THE UNIVERSITY OF MANITOBA

DETECTION AND ESTIMATION OF CEREAL AND OILSEED FLOURS IN WHEAT-BASED COMPOSITE FLOURS

by

ROBERTA GAIL THOMPSON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE

DEPARTMENT OF PLANT SCIENCE

WINNIPEG, MANITOBA

MAY, 1976

"DETECTION AND ESTIMATION OF CEREAL AND OILSEED FLOURS IN WHEAT-BASED COMPOSITE FLOURS"

by

ROBERTA GAIL THOMPSON

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT

Thompson, Roberta Gail, M.Sc., The University of Manitoba, May, 1976. Detection and Estimation of Cereal and Oilseed Flours in Wheat-Based Composite Flours.

Major Professor: Dr. W. Bushuk.

Some biochemical properties of corn, millet, sorghum, soybean and wheat flours were investigated in order to identify constituents that would be useful for detecting and estimating non-wheat flours in admixtures with wheat flour. Proximate analyses, amino acid analyses and protein solubility fractionations revealed significant differences in the compositions of these flours. Protein, fibre, ash, and certain amino acids (lysine, arginine, aspartic acid, glutamic acid, proline, alanine and leucine) were highly correlated with percent non-wheat flour in wheat-based composite flours. These parameters may be useful for estimating blend proportions once the types of flours present in a composite have been identified by more specific methods. Thin-layer isoelectric focusing in polyacrylamide gel (pH range 4-6) identified a protein zone specific to soybean flour. The densitometric area of this zone (pI 4.65) was directly proportional to percent soybean flour in soybean-wheat flour blends containing 0-30% soybean flour. Sugar analysis by ion-exchange chromatography indicated that soybean flour could be detected in wheat flour on the basis of its stachyose content. The proportion of stachyose in flour extracts was linearly related to the soybean flour content of soybean-wheat flour blends containing 0-100% soybean flour. Further investigation is required to identify constituents of corn, millet and sorghum flours that can be used to detect and estimate these cereal flours in wheat-based composite flours.

ACKNOWLEDGMENTS

I am pleased to have had Dr. Walter Bushuk as my advisor, and wish to acknowledge his direction in conceptualizing the project, his support during the research and his guidance in the preparation of this manuscript.

I also thank Drs. D. E. LaBerge and A. W. MacGregor of the Grain Research Laboratory, Canadian Grain Commission, for providing the facilities for sugar chromatography, nitrogen analyses and isoelectric focusing, and especially for their many helpful discussions during the course of this study.

The assistance of Dr. R. Rohringer, Canada Agriculture Research Station, Winnipeg, in obtaining densitometric measurements is gratefully acknowledged.

This study was supported by an operating grant from Canada Agriculture, Research Branch.

TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd.)

LIST OF FIGURES

LIST OF TABLES

I. INTRODUCTION

In 1964 the Food and Agriculture Organization of the United Nations initiated a composite flour research program. The aim of this project has been "to determine through intensive research whether it is possible to produce a wide range of acceptable, high quality, nutritious bakery, confectionary and paste goods, from flours and starches other than wheat that can be, or are being processed in the major wheat-importing developing countries. When this is achieved, and such products become part of an established dietary pattern, the potential savings in foreign exchange could be considerable. Further, the stimulation of local agriculture and industry, through creating a growing demand for substantial quantities of raw materials, should have a most beneficial effect on the economies of many countries now dependent on outside sources for one of their basic foodstuffs" (Asselbergs 1973).

As a result of this program numerous cereal, root and oilseed flours have been investigated as potential replacements for part or all of the wheat flour in bread, biscuits and pasta products. Most of the research on composite flours has been directed toward solving the technological problems of producing acceptable baked products from materials that do not form the viscoelastic doughs that are required for most baked goods. Other studies have been concerned with the marketing of composite flours and their products, and with improving nutritional quality through incorporation of high protein oilseed flours or protein isolates.

As composite flours become more widely used it will be necessary to be able to identify and quantitate various cereal and oilseed flours in admixture with wheat flour. The purpose of this study was to investigate some of the biochemical properties of several flours that have been used successfully

in the composite flour program (corn, millet, sorghum and soybean), and to identify biochemical characteristics that could be used to detect and estimate each of these flours in admixture with wheat flour.

II. LITERATURE REVIEW

A. General

Research on the analysis of composite flours has concentrated on two types of flour mixtures: firstly, determination of common wheat or barley flour in durum wheat semolina, and secondly, determination of soybean flour in wheat flour.

In France and Italy, the presence of any other cereal in durum semolina intended for pasta production is considered an adulteration. Therefore, considerable effort has been made to devise tests to detect non-durum components in semolina and pasta products.

One of the first tests for determining the amount of common wheat in durum semolina was proposed by Matveef (1952) and involved measuring sitosterol palmitate. The test was based on an observation by Walde and Mangels (1930) that a material, thought to be an ester of sitosterol, could be crystallized from ether extracts of common wheat flour but not from extracts of durum wheat flour. To further investigate Matveef's test, Gilles and Young (1964) used quantitative thin layer chromatography to separate and identify the sitosterol esters in common wheat, durum wheat, barley, rye, oats, corn and soybeans. Although palmitate, oleate, linoleate and linolenate esters of sitosterol were present in all samples, sitosterol palmitate was a major component of common wheat, barley and rye lipids, but was a minor component in durum wheat, corn, oats and soybean lipids. In extracts from mixtures of common and durum wheat, sitosterol palmitate increased linearly with the proportion of common wheat in the flour mixture. These authors concluded that the technique would be useful for detecting flours of other cereals in admixture with durum semolina.

However, subsequent analyses of numerous varieties of *Triticum aestivum* (common wheat) and *Triticum durum* indicated that the method was not reliable, as some varieties of common wheat contained amounts of sitosterol palmitate similar to those found in durum wheats (Gruener and Bernaerts 1968, Chindemi and Zampa 1969).

Another approach based on differences in lipid composition was proposed by Brogioni and Franconi (1963). They reported that infrared spectroscopy of acetone extracts could be used to quantitatively determine the proportion of common wheat in durum wheat products. Several investigators compared this method with the Gilles-Young modification of Matveef's sitosterol palmitate assay (Montefredine and Salvioni 1967, Salvioni 1968, Zampa and Chindemi 1968), and concluded that both tests were limited by varietal differences in lipid composition. Better results were obtained if both tests were used than with either method used alone.

Other investigators attacked the problem of detecting pasta adulteration using recently-developed techniques of protein chemistry, particularly gel electrophoresis. In 1967, Silano and co-workers reported a method for determining barley flour in durum wheat flour. The proteins in 90% aqueous ethanol extracts of durum wheat flour, barley flour and mixtures of the two cereals were separated by disc electrophoresis on polyacrylamide gel, and determined quantitatively by densitometry. The densitometric area of a protein band specific to barley was found to be directly proportional to the barley content of mixtures containing 2-20% barley flour.

Similarly, several studies showed that the water soluble proteins of common and durum wheats could be readily differentiated on the basis of their electrophoretic behaviour (Feillet and Bourdet 1967, Silano *et al.* 1968) and that this parameter could be used to detect common wheat flour in durum flour and pasta products if the proportion of common wheat was at least 10%

(Resmini 1968, Silano *et al.* 1968, D'Errico *et al.* 1969). However, the method proved difficult to quantitate due to varietal differences in the composition of the water soluble proteins (Garcia-Faure *et al.* 1969, Feillet *et al.* 1972). Modifications were required for use with pasta products dried at high temperatures (Cubadda and Resmini 1968, Resmini 1974) and the presence of eggs in pasta products interfered with this method (Feillet *et al.* 1972).

Recently, Feillet and Kobrehel (1974) demonstrated that estimation of a specific polyphenol oxidase could be used to determine common wheat in durum products. Water soluble proteins were fractionated by gel electrophoresis and then stained with catechol to detect polyphenol oxidase activity. Analysis of 17 varieties of durum wheat and 48 varieties of common wheat indicated that one of the polyphenol oxidase isozymes was specific to *T. aestivum*. This isozyme was determined quantitatively by densitometry, and was directly proportional to the common wheat content of pasta products over the range 3-50% common wheat. In addition, the assay was not disturbed by the drying temperature of pasta up to 70°, milling extraction rate, or the presence of eggs.

Several investigators have applied immunochemical techniques to the problem of detecting other cereals, especially common wheat and barley, in durum products (Cantagalli *et al.* 1969, Liuzzi and Angeletti 1969, Piazzi and Cantagalli 1969, Bracciali 1974). For example, Piazzi and Cantagalli separated the proteins in phosphate-buffered saline extracts of common and durum wheats by gel filtration, and identified two fractions in common wheat extracts that were not present in durum extracts. These fractions were isolated and used to prepare a rabbit antiserum to *T. aestivum* proteins. Cross-reacting antibodies were removed by adsorption to durum wheat proteins. When the purified antiserum was tested by immunodiffusion against extracts of 16 varieties of common wheats a single precipitin band formed, but no

reaction was observed with extracts of 16 varieties of durum wheat. About 5-10% common wheat flour in durum flours and pasta products could be detected by this method (Cantagalli *et al.* 1969, Piazzi and Cantagalli 1969). Using a similar technique, Liuzzi and Angeletti (1969) could detect 5% barley flour in durum wheat flour. Immunodiffusion is a highly specific qualitative test, but quantitative analysis of mixtures by this method has not been reported.

Although electrophoretic and immunochemical techniques have been used extensively to detect durum wheat pasta adulteration, these methods have not been applied to other types of flour mixtures. Several other methods have been proposed for determining soybean flour in cereal products.

In 1934, La Wall and Harrisson noted that increase in pH due to urease activity could be used to detect soybean flour in manufactured foods. Pomeranz (1953) developed a modification of the urease test for semi-quantitatively detecting 0.03-3.0% soybean flour in wheat flour, bread dough and macaroni. However, the test was unreliable with flours that had been heat treated above the temperature of urease inactivation. To avoid this problem, Pomeranz and Miller (1960) suggested a method based on the yellow fluorescence of soybean flour particles when viewed under ultra violet light at low magnification. As little as 0.01% soybean flour could be detected, and the proportion of soybean flour could be estimated by counting the fluorescent particles and comparing to standards of known composition.

In 1971, Ponte and DeStefanis reported that the soybean flour content of bakery ingredients and products could be estimated by determining the tetrasaccharide stachyose and its breakdown product manninotriose. The concentrations of these sugars were determined by quantitative thin layer chromatography and were directly proportional to the soybean flour content of mixtures containing 0.5-6% soybean flour.

The characteristic color of flours from several cereal grains and soybeans was used by Murthy and Dietz (1974) to evaluate the composition of flour blends. Using an Agtron reflectance spectrophotometer, they demonstrated that when two flours are blended the resultant color reading is directly related to the proportion in which the flours are mixed. The compositions of mixtures of common wheat flour with rye or soybean flour, and of durum wheat flour with whole egg solids or soybean flour could be accurately determined from color readings if standards were prepared from the same materials used to prepare the composite blends.

The above review of the literature indicates that several methods can be used to detect the presence of different cereals and soybeans in a flour mixture. Detection of specific proteins, by electrophoresis, immunodiffusion, or enzyme activity, appears to be the most promising analytical approach. Techniques based on differences in lipid or carbohydrate composition, color, or fluorescence can be useful.

The problem of quantitation is common to all of the proposed methods for analyzing composite flours. Accurate determination of one component in a blend can be achieved only if standards are prepared with the same ingredients as the composite. In certain situations, such as control of blending operations in a bakery, where a set of standards could be prepared for each new batch of ingredients, this may not be a severe disadvantage.

Quantitation is more difficult if the nature of the materials in a composite blend is not known. The exact amount of one component probably cannot be calculated accurately as a function of any biochemical characteristic, due to the variability of the characteristic within each species. However, if the range of values for a biochemical characteristic within a species can be established, then a reasonable estimate of the minimum and maximum content of a component in a mixture can be made.

B. Proximate Analyses

The compositions of corn, millet, sorghum, soybean and wheat flours have been reported by many investigators including Watt and Merrill (1963), Dimler (1969), Brekke (1970), Jones and Beckwith (1970), Jones *et al.*(1970), Wolf (1970), and Smith and Circle (1972). Some variability in the results for each species is evident, as chemical composition is affected by variety, and growing, storage and milling conditions. The values presented in Table I are representative of published proximate analyses of these materials. It is apparent from the data in Table I that the amounts of some of the components differ quite widely among species. Accordingly it should be possible to use these components to obtain an estimate of the amounts of different species in a composite blend after the species present have been identified.

Table 1. Published compositions of corn, millet, sorghum, defatted soybean and hard red spring wheat flours (%, dry basis).

	Protein[1]	Lipid	Fibre	Ash	Reference
Corn	8.1	3.0	0.8	0.9	Watt and Merrill 1963
Millet	11.4	4.9	0.7	-[2]	Jones and Beckwith 1970
Sorghum	9.8	1.3	0.4	0.6	Jones *et al.* 1970
Soybean	46.7	1.0	2.5	6.5	Watt and Merrill 1963
Wheat	11.8	1.1	0.3	0.4	Watt and Merrill 1963

[1] N x 5.7

[2] not determined

C. Amino Acid Analyses

1. Corn

The amino acid composition of a commercially available corn flour was reported by Ewart (1967), but unfortunately other analyses of corn flour are not available for comparison. Earlier analyses of corn grain (Wolfe and Fowden 1957, Bressani and Mertz 1958) do not agree with each other or with Ewart's data for flour. Wolfe and Fowden (1957) noted that considerable variations exist in the amino acid compositions of different varieties of corn, particularly in the values for arginine, histidine, lysine, threonine, valine, leucine and amide nitrogen.

2. Millet

Information on the amino acid composition of proso millet is limited. Complete analyses have been reported by Tkachuk and Irvine (1969) for whole seeds, and by Jones and co-workers (1970) for dehulled grain. The results of these two studies show excellent agreement.

3. Sorghum

The amino acid compositions of three sorghum hybrids and their dry-milled fractions were determined by Jones and Beckwith (1970). No significant differences were observed in the results for the three hybrids. Sorghum flour had essentially the same amino acid composition as the grain.

In a study of fifteen sorghum hybrids, Deyoe and Shellenberger (1965) showed that variety and environmental conditions significantly affect amino acid composition. The results of subsequent studies (Virupaksha and Sastry 1968, Skoch *et al.* 1970, Haikerwal and Mathieson 1971A) confirmed this finding, and are generally within the range of values reported by Deyoe and Shellenberger (1965).

4. Soybean

In 1961 Rackis reported the amino acid composition of dehulled, solvent extracted soybean meal. The results of this study are in general agreement with the data of Tkachuk and Irvine (1969) for defatted whole seeds, although large differences in the values for some amino acids, notably arginine and glutamic acid, are apparent. Partial analyses of defatted soybean flour reported by Inglett (1969) and Meyer (1969) agree with the studies by Rackis (1961) and Tkachuk and Irvine (1969).

5. Wheat

The amino acid composition of hard red spring wheat flour has been reported by many investigators, including Nunnikhoven and Bigwood (1959), McDermott and Pace (1960), Tkachuk (1966), Ewart (1967), and Tkachuk and Irvine (1969).

The results of these analyses are similar, despite sample differences in baking quality, genetic background, and the environmental conditions under which the wheat was grown.

D. Protein Solubility Fractionations

Solubility fractionations have been used to characterize plant proteins since Osborne (1907) first classified proteins according to their solubility in water, salt solutions, aqueous alcohol, and dilute acid or alkali. Ideally a classification scheme should be based on structural aspects, but, as present understanding of protein structure is inadequate for this purpose, classification on the basis of solubility is still used.

Unfortunately the usefulness of the solubility fractionation system is limited by the lack of distinct boundaries between the various solubility groups. Fractionation depends on rigorous control of experimental conditions, and consequently, the results of studies employing different conditions cannot be compared. However, useful information can be obtained from studies in which a single fractionation procedure is used to compare the proteins of different species or varieties.

Protein solubility fractionations have been used extensively to investigate the relationship between protein composition and the breadmaking quality of different cereals and of different varieties of wheat. Ewart (1968) reported that cereal flours that did not form viscoelastic doughs (oats, maize) were characterized by negligible amounts of acetic acid soluble protein and high proportions of insoluble residue protein after successive extractions with 0.04M sodium chloride, water, 70% ethanol and 0.1N acetic acid. In contrast, wheat flour contained the lowest proportion of insoluble residue protein and the highest proportion of glutelin of the flours analyzed. Ewart concluded that the breadmaking quality of cereals is related to the structure of the prolamine and glutelin proteins.

Chen and Bushuk (1970) compared the protein solubility characteristics

of hard red spring wheat, durum wheat, rye and triticale flours. The superior breadmaking quality of hard red spring wheat flour was attributed to low content of water soluble protein and high proportion of insoluble gluten protein compared to the other three species. The study also demonstrated that the solubility distribution of triticale proteins is intermediate between those of its durum wheat and rye parents.

The effect of wheat variety on protein solubility was examined by Orth and Bushuk (1972). Although intervarietal differences were found in all protein classes, the proportions of glutenin and insoluble residue protein showed the greatest variation. Glutenin content was negatively correlated with baking performance, while the proportion of residue protein was positively correlated. Tanaka and Bushuk (1972) showed that within a variety, the protein solubility pattern is independent of protein content.

Solubility fractionations of proteins from other cereals, such as corn and sorghum, have been used to determine the effect of breeding for increased protein, lysine content, or starch composition on protein composition. Boundy and co-workers (1967) fractionated the proteins of three types of corn to determine whether selection for high amylose or high amylopectin influences the quantity and kind of protein in the grain. The dent, waxy, and amylomaize hybrids used in the study were alike in all hereditary factors except the *ae* and *wx* genes which control the amylose and amylopectin contents of the starch. Although protein content varied from 11.0% in waxy corn to 15.3% in high amylose corn the proportion of total nitrogen extracted from the three corns was approximately the same. The solubility characteristics of the dent corn and amylomaize proteins were similar, but waxy maize contained a higher proportion of water and salt soluble proteins and a lower proportion of alcohol soluble proteins than the other two corn types.

Virupaksha and Sastry (1968) used solubility fractionation to compare

the protein composition of five varieties of sorghum grain of both high- and low-protein types. They concluded that the prolamines and glutelins are the major fractions, and that the higher protein content of some varieties is due to an increase in the prolamine fraction.

In contrast, Skoch and co-workers (1970) reported that the distribution of proteins in the soluble fractions was similar for five varieties of sorghum of high and low protein content, and that glutelin was the major fraction in each case. When compared with opague-2 corn protein, sorghum proteins were relatively insoluble. The fractionation procedure used extracted 86.1% of corn proteins, but only 26.4-40.0% of sorghum proteins.

Jones and Beckwith (1970) studied the proteins of three varieties of sorghum and found that the solubility of the prolamine fraction was highly dependent on temperature and on the nature of the alcohol used. At room temperature (ca. 20°), 60% ethanol dissolved only 3% of total protein, whereas 60% butanol at room temperature, or either of these alcohols at 60°, extracted 35-40% of total protein. This finding may explain the different conclusions reached by Skoch and co-workers (1970), who performed all extractions at room temperature, and Virupaksha and Sastry (1968), who extracted prolamines with alcohol at 65°.

Jones and co-workers (1970) fractionated millet proteins by the same method used for sorghum (Jones and Beckwith (1970). The solubility characteristics of the proteins of the two cereals were quite similar. Millet proteins were relatively insoluble in alcohols at room temperature, but solubility of the prolamines was not dependent on the type of alcohol used.

A variety of aqueous solvents has been used to extract proteins from defatted soybean meal, but in most studies only the percentage of total protein extracted by a single solvent is reported. Water, dilute alkali (pH 7-9) or aqueous solutions of sodium chloride (0.5-2M) solubilize 90-95%

of total protein (Smith and Circle 1938, Smith *et al.* 1938, 1966). The pH of the medium had a more dramatic effect on solubility than the nature of the solvent. Only about 10% of total protein can be extracted at pH 4-5, the isoelectric region for the major proteins (Smith and Circle 1938). As soybean proteins are dispersed so readily in most aqueous solutions, fractional extractions of the Osborne type have not been used.

The results of published protein solubility studies are difficult to compare because minor variations in the fractionation procedure can give rise to major differences in results. Accordingly, to make a valid comparison of the results of different studies it is necessary to establish that the fractionation procedures were identical.

E. Isoelectric Focusing

The technique for performing isoelectric focusing in columns of acrylamide gel was reported independently and almost simultaneously by several investigators, including Catsimpoolas (1968) using soybean whey proteins, and Wrigley (1968) using wheat albumins and gliadins. Isoelectric focusing of proteins from other cereals used in this study (corn, millet, sorghum) has not been reported. The method has been used to investigate genotypic variations in wheat proteins (Wrigley 1970, Wrigley and Shepherd 1973) and to isolate and characterize individual soybean whey proteins (Catsimpoolas 1969, Catsimpoolas and Meyer 1969).

Isoelectric focusing in thin layers of acrylamide gel was reported initially by Awdeh and Williamson (1968) and Leaback and Rutter (1968). The main advantage of this technique over isoelectric focusing in gel columns is that many samples can be focused simultaneously on the same gel under identical conditions. This greatly facilitates sample comparisons and simplifies gel preparation, staining, sectioning, pH determinations and photography.

A serious drawback of the original method was insufficient cooling of the gel layer. To prevent overheating the voltage had to be kept low, and consequently, long focusing times were required. Such conditions were conducive to denaturation of the proteins during the experiment. The technique was improved significantly by Vesterberg (1972) who designed an apparatus in which the gel layer rested on a glass plate cooled from beneath by water circulated from a thermostatically controlled bath. This allowed the use of higher voltage without overheating, and shortened the time required to obtain optimal resolution. Separations of cereal or soybean proteins by this technique have not been published.

The advantage of isoelectric focusing over most other separation techniques is that the zones become sharpened as the fractionation proceeds because the forces producing the separation minimize zone spreading due to diffusion. Fractionation of cereal and legume flour proteins by isoelectric focusing may reveal proteins specific to each species that can be used to identify the components of a composite flour. If such marker proteins can be determined quantitatively, an estimate of the proportion of each component in the blend can be made.

F. Sugar Analyses

Wide divergence exists in the results of sugar analyses of cereal grains and soybeans, both in regard to the quantity of specific sugars and the identity of sugars present. This variability arises from two major sources, the analytical methods used and the nature of the materials analyzed.

Variability in early work was primarily due to inadequate extraction procedures and the lack of reliable, precise techniques for quantitative analysis of individual carbohydrates. The advent of paper chromatography in 1951 and the subsequent development of new separation and identification techniques led to considerable progress in carbohydrate analysis. However, results obtained by different methods often differ quite substantially.

With the use of the more reliable techniques for sugar analysis, significant variations in sugar composition among species have become apparent. In addition, recent studies have shown that both variety within a species and environmental conditions influence the sugar composition of seeds (Tollier *et al.* 1968, deMan *et al.* 1975).

The sugar compositions of flours milled from corn, millet, sorghum and soybeans have not been published, therefore, analyses of whole seeds of these species will be discussed in this review.

1. Corn

Free sugars in the corn kernel range from 1-3% of dry matter with sucrose being the most predominant component (Taüfel *et al.* 1960). Raffinose, glucose and fructose have also been identified in corn extracts, but are present in much smaller amounts (Peat *et al.* 1954, Taüfel *et al.* 1960, Bond and Glass 1963).

2. Millet

The sugars of millet grain were investigated by Rakimbaev (1968) using paper chromatography. The major components are glucose, fructose and sucrose. Smaller amounts of rhamnose and galactose, and traces of maltose and raffinose were detected.

3. Sorghum

The free sugar content of mature sorghum grain ranges from 0.9 to 2.0% in normal varieties (Edwards and Curtis 1943) but sugary mutants may contain up to 3.9% sugar (Karper and Quinby 1963). Using paper chromatography, Nordin (1959) identified raffinose, stachyose, sucrose, fructose and glucose in grain sorghum extracts. Watson and Hirata (1960) established that sucrose was the most predominant sugar in all sorghum types. They confirmed the presence of the sugars reported by Nordin (1959), with the exception of stachyose, and in addition, detected maltose.

4. Soybeans

The free sugar content of soybeans varies widely with variety and environmental conditions during growth. Values from 1.6 to 13.3% of dry matter have been reported (Kawamura 1967, Hymowitz *et al.* 1972, deMan *et al.* 1975). The types of sugars present are also a function of variety. Although the major components are usually sucrose, raffinose and stachyose (Kawamura 1967, Hymowitz *et al.* 1972), it appears that only stachyose is present in all varieties. In a study of the sugar compositions of fifty-five varieties of Ontario soybeans, deMan and co-workers (1975) found that fructose was also common to all the varieties analyzed. Fructose has not been detected in any other investigation of soybean sugars. Other sugars which have been identified include melibiose in extracts of whole soybeans (deMan *et al.* 1975) and galactose and manninotriose in defatted meal (Delente and Ladenburg 1972).

5. Wheat

Wheat flour contains 1.4% to 2.6% free sugars (Vaisey and Unrau 1964, Audidier *et al.* 1966, Tollier *et al.* 1968), but the absolute amount is subject to varietal and environmental influences (Tollier *et al.* 1968). The sugars present in wheat flour have been determined by paper chromatography of 70-80% ethanol extracts by several investigators (Koch *et al.* 1951, Williams and Bevenue 1951, MacKenzie 1958, Taüfel *et al.* 1959, Vaisey and Unrau 1964). The largest component is an oligosaccharide fraction first called levosine by Tanret (1891). Vaisey and Unrau (1964) found that this fraction accounted for about 75% of the total ethanol soluble sugars in spring wheat flour. Koch *et al.* (1951) and Williams and Bevenue (1951) demonstrated that levosine was not a single chemical entity, as it could be resolved into 6 to 7 components by paper chromatography. On acid hydrolysis, these components yielded fructose and glucose in varying ratios (White and Secor 1953), suggesting that levosine is a mixture of glucofructans. Glucofructans, or fructosans, are now known to comprise a homologous series of oligosaccharides having increasing numbers of fructose residues with single terminal glucose residues.

Sucrose, raffinose, small amounts of maltose, fructose and glucose were also detected in these paper chromatographic studies. More recent analyses by ion-exchange chromatography have confirmed the presence of these sugars in wheat flour (Abou-Guendia and D'Appolonia 1972, D'Appolonia and MacArthur 1975). Abou-Guendia and D'Appolonia (1972) observed that the first two peaks eluted from the ion-exchange column yielded only fructose and glucose on acid hydrolysis, and concluded that these components corresponded to the glucofructans reported by earlier workers.

III. MATERIALS

The corn, millet and sorghum flours used in this study were commercial products supplied by Grain Process Enterprises Ltd., Scarborough, Ontario. These flours were milled on an experimental Palyi mill. Defatted soybean flour was obtained from Lauhoff Grain Company, Danville, Illinois. Commercial grade hard red spring wheat flour was supplied by Soo Line Mills (1969) Ltd., Winnipeg, Manitoba.

Each non-wheat flour was mixed with wheat flour at four levels, to give a series of composite flours containing 5, 10, 20 and 30% non-wheat flour (dry basis).

All other materials used in this study will be described in the Methods section of this thesis.

IV. METHODS

A. Proximate Analyses

1. Protein content

Determinations were made using the macro Kjeldahl procedure and the nitrogen to protein conversion factor 5.7 (A.A.C.C. 1962).

2. Lipid content

Analyses were made according to Method 14.018 of the Official Methods of Analysis of the Association of Official Analytical Chemists (A.O.A.C. 1975).

3. Fibre content

Crude fibre was determined according to Method 14.020 of A.O.A.C. (1975).

4. Ash content

Determinations were made according to the Approved Method 08-01 of the American Association of Cereal Chemists (A.A.C.C. 1962).

B. Amino Acid Analyses

Amino acid compositions of flours and blends were determined on a Beckman Model 121 automatic amino acid analyzer using the standard 4 hr procedure (Tkachuk 1966). The proteins were hydrolyzed under vacuum with 6N hydrochloric acid for 24 hr at 110°. Concentrations of individual amino acids were computed against a Beckman standard.

C. Protein Solubility Fractionation

Corn, millet, sorghum, soybean and wheat flour proteins were fractionated on the basis of their solubility using the modified Osborne fractionation described by Chen and Bushuk (1970). This procedure separates the proteins into five fractions: water soluble or albumins, salt soluble or globulins, alcohol soluble or prolamines, acetic acid soluble or glutenins, and an insoluble residue.

Nitrogen determinations were made on aliquots (1 to 10 ml) of each soluble fraction by the automated method of Mitcheson and Stowell (1970). The insoluble residues were freeze-dried and ground in a Krupp coffee grinder. Fifty to 100 mg portions were used for nitrogen determinations.

D. Isoelectric focusing

Isoelectric focusing was performed in a thin layer of polyacrylamide gel using the LKB Multiphor apparatus (LKB-Produkter AB, S-161 2S Bromma 1, Sweden).

1. Gel preparation

The gel solution was prepared by mixing 10 ml 40% acrylamide (BDH), 10 ml 0.9% N,N'-methylene bisacrylamide (BDH Aristar) dissolved in 36 ml distilled water, and 3 ml pH 4-6 Ampholine (LKB). The solution was deaerated by evacuation. Riboflavin (0.5 ml of a 0.004% solution) was added immediately before casting the gel. Polymerization was effected by placing the gel layer 10 cm from a fluorescent light for 18 hr.

2. Sample preparation

Two-g samples of wheat flour and soybean-wheat flour blends and 1-g samples of soybean flour were stirred with 10 ml distilled water at room temperature for 1 hr. The extracts were clarified by centrifugation (12,500 x g, 10 min, 20°) and used immediately for isoelectric focusing.

Twice recrystallized ovalbumin (Schwarz-Mann, Orangeburg, N.Y.) was used as an internal standard. The standard solution was prepared by dissolving 5 mg ovalbumin in 1 ml distilled water. Fifty µl portions were used for isoelectric focusing with the flour protein extracts.

3. Isoelectric focusing conditions

After polymerization, the gel was placed on a glass cooling plate maintained at 4°.

Samples (50 µl) were applied to the gel surface using Whatman No. 1 filter paper (2 layers 5 x 15 mm). The papers were placed about 5 mm from the cathode with their long axes perpendicular to the electrode.

The electrode solutions were 1M phosphoric acid at the anode and 1M sodium hydroxide at the cathode. The current was maintained at 14 mA for about 1.5 hr until the maximum voltage of 600 was achieved. Focusing was continued for an additional 2.5 hr, by which time the current decreased to about 8 mA.

4. Detection of protein zones

After focusing, the proteins were visualized by staining (Coomassie Brilliant Blue R250) and destaining the gel at room temperature using the solutions described by Vesterberg (1971). When the background stain was completely removed the gel was placed over a fluorescent light and photographed using a Polaroid 545 Land film holder with Polaroid 4x5 Land film, Type 55 P/N.

Densitometric evaluation of the negatives was performed using a Carl Zeiss Chromatogram Spectrophotometer (Zeiss, Oberkochen, W. Germany) equipped to record percent transmittance. The relative areas of densitographic peaks were obtained by multiplying peak height (percent transmittance) by peak width (mm) at one-half peak height.

5. Determination of the pH gradient

A 1-cm wide section was cut out of the gel immediately after the focusing experiment and divided into 2 mm segments. Each section was homogenized with 0.5 ml freshly boiled, distilled water and allowed to stand overnight. The homogenates were equilibrated to 4° in a thermostated bath and the pH of each was determined using a pH meter equipped with a microelectrode.

E. Sugar Analyses

Free sugars were extracted from the flour samples, separated and determined quantitatively as negatively-charged borate derivatives by anion-exchange chromatography on substituted polystyrene resin as described by LaBerge *et al.*(1973).

V. RESULTS AND DISCUSSION

A. Proximate Analyses

The proximate analysis of the flours was undertaken to characterize the materials used in the study and to determine whether there were any gross differences in their composition which would be useful in the analysis of flour blends. The results of the analyses, expressed as percent of dry matter, are presented in Table 2. These data are not directly comparable to the findings of other studies, as the chemical composition of a flour is affected by varietal and environmental factors, as well as milling conditions. However, with the exception of the lipid content of the corn and millet flours, the data fall within the range of values previously reported for these materials. (Bressani *et al.* 1962, Watt and Merrill 1963, Oke 1965, Dimler 1969, Jones and Beckwith 1970, Jones *et al.* 1970, Wolf 1970, Atlas of Nutritional Data 1971, Smith and Circle 1972).

1. Protein content

Protein content of the flours ranged from 10.1% of corn and millet flours to 53% of the defatted soybean flour. Wheat and sorghum flours had intermediate protein contents of 15.1% and 12.3% respectively.

2. Lipid content

Corn and millet flours were characterized by relatively high proportions of crude fat, 6.8% and 6.5% respectively. Very few analyses of corn and millet flours are available for comparison, but these results are significantly higher than the previously reported values of 3.0% for corn flour (Watt and Merrill 1963) and 4.9% for ground, dehulled proso millet (Jones *et al.* 1970).

In contrast to the high fat content of corn and millet flours, the

Table 2. Composition of corn, millet, sorghum, soybean and wheat flours (%, dry basis).

	Corn	Millet	Sorghum	Soybean	Wheat
Protein[1]	10.1	10.1	12.3	53.0	15.1
Lipid	6.8	6.5	2.0	1.1	1.2
Crude fibre	1.4	1.7	1.0	2.5	0.2
Ash	1.8	2.3	1.0	5.9	0.4

[1] N x 5.7

sorghum, soybean and wheat flours contained less than 2% lipid.

3. Fibre content

Fibre content ranged from 0.2% for wheat flour to 2.5% for soybean flour. The greater proportion of crude fibre in the non-wheat flours indicates less efficient removal of the bran or hull during the milling of these flours.

4. Ash content

The ash content of the cereal flours ranged from 0.4% of wheat to 2.3% of millet. The mineral content of soybean flour (5.9%) was markedly greater than that of the cereal flours. Like fibre content, ash content of the flours reflected varying degrees of milling efficiency.

Proximate analysis of the flour blends was undertaken to determine whether there were observable differences in chemical composition between wheat flour and the composite flours, and to determine whether the protein, lipid, ash and fibre contents of the composite flours were linearly related to the percentage of non-wheat flour in the blend.

The compositions of the flour blends are given in Tables 3 to 6. Although differences in chemical composition were evident, in most cases, at the lower blend levels, the non-specific nature of such data would limit its usefulness in the analysis of an unknown composite flour. The soybean-wheat blends were characterized by high protein contents, but the protein contents of the corn, millet and sorghum blends were within the range normally found in wheat flours. Differences were detectable in ash content in blends containing 5% non-wheat flour, and in fibre content at 20 to 30% non-wheat flour, but these parameters are extremely dependent on the milling of the flours. If used in the analysis of an unknown composite flour, proximate analysis could indicate only that the sample does not have the expected composition of a wheat flour.

The linear regression equation was used to determine the relationship

Table 3. Composition of corn-wheat flour blends (%, dry basis).

	Corn, %					
	0	5	10	20	30	100
Protein[1]	15.1	14.9	14.7	14.3	14.1	10.1
Lipid	1.2	1.1	1.1	1.0	1.9	6.8
Crude fibre	0.2	0.2	0.3	0.4	0.5	1.4
Ash	0.4	0.6	0.6	0.8	0.9	1.8

[1] N x 5.7

Table 4. Composition of millet-wheat flour blends (%, dry basis).

	Millet, %					
	0	5	10	20	30	100
Protein[1]	15.1	15.1	14.8	14.2	13.8	10.1
Lipid	1.2	1.2	1.1	1.1	1.0	6.5
Crude fibre	0.2	0.2	0.2	0.3	0.4	1.0
Ash	0.4	0.6	0.7	0.9	1.0	2.3

[1] N x 5.7

Table 5. Composition of sorghum-wheat flour blends (%, dry basis).

	Sorghum, %					
	0	5	10	20	30	100
Protein[1]	15.1	14.9	14.8	14.7	14.3	12.3
Lipid	1.2	1.2	1.1	1.1	1.0	2.0
Crude fibre	0.2	0.2	0.2	0.3	0.4	1.0
Ash	0.4	0.5	0.6	0.6	0.7	1.0

[1] N x 5.7

Table 6. Composition of soybean-wheat flour blends (%, dry basis).

	Soybean, %					
	0	5	10	20	30	100
Protein[1]	15.1	16.9	18.6	22.6	23.8	53.0
Lipid	1.2	1.2	1.2	1.2	1.2	1.1
Crude fibre	0.2	0.3	0.3	0.6	0.6	2.5
Ash	0.4	0.7	1.0	1.7	1.8	5.9

[1] N x 5.7

between percentage of non-wheat flour and each chemical component (protein, lipid, fibre, ash) for each type of composite flour. Correlation coefficients were calculated and are presented in Table 7. The data for protein, fibre and ash were significant at the .001 level (df = 5, $r \geq .951$) indicating that these components were accurately determined in wheat/non-wheat flour blends. However, lipid content was not so highly correlated with percentage of non-wheat flour in the blend. Blends containing higher fat contents than wheat (corn, millet, sorghum) appeared to have lipid contents lower than wheat flour (Tables 3-5). These results indicate that there was an interaction between the components of the blend which decreased the extractability of lipids from the composite flours. Further investigation is required to determine the nature of this interaction and to develop a method for accurately determining the lipid content of flour blends.

Table 7. Correlation coefficients for linear regression analysis of protein, lipid, fibre and ash content vs. % non-wheat flour.

	Non-wheat flour, 0-100%			
	Corn	Millet	Sorghum	Soybean
Protein	-.995	-.999	-.997	.997
Lipid	.975	.947(.01)	.881(.01)	-.957
Fibre	.998	.996	.996	.992
Ash	.995	.997	.965	.997

The above r values are significant at the .001 level except as otherwise noted in the table.

B. Amino Acid Analyses

The amino acid compositions of corn, millet, sorghum, soybean and wheat flours are presented in Table 8. The results are expressed as grams of amino acid per 100 grams of sample nitrogen. Tryptophan, cysteine, cystine, asparagine and glutamine were not determined, however, the ammonia released on hydrolysis of asparagine and glutamine is indicative of the amounts of these two amino acids. Nitrogen recovery for the five flours was similar, allowing comparison of their amino acid patterns.

Corn, millet and sorghum flours were very similar in amino acid composition. Compared to wheat, they were especially high in the hydrophobic amino acids alanine and leucine but had only half as much proline. These three flours contained more aspartic acid than wheat, but much less glutamic acid and amide nitrogen. Corn and millet were slightly richer than wheat in the basic amino acids lysine and arginine. The remaining amino acids did not vary appreciably in the four cereal flours.

Soybean flour was distinguishable from the cereal flours by its high proportion of basic amino acids, notably lysine and arginine. In addition, soybean flour had the highest aspartic acid content of all the samples. Compared to wheat, soybean flour was low in proline, glutamic acid and amide nitrogen.

Comparison of the data obtained in this study with previously published amino acid compositions was complicated by several factors. Different varieties or composite samples were used in each study, and within each species the samples analyzed varied widely in protein content. Whole seeds rather than flours were used in most studies of corn, millet and sorghum proteins, although a few analyses of dehulled or milled grain have been reported. In view of these differences in the materials analyzed it was

Table 8. Amino acid compositions of corn, millet, sorghum, soybean and wheat flours (g amino acid/100 g sample N).

	Corn	Millet	Sorghum	Soybean	Wheat
Lysine	17.6	14.3	11.6	40.5	13.2
Histidine	14.7	13.7	12.9	16.1	12.3
Ammonia	11.7	18.0	17.2	12.8	23.7
Arginine	28.4	26.1	18.3	46.0	20.0
Aspartic acid	40.4	40.7	42.6	75.9	26.9
Threonine	20.5	19.7	18.5	24.8	16.6
Serine	26.6	38.2	24.4	32.0	26.2
Glutamic acid	113.0	136.3	139.6	123.1	224.8
Proline	47.7	39.4	45.5	29.2	82.2
Glycine	23.0	18.3	18.5	26.7	21.8
Alanine	43.6	61.6	59.1	27.4	17.8
Valine	28.3	31.9	31.5	30.8	26.2
Methionine	9.6	11.9	9.1	6.7	7.9
Isoleucine	20.9	24.9	25.7	29.3	23.6
Leucine	71.4	72.3	89.7	50.2	44.3
Tyrosine	19.5	16.6	22.5	22.7	16.0
Phenylaline	27.4	32.4	32.6	30.3	32.0
N, % recovery	81.97	91.59	88.59	94.30	93.44

difficult to assess the significance of variations in their amino acid compositions. However, an attempt was made to relate the data of this study to the results of similar experiments reported in the literature.

The amino acid composition of corn flour determined in this study was in agreement with the microbiological analysis of whole kernels by Bressani and Mertz (1958) but not with other published analyses of corn grain (Wolfe and Fowden 1957, Atlas of Nutritional Data 1971) or Ewart's (1967) analysis of corn flour.

The data for millet flour was compared with that reported for ground whole seeds (Tkachuk and Irvine 1969, Jones *et al.* 1970, Atlas of Nutritional Data 1971). The values for lysine, arginine and glycine found in this study were significantly higher than those previously reported, while values for tyrosine and methionine were lower.

The amino acid composition of sorghum flour was within the range of values reported for whole grain by Deyoe and Shellenberger (1965), Virupaksha and Sastry (1968), Jones and Beckwith (1970) and Skoch *et al.* (1970), but did not agree with the composition given by the Atlas of Nutritional Data (1971). Jones and Beckwith (1970) also determined the amino acid composition of sorghum flour, but they reported significantly lower amounts of the basic amino acids and methionine than were found in this study.

The data for soybean flour agreed with previously reported partial analyses of defatted soy flour (Inglett 1969, Meyer 1969) as well as with analyses of defatted whole seeds (Tkachuk 1969) and dehulled, solvent extracted whole meal (Rackis 1961). However, many discrepancies are apparent on comparing the results of this study to the partial analysis of solvent extracted soybean flour reported in the Atlas of Nutritional Data (1971).

The amino acid composition of the wheat flour used in this study was consistent with analyses of hard red spring wheat flours reported by other

workers. (Nunnikhoven and Bigwood 1959, McDermott and Pace 1960, Tkachuk 1966, Ewart 1967, Tkachuk and Irvine 1969).

Amino acid analyses of the flour blends were undertaken to determine whether differences in amino acid composition were observable at the levels of non-wheat flour used in this study, and to determine whether the proportions of individual amino acids were linearly related to the percentage of non-wheat flour in the blends.

The amino acid compositions of the flour blends are presented in Tables 9-12. For blends of corn, millet and sorghum flours with wheat flour, the amino acids that reflect the changing blend proportions most markedly are aspartic acid, glutamic acid, proline, alanine and leucine.

For soybean-wheat flour blends the significant amino acids are lysine, arginine, aspartic acid, glutamic acid and proline. In most cases, changes in the levels of these amino acids, in excess of the error of analysis, are observable even at the level of 5% non-wheat flour.

To determine whether the proportions of these key amino acids were directly related to the percentage of non-wheat flour in the blend, the data were analyzed using the linear regression equation. Correlation coefficients were calculated and are presented in Table 13. In each case the correlation was significant at the .01 level ($df = 5$, $r \geq .874$).

Table 9. Amino acid compositions of corn-wheat flour blends (g amino acid/100 g sample N).

	Corn, %					
	0	5	10	20	30	100
Lysine	13.2	13.2	13.1	13.8	13.8	17.6
Histidine	12.3	12.6	12.8	13.1	13.2	14.7
Ammonia	23.7	23.0	21.9	21.7	20.9	11.7
Arginine	20.0	22.3	23.0	23.0	23.1	28.4
Aspartic acid	26.9	25.9	27.2	27.2	32.2	40.4
Threonine	16.6	16.8	16.7	16.9	17.2	20.5
Serine	26.2	28.9	28.7	28.4	28.4	26.6
Glutamic acid	224.8	229.0	222.0	207.8	199.9	113.0
Proline	82.2	70.4	69.0	68.1	64.9	47.7
Glycine	21.8	20.9	20.8	20.7	21.0	23.0
Alanine	17.8	18.0	19.0	20.7	23.2	43.6
Valine	26.2	24.1	24.3	24.1	24.5	28.3
Methionine	7.9	9.5	6.9	8.5	3.1	9.6
Isoleucine	23.6	21.3	21.0	20.4	20.9	20.9
Leucine	44.3	43.6	44.5	45.8	48.2	71.4
Tyrosine	16.0	17.5	17.9	16.9	17.4	19.5
Phenylaline	32.0	30.7	30.8	28.7	29.2	27.4
N, % recovery	93.44	92.36	90.95	89.72	89.01	81.97

Table 10. Amino acid compositions of millet-wheat flour blends (g amino acid/100 g sample N).

	Millet, %					
	0	5	10	20	30	100
Lysine	13.2	12.3	12.4	12.7	12.7	14.3
Histidine	12.3	12.3	12.5	12.7	12.7	13.7
Ammonia	23.7	24.7	24.4	24.1	23.3	18.0
Arginine	20.0	21.1	21.3	22.4	23.5	26.1
Aspartic acid	26.9	24.3	27.6	26.6	28.5	40.7
Threonine	16.6	16.0	16.1	16.7	17.0	19.7
Serine	26.2	28.0	28.2	29.6	30.3	38.2
Glutamic acid	224.8	220.2	215.6	212.7	204.4	136.3
Proline	82.2	70.0	67.1	77.3	61.6	39.4
Glycine	21.8	20.0	19.9	20.0	19.9	18.3
Alanine	17.8	18.2	20.0	23.8	27.0	61.6
Valine	26.2	23.4	23.8	25.2	25.5	31.9
Methionine	7.9	9.0	5.5	9.3	8.3	11.9
Isoleucine	23.6	21.0	20.9	21.6	21.3	24.9
Leucine	44.3	42.2	42.7	46.2	47.8	72.3
Tyrosine	16.0	16.4	16.8	17.3	17.8	16.6
Phenylalanine	32.0	26.7	29.5	30.4	30.3	32.4
N, % recovery	93.44	91.00	90.74	93.76	91.70	91.59

Table 11. Amino acid compositions of sorghum-wheat flour blends (g amino acid/100 g sample N).

	Sorghum, %					
	0	5	10	20	30	100
Lysine	13.2	12.4	12.6	12.6	12.0	11.6
Histidine	12.3	12.5	12.7	12.6	12.6	12.9
Ammonia	23.7	24.1	24.1	24.0	23.2	17.2
Arginine	20.0	21.2	22.0	21.3	21.0	18.3
Aspartic acid	26.9	25.1	26.2	26.7	28.5	42.6
Threonine	16.6	16.4	16.6	16.5	16.9	18.5
Serine	26.2	28.2	28.8	28.2	28.2	24.4
Glutamic acid	224.8	211.5	224.5	216.2	203.4	139.6
Proline	82.2	70.3	69.3	69.1	64.5	45.5
Glycine	21.8	20.2	20.7	20.1	19.6	18.5
Alanine	17.8	19.0	20.4	22.4	27.8	59.1
Valine	26.2	24.2	24.8	24.8	25.1	31.5
Methionine	7.9	9.1	8.2	8.9	8.5	9.1
Isoleucine	23.6	21.0	21.7	21.3	21.6	25.7
Leucine	44.3	43.7	45.5	46.9	53.2	89.7
Tyrosine	16.0	17.0	17.9	17.5	19.0	22.5
Phenylalanine	23.0	30.3	30.8	30.3	30.2	32.6
N, % recovery	93.44	90.70	93.12	92.19	91.39	88.59

Table 12. Amino acid compositions of soybean-wheat flour blends (g amino acid/100 g sample N).

	Soybean, %					
	0	5	10	20	30	100
Lysine	13.2	16.1	20.1	25.5	26.4	40.5
Histidine	12.3	12.8	13.7	14.5	14.3	16.1
Ammonia	23.7	22.8	21.9	20.0	18.0	12.8
Arginine	20.0	24.7	28.5	33.5	34.5	46.0
Aspartic acid	26.9	30.8	38.6	48.6	50.5	75.9
Threonine	16.6	17.1	18.4	20.5	21.2	24.8
Serine	26.2	28.6	29.7	30.7	30.8	32.0
Glutamic acid	224.8	210.4	198.5	161.9	168.5	123.1
Proline	82.2	68.1	71.6	61.0	52.7	29.2
Glycine	21.8	21.0	22.1	23.4	23.7	26.7
Alanine	17.8	18.3	19.8	21.9	22.2	27.4
Valine	26.2	24.5	25.9	27.2	27.6	30.8
Methionine	7.9	8.5	8.6	8.2	8.1	6.7
Isoleucine	23.6	21.6	22.8	24.3	25.0	29.3
Leucine	44.3	41.7	43.4	45.5	46.0	50.2
Tyrosine	16.0	17.3	18.2	18.9	19.7	22.7
Phenylalanine	32.0	29.9	30.8	31.4	31.4	30.3
N, % recovery	93.44	91.77	94.76	93.96	92.89	94.30

Table 13. Correlation coefficients for linear regression analysis of amino acids vs. % non-wheat flour.

	Non-wheat flour, 0-100%			
	Corn	Millet	Sorghum	Soybean
Lysine	-	-	-	.960
Arginine	-	-	-	.931
Aspartic acid	.956	.969	.978	.960
Glutamic acid	-.996	-.996	-.979	-.904
Proline	-.936	-.923	-.949	-.953
Alanine	.995	.996	.996	-
Leucine	.986	.985	.991	-

C. Protein Solubility Fractionations

The results of the modified Osborne fractionation of corn, millet sorghum, soybean and wheat flour proteins are presented in Table 14 and Figure 1. The data are expressed as percent of recovered nitrogen. As indicated in Table 14, nitrogen recovery was similar for the five flours, so comparison of their solubility patterns is justifiable. However, these results are not directly comparable with the findings of other protein solubility fractionations reported in the literature as different experimental conditions were used in each case (Boundy *et al.* 1967, Ewart 1968, Virupaksha and Sastry 1968, Jones and Beckwith 1970, Jones *et al.* 1970, Skoch *et al.* 1970, Haikerwal and Mathieson 1971B).

1. Water soluble fraction (albumins)

The water soluble fraction (albumins) represented a similar proportion of the total recovered nitrogen in each of the four cereal flours. This fraction accounted for 6.1% of sorghum protein and about 9.5% of corn and millet proteins. Thirteen percent of the wheat flour nitrogen was water soluble. This agrees with Chen and Bushuk (1970), who, using the same fractionation method, reported 11.9% albumin in Manitou wheat flour, but is significantly higher than the range of values for 26 varieties reported by Orth and Bushuk (1972).

The water soluble protein content of soybean flour was not determined. In the fractionation procedure used, the water soluble fraction is obtained by dialyzing the initial salt extract against distilled water and removing the precipitated salt soluble proteins by centrifugation. The water soluble proteins remain in the supernatant solution. Dialysis of the soybean flour salt extract produced a suspension that could not be resolved into two fractions

Table 14. Nitrogen content of flour fractions (% of total recovered N).

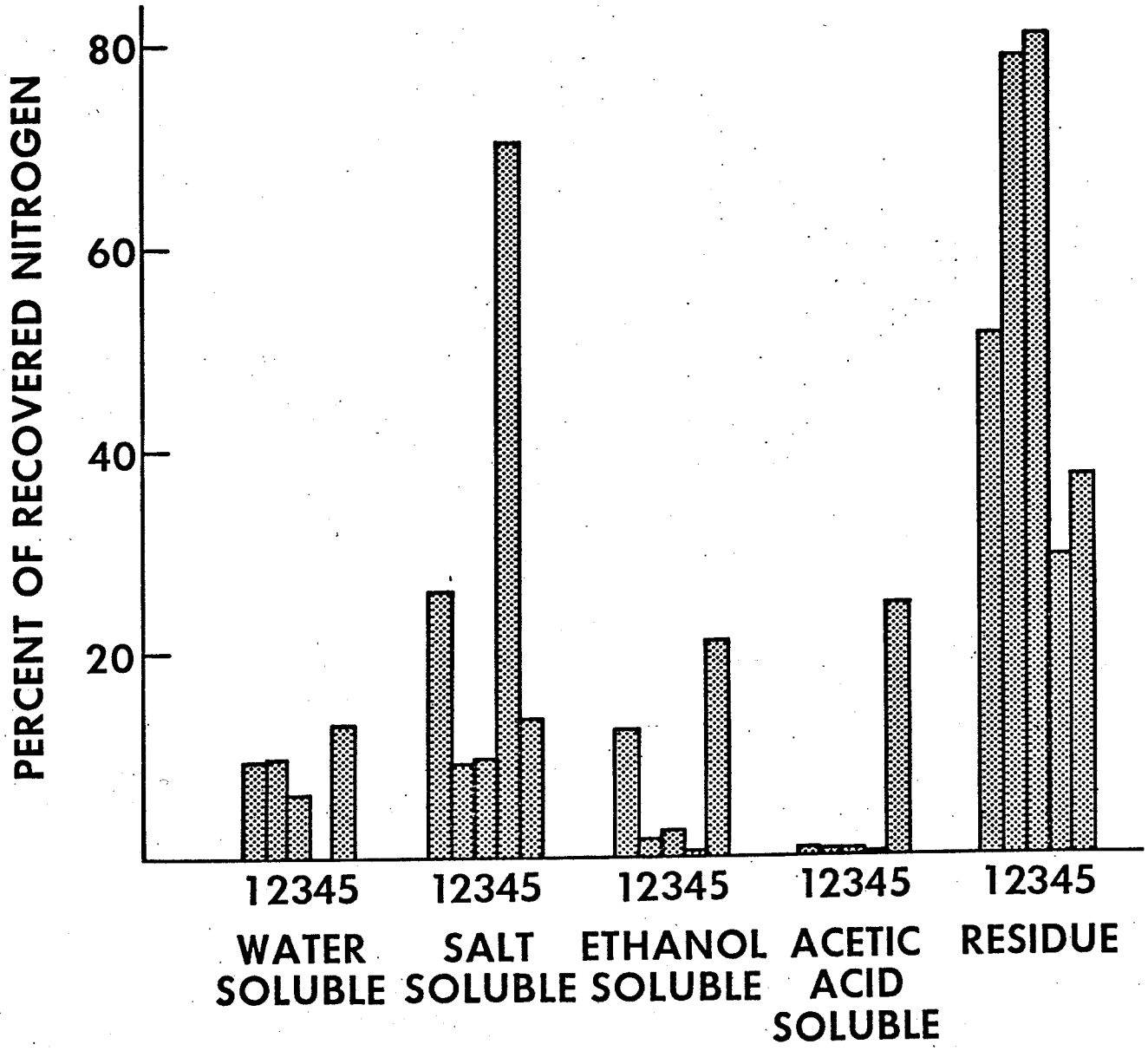
Fraction	Corn	Millet	Sorghum	Soybean	Wheat
Water soluble	9.4	9.6	6.1	-[1]	13.0
0.5M NaCl soluble	26.1	9.2	9.8	70.1	13.7
70% ethanol soluble	12.4	1.8	2.6	0.3	21.1
0.05M acetic acid soluble	0.8	0.7	0.7	0.2	14.9
Residue	51.3	78.7	80.8	29.4	37.3
N, % recovery	101.2	92.9	89.2	101.6	92.1

[1] Water soluble proteins of soybean flour could not be separated from the salt soluble proteins by this fractionation method (see text).

Figure 1.

Nitrogen content of flour fractions

1. Corn flour
2. Millet flour
3. Sorghum flour
4. Soybean flour
5. Hard red spring wheat flour


PERCENT OF RECOVERED NITROGEN
80
60
40
20
12345
12345
12345
12345
12345
WATER SOLUBLE
SALT SOLUBLE
ETHANOL SOLUBLE
ACETIC ACID SOLUBLE
RESIDUE

by this technique. The protein removed during the initial salt extraction was designated the salt soluble fraction although it was probably a combination of albumins and globulins.

2. Salt soluble fraction (globulins)

Soybean flour contained the highest proportion of salt soluble protein, 70.1%. Smith and Circle (1938) reported that 0.5M sodium chloride at neutral pH extracted 76% of total soybean nitrogen. The slightly lower value obtained in this study is probably due to decreased solubility of the 11S and 15S components of soybean protein at low temperature (Wolf and Briggs 1956).

Of the cereal flours, corn had the largest salt soluble fraction, 26.1%. Published values for corn globulins, although not obtained under identical experimental conditions, are significantly lower. Ewart (1968) reported that 9.9% of corn flour nitrogen was soluble in 0.04M sodium chloride. Similarly, Boundy and co-workers (1967) found that 0.5M sodium chloride extracted about 7% of the protein of defatted corn grain.

Millet and sorghum flours had very similar proportions of salt soluble nitrogen. About 9-10% of the protein of these cereals was soluble in 0.5M sodium chloride.

The globulin fraction of wheat flour accounted for 13.7% of recovered nitrogen. This is significantly greater than the values for globulin reported by Chen and Bushuk (1970) and Orth and Bushuk (1972). This lack of agreement may be attributable to differences in the methods used to report the nitrogen content of this fraction. Chen and Bushuk (1970) and Orth and Bushuk (1972) measured the nitrogen content of the salt soluble fraction directly, by determining the nitrogen content of the precipitate obtained upon dialysis of the initial salt extract against distilled water. However, in this study, the nitrogen content of the globulin fraction was determined by difference; the nitrogen content of the water soluble fraction obtained

after dialysis was subtracted from that of the original salt extract. The previously reported values for the globulin fraction are probably lower than those reported in this study due to losses of nitrogenous material during dialysis of the salt extracts.

3. Ethanol soluble fraction (prolamines)

Millet, sorghum and soybean proteins are relatively insoluble in 70% ethanol. The prolamines of these flours account for less than 3% of recovered nitrogen. The data for millet and sorghum flours support the findings of Jones and Beckwith (1970) and Jones *et al.* (1970) who reported that approximately 3% of millet and sorghum proteins are soluble in 60% ethanol at room temperature.

In contrast to the other flours, corn and wheat flours contained significant proportions of alcohol soluble protein. About 12% of the corn protein and 21.1% of the wheat protein was soluble in 70% ethanol. Other investigators using the same solvent have reported higher proportions of prolamine; 15.8-22.6% for corn (Boundy *et al.* 1967, Ewart 1968) and approximately 28-40% for wheat (Chen and Bushuk 1970, Orth and Bushuk 1972).

4. Acetic acid soluble fraction (glutelins)

The most striking feature of the solubility fractionation is that all the flour proteins, with the exception of wheat proteins, are virtually insoluble in 0.05M acetic acid. The value for corn glutelin concurs with Ewart's (1968) finding that only 0.3% of corn flour protein is soluble in 0.1M acetic acid. Most other investigators have used dilute alkali rather than dilute acid to extract glutelins, and have obtained much higher values for this fraction. Boundy and co-workers (1967) classified 26% of corn protein as glutelin based on extraction with 0.1M sodium hydroxide. Studies on sorghum proteins indicate that they are also much more soluble in dilute alkali than dilute acid (Virupaksha and Sastry 1968, Skoch *et al.* 1970,

Haikerwal and Mathieson 1971B).

In contrast to the negligible acetic acid soluble contents of the other flours, 14.9% of wheat flour nitrogen was soluble in dilute acetic acid. This is in reasonable agreement with Chen and Bushuk's (1970) value of 16.6% for Manitou wheat flour, and is within the range of values for 26 varieties reported by Orth and Bushuk (1972).

5. Residue

Soybean flour had the lowest proportion of nitrogen remaining in the residue fraction, 29.4%. Briggs and Mann (1950) reported that distilled water dissolves as much as 95% of soybean protein and that solubility decreases with increasing molarity of salts. The initial extraction with 0.5M sodium chloride dissolved 70.1% of the soybean protein, therefore, much of the protein remaining in the residue was probably albumin insolubilized by the salt used in the first extraction of the fractionation procedure.

The high nitrogen content of the millet and sorghum residues indicates the relative insolubility of the proteins of these cereals in standard aqueous solvents.

Corn proteins were significantly more soluble in this system than the millet and sorghum proteins. As these three flours had similar albumin and glutelin contents, the greater solubility of corn proteins was due to larger globulin and prolamine fractions.

Wheat flour had the largest ethanol soluble and acetic acid soluble fractions and, therefore, the smallest residue fraction of the cereal flours. The wheat flour residue contained 37.3% of the recovered nitrogen, which compares favorably with previously reported data (Chen and Bushuk 1970, Orth and Bushuk 1972).

D. Isoelectric Focusing

Isoelectric focusing of flour proteins was undertaken to identify proteins specific to each of the cereal and legume flours that could be used to detect and estimate these flours in admixture with wheat flour.

Figure 2 shows the separation of proteins extracted from soybean flour, wheat flour, and soybean-wheat flour blends. The arrow indicates a protein zone specific to soybean flour (Sample 6). By this technique it was not possible to detect any component of the wheat flour extract (Sample 7) having the same isoelectric point. The specific soybean protein zone could be detected in extracts of soybean-wheat flour blends containing 5% soybean flour (Sample 2) and was present in increasing amounts in extracts of blends containing 10, 20 and 30% soybean flour (Samples 3, 4 and 5 respectively).

To determine whether the amount of the specific soybean flour protein was proportional to percent soybean flour in soybean-wheat flour blends, a photographic negative of the stained gel layer was evaluated by densitometry. Figure 4 shows the densitometer tracing for an extract of the 20% soybean - 80% wheat flour blend, superimposed on the pH gradient in the gel layer after isoelectric focusing. The relative area of the protein zone that focused at pH 4.65 was calculated for each sample. The data are given in Table 15 and presented graphically in Figure 3. Linear regression analysis and subsequent calculation of the correlation coefficient ($r = .999$) indicated that the relationship between relative densitometric area of this protein zone and percent soybean flour in soybean-wheat flour blends was significant at the .01 level.

Isoelectric focusing of corn, millet and sorghum flour proteins was less successful. Whereas water extracts of wheat and soybean flours were consis-

Figure 2.

Separation of proteins by thin layer isoelectric focusing in polyacrylamide gel, pH range 4 to 6.

1. Ovalbumin

2-5. Water soluble proteins extracted from soybean-wheat flour blends containing 5,10,20 and 30% soybean flour, respectively.

6. Water soluble proteins extracted from soybean flour.

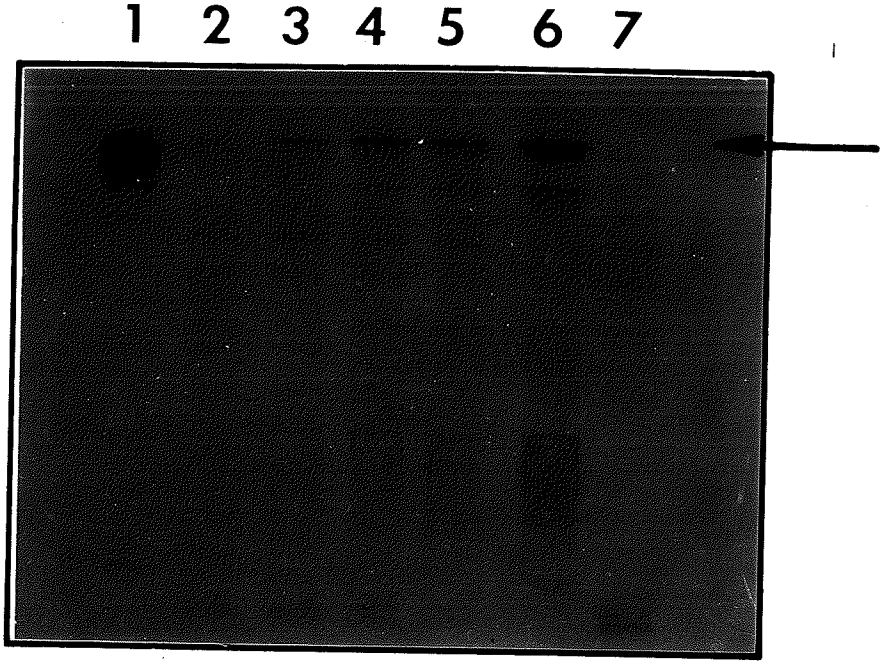
7. Water soluble proteins extracted from wheat flour.

The arrow indicates the major protein zone specific to soybean flour.

The anode was at the top of the photograph.

Figure 3.

Relative densitometric area of the major soybean protein zone as a function of percent soybean flour in soybean-wheat flour blends.


1 2 3 4 5 6 7

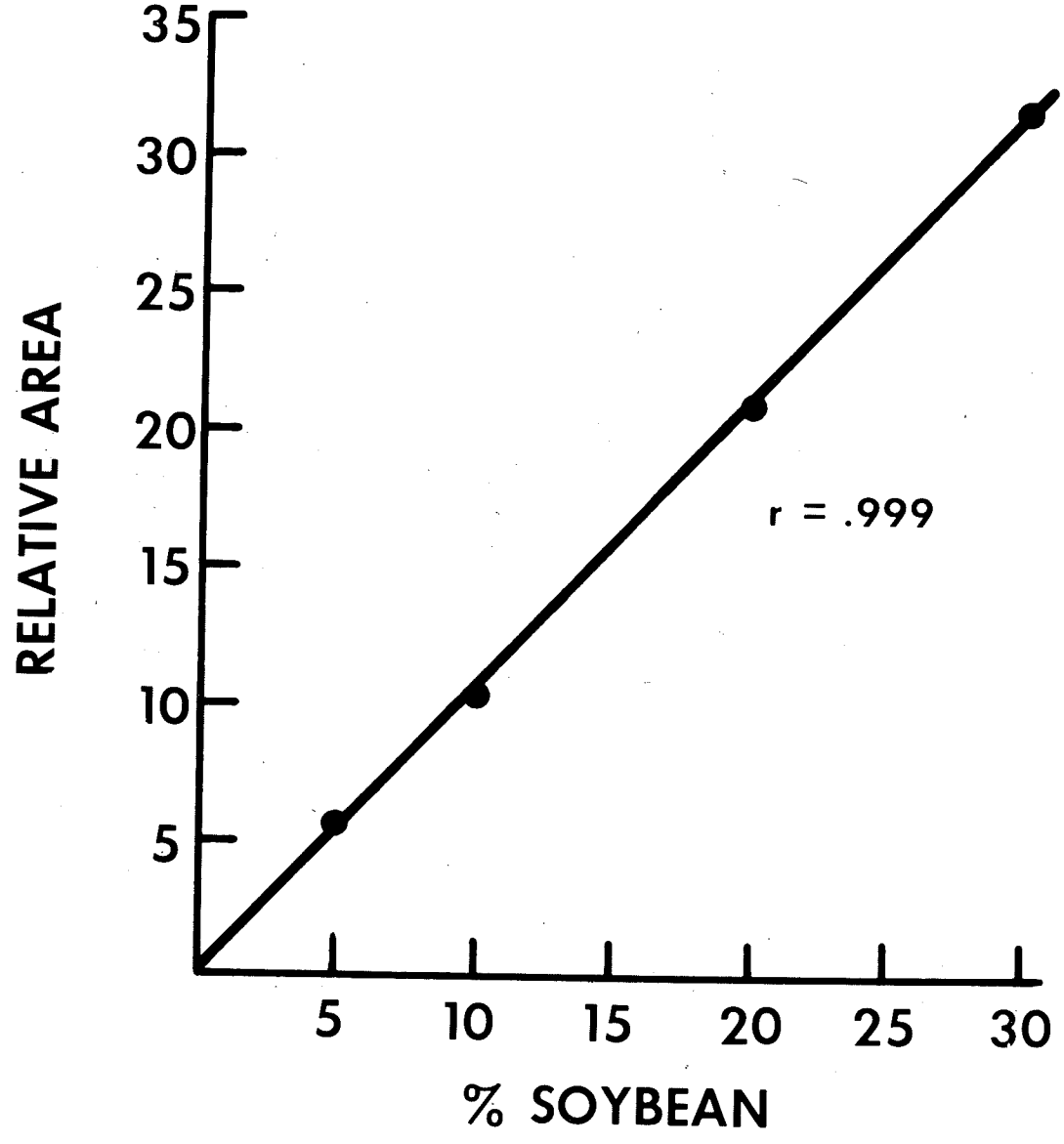
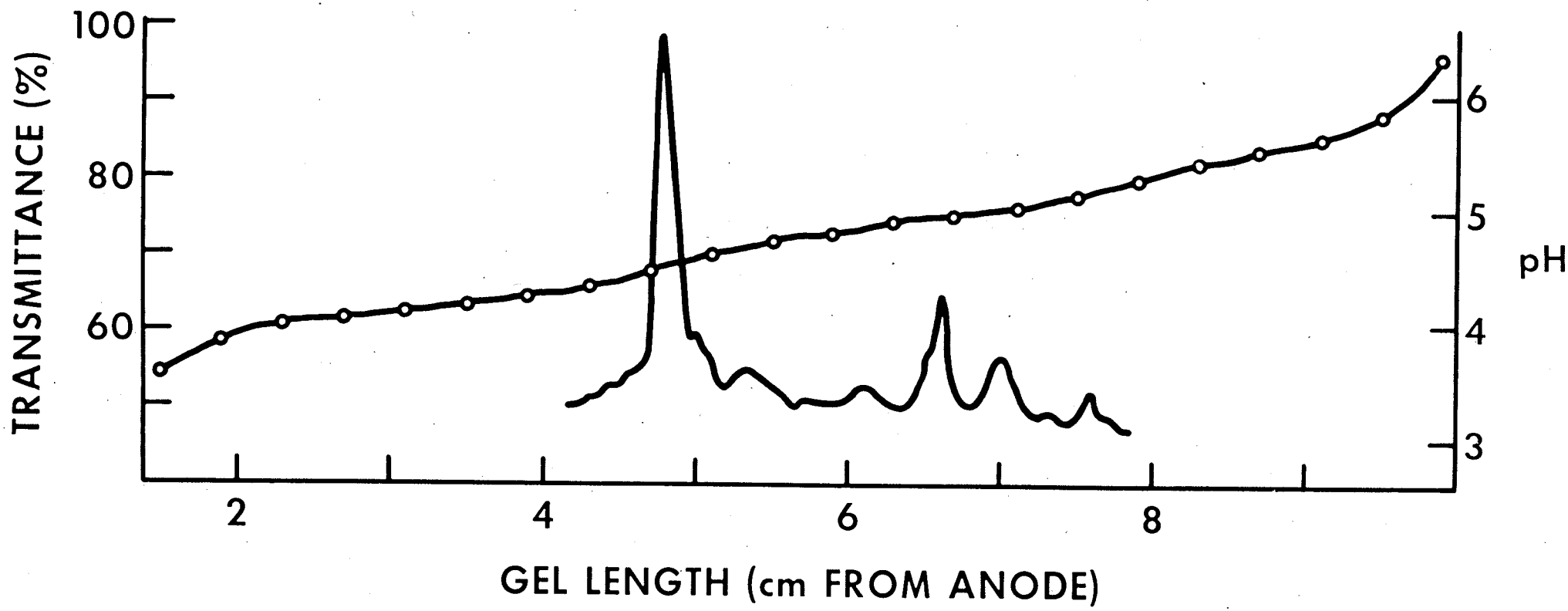

RELATIVE AREA
35
30
25
20
15
10
5
r = .999
5 10 15 20 25 30
% SOYBEAN

Table 15. Relative densitometric area of the major soybean protein zone obtained after thin-layer isoelectric focusing of extracts of soybean-wheat flour blends.

	Soybean, %				
	0	5	10	20	30
Relative Area	0	5.59	10.45	20.88	31.68

Figure 4.

Thin layer isoelectric focusing in the pH range 4 to 6 of water soluble proteins extracted from a 20% soybean-80% wheat flour blend. The densitometer tracing is of a photographic negative obtained after staining the electrofocused gel layer. The closed circles represent the pH gradient in the gel layer after isoelectric focusing.


100
80
60
TRANSMITTANCE (%)
2
4
6
8
GEL LENGTH (cm FROM ANODE)
6
5
4
3
pH

tently well resolved by isoelectric focusing, aqueous extracts of corn, millet and sorghum flours were streaky and poorly resolved. Extracts prepared using many other solvents including 70% aqueous ethanol, 65% aqueous t-butanol, sodium citrate (0.02M, pH6), buffered detergent (Bjerrum and Bøg-Hansen 1975) and alkaline copper sulfate (Mertz and Bressani 1957) did not give satisfactory results. Dialysis and treatment of the extracts with ion-exchange resin did not improve resolution on isoelectric focusing.

Further investigation is required to establish sample preparation techniques that will allow optimal isoelectric focusing of corn, millet and sorghum flour proteins. Once this is accomplished it may be possible to identify specific protein components that would be useful in analyzing composite flours.

E. Sugar Analyses

The sugars in 80%-ethanol extracts of corn, millet, sorghum, soybean and wheat flours were separated as their negatively-charged borate derivatives on substituted polystyrene resin (LaBerge *et al.* 1973) and identified by comparing their elution times with those of known standards. A typical chromatographic separation of the sugars in a standard solution containing 50 µg of each sugar is shown in Figure 5. Chromatograms depicting the separation of sugars in 0.1 ml portions of sorghum, millet, corn and wheat extracts are shown in Figures 6, 7, 8 and 10, respectively. Figure 9 represents the separation of the sugars in 0.025 ml soybean extract.

The sugar compositions of the corn, millet and sorghum flour extracts were extremely similar. Sucrose was the major component, occurring together with smaller proportions of raffinose and glucose. A trace amount of an unidentified sugar was detected in the corn flour extract.

Although the sugar compositions of corn, millet and sorghum flours have not been published, a few analyses of whole grain are available for comparison. In addition to sucrose, raffinose and glucose, fructose has been identified in each of these cereals (Peat *et al.* 1954, Nordin 1959, Taüfel *et al.* 1960, Watson and Hirata 1960, Bond and Glass 1963, Rakimbaev 1968). In addition, Rakimbaev (1968) detected rhamnose, galactose and maltose in millet. Stachyose (Nordin 1959) and maltose (Watson and Hirata 1960) have been found in sorghum grain.

Sucrose, raffinose and stachyose were the principal sugars in soybean flour extract (Figure 9). This finding is in agreement with previous analyses of whole seeds (Hymowitz *et al.* 1972, Tanusi *et al.* 1972, deMan *et al.* 1975) and of defatted soybean meal (Kasai and Kawamura 1966, Kawamura 1967, Delente and

Figure 5.

Separation of standard sugars (50 μg) by ion-exchange chromatography.

Figure 6.

Separation by ion-exchange chromatography of sugars extracted from sorghum flour.

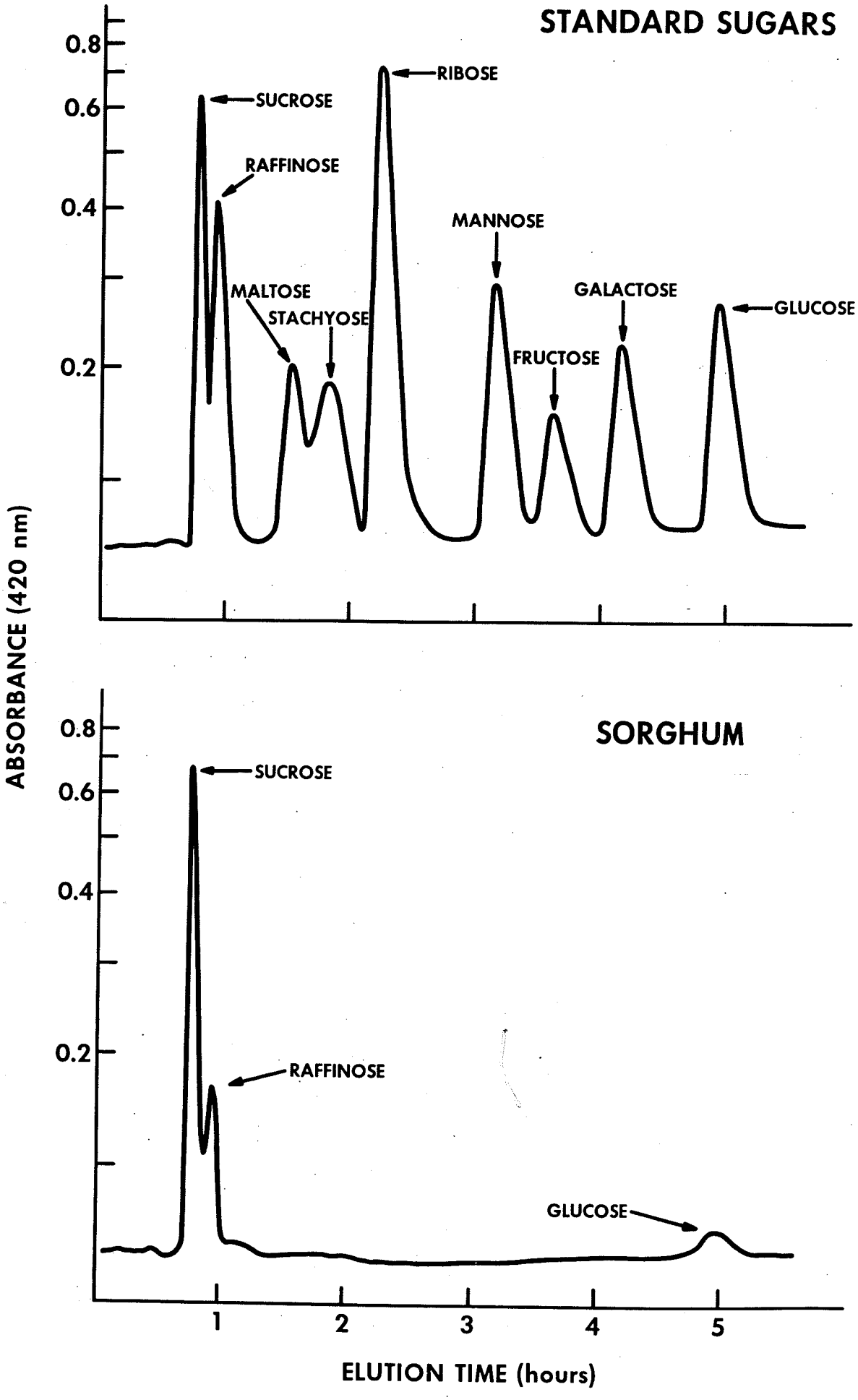

STANDARD SUGARS
0.8
0.6
0.4
0.2
SUCROSE
RAFFINOSE
MALTOSE
STACHYOSE
RIBOSE
MANNOSE
FRUCTOSE
GALACTOSE
GLUCOSE
SORGHUM
SUCROSE
RAFFINOSE
GLUCOSE
ABSORBANCE (420 nm)
1
2
3
4
5
ELUTION TIME (hours)

Figure 7.

Separation by ion-exchange chromatography of sugars extracted from millet flour.

Figure 8.

Separation by ion-exchange chromatography of sugars extracted from corn flour.

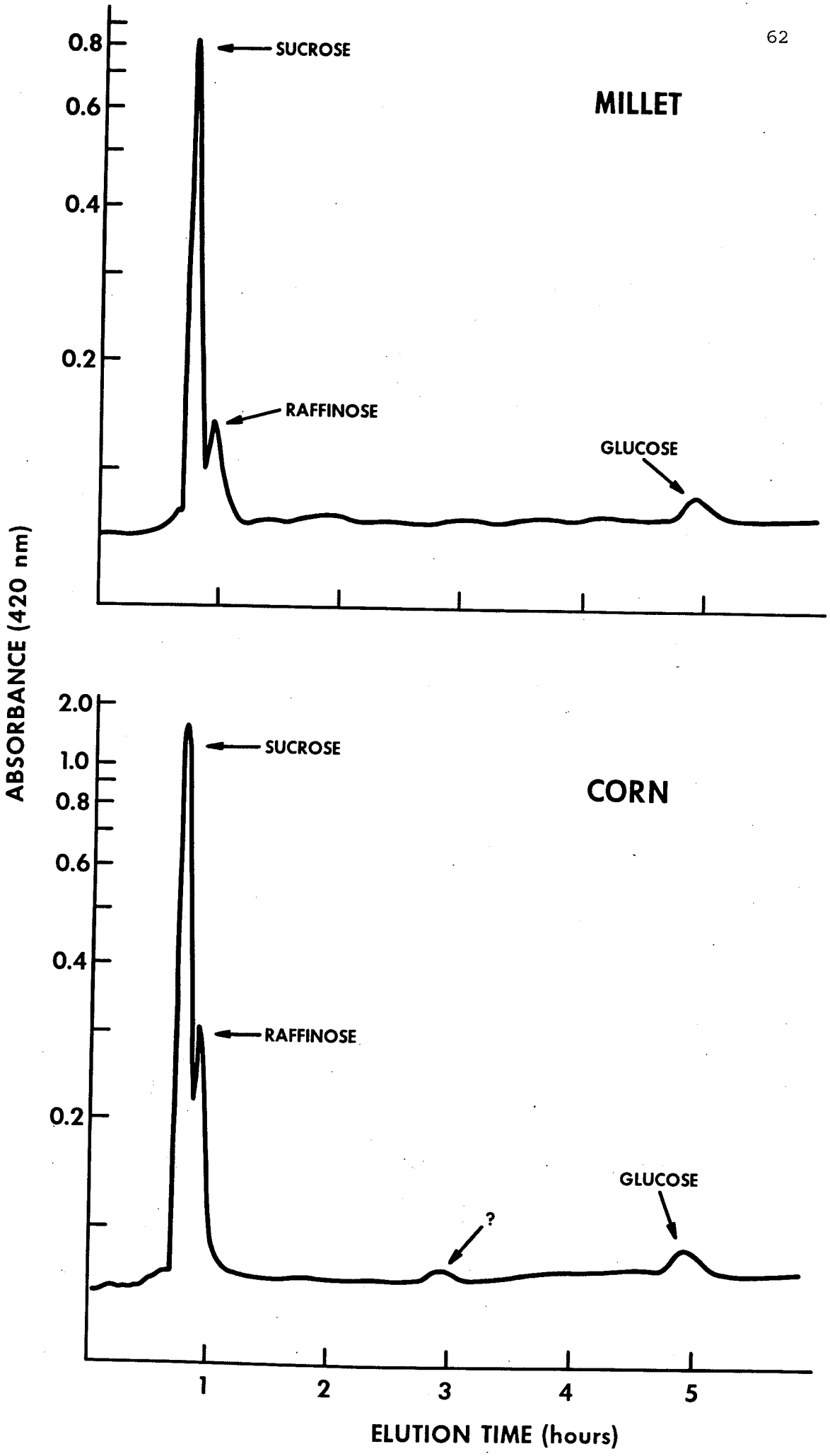
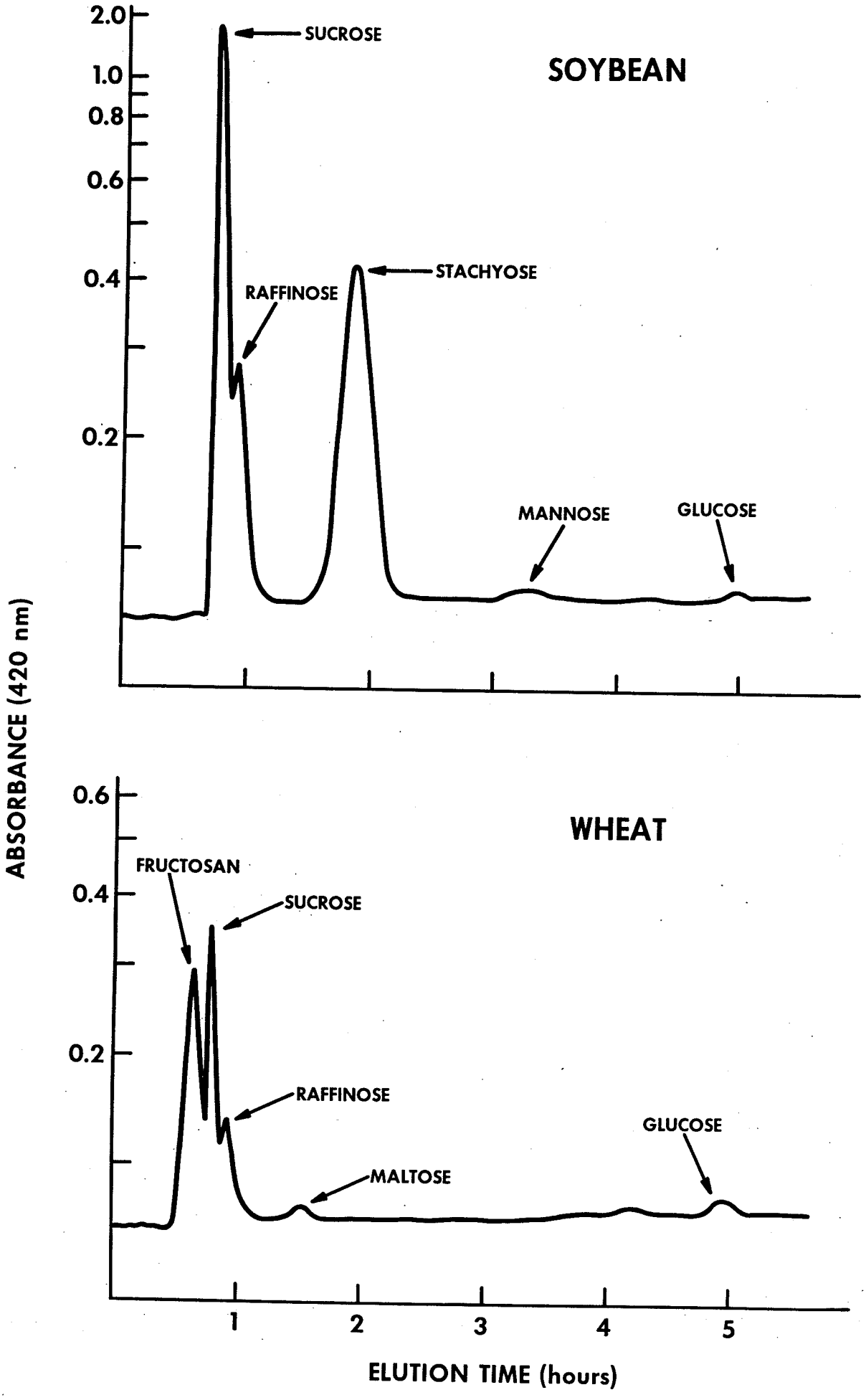

MILLET
SUCROSE
RAFFINOSE
GLUCOSE
0.8
0.6
0.4
0.2
CORN
SUCROSE
RAFFINOSE
GLUCOSE
?
2.0
1.0
0.8
0.6
0.4
0.2
1
2
3
4
5
ABSORBANCE (420 nm)
ELUTION TIME (hours)

Figure 9.

Separation by ion-exchange chromatography of sugars extracted from soybean flour.

Figure 10.

Separation by ion-exchange chromatography of sugars extracted from wheat flour.


SOYBEAN
SUCROSE
STACHYOSE
RAFFINOSE
MANNOSE
GLUCOSE
2.0
1.0
0.8
0.6
0.4
0.2
WHEAT
FRUCTOSAN
SUCROSE
RAFFINOSE
MALTOSE
GLUCOSE
0.6
0.4
0.2
1
2
3
4
5
ABSORBANCE (420 nm)
ELUTION TIME (hours)

Ladenburg 1972). In this study traces of mannose and glucose were identified in soybean flour extracts, however, several sugars reported by other workers were not detected. These include verbascose (Kasai and Kawamura 1967), galactose and manninotriose (Delente and Ladenburg 1972), fructose and melibiose (deMan *et al.* 1975).

The wheat flour extract contained a fructosan component, sucrose, raffinose and small amounts of maltose and glucose (Figure 10). This is in general agreement with the findings of previous investigations (Abou-Guendia and D'Appolonia 1972, Cerning and Guilbot 1973, D'Appolonia and MacArthur 1975) with the exception that fructose was also identified in each of these studies. The fructosan was not identified conclusively due to lack of suitable standard sugars. However, this component probably corresponds to the glucofructan observed in wheat flour extracts by Abou-Guendia and D'Appolonia (1972), and in whole kernel extracts by Cerning and Guilbot (1973).

The results of the sugar analyses indicated that this would not be a useful method for identifying corn, millet or sorghum in wheat flour because the sugar compositions of these materials were similar. However, soybean flour contained a significant amount of stachyose, a sugar not detected in wheat flour. Ponte and DeStefanis (1971) reported that the concentrations of stachyose and its breakdown product manninotriose, as determined by quantitative thin-layer chromatography, were useful in determining the proportion of soybean flour in wheat flour and yeast leavened bakery products. Consequently, soybean-wheat flour blends were analyzed by ion-exchange chromatography to determine whether stachyose content was linearly related to percent soybean flour over the range used in this study. As an example of the separation of sugars extracted from soybean-wheat flour blends, the chromatogram for the blend containing 20% soybean flour is shown in Figure 11.

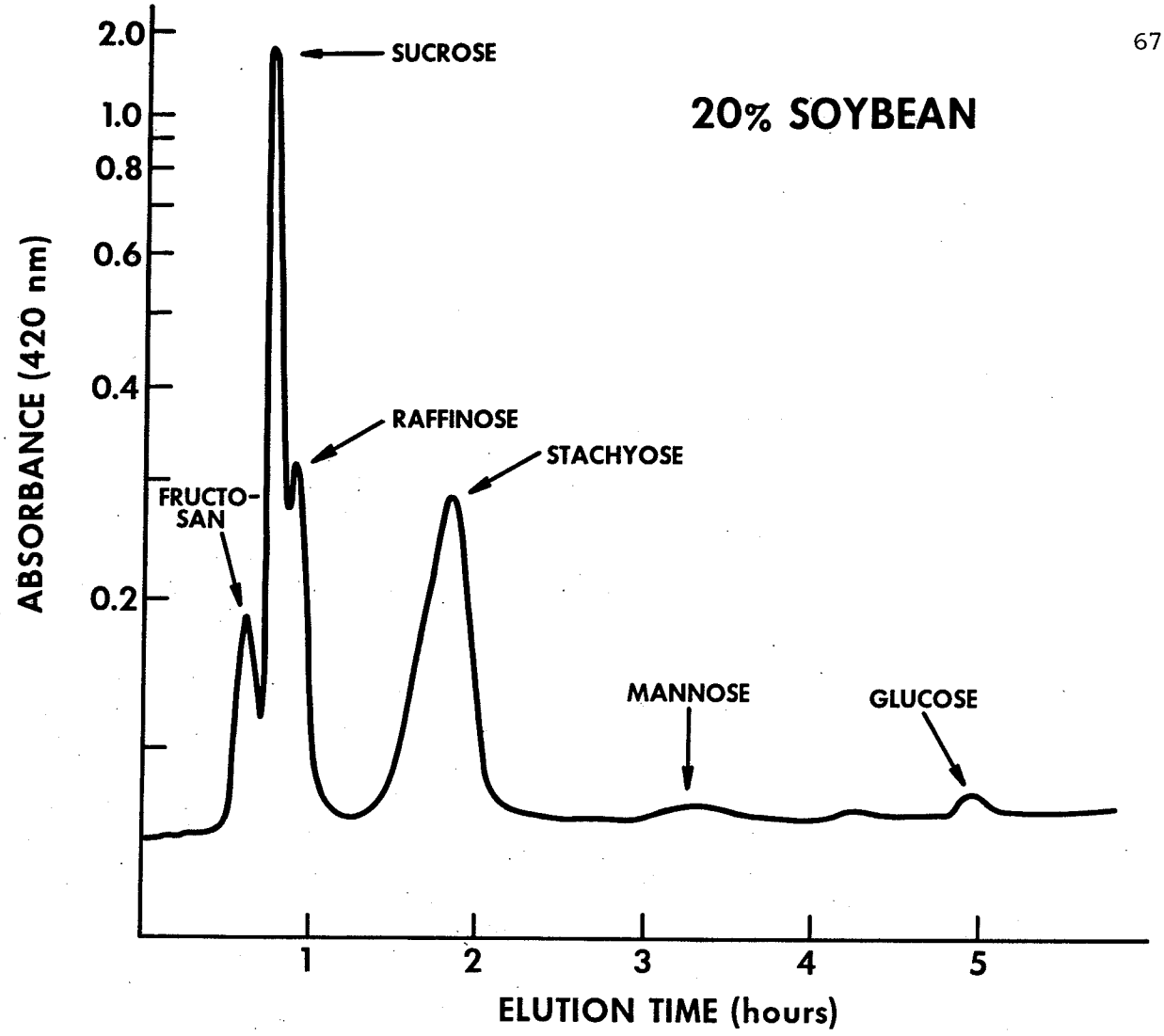
Stachyose was determined quantitatively by calculating the ratio of the

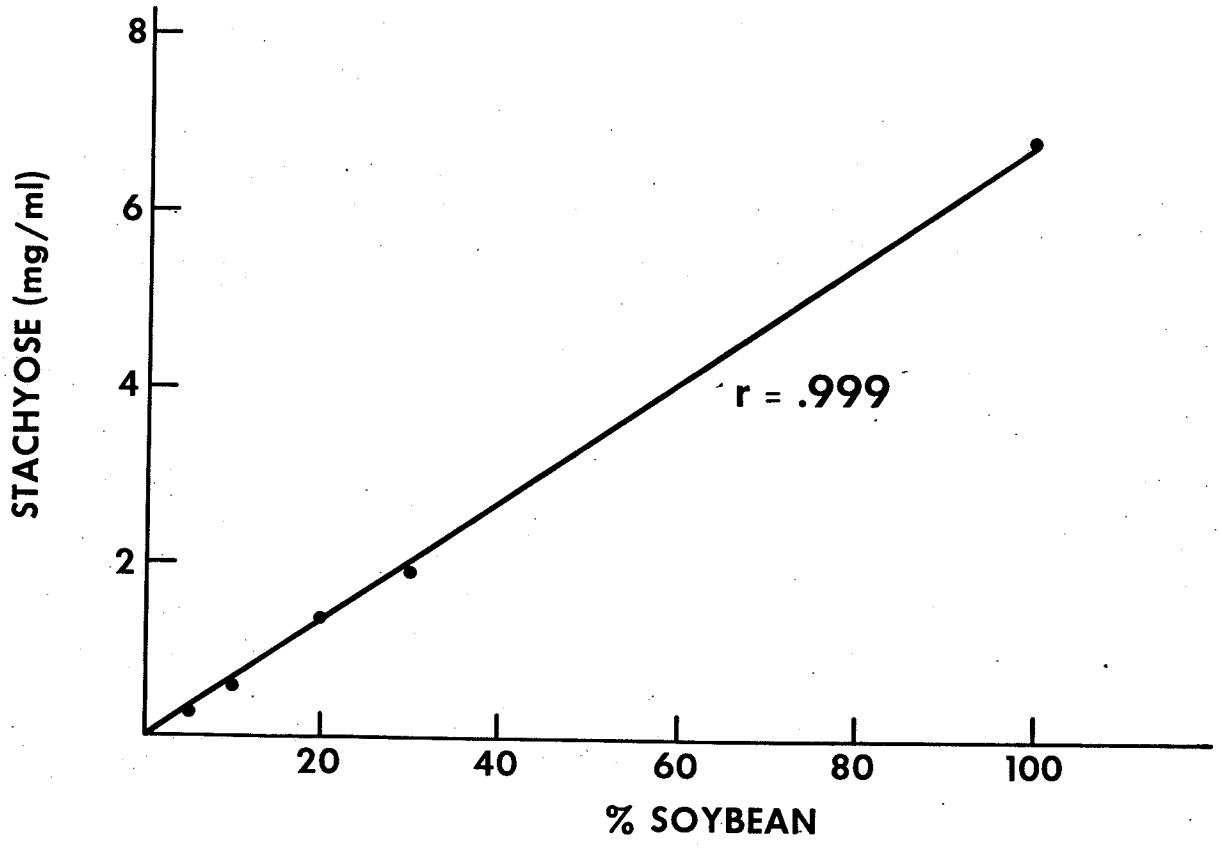
Figure 11.

Separation by ion-exchange chromatography of sugars extracted from a 20% soybean-80% wheat flour blend.

Figure 12.

Stachyose content of flour extracts as a function of percent soybean flour in soybean-wheat flour blends.


20% SOYBEAN
SUCROSE
RAFFINOSE
STACHYOSE
FRUCTO-
SAN
MANNOSE
GLUCOSE
ABSORBANCE (420 nm)
2.0
1.0
0.8
0.6
0.4
0.2
1
2
3
4
5
ELUTION TIME (hours)


STACHYOSE (mg/ml)
8
6
4
2
r = .999
20
40
60
80
100
% SOYBEAN

area under the peak relative to the area under the stachyose standard curve. The area under the curve was obtained by multiplying the peak height absorbance by peak width in mm at one-half peak height absorbance.

The stachyose content of the soybean-wheat flour blends is given in Table 16 and presented graphically in Figure 12. The data were analyzed using the linear regression equation. A very high correlation (r = .999) was found between stachyose content and percent soybean flour in the blend.

Although detection of stachyose may indicate the presence of soybean flour in a composite, its usefulness for predicting the proportion of soybean flour in a blend might be limited by the fact that stachyose concentration varies dramatically with variety (Kawamura 1967, Hymowitz *et al.* 1972, deMan *et al.* 1975). However, if the stachyose content of the soybean flour used to prepare the blend is known, then this parameter can be used to determine the amount of soybean flour in a composite flour with considerable degree of accuracy.

Table 16. Stachyose content (mg/ml) of soybean-wheat flour blends.

	Soybean, %					
	0	5	10	20	30	100
Stachyose	0	.265	.565	1.374	1.900	6.762

VI. SUMMARY

This study has investigated some of the biochemical properties of corn, millet, sorghum, soybean and wheat flours in order to identify constituents that may be useful in detecting and estimating non-wheat flours in admixtures with wheat flour.

Proximate analyses, amino acid analyses and protein solubility fractionations reveal significant differences in the chemical compositions of the flours. The differences detected by these assays are in the proportions of the constituents present, rather than the types of constituents detected. Consequently, such analyses are not sufficiently specific to identify other cereal or legume flours in admixture with wheat flour, but they can indicate whether the composition of a flour sample is within the range of values expected for wheat flour. The proportions of protein, fibre, ash, and a number of amino acids (lysine, arginine, aspartic acid, glutamic acid, proline, alanine and leucine) are highly correlated with percent non-wheat flour in composite blends. These analyses may be useful for estimating the proportion of non-wheat flour in a composite, if the identity of the flours in a composite can be determined by another method.

Thin layer isoelectric focusing of flour proteins identified a protein zone specific to soybean flour. The densitometric area of the zone that focused at pH 4.65 was directly proportional to percent soybean flour in soybean-wheat flour blends containing 5 to 30% soybean flour. Protein zones specific to corn, millet and sorghum flour proteins were not identified by this technique.

Sugar analyses by ion-exchange chromatography indicated that corn, millet and sorghum flours were not identifiable in admixture with wheat flour

on the basis of their sugar compositions. In contrast, soybean flour contained a high proportion of stachyose, a sugar not detected in wheat flour. Stachyose content was directly proportional to the soybean content of soybean-wheat flour blends containing 0 to 100% soybean flour.

Thus, soybean flour can be detected in wheat flour by detecting the presence of stachyose or by identifying a specific soybean protein component. The high correlation of these parameters with percent soybean flour in soybean-wheat blends of known composition indicates that these constituents could be used to estimate the proportion of soybean flour in blends of unknown proportions. If the variability of these constituents in soybean and wheat flours from different sources can be established, these assays could be used to estimate the minimum and maximum soybean flour content of soybean-wheat flour blends with a reasonable degree of accuracy. Further investigation is required to identify constituents that can be used for detecting and estimating corn, millet and sorghum flours in admixture with wheat flour.

VII. LITERATURE CITED

1. ABOU-GUENDIA, M. and D'APPOLONIA, B. L. 1972. Changes in carbohydrate components during wheat maturation. I. Changes in free sugars. Cereal Chem. 49: 664-676.
2. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1962. Approved Methods. St. Paul, Minnesota.
3. ASSELBERGS, E. A. 1973. The FAO composite flour programme. Composite Flour Programme. Vol. 1. ed. R. P. Chatelant. Food and Agriculture Industries Service, Agricultural Services Division, Food and Agriculture Organization of the United Nations, Rome, Italy.
4. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1975. Official Methods of Analysis. Washington, D.C.
5. ATLAS OF NUTRITIONAL DATA ON UNITED STATES AND CANADIAN FEEDS. 1971. National Academy of Sciences, Washington, D.C.
6. AUDIDIER, Y., *et al.* 1966. Carbohydrates, damaged starch, and amylase activities in air classified flours. Ind. Aliment. Agr. 83: 519-527.
7. BJERRUM, O. J. and BØG-HANSEN, T. C. 1975. Immunochemical gel precipitation techniques for analysis of membrane proteins. Membrane Methods. ed. A. H. Maddy. Chapman and Hall, London.
8. BOND, A. B. and GLASS, R. L. 1963. The sugars of germinating corn. Cereal Chem. 40: 459-466.
9. BOUNDY, J. A., *et al.* 1967. Protein composition of dent, waxy and high-amylose corns. Cereal Chem. 44: 160-169.
10. BRACCIALI, A., *et al.* 1974. Determination of soft wheat in semolina and macaroni products by two immunochemical methods. Boll. Lab. Chim. Prov. 25: 232-248. (CA 82: 168921t).

11. BREKKE, O. L. 1970. Corn dry milling industry. Corn: culture, processing, products. ed. G. E. Inglett. Avi Publishing Co., Inc. Westport, Conn.

12. BRESSANI, R. and MERTZ, E. T. 1958. Studies on corn proteins. IV. Protein and amino acid content of different corn varieties. Cereal Chem. 35: 227-235.

13. BRIGGS, D. R. and MANN, R. L. 1950. An electrophoretic analysis of soybean protein. Cereal Chem. 27: 243-257.

14. BROGIONI, M. and FRANCONI, U. 1963. Indagi spettrofotometriche nell' infrarosso sulgi sfarinata di frumento e sulle paste alimentari. Boll. Lab. Chim. Prov. 14: 135-160 (CA 59: 12084e).

15. CANTAGALLI, P., *et al.* 1969. Immunochemical method for the identification and determination of soft wheat in alimentary flours and pastes. Ann. Ist. Super. Sanita 5: 628-630. (CA 73: 10830w).

16. CATSIMPOOLAS, N. 1968. Micro isoelectric focusing in polyacrylamide gel columns. Anal. Biochem. 26: 480-482.

17. CATSIMPOOLAS, N. 1969. Isolation of soybean lipoxidase by isoelectric focusing. Arch. Biochem. Biophys. 131: 185-190.

18. CATSIMPOOLAS, N. and MEYER, E. W. 1969. Isolation of soybean hemagglutinin and demonstration of multiple forms by isoelectric focusing. Arch. Biochem. Biophys. 132: 279-285.

19. CERNING, J. and GUILBOT, A. 1973. Changes in the carbohydrate composition during development and maturation of the wheat and barley kernel. Cereal Chem. 50: 220-232.

20. CHEN, C. H. and BUSHUK, W. 1970. Nature of proteins in *Triticale* and its parental species. I. Solubility characteristics and amino acid composition of endosperm proteins. Can. J. Plant Sci. 50: 9-14.

21. CHINDEMI, A. and ZAMPA, O. 1969. Determination of sitosterol palmitate in different varieties of *Triticum aestivum* and *Triticum durum*. Ann.

Ist. Super. Sanita 5: 395-398. (CA 73: 108296x).

22. CIRCLE, S. J. and SMITH, A. K. 1972. Processing soy flours, protein concentrates and protein isolates. Soybeans: Chemistry and Technology Vol. I. ed. A. K. Smith and S. J. Circle. Avi Publishing Co., Inc., Westport, Conn.

23. CUBADDA, R. and RESMINI, P. 1968. Use of electrophoresis for studying soft wheat in food pastes dried at various temperatures. Quad. Nutr. 28: 81-88. (CA 72: 109877d).

24. D'APPOLONIA, B. L. and MacARTHUR, L. A. 1975. Comparison of starch, pentosans, and sugars of some conventional height and semi-dwarf hard red spring wheat flours. Cereal Chem. 52: 230-239.

25. DELENTE, J. and LADENBURG, K. 1972. Quantitative determination of oligosaccharides in defatted soybean meal by gas-liquid chromatography. J. Food Sci. 37: 372-374.

26. deMAN, J. M., STANLEY, D. W. and RASPER, V. 1975. Composition of Ontario soybeans and soymilk. Can. Inst. Food Sci. Technol. J. 8: 1-8.

27. D'ERRICO, A. M. *et al.* 1969. Electrophoretic study of cereals. III. Rapid method for the determination of soft wheat in alimentary flours and pastes. Ann. Ist. Super. Sanita 5: 425-431. (CA 73: 108301v).

28. DEYOE, C. W. and SHELLENBERGER, J. A. 1965. Studies on the amino acids and proteins in sorghum grain. J. Agr. Food Chem. 13: 446-450.

29. DIMLER, R. J. 1969. Oilseed protein sources and potentials: soybeans. Am. Inst. Chem. Eng. 65: 22-29.

30. EDWARDS, W. M. and CURTIS, J. J. 1943. Grain sorghums, their products and uses. Northern Regional Research Laboratory, USDA, Peoria, Ill. ACE-193, NM-229.

31. EWART, J. A. D. 1967. Amino acid analyses of cereal flour proteins. J. Sci. Food Agr. 18: 548-552.

32. EWART, J. A. D. 1968. Fractional extraction of cereal flour proteins. J. Sci. Food Agr. 19: 241-245.

33. FEILLET, P. and BOURDET, A. 1967. Composition protéique et charactéristiques génétique des blés. Bull. Soc. Chim. Biol. 49: 1273-1283. (CA 68: 57359g).

34. FEILLET, P., KOBREHEL, K. and RODRIGUEZ, F. 1972. Recherche et dosage des produits de blé tendre dans les pâtes alimentaires par électrophorèse des protéines solubles. Ann. Technol. Agr. 21: 17-24. (CA 78: 70295f).

35. FEILLET, P. and KOBREHEL, K. 1974. Determination of common wheat content in pasta products. Cereal Chem. 51: 203-209.

36. GARCIA-FAURE, R., MERCK-LUENGO, J. G. and GARCIA-OLMEDO, F. 1969. Detection of flour or farina from *Tritucum aestivum* in macaroni, by starch gel electrophoresis of water-soluble proteins. Cereal Chem. 46: 621-625.

37. GILLES, K. A. and YOUNG, V. L. 1964. Evaluation of durum wheat and durum products. II. Separation and identification of the sitosterol esters of semolina. Cereal Chem. 41: 502-513.

38. GRUENER, M. and BERNAERTS, M. J. 1968. Structure of wheat sterol esters and differentiation between soft and hard wheat. Getreide Mehl 18: 69-74. (CA 70: 103687d).

39. HAIKERWAL, M. and MATHIESON, A. R. 1971A. The protein content and amino acid composition of sorghum grain. Cereal Chem. 48: 690-699.

40. HAIKERWAL, M. and MATHIESON, A. R. 1971B. Extraction and fractionation of proteins of sorghum kernels. J. Sci. Food Agr. 22: 142-145.

41. HEPBURN, F. N., LEWIS Jr., E. W. and ELVEHJEM, C. A. 1957. The amino acid content of wheat, flour, and bread. Cereal Chem. 34: 312-322.

42. Hymowitz, T., *et al.* 1972. Relationship between the content of oil, protein and sugar in soybean seed. Agron. J. 64: 613-616.

43. INGLETT, G. E., *et al.* 1969. Using a computer to optimize cereal based food composition. Cereal Sci. Today 14: 69-74.

44. JONES, R. W. and BECKWITH, A. C. 1970. Proximate composition and proteins of three grain sorghum hybrids and their dry-mill fractions. J. Agr. Food Chem. 18: 33-36.

45. JONES, R. W., *et al.* 1970. Protein composition of proso millet. J. Agr. Food Chem. 18: 37-39.

46. KARPER, R. E. and QUINBY, T. R. 1963. Sugary endosperm in sorghum. J. Hered. 54: 121-126.

47. KASAI, T. and KAWAMURA, S. 1966. Soybean oligosaccharides. Isolation by gel filtration and identification by acetylation. Kagawa Daigaku Nogakubu Gakuzyutu Kokoku 18: 9-15. (CA 67: 89886x).

48. KAWAMURA, S. 1967. Quantitative paper chromatography of sugars of defatted soybean meal. Kagawa Daigaku Nogakubu Gakujutsu Hokoku 18: 132-137. (CA 69: 33522z).

49. KOCH, R. B., GEDDES, W. F. and SMITH, F. 1951. The carbohydrates of the Gramineae. I. The sugars of the flour of wheat. Cereal Chem. 28: 424-430.

50. LaBERGE, D. E., MacGREGOR, A. W. and MEREDITH, W. O. S. 1973. Changes in the free sugar content of barley kernels during maturation. J. Inst. Brew. 79: 471-477.

51. LaWALL, C. H. and HARRISSON, J. W. E. 1934. Method for the detection of soybean flour in manufactured foods. J. Assoc. Off. Agr. Chem. 17: 329-334.

52. LEABACK, D. H. and RUTTER, A. C. 1968. A new technique for the electrophoresis of proteins. Biochem. Biophys. Res. Commun. 32: 447-453.

53. LIUZZI, A. and ANGELETTI, P. U. 1969. Application of immunodiffusion in detecting the presence of barley in wheat flour. J. Sci. Food Agr. 20: 207-209.

54. MacKENZIE, R. M. 1958. The utilization of flour carbohydrates in panary fermentation. Soc. Chem. Ind. Monograph No. 3.

55. MATVEEF, M. 1952. Détection des farines de blé tendre dans les semoules et pâtes alimentaires. C. R. Acad. Agr. Fr. 39: 658.

56. McDERMOTT, E. E. and PACE, J. 1960. Comparison of the amino acid composition of the protein in flour and endosperm from different types of wheat, with particular reference to variation in lysine content. J. Sci. Food Agr. 11: 109-115.

57. MERTZ, E. T. and BRESSANI, R. 1957. Studies on corn proteins. I. A new method of extraction. Cereal Chem. 34: 63-69.

58. MEYER, E. W. 1969. Soy-protein products for food. Conf. Protein-rich Food Products from Oilseeds. USDA, New Orleans. ARS-72-71.

59. MITCHESON, R. E. and STOWELL, K. C. 1970. Application of new analytical techniques to routine malt analysis. I. Determination of barley and malt nitrogen content using an auto-analyzer technique. J. Inst. Brew. 76: 335-339.

60. MONTEFREDINE, A. and SALVIONI, C. 1967. The Matveef and Brogioni-Franconi methods in the differentiation between hard wheat and soft wheat products. Boll. Lab. Chim. Prov. 18: 436-445. (CA 68: 3825lz).

61. MURTHY, P. R. and DIETZ, J. H. 1974. Agtron in evaluation of cereal flours. Cereal Chem. 51: 126-133.

62. NORDIN, P. 1959. Sorghum grain. The soluble sugars. Trans. Kansas Acad. Sci. 62: 212-215. (BA 36: 21783).

63. NUNNIKHOVEN, R. and BIGWOOD, E. J. 1959. Variation de la composition en acides aminés de la farine de froment en fonction du pourcentage de son extraction du grain de blé. Nutrito et Dieta 1: 177-191.

64. OKE, O. L. 1965. Chemical studies on some Nigerian cereals. Cereal Chem. 42: 299-302.

65. ORTH, R. A. and BUSHUK, W. 1972. A comparative study of wheats of diverse baking qualities. Cereal Chem. 49: 268-275.

66. OSBORNE, T. B. 1907. The proteins of the wheat kernel. Carnegie Inst. Wash. Publ. No. 84.

67. PEAT, S., WHELAN, W. J. and TURVEY, J. R. 1956. The soluble polyglucose of sweet corn (*Zea mays*). J. Chem. Soc. 2317-2322.

68. PIAZZI, S. E. and CANTAGALLI, P. 1969. Immunochemical analysis on soluble proteins of wheat. Cereal Chem. 46: 642-646.

69. POMERANZ, Y. 1953. The detection and estimation of small amounts of untreated soybean flour in cereals. Bull. Research Council of Israel 3: 252-253. (CA 49: 9174h).

70. POMERANZ, Y. and MILLER, G. D. 1960. Detection and estimation of soy flour in wheat flour. J. Assoc. Off. Agr. Chem. 43: 442-444.

71. PONTE Jr., J. G. and DeSTEFANIS, V. A. 1971. Detection of soy flour in bakery products and ingredients. Cereal Science Today 16: 307-308.

72. RACKIS, J. J., *et al.* 1961. Amino acids in soybean hulls and oil meal fractions. J. Agr. Food Chem. 9: 409-412.

73. RADOLA, B. J. 1973. Isoelectric focusing in layers of granulated gels. I. Thin-layer isoelectric focusing of proteins. Biochim. Biophys. Acta 295: 412-428.

74. RAKIMBAEV, I. R. 1968. Composition of sugars in millet grain. Prikl. Biokhim. Mikrobiol. 4: 227-229. (CA 69: 8899v).

75. RESMINI, P. 1968. Un nuovo metodo per indentificare e dosage gli sfarinati di grano tenero presenti in quello di grano duro e nelle pasti alimentari. Tec. Molitoria 19: 145-169. (CA 70: 1896lu).

76. RESMINI, P. 1974. Protein denaturation in some macaroni products and determination of their soft wheat content by electrophoresis. Getreide, Mehl Brot 28: 234-236. (CA 82: 41903h).

77. SALVIONI, C. 1968. Determination of soft wheat in flour and alimentary

pastes. Boll. Lab. Chim. Prov. 19: 696-704. (CA 70: 86324p).

78. SILANO, V., *et al.* 1967. Gel electrophoresis of cereal proteins. I. Qualitative and quantitative analysis of barley in wheat flours and macaronis. Ann. Ist. Super. Sanita 3: 753-767. (CA 69: 26081b).

79. SILANO, V., *et al.* 1968. Indajini elettroferetiche sui cereali. II. Riconoscimento e dosaggio del grano tenero in sfarinati e pasti alimentari. Ann. Ist. Super. Sanita 4: 641-643. (CA 71: 48353k).

80. SKOCH, L. V., *et al.* 1970. Protein fractionation of sorghum grain. Cereal Chem. 47: 472-481.

81. SMITH, A. K. and CIRCLE, S. J. 1938. Peptidization of soybean proteins. Extraction of nitrogenous constituents from oil-free meal by acids and bases with and without added salts. Ind. Eng. Chem. 30: 1414-1418.

82. SMITH, A. K., CIRCLE, S. J. and BROTHER, G. H. 1938. Peptidization of soybean proteins. The effect of neutral salts on the quantity of nitrogenous constituents extracted from oil-free meal. J. Am. Chem. Soc. 60: 1316-1320.

83. SMITH, A. K., *et al.* 1966. Nitrogen solubility index, isolated protein yield, and whey nitrogen content of several soybean strains. Cereal Chem. 43: 261-270.

84. TANAKA, K. and BUSHUK, W. 1972. Effect of protein content and wheat variety on solubility and electrophoretic properties of flour proteins. Cereal Chem. 49: 247-257.

85. TANRET, C. 1891. On the levosine, new principle for some cereals. Bull. Soc. Chim. (France) 5: 724-731.

86. TANUSI, S., KASAI, T. and KAWAMURA, S. 1972. Determination of oligosaccharides in some edible legume seeds. J. Jap. Soc. Food and Nutr. 25: 25-27. (CA 77: 3981w).

87. TAÜFEL, K., ROMMINGER, K. and HIRSCHFELD, W. 1959. Oligosaccharides

of cereals and flour. Z. Lebensmittel-Untersuch. Forsch. 109: 1-12.

88. TAÜFEL, K., STEINBACH, K. J. and HARTMAN, B. 1960. Uberdie niederen saccharide des maises und ihr verhalten während lagerung und Keimung. Nahrung 4: 452-465.

89. TOLLIER, M. T., GUILBOT, A. and CERNING, J. 1968. Influence of the variety and growing conditions on the carbohydrate composition of wheat and flour. Unpublished work cited in Wheat: Production and Utilization. ed. G. E. Inglett. Avi Publishing Co., Inc. Westport, Conn.

90. TKACHUK, R. 1966. Amino acid composition of wheat flours. Cereal Chem. 43: 207-223.

91. TKACHUK, R. and IRVINE, G. N. 1969. Amino acid compositions of cereals and oilseed meals. Cereal Chem. 46: 206-218.

92. VAISEY, M. and UNRAU, A. M. 1964. Flour composition. Chemical constituents of flour from cytologically synthesized and natural cereal species. J. Agr. Food Chem. 12: 84-86.

93. VESTERBERG, O. 1971. Staining of protein zones after isoelectric focusing in polyacrylamide gels. Biochim. Biophys. Acta 243: 345-348.

94. VESTERBERG, O. 1972. Isoelectric focusing of proteins in polyacrylamide gels. Biochim. Biophys. Acta 257: 11-19.

95. VIRUPAKSHA, T. K. and SASTRY, L. V. S. 1968. Studies on the protein content and amino acid composition of some varieties of grain sorghum. J. Agr. Food Chem. 16: 199-203.

96. WALDE, A. W. and MANGELS, C. E. 1930. Variation in properties of acetone extracts of common and durum wheat flours. A preliminary report. Cereal Chem. 7: 480-486.

97. WATSON, S. A. and HIRATA, Y. 1960. Carbohydrates in sorghum grain. Sorghum Newsletter 3: 6-7.

98. WATT, B. K. and MERRILL, A. L. 1963. Composition of foods. Washington,

D.C. Consumer and Food Economics Research Division, Agricultural Research Service, United States Department of Agriculture. (USDA Handbook No. 8).

99. WHITE, L. M. and SECOR, G. E. 1953. Occurrence of two similar homologous series of oligosaccharides in wheat flour and wheat. Arch. Biochem. Biophys. 43: 60-66.

100. WILLIAMS, K. T. and BEVENUE, A. 1951. The chromatographic examination of sugars in wheat flour. Cereal Chem. 28: 416-423.

101. WOLF, W. J. 1970. Soybean proteins: their functional, chemical and physical properties. J. Agr. Food Chem. 18: 969-976.

102. WOLF, W. J. and BRIGGS, D. R. 1956. Ultracentrifugal investigation of the effect of neutral salts on the extraction of soybean proteins. Arch. Biochem. Biophys. 63: 40-49.

103. WOLFE, M. and FOWDEN, L. 1957. Composition of the protein of whole maize seeds. Cereal Chem. 34: 286-295.

104. WRIGLEY, C. W. 1968. Gel electrofocusing - a technique for analyzing multiple protein samples by isoelectric focusing. Sci. Tools 15: 17-23.

105. WRIGLEY, C. W. 1970. Protein mapping by combined gel electrofocusing and electrophoresis: application to the study of genotypic variations in wheat gliadins. Biochem. Genet. 4: 509-516.

106. WRIGLEY, C. W. and SHEPHERD, K. W. 1973. Electrofocusing of grain proteins from wheat genotypes. Ann. N. Y. Acad. Sci. 209: 154-162.

107. ZAMPA, O. and CHINDEMI, A. 1968. Gilles-Youngs and Brogioni-Franconi determinations of powdered soft wheat in flour and alimentary pastes. Boll. Lab. Chim. Prov. 19: 687-695. (CA 70: 86301d).