion ratios showed significant decreases with the use of either type of whey proteins, especially at the 10% and 15% ratios. With the use of soy proteins this ratio decreased with the use of unhydrolyzed proteins. With the whey proteins apparent density and overall expansion ratio were found to have a negatively

linear relationship, all of which have a lower overall expansion and higher apparent density than corn meal. The overall expansion ratio and porosity have a direct linear relationship and corn meal has a higher overall expansion ratio and porosity than either of the whey proteins.

Breaking strength showed the most significant difference between the unhydrolyzed and hydrolyzed proteins. With the use of unhydrolyzed proteins the breaking strength went extremely higher than the corn meal, in many cases by two or three times. By adding hydrolyzed proteins the breaking strength of the extrudates went lower than that of corn meal. For unhydrolyzed proteins breaking strength and radial expansion ratio were found to have a negative linear relationship. With the use of hydrolyzed protein there is a non-directional linear relationship.

Conclusions

The addition of proteins into extruded snack foods has been avoided because of the detrimental effects to sensory characteristics. This study confirms what has been found in previous studies using other types of proteins. Many of the desirable characteristics of starch extrusion can be kept if the proteins incorporated are hydrolyzed proteins. Although the isolated soy proteins were only tested in a very general manner, they showed almost identical results to that of the whey proteins. The fact that this has been shown for more than just one type of protein gives hope for the opportunity of protein incorporation into low moisture expanded snack foods. More research does need to be done to determine the reasoning behind what is happening.

References

- Adler-Nissen, J. (1986). *Enzymatic hydrolysis of food proteins*. London and New York, NY: Elsevier Applied Science Publishers.
- Areas, J. A. G. (1992) Extrusion of food proteins. *Critical Reviews in Food Science and Nutrition*, 32 (4), 365-392.
- Choudhury, G. S., & Gautam, A. (2003) Hydrolyzed fish muscle as a modifier of rice flour extrudate characteristics. *Journal of Food Science*, 68(5), 1713-1721.
- Choudhury, G. S., Gogoi, B. K., & Oswalt, A. J. (1998) Twin-screw extrusion of pink salmon muscle and rice flour blends: effects of kneading elements. *Journal of Aquatic Food Products Technology*, 7(2), 69-91.
- Frokjaer S. (1994). Use of hydrolysates for protein supplementation. *Food Technology*, 48(10), 86-88.
- Loimaranta V., Laine M., Soderling E., Vasara E., Rokka S., Marnila P., Korhonen H., Tossavainen O., & Tenovuo J. (1999). Effects of bovine immune and non-immune whey preparations on the composition and pH response of human dental plaque. *European Journal of Oral Science*, 107(4), 244-250.
- Maga, J. A., & Reddy, T. (1985) Co-extrusion of carp (*Cyprinus carpio*) and rice flour. *Journal of Food Processing and Preservation*, 9, 121-128.
- Nelson, L. A., Colker, C. M., Kalman, D. S., & Swain, M. (2002). A double blind comparative pilot trial evaluating the effect of whey protein isolate and isolated soy protein in healthy adults. July 30, 2003.
<http://www.wheyoflife.org/news/soyvswhey.pdf>.

- Onwulata, C. I., Konstance, R. P., Smith, P. W., & Hilsinger, V. H. (1998) Physical Properties of extruded products as affected by cheese whey. *Journal of Food Science* 63(5), 814-818.
- Onwulata C. I., Smith P. W., & Konstance R. P. (2001) Incorporation of whey products in extruded corn, potato or rice snacks. *Food Research International*. (8), 679-687.
- Pins, J. J., & Keenan, J. M. (2002) The antihypertensive effects of a hydrolyzed whey protein isolate supplement (Biozate 1®). 11th International Congress of Cardiovascular Pharmacotherapy. Montreal, Quebec, Canada.
- Schmidl, M. K., Taylor, S. L., & Nordlee, J. A. (1994). Use of hydrolysates-based products in special medical diets. *Food Technology*, 48(10),77-85.
- Snack Food Association. (2000) State of the industry report 2000. *Snack Food & Wholesale Bakery*, 89(6), SI35-SI49.
- Snack Food Association. (2001) State of the industry report 2001. *Snackfood & Wholesale Bakery*, 90(6), SI37-SI49.
- Snack Food Association. (2002) State of the industry report 2002. *Snackfood & Wholesale Bakery*, 91(6), SI46-SI71.
- Trimetrex. (1992) *Axum Technical Graphics and Data Analysis*. 444 NE Ravenna Boulevard, Suit 10, Seattle, WA.
- Ziegler, F., Nitenberg, G., Coudray-Lucas, C., Lasser, P., Giboudeau, J., & Cynober, L. (1998). Pharmacokinetic assessment of an oligopeptide-based enteral formula in abdominal surgery patients. *American Journal of Clinical Nutrition*, 67, 124-128.