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I am submitting herewith a thesis written by Scotty A. Devine entitled "Function Properties of Lady Godiva (Cucurbita pepo) Pumpkin Seed Meal." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

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FUNCTION PROPERTIES OF LADY GODIVA (CUCURBITA PEPO) PUMPKIN SEED MEAL

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Scotty A. Devine

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ABSTRACT

The objective of this investigation was to analyze the chemical and functional properties of a flour made from the defatted meal of pumpkin seeds from the cultivar Lady Godiva (Cucurbita pepo L.) in order that its potential as a novel food source might be evaluated.

The proximate composition of two crop years (1978 and 1979) was determined. Defatted flour of pumpkin seed 1978 and 1979 had 8.91 and 4.56 percent moisture, 62.21 and 61.90 percent crude protein, 8.97 and 9.23 percent ash, 8.41 and 4.53 percent total lipid, 3.57 and 3.96 percent crude fiber, and 7.93 and 15.82 percent nitrogen-free extract.

The mineral content (K, Ca, Mg, Na, Fe, and Zn) of the two flours was determined by atomic absorption spectrophotometry. Calcium, iron, and potassium (279, 16, and 1500 mg/100g, respectively) in the pumpkin seed meals were found at levels comparable to the levels found in the soy reference. The levels of magnesium, sodium, and zinc (540.9, 144.9, and 22.9 mg/100g, respectively) in the pumpkin seed meal were found in much greater levels than in the soy concentrate (269, 6, and 6.5, respectively).

Nitrogen solubility profiles of the defatted pumpkin seed meals showed pumpkin 78 acquiring its isoelectric point at pH 3 and its maximum solubility at pH 10. Pumpkin 79 also acquired its isoelectric point at pH 3 and its maximum solubility was at pH 10.

Water holding capacity revealed pumpkin 79 capable of holding 4.85 grams water/gram flour, which was not significantly different (0.05 level) from the 4.47 grams water/gram flour that a soy concentrate (used as a reference in this study) was capable of holding.

The whippability value for pumpkin 79 (194.30 percent volume increase) was significantly different (0.01 level) from soy concentrate (118.66 percent volume increase). The foam produced by each of the flours was very stable.

Pumpkin 78 was found to be significantly lower than pumpkin 79 in water holding capacity and whippability. This difference was thought to be due to the longer storage time of pumpkin 78 and the initially higher lipid content of pumpkin 78. Thus it was not included in the comparison between pumpkin seed meal and soy concentrate on water holding capacity and whippability.

The mean value of oil holding capacity for pumpkin 78 and 79 was 4.26 grams oil/gram flour, and oil holding capacity for the concentrate was 3.05 grams oil/gram flour. The value for the pumpkin flour was significantly greater than the soy concentrate at the 0.01 level.

The results of emulsifying capacity (EC) showed pumpkin 78 and 79 were able to emulsify approximately the same amount of oil (67.14 ml oil/g flour and 67.85 ml oil/g flour, respectively). The soy concentrate was found to emulsify a significantly smaller amount of safflower oil than pumpkin 78 or 79. The EC for soy was 32.94 ml oil/g flour.

Heat stability of water extracts of both pumpkin seed meal and soy concentrate proved to be very stable even after one hour in boiling water. It is felt that the functional property of heat stability deserves further scrutiny.

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CHAPTER I

INTRODUCTION

In many developing countries (and developed countries) there is a substantial need to increase the food supply to keep pace with the population growth. Exploiting novel sources of food is one method of ameliorating the disparity between population and food resources (Betchart *et al.*, 1979).

In recent years pumpkin seeds have become a promising new source of food protein. The U.S.D.A., in 1972, released Lady Godiva, a naked-seeded pumpkin cultivar, and stated that yields of 1,450 kg/ha were high enough to allow production solely for the seeds (Robinson, 1975). Even though the protein content of the seed ranges between 30 and 35 percent, the major appeal of the Lady Godiva naked seed is its oil content, which ranges between 40 and 46 percent (Robinson, 1975). Nevertheless, the press cake remaining after oil extraction is much too valuable a protein source to be neglected.

Functional properties (nitrogen solubility, water-holding capacity, oil-holding capacity, whippability, foam stability, emulsification capacity, heat stability) and physical properties, rather than the nutritional value, of protein in protein-containing products will largely determine their acceptability as ingredients in prepared food (Johnson, 1970).

Because of the lack of reported information on the defatted pumpkin seed meal of *C. pepo* L. (Lady Godiva cultivar), this study was

undertaken to characterize the basic chemical composition of the defatted meal and the functional properties since these must be determined before the food potential of the crop can be evaluated.

CHAPTER II

LITERATURE REVIEW

I. REVIEW OF PUMPKINS

The Fourth FAO World Food Survey (FAO, 1978) indicated that food supplies in many developing countries would need to increase substantially in the future to keep pace with population growth as well as improve the nutritional status of the population. Exploiting novel sources of food is one method of ameliorating the disparity between population and food resources (Betschart *et al.*, 1979).

In recent years pumpkin seeds have become a promising new source of food protein. However, some difficulty has been incurred in removing the pericarp covering the cotyledons. Research into the problem of producing seeds devoid of exterior pericarp has produced the Lady Godiva cultivar of *Cucurbita pepo* L. However, the seeds still have a thin green intugument (seed coat) covering the cotyledons.

Curtis (1948) reported that the best experimental lines of naked-seeded squash (*Cucurbita pepo* L.) produced about 1,450 kg/ha as compared with about 400 kg/ha for unimproved genotypes. The U.S.D.A. released Lady Godiva, a naked-seeded pumpkin cultivar, in 1972 and stated that yields were high enough to allow production solely for the seeds (Robinson, 1975).

A review of the literature on the pumpkin itself is very interesting. All pumpkins and squashes are of the curcurbit family, *Curcurbitaceae*. In this family also are watermelons, cucumbers, muskmelons,

and gourds. Botanically, there is no distinction between "squash" and "pumpkin." Both pumpkin and squash varieties are found in the species Cucurbita pepo, Cucurbita moschata, C. maxima, and in at least one classification is in C. mixta (Seelig, 1970). The word "pumpkin" is a culinary term, so it is difficult to find a true definition of the word. Whitaker and Bohn (1950) suggested that "pumpkin" be defined "as the edible fruit of any species of Curcurbita utilized when ripe as forage, as a table vegetable or in pies; flesh somewhat coarse and/or strongly flavored; hence not generally served as a baked vegetable."

The name appears to come from the Greek "pepon" or "large melon" by way of the French which converts the word "pepon" to "popon" and then in nasalized form to "pompon." This eventually became "pumpion" and the ending was converted to "kin" in the American colonies and became "pumpkin" (Onions, et al., 1966).

The history of pumpkins and squashes reveals that Cucurbita pepo, C. moschata, and C. maxima are undoubtedly of American origin. Erwin (1931) stated that he was able to identify fragments of stems, seeds and fruits of C. pepo and C. moschata recovered from the cliff dweller ruins of the southwestern United States. With the help of archaeologists, Erwin determined that some of the material was from the basket makers, whose civilization antedates that of the cliff dwellers; indeed, they were probably the oldest agricultural people of whom there is any record on the North American continent. Varilov (1935), from Seelig (1970), believes that C. moschata originated in the Mexican-Central American region and that C. maxima originated in the Peruvian-Colombian-Ecuadorean area.

II. PUMPKIN PRODUCTION IN THE UNITED STATES

The states having the greatest acreage in pumpkins--whether for fresh market or processing--are Illinois, California, New Jersey, New York, Ohio, Michigan, Delaware, Pennsylvania, Indiana and Minnesota, with Illinois having more than twice the acreage of the second state, California (Anon., 1964). The growing conditions for pumpkins and squashes in these states are better suited than in most other states. Some of the growing conditions are: a well-drained soil, plenty of organic matter in the soil, ability of the soil to retain moisture during periods when rainfall is deficient, and a soil of medium texture is best for maximum yields. Pumpkins and squashes do best on soils that are slightly acid or nearly neutral; good yields are produced on some of the slightly alkaline soils of the West. Extremely acid soils should be avoided (Seelig, 1970). Pumpkins are also grown commercially in parts of Eastern Europe, Asia, Africa, South America, and China.

III. FUNCTIONAL PROPERTIES OF OIL SEED PROTEINS

Although there has been much literature reported on the pumpkin itself, there has been very little information reported on the seeds and the functional properties of the seed protein, such as nitrogen solubility, water absorption, fat absorption, emulsification, whippability, foam stability, and protein heat stability. It is these functional properties that will largely determine the acceptability of pumpkin seed protein as an ingredient in prepared foods.

There has been a considerable amount of information published on the functional properties of soybean, cottonseed, sunflower, safflower,

and rapeseed. Methods used to evaluate the functional properties of the various seeds listed are very similar and can be used to evaluate the functional properties of the pumpkin seed meal.

Nitrogen solubility or dispersibility of soy protein is a physiochemical property that is related to other functional properties and is, therefore, usually the first property to be studied in a systematic investigation of physical properties (Hutton and Campbell, 1977a; Mattil, 1971; Hermansson, 1973; Wu and Inglett, 1974). Generally as ionic strength, pH, and temperature increase, solubility increases. When particle size and processing decrease, solubility increases; these factors have been reported to affect the solubility of soy protein (Johnson, 1970; Anderson et al., 1973; Hermansson, 1973; Lin et al., 1974; Hermansson and Aksson, 1975). Nitrogen solubility profiles provide an indication of the potential application in liquid and beverage systems by looking at the solubility at the desired pH. It suggests general functional potential for properties dependent upon dispersion and/or solubility, and reflects the effects of processing conditions which partially or totally insolubilize the protein (Betschart et al., 1979).

The ability of soy protein and other vegetable proteins to bind water is attributed to the protein content, pH, temperature, and to a number of other factors (Hutton and Campbell, 1977a; Kilara et al., 1972; Hermansson and Aksson, 1975; Betschart et al., 1979). Wolf and Cowan (1971) reported the pH-water retention curve of soy protein followed the pH-solubility curve, and they found that both solubility and water retention were minimal at the isoelectric point (4.5) and increased as

the pH diverged from this point.Hermansson and Aksson (1975) reported that the increase in water-holding properties with the addition of salt is regarded as an effect of the binding of chloride ions to the structure-forming meat proteins. When chloride ions are bound to proteins at pHs above the isoelectric point, the net negative charge is increased, and thereby the repulsive forces, meaning that more water can be imbibed in the protein network. Lin et al. (1974) reported that the water absorption capacity of sunflower protein concentrates increased as the protein solubility index (PSI) became lower. The results of temperature effect showed that heat denaturation did not lower the water-imbibing capacities of sunflower proteins, but instead improved these properties. A similar effect was reported on soy proteins (Anon., 1964).

Childs and Park (1976) reported that by acylating glandless cottonseed flour with either succinic or acetic anhydride its water-holding capacity was increased greatly. Succinylated cottonseed flour bound 1.5 times more water than unmodified glandless flour, and acetylated flour bound greater than 2.0 times more water.

Carbohydrates are thought to be able to compete with protein for the available water (Hutton and Campbell, 1977a; Barman et al., 1977; Eastwood et al., 1976). Kilara et al. (1972) reported that sunflower samples absorbed water up to 40 percent of its seed weight, while rapeseed and soybean took up 60 percent. These variations were thought to be due to differences in type and quantity of carbohydrate material.

The ability of some foods to absorb fat improves their mouthfeel and flavor retention. Fat absorption data for soy protein products are

meager and the mechanism of fat absorption or binding has not been elucidated (Hutton and Campbell, 1977b). Even though data on fat absorption for soy protein is very minute, Lin et al. (1974) has reported data on fat absorption clearly showing that soy products have oil absorption values ranging from 84.4 - 154.5 percent of their weight at 14 percent moisture, while oil absorption values of sunflower products extended from 207.8 percent for the flour to 256.7 percent for the isolate. Contrary to the absorption of water, all sunflower products bound more oil than the soy products. In this regard, structurally, the sunflower proteins could be more lipophilic than the soy proteins. Although, the mechanism of fat absorption by proteins is unclear, as mentioned earlier, it seems likely that sunflower proteins contain numerous nonpolar side chains that have been believed to bind the paraffin chains (hydrocarbon chains) of fats (Przylecki et al., 1935), thereby contributing to higher absorption of oil.

Emulsifying capacity of proteins is very important in their use in foods, such as salad dressings and comminuted meat products (Hutton and Campbell, 1977b; Crenwelge et al., 1974). Soy protein is thought to play two roles in emulsification: 1) they aid in the formation of oil-in-water emulsions, and 2) they stabilize the emulsions once formed (Wolf and Cowan, 1971). Proteins lower surface tension and collect at oil-water interfaces (Hutton and Campbell, 1977b). A stabilizing effect of soy protein in emulsions, thus possibly, results from the protective barrier around fat droplets, preventing their coalescence (Wolf and Cowan, 1971; Lin et al., 1974).

Emulsification capacity of oilseed proteins has been reported to increase as protein solubility, pH, and protein concentration increase (Hutton and Campbell, 1977b; Betschart et al., 1979; Webb et al., 1970; Lin et al., 1974; Crenwelge et al., 1974; Franzen and Kinsella, 1976; Yasumatsu et al., 1972). Another factor that has been reported to influence emulsifying ability of water soluble proteins is the shape of the molecule. This was evidenced by a small change in emulsifying capacity corresponding to the slight change in shape of the protein as measured by viscosity (Carpenter and Saffle, 1965).

The methods used in determining emulsion inversion have been subject to a lack of precision. Several methods for determining the endpoint, or collapse of emulsions, have been employed. Carpenter and Saffle (1965), Pearson et al. (1965), and Christian and Saffle (1967) visually observed the abrupt decrease in viscosity associated with emulsion inversion. Electrical conductivity was used by Webb et al. (1970) and Crenwelge et al. (1974) to determine emulsion endpoints.

Functional properties which are related to surface characteristics include foaming capacity or whippability, fat binding capacity, and emulsification activity and stability (Betschart et al., 1979).

Foam capacity is useful in food systems which require aeration for textural, esthetic, and/or leaving purposes (Betschart et al., 1979; Yasumatsu et al., 1972; Lawhon et al., 1972).

Lin et al. (1974) reported that both sunflower flour and protein concentrate increased in whipping volume by about 230 percent, as compared to 70 percent for the soy flour and 1970 percent for one of the soy concentrates. However, the sunflower and soy protein isolates

had the same whippability, although the protein content of sunflower flour was about 35 percent lower than that of the isolate. The results indicate that constituents other than proteins may aid in the formation of whipped foam (Lin et al., 1974).

Foam stability is important since success of a whipping agent depends on its ability to maintain the whip as long as possible (Lin et al., 1974; Franzen and Kinsella, 1976).

Lin et al. (1974) also reported that soy flour and protein concentrates produced less stable foams than their sunflower counterparts. However, foams from both soy isolates were very stable.

Heat treatment is an important part of food processing and also of the production of protein concentrates and isolates (Hagerdal and Martins, 1976).

"Denaturation may be defined in general terms as any modification of the secondary, tertiary, or quaternary structure of the protein molecule, that does not break covalent bonds. A change in protein structure is usually associated with changes in physical-chemical and functional properties" (Wu and Inglett, 1974).

Pour-El and Peck (1973) studied a series of "native" defatted soy flakes which were subjected to both dry heat (130° C) and wet heat (steaming at 100° C) at different times, and upon analysis of protein solubility, by two accepted methods, found that wet heat had a harsher effect than dry heat. General functionality dropped with the length of treatment for either.

CHAPTER III

MATERIALS AND METHODS

I. SOURCE AND PREPARATION OF PUMPKIN SEED MEAL AND SOY PROTEIN CONCENTRATE

Pumpkin seeds from Cucurbita pepo L., Lady Godiva cultivar, were used in this study. The pumpkins were grown on the Plant Science farm in Knox County during the summers of 1978 and 1979. They were harvested in September 1978 and August 1979. The pumpkins were cut in half to remove the seeds which were rinsed in cold water, dried, placed in plastic bags, flushed with nitrogen, and then frozen until needed.

The pumpkin seed meal was prepared from the seeds harvested from each of the two years using the following procedure: 1. The seeds were ground in a Viking hammer mill while still frozen. This was done to prevent gumming in the mill, due to an excess release of oil from the seeds at room temperature. 2. The fat in the meal was extracted in a Soxhlet apparatus for 16 hours using hexane as the solvent. 3. The meal was desolventized in air and heated in a vacuum oven for 16 hours at 65 - 75° C, and reground in a Wiley mill to pass a U.S. 80-mesh screen (Kilara et al., 1972). The two meal types (1978 and 1979) were stored in plastic bags, flushed with nitrogen, and kept frozen at -17° C until evaluated.

The soy protein concentrate (≈70 percent protein) used as a reference was PROCON Soy Protein Concentrate, a product of A. E. Staley Mfg. Company.

II. CHEMICAL ANALYSES OF PUMPKIN SEED MEAL AND SOY PROTEIN CONCENTRATE

A. Determination of Proximate Analysis

1. Crude protein. Total nitrogen was determined by the modified Kjeldhal method (A.O.A.C., 1975). Six samples consisting of two grams each of pumpkin meal 1978 and 1979 and soy concentrate were analyzed for total nitrogen content. Percentage of crude protein for the pumpkin meal was calculated by multiplying percentage of nitrogen x 5.65 (Robinson, 1975), however, the crude protein for soy concentrate was calculated by multiplying percentage of nitrogen x 6.25 (Hutton and Campbell, 1977a). Results were on a dry weight basis.

2. Total lipid. Percentage of total lipid in the pumpkin meal and soy concentrate was determined by using a chloroform-methanol lipid extraction procedure (Melton *et al.*, 1979). Due to a limited supply of pumpkin seed meal and the large amount of sample required for each run, only three samples were conducted for each pumpkin meal and soy concentrate.

3. Moisture. Moisture content of the pumpkin seed meal and soy concentrate was determined by drying two gram samples in disposable aluminum pans in a vacuum oven at 65° - 75° C and 24 torr mercury overnight, cooled, and weighed (A.O.A.C., 1975). Nine samples of each pumpkin meal and soy concentrate were analyzed. Percentage of moisture was calculated.

4. Ash. Ash content was determined by ashing six, two gram samples of each pumpkin seed meal and soy concentrate in a muffle furnace at 550° C for four hours (A.O.A.C., 1975). Percentage ash was calculated after obtaining a constant weight.

5. Crude fiber. Crude fiber content of the 1978 and 1979 pumpkin seed meal and soy concentrate was determined by digesting two gram samples with one gram asbestos in 1.25 percent sulfuric acid solution, and then in 1.25 percent sodium hydroxide solution. The dried residue remaining after digestion was weighed and recorded, and then incinerated in a muffle furnace at 600° C for 30 minutes, cooled in a desiccator and reweighed (A.O.A.C., 1975). Three samples for each pumpkin seed meal and soy concentrate were analyzed.

6. Nitrogen free extract. The nitrogen free extract was determined by subtracting the percentage of crude protein, moisture, crude lipid, ash, and crude fiber from 100 percent (Post, 1979).

B. Mineral Determination

Defatted pumpkin seed meal (78 and 79) samples (1.000g) were weighed into clean, dry porcelain crucibles. The samples were then covered with concentrated (16N) nitric acid, and let stand for 30 minutes. This was to digest as much fiber as possible before incineration. A blank was prepared in the same manner. A Sybron/Thermolyne electric muffle furnace was used to incinerate the samples at 550° C for 3 hours, to constant weight. After cooling in a desiccator and weighing, the ash was dissolved in 5 ml of 12N HCl and diluted with 25 ml of deionized water (Post, 1979). The samples were then diluted further (if necessary) to bring the concentration of each element into suitable instrument reading range. Soy samples and a blank were prepared in the same manner as the pumpkin seed meal, except they were not digested in 16N nitric acid. The final dilutions contained about 0.75 percent (v/v) of 5 percent (w/v) lanthanum to overcome interference

created by some elements in atomic absorption spectrophotometry (AAS) analysis. The samples were then analyzed by AAS for the elements calcium, potassium, magnesium, sodium, iron, and zinc.

All analyses were performed with a Perkin-Elmer model 1500 atomic absorption spectrophotometer. A standard air-acetylene burner and single element hollow-cathode lamps were used for all the elements (Galvao et al., 1976). The settings for the instrument and other experimental conditions were in accordance with the manufacturer's specifications (Anon., 1968).

C. Determination of Functional Properties

1. Nitrogen solubility. Nitrogen solubility for the pumpkin seed meal 1978 and 1979 and soy concentrate was determined according to a modification of the procedure of Franzen and Kinsella (1976). Modification involved the use of 0.5 gram samples being added to 50 ml deionized water with varying pHs from 2-11, 0.1N NaOH or 0.1N HCl was used to adjust the pH. Each solution was mixed for 1 minute on a Flexa mix (Fisher) at a speed setting of 5. The pH of each solution was checked after 1 minute and readjusted to its original pH, and allowed to mix for five more minutes. The solutions were then centrifuged at 2000 x G for 10 minutes. Aliquots (2 ml) of the supernatant were diluted with 8 ml of a biuret reagent, shaken, and allowed to stand for 30 minutes. After 30 minutes, the absorbance of the solutions were read on a spectrophotometer (Hitachi model 100-60) at 550 nm against a deionized water/biuret reagent blank.

A standard curve of absorbance versus g soluble N either in the pumpkin seed meal or soy concentrate were preapred as follows. One

gram of each protein source was placed in a beaker with 50 ml of deionized water with the pH adjusted to the desired point (pH 10 for pumpkin seed meal and pH 11 for soy concentrate). The rest of the procedure was the same as in preparing the 1 percent solutions. From these 2 percent standard solutions, dilutions to 1.0, 0.5, 0.25, 0.125 and 0.0625 percent were made. Two ml aliquots of these solutions were mixed with 8 ml of biuret reagent and the absorbance of each solution (2-0.0625 percent) was read after 30 minutes. The grams of nitrogen in each 2 percent standard solution was determined by Kjeldahl analysis and each succeeding standard solution was assigned a nitrogen value one-half of the preceding one. Using linear regression ($y = mx + b$), the absorbance value of the three protein meals were translated into g soluble N. These values were then divided by the total amount protein N available for each respective meal and multiplied by 100.

The biuret reagent was prepared by dissolving 3 grams of sodium tartrate and 0.75 grams of copper sulfate ($CuSO_4$) in 250 ml of deionized water. This solution was then added to 150 ml of 10 percent aqueous NaOH with constant stirring, and the final volume was adjusted to 500 ml with deionized water.

2. Whippability and foam stability. Whippability and foam stability were measured according to the method of Lin *et al*. (1974). This was done by weighing a 6 gram sample into a stainless steel mixing bowl containing 200 ml of deionized water. The solid material was dispersed in water with a spatula and the suspension was whipped for 6 minutes using a food mixer (Scovill Hamilton Beach) at a speed set for whipping. Volumes were recorded before and after whipping in a

1000 ml graduated cylinder, and the percent volume increase due to whipping was calculated according to the method of Lawhon and Cater (1971) and Lawhon et al. (1972). After the total volume of whip was measured, the volume of foam in the standing cylinder also was recorded for foam stability studies at 1, 10, 30, and 60 minutes after whipping. Three replications were conducted for whippability and foam stability.

The percent volume increase on whipping was calculated by the following method:

$$\% \text{ volume increase} = \frac{\text{vol. after whipping} - \text{vol. before whipping}}{\text{vol. before whipping}} \times 100$$

All whipping was carried out on an as-is pH basis, where pumpkin seed meal pH = -6.5 and soy concentrate pH = 7.2.

3. Water holding capacity (WHC). The water holding capacity of the pumpkin seed meals and soy concentrate was determined by using a modification of the procedure of Childs and Park (1976). The modification involved the use of deionized water in the place of 0.02M citrate buffer.

The WHC was carried out by placing 0.1 gram sample in a dried, weighed 15 ml glass centrifuge tube, and then 5 ml of deionized water was added. The tube was then agitated on a vortex mixer, at a speed setting of 1, for 2 minutes, and then centrifuged for 15 minutes at 1000 x G. The supernatant was decanted and discarded. The pellet was weighed, and the weight of water bound per gram flour was calculated as water holding capacity.

Water bound per gram flour = (wt. of tube + sample after centrifuging) - (wt. of tube + sample before centrifuging) x 10

Three replications were conducted.

4. Oil holding capacity (OHC). Oil holding capacity was determined with the same procedure used in water holding capacity, except pure safflower oil (Hollywood Health Foods) was used instead of deionized water. The sample size was 0.1 gram. The difference in weight between the bound oil samples and meal or concentrate was calculated as OHC (Childs and Park, 1976).

Oil bound per gram flour = (wt. of tube + sample after centrifuging) - (wt. of tube + sample before centrifuging) x 10

There were three replications.

5. Emulsification capacity (EC). Emulsification capacity was determined by using the method of Webb et al. (1970) and Post (1979). A plastic blender jar of 250 ml capacity was used for EC determination. Two small holes (in opposing corners) were drilled through the bottom of the jar for the insertion of two copper electrodes. One large hole (near the center of the jar) was also drilled for the addition of oil.

A 1.0 gram sample was blended into 30 ml of 5 percent NaCl solution for 30 seconds at 12,000 rpm, or at Frappè on an Osterizer Galaxy blender. Then 20 ml of pure safflower oil (the same as in OHC) was added from a burette and blended for 30 seconds at 12,000 rpm. With continuous blending, oil was added at the initial rate of 2 ml/min until the emulsion broke. The emulsion was determined to be broken by observing a sudden sharp increase in the DC resistance being

measured by a volt-ohm meter (Micronta Multitester). The volume of oil was recorded at the emulsion breakpoint and calculated as emulsifying capacity (ml oil/gram sample).

6. Heat stability. The effect of temperature on the solubility of pumpkin seed meal from both years and soy concentrate was determined by preparing a 1 percent solution of each flour in deionized water at each flour's maximum solubility pH; pumpkin seed meal 78 and 79 at pH 10 and soy concentrate at pH 11. Each solution was stirred 1 minute with a magnetic stirring rod, after which the pH was checked to make any adjustment to maintain the original pH. The solutions were then allowed to stir for five more minutes.

Each solution was then poured into a separate centrifuge tube and centrifuged at 2000 x G for 10 minutes. The supernatant for each solution was decanted and poured into three test tubes to give a total of nine test tubes. The solutions were then heated in boiling water for 7.5, 15.0, 30.0 and 60 minutes. Change in the solubility due to heating was determined by the sight of any denatured protein precipitating out of solution.

IV. STATISTICAL ANALYSIS

All the data were averaged and means reported. The data for nitrogen solubility, water holding capacity, oil holding capacity, whippability, and emulsifying capacity were analyzed by orthogonal comparison using the Statistical Analysis System (SAS) (Barr and Goodnight, 1976) and the computer center of The University of Tennessee, Knoxville.

CHAPTER IV

RESULTS AND DISCUSSION

I. CHEMICAL ANALYSES OF PUMPKIN SEED MEAL AND SOY CONCENTRATE

After processing the pumpkin seeds from both years (1978 and 1979) to obtain a defatted meal, each had a slight green discoloration which was due to a thin green integument on the seeds. This green layer could probably be removed more efficiently by industry through a chemical or mechanical treatment. Nevertheless, all of the experiments in this study were carried out with meal which had the green discoloration.

The proximate composition for each defatted pumpkin meal and the soy concentrate is presented in Table 1 on a dry weight basis. Pumpkin 78 and pumpkin 79 had 8.91 and 4.56 percent moisture, 62.21 and 61.90 percent crude protein, 8.97 and 9.23 percent ash, 8.41 and 4.53 percent total lipid, 3.57 and 3.96 percent crude fiber, and 7.93 and 15.82 percent nitrogen free extract, respectively. Soy concentrate had 4.82 percent moisture, 69.90 percent crude protein, 5.87 percent ash, 0.67 percent total lipid, 2.93 percent crude fiber, and 15.81 percent nitrogen free extract. These respective values are in fairly close accordance with published values for pumpkin seed meal (Robinson, 1975; Adams and Richardson, 1977) and soy concentrate (Technical Data Sheet Staley Protein Division). When the results of the pumpkin seed meal are compared to those of soy, two factors (crude protein and fat)

Table 1

Composition of Defatted Pumpkin Seed Meal and Soy Concentrate

Description	Pumpkin 1978 Concentrate	Pumpkin 1979 Concentrate	Soy Concentrate
Moisture[a], %	8.91 ± 0.97	4.56 ± 0.25	4.82 ± 1.55
Protein[a], %	62.21 ± 2.30	61.90 ± 0.79	69.90 ± 3.52
Ash[a], %	8.97 ± 0.27	9.23 ± 0.07	5.87 ± 0.20
Total lipid[b], %	8.41 ± 0.41	4.53 ± 0.28	0.67 ± 0.01
Crude fiber[b], %	3.57 ± 0.53	3.96 ± 0.10	2.93 ± 0.03
Nitrogen-free[c] extract	7.93 ± 3.35	15.82 ± 1.15	15.81 ± 4.96

[a]Mean of nine samples

[b]Mean of three samples

[c]100-(Moisture + protein + ash + fat + fiber)

are of significant interest. Crude protein is of interest because it is a well-known fact that soy concentrate is a high protein source (≃70 percent) and defatted pumpkin seed meal is not far behind with approximately 62 percent protein, thus it can be added to the list of high protein vegetable products. The other factor of interest is the percent crude fat, which shows pumpkin seed meal with 8.41 and 4.53 percent total lipid while soy concentrate only contains 0.67 percent. It is obvious from these results that the soy concentrate had a much more efficient fat extraction method than the defatted pumpkin seed meal. This large amount of residual lipid in the defatted pumpkin seed meal could possibly cause it to become more unstable in storage than the soy concentrate.

The data for mineral analysis in Table 2 are presented in mg/100 g of flour on dry weight basis. The desired mineral analysis for soy PROCON 2000 was provided by the Technical Data Sheet from Staley Protein Division.

Calcium, iron, and potassium (279, 16, and 1500 mg/100 g, respectively) in the pumpkin seed meal were found at levels comparable to the levels found in the soy reference (285, 13.5, and 1450 mg/100 g, respectively), as stated by Staley Protein Division.

The levels of magnesium, sodium, and zinc (540.9, 144.9, and 22.9 mg/100 g, respectively) in the pumpkin seed meal were found in much greater levels than in the soy concentrate (269, 6, and 6.5 mg/100 g, respectively). The level of magnesium and zinc in the pumpkin seed meal may serve as a nutritional advantage over soy concentrate, but the high level of sodium (144.9 mg/100 g) may be a distinct disadvantage with present day food trends calling for reduced sodium intake.

Table 2

Mineral Determination Defatted Pumpkin Seed Meal 1978 and 1979 and Soy Concentrate

Element	Pumpkin 78[a] (mg/100g)[b]	Pumpkin 79[a] (mg/100g)[b]	Total Mean (mg/100g)[b]	Soy Concentrate[c] (mg/100g)
Calcium	290.45 ± 43.84	269.25 ± 26.97	279.85 ± 34.56	285
Iron	11.53 ± 1.71	20.85 ± 1.92	16.19 ± 5.36	13.5
Potassium	1248.81 ±117.84	1752.12 ± 91.95	1500.46 ± 291.43	1450
Magnesium	534.56 ± 31.09	547.28 ± 30.88	540.91 ± 28.57	269
Sodium	144.90 ± 6.42	145.69 ± 16.50	144.89 ± 11.23	6
Zinc	25.80 ± 1.13	20.05 ± 1.73	22.92 ± 4.41	6.5

[a]Mean value of nine observations

[b]Dry weight basis

[c]Values from Staley Protein Division for PROCON Soy Concentrate

II. DETERMINATION OF FUNCTIONAL PROPERTIES

1. Nitrogen Solubility

It has been reported that nitrogen solubility is a physiochemical property and is related to other functional properties, thus it was the first property to be tested (Hermansson *et al.*, 1974; Hutton and Campbell, 1977a; Lawhon and Cater, 1971).

Using the procedure of Franzen and Kinsella (1976), the solubility of soy concentrate and the two pumpkin concentrates (1978 and 1979) were analyzed at varying pH (2-11) with the spectrophotometer by reading absorbance at 550 nm of 1 percent solutions. From this test it was observed that soy protein concentrate (the reference in this study) obtained its maximum absorbance at pH 11 and decreased with decreasing pH, and obtained its minimum absorbance or its isoelectric point (IEP) at pH 4, but then increased in absorbance to the final pH of 2. This is in agreement with the literature on solubility of soy protein (Crenwelge *et al.*, 1974; Kilara *et al.*, 1972; Franzen and Kinsella, 1976). Pumpkin 78 and 79's maximum absorbance was found to be at pH 10. Both pumpkin 78 and 79's IEP occurred at pH 3, and increased absorbance was observed to the final pH of 2. From this information, the pH of soy's maximum absorbance (pH 11) and pH of both pumpkin meals (pH 10) were used to make 2 percent standard solutions. From the dilutions of the 2 percent standard solutions, straight lines were obtained, as would be expected by the Beer-Lambert law, when absorbance was plotted against concentration. By using these graphs and linear regression ($y = mx + b$), the percent soluble protein was determined for the three protein sources at varying pHs.

From Figure 1 it can be seen that both pumpkin 78 and 79 are very much alike, except at pH 7 where pumpkin 79 is significantly (0.05 level) more soluble than pumpkin 78. The nitrogen solubility curves show that pumpkin 78 and 79 and soy are very similar except at pHs 7, 9, and 10 where an orthogonal comparison shows a significant amount of difference between pumpkin and soy (refer to Table 3 for the specific amount of significant difference).

Some of the useful information that can be drawn from nitrogen solubility curves is the correlation between it and certain functional properties (discussed later), the ability to determine protein solubility at a specific pH, which allows one to determine the pHs necessary to extract proteins and the pHs necessary to precipitate the proteins to obtain an isolate, and nitrogen solubility curves can be used as an indication of protein quality, as traditionally done by the cottonseed processing industry (Lawhon and Cater, 1971). An attempt was made to make a pumpkin seed protein isolate using the above mentioned procedure, however, the yield was insufficient to produce a working amount of isolate.

The ability of proteins to imbibe water is an important functional property in the respect that it imparts moisture to many food products.

Water uptake by soy products has been attributed primarily to the protein content, but is also affected by a number of other factors, one of which is pH (Hutton and Campbell, 1977a). Wolf and Cowan (1971) showed that the pH-water retention curve of soy proteins followed the pH-solubility curve. The pH, however, was not taken into consideration in this study. Water holding capacity was observed under the as-is pH basis with deionized water.

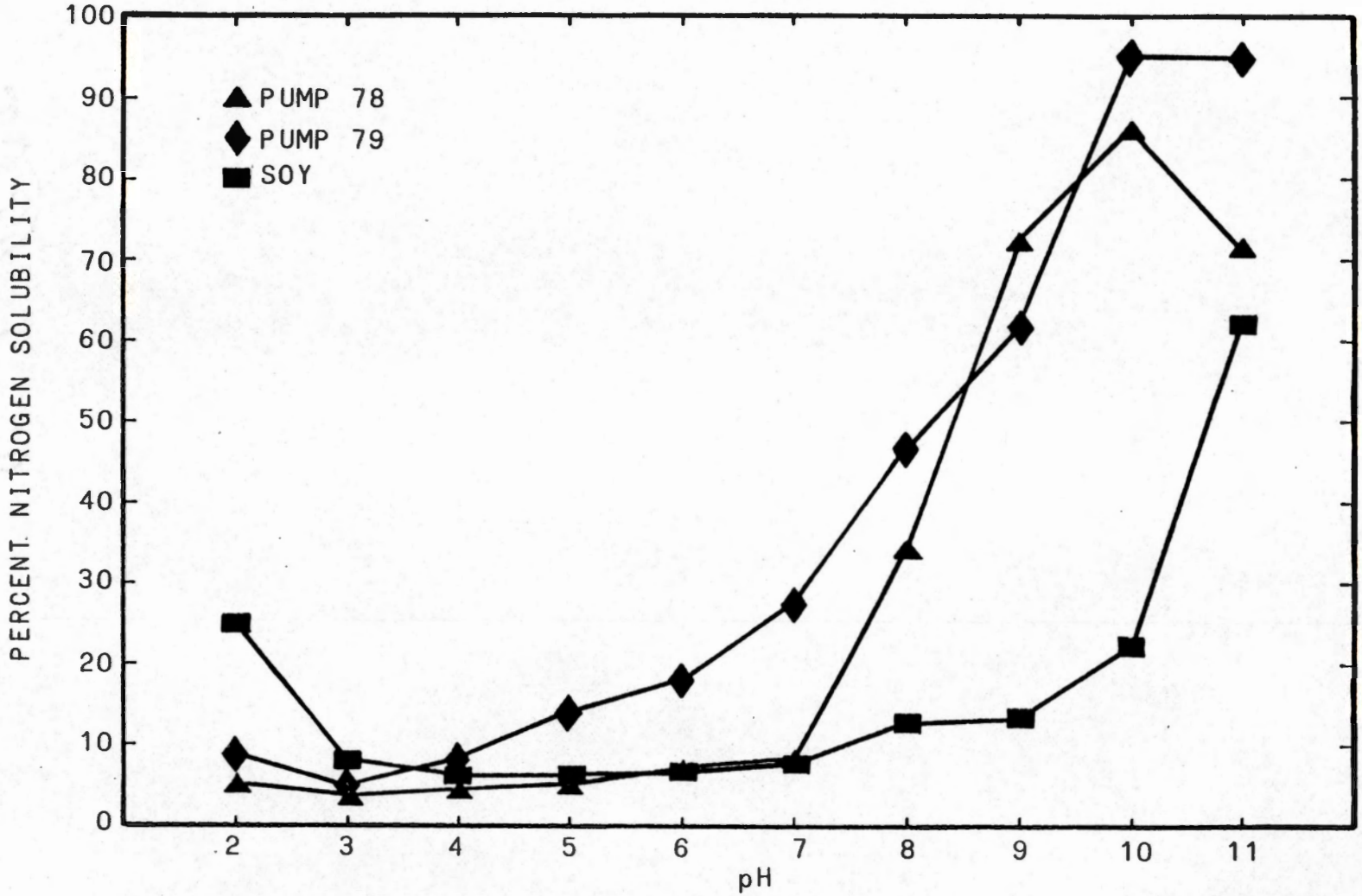

Figure 1

Percent Nitrogen Solubility of Pumpkin Seed Meal and Soy Concentrate at Varying pH

Table 3

F-Ratios of Orthogonal Comparison for Nitrogen Solubility of Pumpkin Seed Meal 78 and 79, and Soy Concentrate at Different pH Values

pH	Source	F-Ratios	pH	Source	F-Ratios
2	C1	0.31^{ns}	2	C2	9.81^{ns}
3	C1	0.04^{ns}	3	C2	0.48^{ns}
4	C1	1.60^{ns}	4	C2	0.00^{ns}
5	C1	6.03^{ns}	5	C2	1.05^{ns}
6	C1	13.41^{ns}	6	C2	5.10^{ns}
7	C1	51.61*	7	C2	19.55*
8	C1	0.83^{ns}	8	C2	5.59^{ns}
9	C1	1.58^{ns}	9	C2	53.98*
10	C1	2.68^{ns}	10	C2	197.42**
11	C1	3.10^{ns}	11	C2	9.02^{ns}

C1 = Pumpkin 78 compared to pumpkin 79

C2 = Pumpkin 78 + 79 compared to soy concentrate

*Significant at the 0.05 level

**Significant at the 0.01 level

^{ns}Not significant at the 0.05 level

The mean values of water holding capacity (WHC) for both pumpkins and soy concentrate are presented in Table 4. Pumpkin 79 ranked first in water holding capacity, binding 4.85 g of water per gram of meal at 4.56 percent moisture. Pumpkin 78 ranked last in water holding capacity, binding 3.61 g of water per gram of meal at 8.91 percent moisture. When pumpkin 78 and 79 were statistically analyzed for WHC (Table 5) there was a significant difference at the 0.01 level. It is felt that this significant difference was caused by the greater initial moisture content of pumpkin 78. Soy concentrate ranked second in water holding capacity, binding 4.47 g water per gram of concentrate. This is somewhat higher than the 3.5 g water per gram of concentrate reported in the Technical Data Sheet for PROCON. When the WHC values for pumpkin 78 and 79 were combined and statistically analyzed against the WHC value for soy, there was no significant difference as seen from the F-ratios for water holding capacity on Table 5.

From the literature on WHC, it is obvious that pumpkin seed meal has a better ability to bind water than sunflower (DE-90) at 2.03 g water/g flour or safflower at 1.84 g water/g isolate, and is comparable to soy concentrate (Lin et al., 1974; Betschart et al., 1979).

Data on whipping properties of pumpkin 1978 and 1979 and soy concentrate were expressed as volume increase due to whipping without addition of sugar (Table 6). The data on Table 6 are means of three replicates, which were carried out at as-is pH using deionized water. Both pumpkin 1979 and soy had whipping volume increases well over 100

Table 4

Mean Values for Water Holding Capacity, Oil Holding Capacity, and Emulsifying Capacity for Pumpkin Seed Meal and Soy[a] Concentrate

Type of Material	Water Holding Capacity Grams Water/Gram Flour	Oil Holding Capacity Grams Oil/Gram Flour	Emulsifying Capacity ml Oil/Gram Flour
Pumpkin Seed 1978 Concentrate	3.61 ± 0.37[b]	4.06 ± 0.25[bc]	67.14 ± 0.70[b]
Pumpkin Seed 1979 Concentrate	4.85 ± 0.12[c]	4.46 ± 0.42[b]	67.85 ± 3.19[b]
Soy Concentrate	4.47 ± 0.13[b]	3.05 ± 0.53[c]	32.94 ± 0.84[c]

[a]Mean of nine samples

[b,c]Means with the same letter in the same column are not significantly different at 0.05 level.

Table 5

F-Ratios of Orthogonal Comparison for Functional Properties of Pumpkin 78, Pumpkin 79 and Soy Concentrate

Source	D.F.	F-Ratios			
		Water Holding Capacity	Oil Holding Capacity	Whippability	Emulsification Capacity
C1[a]	1	36.45**	3.45[ns]	289.64**	0.19[ns]
C2[b]	1	1.80[ns]	42.96**	1.75[ns]	596.97**
Rep.	2	0.63[ns]	5.59[ns]	8.02*	0.85[ns]

[a]C1 = Pumpkin 78 compared to pumpkin 79

[b]C2 = Pumpkin 78 + 79 compared to soy concentrate

*Significant at the 0.05 level

**Significant at the 0.01 level

[ns]Not significant at the 0.05 level

Table 6

Mean Values of Whippability and Foam Stability[a]

Source	Vol. Increase on Whipping Percent	Volume of Foam after Whipping, ml			
		1 min	10 min	30 min	60 min
Pumpkin 1978 Concentrate	19.49[b]	44.47	43.33	40.00	29.00
Pumpking 1979 Concentrate	194.30[c]	496.33	450.67	431.00	399.67
Soy Concentrate	118.66[d]	301.33	279.00	270.67	263.00

[a]Mean of nine samples

[b,c,d]Means with the same letter in the same column are not significantly different at the 0.05 level

percent (194.30 percent and 118.66 percent, respectively), which is an indication of high whipping potential. However, pumpkin 1978 had a very low volume increase of 19.49 percent.

When these values were statistically analyzed, there was a significant difference at the 0.01 level between pumpkin 1978 and 1979, as seen from the F-ratios for whippability on Table 5. Yasumatsu et al. (1972) reported observing a large negative correlation coefficient between whipping properties and fat content. This could very well explain why pumpkin 78's whippability was significantly smaller (0.01 level) than pumpkin 79. Upon examining the lipid content of pumpkin 78 and 79 (Table 1) it can be seen that the lipid content of pumpkin 78 is almost twice that of pumpkin 79. There was a significant difference at the 0.05 level between the replications, however, the cause of this difference is not known. When the whippability values for pumpkin 78 and 79 were combined and statistically analyzed against soy, there was no significant difference, as seen from the F-ratios of Table 5. This is inevitably due to the low whipping volume of pumpkin 78.

Foam stability is important since success of a whipping agent depends on its ability to maintain the whip as long as possible (Lin et al., 1974). As seen in Table 6, foams from pumpkin 79 and soy were much larger than the foam from pumpkin 78, which would be expected from the whippability data. Nevertheless, all of the foams were very stable.

From the data obtained from the whippability study for pumpkin 79 and from the literature on whippability of other oilseeds (sunflower, 230 percent; soy, 170 percent; cottonseed, 438 percent;

rapeseed, 400 percent; and safflower, 400 percent) (Lin et al., 1974; Lawhon et al., 1972; Lawhon et al., 1971; and Hermansson et al., 1974), it is apparent that defatted pumpkin seed meal could possibly become a competetive whipping agent.

Oil holding capacity (OHC) is a functional property of food products that has been attributed to the protein content (Hutton and Campbell, 1977b). The absorption of some oil by food is reported to improve mouth feel and flavor retention (Kinsella, 1976).

The mean sources of oil holding capacity for all the sources used are presented in Table 4. Pumpkin 78 and 79 absorbed 4.06 and 4.46 g oil/f flour, respectively, while soy concentrate absorbed only 3.05 g oil/g flour which is in fairly close agreement with the Technical Data Sheet for PROCON at 2.5 g oil/g flour. All OHCs were done on the as-is moisture and pH basis.

When the OHC values for pumpkin 78 and 79 were statistically analyzed against soy concentrate, there was a significant difference at the 0.01 level, as can be seen from the F-ratios of Table 5. By reexamining the mean values of OHC from Table 4, it can be seen that pumpkin seed meal has the better oil holding capacity. After reviewing the literature on oil holding capacity, it appears that defatted pumpkin seed meal (79 crop at 446 percent) has a better oil holding capacity than sunflower concentrate at 254.9 percent, soy concentrate at 133 percent (Lin et al., 1974), soy concentrate at 305 percent in this study, and better than safflower isolate at 188 percent (Betschart et al., 1979).

The results on emulsification capacity (EC) (Table 4) are based on the amount (ml) of pure safflower oil emulsified in 30 ml of 5 percent

NaCl solution and 1 gram of meal on the as-is moisture basis. Pumpkin 78 and 79 were not significantly different (0.05 level) for EC (67.14 ml/g and 67.85 ml/g, respectively). Soy concentrate was found to emulsify a significantly smaller (0.01 level) amount of safflower oil than pumpkin 78 or 79. The EC for soy was 32.94 ml/g. Table 5 contains the statistical analysis for emulsification capacity.

Yasumatsu et al. (1972) reported that all emulsifying properties of soy correlated positively with the amount of dispersible nitrogen.

By examining the nitrogen solubility profile on Figure 1, it can be seen that pumpkin 78 and soy both share approximately the same solubility at or near neutrality, while pumpkin 79 is approximately 3 times more soluble at the same pH. The solubilities of pumpkin 78 and 79 are significantly different at pH 7, but yet are not significantly different in relation to EC. Pumpkin 78 and soy share the same solubility at pH 7, but are significantly different when related to emulsifying capacity. This would indicate that some other factor besides nitrogen solubility is causing the significant difference between pumpkin 78 and 79 and soy concentrate. Again it could be the initially higher lipid content of pumpkin 78 that is causing the difference. However, it does appear that pumpkin seed meal is a better emulsifying agent than the PROCON soy concentrate.

The data for protein heat stability is presented in Table 7 with a "+" sign indicating precipitation of denatured protein and a "-" sign indicating an absence of precipitate over the indicated time periods. As shown in Table 7, all of the protein extracts (2 percent meal wt/v) were stable even after one hour in boiling water.

Table 7

Protein Heat Stability of Pumpkin and Soy Extracts

Source	Time				
	7.5 min	15.0 min	22.5 min	30 min	60 min
Pumpkin 78	-	-	-	-	-
Pumpkin 79	-	-	-	-	-
Soy	-	-	-	-	-

+precipitate present

-precipitate absent

After reviewing the literature more closely, it is felt that Pour-El and Peck (1973), as quoted in Wu and Inglett (1974), have a more reliable method for studying heat stability than the one devised in this study. Pour-El and Peck's (1973) method entails the heating of a series of native defatted soy flakes with both dry heat (130° C) and wet heat (steaming at 100° C) for different times. The samples are then analyzed for protein solubility. By this method, it was found that wet heat had a harsher effect than dry heat. General functionality dropped with the length of treatment for either.

CHAPTER V

SUMMARY

Very little research has been conducted in analyzing the functional properties of defatted pumpkin seed meal. Consequently, this study was undertaken to analyze a flour made from a defatted pumpkin seed meal.

Pumpkin seeds from the cultivar Lady Godiva (Cucurbita pepo L.) were used in making a flour for each crop year of 1978 and 1979. A soy protein concentrate was used as a reference.

Water holding capacity revealed pumpkin 79 capable of holding 4.85 grams water/gram flour, which was not significantly different (0.05 level) from the 4.47 grams water/gram flour that the soy concentrate was capable of holding.

The whippability value for pumpkin 79 (194.30 percent volume increase) was significantly different (0.01 level) from soy concentrate (118.66 percent volume increase). The foam produced by each of the flours was very stable.

Pumpkin 78 was found to be significantly lower than pumpkin 79 in water holding capacity and whippability. This difference was thought to be due to the longer storage time of pumpkin 78 and the initially greater lipid content of pumpkin 78. Thus it was not included in the comparison between pumpkin seed meal and soy concentrate on water holding capacity and whippability.

The mean value of oil holding capacity for pumpkin 78 and 79 was 4.26 grams oil/gram and oil holding capacity for the concentrate

was 3.05 grams oil/gram flour. The value for the pumpkin flour was significantly greater than the soy concentrate at the 0.01 level.

The results of emulsifying capacity (EC) showed pumpkin 78 and 79 were able to emulsify approximately the same amount of oil (67.15 ml oil/g flour and 67.85 ml oil/g flour, respectively). The soy concentrate was found to emulsify a significantly smaller amount of safflower oil than pumpkin 78 or 79. The EC for soy was 32.94 ml oil/g flour.

Heat stability of water extracts of both pumpkin seed meal and soy concentrate proved to be very stable even after one hour in boiling water. It is felt that the functional property of heat stability deserves further scrutiny.

The mineral determination of the pumpkin seed meal found calcium, iron, and potassium at the levels of 279, 16, and 1500 mg/100 g, respectively. These were at levels comparable to the soy reference at 285, 13.5, and 1450 mg/100 g, respectively. The levels of magnesium, sodium, and zinc were 540.9, 144.9, and 22.9 mg/100 g, respectively for the pumpkin seed meal, which were in a greater abundance than in the soy concentrate (269, 6, and 6.5 mg/100 g, respectively). The level of magnesium and zinc in the pumpkin seed meal may serve as a nutritional advantage over soy concentrate, but the high level of sodium (144.9 mg/100 g) may be a distinct disadvantage with present day food trends calling for reduced sodium intake.

It has been reported by Johnson (1970) that the functional and physical properties, rather than the nutritional value, of protein in protein-containing products will largely determine their acceptability

as ingredients in prepared foods. From this study it has been shown that defatted pumpkin seed meal possesses both functional and physical properties equal to or greater than those of the widely used soy protein concentrate.

Defatted pumpkin seed meal may be a very competitive vegetable protein; however, to determine how an ingredient will function in a food system, it is necessary to incorporate that ingredient into a food formulation and produce the finished product.

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