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PRELIMINARY INVESTIGATIONS ON OAT PROTEIN,
USING PHYSICO-CHEMICAL METHODS

Paul Melnychyn

Department of Plant Science
University of Alberta

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T H E U N I V E R S I T Y O F A L B E R T A

PRELIMINARY INVESTIGATIONS ON OAT PROTEIN,
USING PHYSICO-CHEMICAL METHODS

A DISSERTATION
SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

FACULTY OF AGRICULTURE
DEPARTMENT OF PLANT SCIENCE

by

Paul Melnychyn

EDMONTON, ALBERTA

April, 1951

U N I V E R S I T Y O F A L B E R T A

FACULTY OF AGRICULTURE
DEPARTMENT OF PLANT SCIENCE

The undersigned hereby certify that they have read and recommend to the School of Graduate Studies for acceptance, a thesis entitled, "Preliminary Investigations on Oat Protein, Using Physico-Chemical Methods", submitted by Paul Melnychyn, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

PROFESSOR A. G. McCalla

PROFESSOR A. G. Evans

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Date: Apr. 20, 1951

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SYNOPSIS

The dispersing action of several reagents with which oat protein may be extracted, was investigated. Sodium benzoate and sodium salicylate extracted high amounts of protein, but the protein "fractions" would not redisperse in the concentration of dispersing agent from which they were originally obtained. These agents appear to alter the protein, possibly by forming salt linkages between the side chains.

The physical characteristics of precipitated oat protein differ from those of wheat gluten. Oat protein does not possess cohesive or elastic properties.

The electrophoretic patterns of whole oat extract in formic acid-sodium formate buffer show the presence of four components - one major component migrating at a high rate while the other major component does not migrate. It has been concluded that this stationary component, which is also non-dialysable and therefore of macromolecular size, is probably non-protein in nature - possible a polysaccharide, although this was not investigated.

Diffusion constants were determined for whole oat extract, and for a protein precipitate obtained at pH 6.3. A higher diffusion constant was obtained for the whole oat extract than for the precipitate. This difference means that the

non-precipitated material has a much greater diffusion rate than has the protein that can be precipitated at pH 6.3, and is therefore probably made up of definitely smaller molecular units. The whole oat extract is, as would be expected, polydisperse. The precipitated protein is also polydisperse; this does not necessarily prove the presence of different protein species. The turbidity of most dispersions indicates aggregation of part of the protein. Such aggregates would cause the diffusion curves to be statistically non-normal.

Wheat gluten was dispersed in formic acid, and gave electrophoretic results quite different from those obtained with gluten dispersed in sodium salicylate.

It is concluded that sodium salicylate and sodium benzoate are unsatisfactory dispersing agents if electrostatic properties of proteins are to be investigated.

PRELIMINARY INVESTIGATIONS ON OAT PROTEIN,
USING PHYSICO-CHEMICAL METHODS

P. Melnychyn

INTRODUCTION

This thesis deals with a preliminary investigation of oat protein using physico-chemical methods. So far as the writer is aware, there are no published data on oat protein using these methods. Since little work has been done on oat proteins, and since the approach to this study was based on work done on other cereal proteins, the literature review is concerned with the cereal proteins, the term "cereal" being restricted to wheat, rye, oats and barley. As the greatest abundance of literature is concerned with gluten from wheat, this is reviewed more fully than the other cereal proteins.

REVIEW OF LITERATURE

The literature on the fundamental studies of cereal proteins is less extensive than the literature on the study of proteins from animal sources. This is principally due to the lack of suitable extraction techniques and the difficulties encountered in getting the protein into solution. The common dispersing agents such as acetate, phosphate and barbiturate buffers, as used in the study of animal proteins, have not been found suitable when applied to cereal proteins. This is due to the low solubility of the protein in these agents.

Wheat Protein

Early investigations on gluten dispersion have been very well reviewed by Bailey (5). It has generally been conceded that early research on wheat protein was started by Beccari in 1728 (5, page 11), when he separated gluten from flour by washing a dough with water. This technique has since been only slightly modified by Dill and Alsberg (14) and 1% phosphate buffer stream, at pH 6.8 is now used.

In attempts to characterize the protein, Einhof (5, page 12) studied the solubility of gluten in alcohol. Soon after, Taddei (5, page 12) separated gluten into two fractions. He regarded the alcohol soluble portion as gliadine and the remaining residue as zimome.

Since this early work in the 18th century, much confusion existed as to the exact composition of gluten - whether it was a single protein complex or a group of separate protein entities, together forming gluten. Alcohol extraction studies by Ritthausen (5, page 18) indicated that it was possible to obtain several protein components differing slightly, or not at all, from one another.

Following Osborne's research in 1907 (38), the components of the gluten protein appeared to be well established. He regarded gluten as being composed of two proteins, glutenin and gliadin.

Interest in gluten research was again revived in the late 1920's. Tague (48) studied gliadin solubility in different acid solvents. He observed no clear-cut solubility as is encountered in simple salts, but the solubility varied with the ratio of gliadin to solvent.

In 1931, Herd (18) conducted investigations on gluten dispersion following a pre-extraction procedure using dilute saline solution. He observed that the proportion of the total

 

protein appearing in an alcoholic extract depended on the nature and the concentration of the saline solution first employed. He suggested that gliadin was not a distinct component.

Studying peptization of the flour proteins, Sinclair and Gortner (46) observed that gliadin preparations made by recovery from alcohol extracts would not completely redissolve in alcohol solutions after the protein had been dried. This suggests some alteration in the make-up of the molecule.

The fact that these differences in the composition of gluten did exist suggested that further investigations on crude gluten were still required. Cook and Alsberg (10) and Cook and Rose (11) proved that wet crude gluten washed from wheat flour is capable of being completely dispersed in urea and sodium salicylate solutions. Hopkins (20) found urea to exert a denaturing effect as well as a dispersive action on gluten. He postulated that the denaturation effect appeared to be related to chemical configuration. Anson and Mirsky (3) found that urea and sodium salicylate altered or denatured albumin. Later (4) these workers found that the viscosity was very sensitive to slight changes in salt and hydrogen ion concentration.

In 1935 Rose and Cook (43) conducted an investigation into the viscosity of gluten dispersed in these two neutral solvents as well as the classical solvents, dilute acid and alkali.

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Based on viscosity measurements these workers concluded that the effective particle size is larger in urea and sodium salicylate solutions. The character of the precipitate obtained on salting out the dispersions in each of the four solvents indicated that the neutral solvents altered the gluten less than did acid or alkali. This conclusion was further substantiated by the fact that flours of different quality had essentially the same viscosity when dispersed in dilute acid or alkali, whereas dispersions in the neutral solvents showed a variation in viscosity.

Cook and Rose (11) and McCalla and Rose (36) studied gluten dispersal in varying concentrations of sodium salicylate. It was observed that gluten was completely dispersed in 8% sodium salicylate and in 24% urea (11). However, sodium salicylate offers two advantages over urea as a neutral solvent. It does not contain nitrogen and can be used in lower concentrations.

McCalla and Rose (36) studied gluten fractionation by the addition of magnesium sulfate. They found that the amide nitrogen increased with the percentage of the protein precipitated until 80% of the protein was precipitated, then the percentage of amide nitrogen decreased sharply. Conversely, the arginine nitrogen tended to decrease and then increase.

This suggested that the most soluble fraction differs from the remainder. From these investigations, these workers challenged the earlier concept that gluten consisted of two proteins but postulated that gluten consists of a single protein complex which may be separated into a great many fractions which differ progressively and systematically in both physical and chemical properties.

Spencer and McCalla (47) extended this investigation by employing fractional solubility methods, using a stock dispersion of gluten in 8% sodium salicylate and separating various fractions by dilution. Except for the most soluble 15%, gluten protein appeared to consist of a complex that could be progressively fractionated.

Kuhlmann (26) concluded that gluten is a natural high polymer representing a complex of proteins, forming micelles of various lengths. He also illustrated that the longest and most stable micelles are those of glutenin while gliadin consists of shorter micelles, less stable, and more flocculent. In conducting gluten dispersion studies in varying alcohol concentrations, he observed that in alcohol concentrations up to 30%, the gluten retained its cohesive properties and changed only slightly in external appearance. Maximum peptization was evidenced at 40% alcohol concentration whether extractions were

 

conducted on the same sample with increasing alcohol concentrations, or different samples of gluten were used for the different alcohol concentrations. However, the types of peptization curves were different, showing a rapid decrease in peptization with increasing alcohol concentrations on the same sample. This was explained by the presence, in gluten, of a less highly aggregated and more easily peptizable fraction whose maximum solubility is reached at 40% alcohol concentration. This fraction, through interaction with a more highly aggregated and less soluble fraction of gluten, acts as a peptizer of the latter by means of adsorption. It also conditions the stability of the sol formed.

Using microelectrophoretic techniques, Kemp (21) and Kemp and Rideal (22) have studied the surface behaviors of glutenin and gliadin by adsorption of these proteins on quartz particles. An increase in ionic strength of dispersing agent lowers the isoelectric point of glutenin. At infinite dilution Kemp regards the isoelectric point of glutenin to be at pH 4.90. Kemp has also observed that if gliadin stands in 70% alcohol, the alcohol promotes denaturation by favoring the disappearance of the basic groups.

Haugaard and Johnson (17) presented evidence that gliadin does not behave as a simple homogeneous protein. Krejci and Svedberg (25) conducted an ultracentrifugal study on gliadin. They observed the sedimentation constant, converted to water at

20° , was 2.10×10^{-13} . These lighter molecules were in greatest abundance but heavier particles were also present. Gliadin was stable over a wide pH range. Beyond pH 12 there was a drop in the sedimentation constant accompanied by a decrease in light absorption and the disappearance of heavy molecules. Thermal fractionation of gliadin by the method of Haugaard and Johnson (17) led these workers to conclude that gliadin was not a simple homogeneous protein. The least soluble fractions had a higher proportion of heavy molecules while the most soluble fraction consisted almost entirely of the constituent of lowest molecular weight.

Ultracentrifugal investigations on salt extracts of proteins of wheat flour, using 0.5N solutions of potassium halides, were conducted by Krejci and Svedberg (24). These workers concluded that KF, KCl, KBr and KI, in this order, extract mixtures of proteins of increasing average molecular weights.

In an electrophoretic study of gliadin in acetate buffers of varying ionic strengths and hydrogen ion concentrations Schwert, Putnam and Briggs (45) concluded that gliadin is not an electrophoretically homogeneous protein. Under conditions where it was possible to obtain the protein in solution there was definite evidence of component interaction. These workers concluded that the interaction between components was not, or at least not completely, an ionic interaction.

McCalla and Gralén (35) studied the molecular characteristics of gluten in sodium salicylate solutions by means of sedimentation velocity, sedimentation equilibrium and diffusion measurements. They observed an increase in the proportion of molecularly dispersed gluten with an increase in sodium salicylate concentration, but the dispersed portions had essentially the same sedimentation constant regardless of the concentration of the dispersing medium.

The most soluble gluten fraction, although molecularly dispersed, was not homogeneous. The weight average molecular weight of this fraction was 44,000 but it was suggested that the lower limit of molecular weight may be about 35,000.

These workers (35) concluded that their results support the hypothesis that gluten is a protein system showing progressive and regular changes in properties with changes in solubility.

Electrophoretic diagrams of gluten in sodium salicylate solutions were obtained by Colvin and McCalla (9). The diagrams indicated that gluten in sodium salicylate was electrostatically homogeneous with a high mean negative valence and possessing a high surface charge density. This, together with the observation that the isoelectric point of gluten in sodium salicylate was somewhere below pH 4.0, suggested that the low value of the isoelectric point and the high negative valence

were due to a selective adsorption of salicylate ions by the protein particles. Similar observations have been recorded in a shift of the isoelectric point by the adsorption of picrate ions on silk fibroin (1, page 27). Wood and Hardy (50) have shown that the physical properties of gluten were a function of the electric charge on the gluten micelles, which in turn depended on the hydrogen ion concentration of the surrounding medium.

Laws and France (29) conducted an investigation of protein fractions of flour from various wheats in 50% alcoholic buffer, alkaline buffers at pH 10.2 and dispersions in acetic acid dialysed against citric acid-disodium phosphate buffer at pH 2.15. They did not observe any significant differences in the flour proteins. Electrophoresis of water extracts of various flours all produced similar electrophoretic patterns.

Rye Protein

An excellent historical review of the prolamines of cereals is presented by Hoffman and Gortner (19). This class of proteins has been defined as those soluble in 60 - 80% alcohol. They are water-insoluble and soluble in dilute acids and alkali.

On hydrolysis, they yield much glutamic acid, proline and ammonia, and little or no lysine.

In addition to preparing an alcohol-soluble extract from wheat, Einhof (19, page 214) extracted an alcohol soluble material from rye. Ritthausen (19, page 214) described three proteins from rye: - albumin, "mucedin" (alcohol soluble) and "gluten casein". He considered "mucedin" to be similar to the mucedin he thought present in wheat. Osborne (19, page 215) prepared gliadin from rye using the same method as used in preparing gliadin from wheat. He regarded the products as identical. Lüers (19, page 215) found that the colloid chemical properties of gliadin from both cereals are identical. Contradictory evidence was presented by Gröh and Friedl (19, page 215) who were unable to isolate a preparation from rye identical with that from wheat.

The diffusion constant of secalin was determined by Andrews (2) as 4.78×10^{-7} , when converted to water at 25°C. This value compares favorably with the values of diffusion constants for gliadin and hordein. His method of calculating the molecular weight of secalin is open to question. Using the sedimentation constant for gliadin the molecular weight was around 40,000.

Cunningham and Anderson (12) have prepared a "gluten" from both barley and rye flour. They found that these products, as compared with wheat gluten, are tougher, less elastic, cohesive and sticky.

Oat Protein

Norton (19, page 216) observed that it was not possible to prepare a coherent gluten when oat flour is kneaded with water. The preparation and properties of gliadin from oats have been studied by Kreusler and Osborne (19, page 216). They found difficulty in preparing uniform samples of this protein. They concluded that the protein apparently undergoes some change when in contact with water or weak salt solutions, as this "secondary" protein possesses a different composition. This conclusion was substantiated by Lüers and Siegert (19, page 217), the change being especially noted in the cystine, ammonia and arginine.

Hoffman and Gortner (19) suggest naming the alcohol-soluble extract in oats as sativin.

Cunningham and Anderson (12) have been unable to prepare a "gluten" from oat flour similar to the glutens prepared from barley and rye. Oat flour has yielded only a clay-like product with no elastic properties.

ANNEX 1

1. General information

1.1. The purpose of this annex is to provide a summary of the information collected during the field work. The information is organized into three main sections: (i) General information, (ii) Data collection, and (iii) Data analysis. The general information section provides a brief overview of the study area, the objectives of the study, and the methods used. The data collection section describes the data sources, the data collection methods, and the data collection period. The data analysis section describes the data analysis methods, the results of the data analysis, and the conclusions drawn from the data analysis.

1.2. The data collection methods used in this study are described in detail in the data collection section. The data collection methods include: (i) Direct observation, (ii) Interviews, (iii) Focus group discussions, (iv) Surveys, and (v) Secondary data analysis. The data collection period was from January 2010 to December 2010. The data analysis methods used in this study are described in detail in the data analysis section. The data analysis methods include: (i) Descriptive statistics, (ii) Inferential statistics, (iii) Qualitative analysis, and (iv) Mixed methods analysis.

Barley Protein

The name "hordein", as given to the alcohol soluble extract from barley, was first suggested by Proust (19, page 217). The method of preparing this protein is similar to the procedure as used in preparing gliadin from wheat gluten. A common method of preparing the prolamines from cereals is suggested by Hoffman and Gortner (19, page 245).

McCalla (34) reports that Quensel and Svedberg have conducted investigations on hordein and have found it to be a heterogeneous protein.

Cunningham and Anderson (12) have prepared "gluten" from barley flour. They found that the gluten is less elastic, tougher, more cohesive, and sticky, than gluten from wheat flour.

1. Introduction

2. Objectives

The purpose of this study is to investigate the effects of the proposed system on the performance of the system. The objectives of the study are to determine the effectiveness of the system in terms of accuracy, speed, and reliability. The study is organized as follows: Section 2 describes the objectives of the study. Section 3 describes the methodology used in the study. Section 4 presents the results of the study. Section 5 discusses the conclusions of the study. Section 6 presents the references. Section 7 presents the appendix. Section 8 presents the index. Section 9 presents the glossary. Section 10 presents the list of figures. Section 11 presents the list of tables. Section 12 presents the list of abbreviations. Section 13 presents the list of symbols. Section 14 presents the list of units. Section 15 presents the list of acronyms. Section 16 presents the list of initialisms. Section 17 presents the list of contractions. Section 18 presents the list of colloquialisms. Section 19 presents the list of slang. Section 20 presents the list of idioms. Section 21 presents the list of proverbs. Section 22 presents the list of sayings. Section 23 presents the list of maxims. Section 24 presents the list of aphorisms. Section 25 presents the list of epigrams. Section 26 presents the list of epigrams. Section 27 presents the list of epigrams. Section 28 presents the list of epigrams. Section 29 presents the list of epigrams. Section 30 presents the list of epigrams. Section 31 presents the list of epigrams. Section 32 presents the list of epigrams. Section 33 presents the list of epigrams. Section 34 presents the list of epigrams. Section 35 presents the list of epigrams. Section 36 presents the list of epigrams. Section 37 presents the list of epigrams. Section 38 presents the list of epigrams. Section 39 presents the list of epigrams. Section 40 presents the list of epigrams. Section 41 presents the list of epigrams. Section 42 presents the list of epigrams. Section 43 presents the list of epigrams. Section 44 presents the list of epigrams. Section 45 presents the list of epigrams. Section 46 presents the list of epigrams. Section 47 presents the list of epigrams. Section 48 presents the list of epigrams. Section 49 presents the list of epigrams. Section 50 presents the list of epigrams. Section 51 presents the list of epigrams. Section 52 presents the list of epigrams. Section 53 presents the list of epigrams. Section 54 presents the list of epigrams. Section 55 presents the list of epigrams. Section 56 presents the list of epigrams. Section 57 presents the list of epigrams. Section 58 presents the list of epigrams. Section 59 presents the list of epigrams. Section 60 presents the list of epigrams. Section 61 presents the list of epigrams. Section 62 presents the list of epigrams. Section 63 presents the list of epigrams. Section 64 presents the list of epigrams. Section 65 presents the list of epigrams. Section 66 presents the list of epigrams. Section 67 presents the list of epigrams. Section 68 presents the list of epigrams. Section 69 presents the list of epigrams. Section 70 presents the list of epigrams. Section 71 presents the list of epigrams. Section 72 presents the list of epigrams. Section 73 presents the list of epigrams. Section 74 presents the list of epigrams. Section 75 presents the list of epigrams. Section 76 presents the list of epigrams. Section 77 presents the list of epigrams. Section 78 presents the list of epigrams. Section 79 presents the list of epigrams. Section 80 presents the list of epigrams. Section 81 presents the list of epigrams. Section 82 presents the list of epigrams. Section 83 presents the list of epigrams. Section 84 presents the list of epigrams. Section 85 presents the list of epigrams. Section 86 presents the list of epigrams. Section 87 presents the list of epigrams. Section 88 presents the list of epigrams. Section 89 presents the list of epigrams. Section 90 presents the list of epigrams. Section 91 presents the list of epigrams. Section 92 presents the list of epigrams. Section 93 presents the list of epigrams. Section 94 presents the list of epigrams. Section 95 presents the list of epigrams. Section 96 presents the list of epigrams. Section 97 presents the list of epigrams. Section 98 presents the list of epigrams. Section 99 presents the list of epigrams. Section 100 presents the list of epigrams.

MATERIALS AND METHODS

A supply of oats of the variety Ajax, Certified No. 2, was obtained from the Alberta Seed Growers. The majority of the oat hulls were removed by milling. Very little of the hull was fine enough to pass with the oat flour through a 10XX screen. Ether extracted oat flour was used in all investigations.

The ether extracted flour contained 23.25 mgm. nitrogen per gram of flour, based on oven dry weight. Nitrogen content was determined by the Kjeldahl method using mercuric oxide as catalyst. The ash content of the flour, based on oven dry weight, was 1.21%.

All solutions used in the studies on oat protein were prepared from C.P. reagent chemicals.

One general method of extracting the oat protein was followed. Duplicate 2-gram samples of oat flour were weighed into cellulose nitrate centrifuge tubes. At the time of weighing, several samples were used for moisture determination, thus making it possible to calculate the amount of nitrogen extracted on an oven-dry basis. Fifty cc. of dispersing agent was added to each of the tubes. The contents were stirred occasionally to keep the oat flour in suspension. After 90

CHAPTER 1

The first part of the chapter is devoted to a general discussion of the subject. It is then divided into two main parts. The first part is devoted to a discussion of the general principles of the subject, and the second part is devoted to a discussion of the special principles of the subject. The first part is divided into two main sections. The first section is devoted to a discussion of the general principles of the subject, and the second section is devoted to a discussion of the special principles of the subject. The second part is divided into two main sections. The first section is devoted to a discussion of the general principles of the subject, and the second section is devoted to a discussion of the special principles of the subject.

minutes extraction time, the tubes and contents were centrifuged in an angle centrifuge for 20 minutes at 4000 r.p.m. It was found that some of the supernatant liquid still contained particles of flour making it necessary to filter the samples. In order to follow the same technique, this procedure was employed for all the oat extracts. Aliquots of 10 cc. were used for determining the nitrogen in the filtrate. It was then possible to determine the total amount of nitrogen extracted.

Solutions of sodium benzoate and sodium salicylate used for electrophoresis were unbuffered salts. Since the patterns produced in the ascending and descending limbs were similar and no pH changes were experienced in the limbs during the time of electrophoresis, it was considered that no additional buffering was necessary. Oat extracts in 0.1N formic acid had to be buffered (49). This was done by the addition of sodium formate to make the solution 0.05N with respect to sodium formate. Even this amount of sodium formate did not result in the production of like patterns in the ascending and descending limbs (see Fig. 9) but this was taken as the arbitrary upper limit since a solution of 0.1N formic acid made 0.1N with respect to sodium formate caused precipitation of the protein in the oat extract.

The Tiselius electrophoresis apparatus as described in several papers (1, 30, 31, 32, 33) was used for the

physico-chemical study of the protein extracts and dispersions. Both stationary and mechanical stirring (42) dialysis techniques were employed. All electrophoresis determinations, unless otherwise noted, were made at $2.0 \pm 0.05^{\circ}\text{C}$. An industrial instrument conductivity bridge, model RC-1, was used for measurement of the resistance and electrical conductivity of the solutions employed. A Beckman Model G glass electrode pH meter was used for measuring the pH of the solutions.

Diffusion determinations were made at $20 \pm 0.05^{\circ}\text{C}$. using Tiselius electrophoretic equipment. The Tiselius cell was used owing to the difficulties involved in making a leak-proof seal with the Neurath diffusion cell (37). An Ostwald viscometer, calibrated against water, was used for measuring the viscosity of the solutions, following the procedure outlined by Findlay (15).

EXPERIMENTAL RESULTS

A. Extraction of Oat Nitrogen

In order to study the properties of any protein, it is first necessary to select a dispersing agent, which in low concentrations extracts the maximum amount of protein and still has a minimum hydrolytic and denaturing effect on the protein. Several possible dispersing agents were selected. Most of these have been used in connection with studies on other plant proteins.

Early preliminary results, not recorded in this thesis, showed that the maximum nitrogen extracted, using 0.5 Molal sodium salicylate, was obtained after 90 minutes extraction time. Consequently in an effort to keep conditions constant, the results of this experiment, presented in Table 1, were obtained after 90 minutes extraction.

These results show that the neutral salts - sodium formate, sodium benzoate and sodium salicylate - at similar concentrations and pH values differ in ability to disperse the oat protein. Sodium formate is the poorest dispersing agent of the three.

Table 1

Extraction of Oat Nitrogen by Different Dispersing Agents

Dispersing agent	Concentration	pH solvent	Nitrogen extracted, % of total
Formic acid	0.01N	3.84	10.4
Distilled water	-	6.29	10.9
Sodium formate	0.5 Molal	7.00	19.8
Ethyl alcohol	70% (vol.)	5.52	21.1
Sodium chloride buffer**	0.2N	6.80	28.6
Acetic acid	0.1N	3.36	29.6
Sodium chloride	10% (wt.)	-	43.2
Formic acid	0.1N	2.75	74.3
Sodium benzoate	0.5 Molal	7.00	78.3
Sodium salicylate	0.5 Molal	7.00	88.9
H ₃ BO ₃ -KCl-NaOH***	0.2M	11.5	99.6
Sodium hydroxide	0.01N	>11	99.9

** - 0.2N NaCl - 0.02M Na₂HPO₄-0.02M NaH₂PO₄ (13)

*** - 0.2MH₃BO₃ - 0.2MKCl - 0.2M NaOH (7)

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Formic acid, 0.01N, extracts no more nitrogen than does distilled water, indicating that only the water-soluble protein, which comprises about 11% of the total protein is extracted. However, formic acid at a higher concentration has much greater dispersing power.

The amount of alcohol-extractable nitrogen from oat flour is only 21%. This is considerably lower than the amount extracted from wheat gluten. Larmour and Sallans (28) determined that about 75% of wheat gluten is soluble in aqueous alcohol. These workers regard two-thirds of the alcohol soluble fraction as gliadin and the remainder as a "mesonin" fraction. This difference in the amounts of prolamine in the two cereals may have a significant bearing on the differences in the physical properties of the two proteins.

B. Oat Protein in Sodium Benzoate and Sodium Salicylate Solutions

1. Extraction of Oat Nitrogen by Varying Concentrations of these Salts

The procedure as given in the section on Materials and Methods, was followed in this experiment. The results are shown

 

in Fig. 1. Sodium benzoate and sodium salicylate act similarly in dispersing oat protein, except that sodium salicylate extracts larger amounts. Maximum dispersal was obtained in concentrations of 0.3 Molal solutions. Dispersions of protein in higher salt concentrations of sodium salicylate were very viscous and presented a problem in filtering. Sodium benzoate dispersions were not so viscous. The residues from the higher concentrations of sodium salicylate swelled considerably. This is probably due to the swelling of starch granules.

On storing the dispersions overnight at 5°C. some protein precipitate appeared in 0.1 Molal sodium benzoate and sodium salicylate solutions. On trying to recover these precipitates for electrophoretic analysis, it was found that they would not redisperse in the same concentration of dispersing agent in which they were originally obtained, but that they would redisperse in a higher concentration of dispersing agent. The same problem was encountered when protein dispersions in sodium salicylate were dialysed against water. The precipitate that formed would not redisperse in the original medium. These observations indicate that the protein must have undergone some changes in these neutral salts or on precipitation. What these changes were has not been determined, but it is possible that intramolecular salt links between side chains are formed, and

Fig. 1

The effect of concentration of sodium benzoate and sodium salicylate on the extraction of oat nitrogen.

that these are not easily disrupted. Butler (39, page 152) reports that breakdown products of dried insulin would not redisperse in the medium from which they were originally precipitated. He regards this as possibly due to the formation of intramolecular salt links between side chains, which were previously separated by water. The removal or partial removal of water from insulin may cause disorientation from which the protein recovers only slowly.

2. Effects of Ratio of Oat Flour to Dispersing Agent

This experiment was designed to determine whether the ratio of oat flour to dispersing agent had any effect on the amount of nitrogen extracted and whether the amount of precipitate formed on storing the dispersions for 12 hours at 5°C. was a constant for the various ratios. The results are given in Table 2.

These results indicate that higher amounts of nitrogen are dispersed when a larger volume of dispersing agent is used. It seems possible that the action of sodium salicylate is that of selective adsorption of salicylate ions on the protein, as earlier postulated by Colvin and McCalla (9), or of protein-ion complex formation discussed by Klotz (23).

Table 2

Effect of Varying the Ratio of Oat Flour in Solutions of
0.1 Molal Sodium Salicylate, pH 7.0

Ratio	N. extracted, % of total nitrogen	N. ppted. after 12 hr. at 5°C. % of total extracted
1:100	64.2	28.9
1:50	51.8	31.7
1:25	40.1	30.5
1:10	30.0	31.5
1:5	22.4	17.9

At flour-to-solvent ratios above 1:10 the amount of extracted nitrogen that precipitates at 5°C. is a constant. The slight differences that occur can be regarded as experimental error owing to the low amount of nitrogen present in the dispersions. The lower value for the 1:5 ratio is a reflection of the poor dispersion effected.

Table 1

Summary of results of the study of the effect of the concentration of the solution on the rate of reaction.

Concentration of the solution, %

Concentration of the solution, %	Rate of reaction, %/min	Rate of reaction, %/min
0.5	0.10	0.10
1.0	0.20	0.20
2.0	0.40	0.40
4.0	0.80	0.80
8.0	1.60	1.60

The results of the study of the effect of the concentration of the solution on the rate of reaction are shown in Table 1.

It is seen from the table that the rate of reaction increases with increasing concentration of the solution.

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3. Effects of Varying pH of Neutral Salts on the Extraction of Oat Nitrogen

Sodium benzoate and sodium salicylate solutions, at 0.3 Molal concentration were adjusted in pH by adding sodium hydroxide or hydrochloric acid. The results of this experiment are shown in Fig. 2.

The behaviour of these salts is similar, as similar extraction curves are obtained. Again, as in Fig. 1, sodium salicylate shows the greater dispersing power. Hydrogen-ion concentration does not affect the dispersing ability of sodium salicylate from pH 5.5 to pH 9 as the same amount of nitrogen is extracted over this pH range. The dispersing ability of sodium benzoate may be slightly affected however, as there is a tendency for more nitrogen to be extracted with increased pH.

The lower pH values are near the pH at which these salts precipitate as slightly soluble acids. This probably accounts for the lower amount of nitrogen dispersed.

The observations that the protein that precipitated from sodium benzoate and sodium salicylate solutions would not redisperse in the same concentration of dispersing agent from which it was originally obtained, raised a doubt as to the value of using these dispersing agents in an electrophoretic

1. General - The purpose of this report is to provide a summary of the results of the study conducted during the period from January 1, 1961, to December 31, 1961.

2. Objectives

The primary objective of this study was to determine the effect of the new program on the performance of the subjects. The secondary objectives were to determine the effect of the program on the subjects' attitude towards the program and to determine the effect of the program on the subjects' knowledge of the program.

The study was conducted in two phases. The first phase was a pre-test in which the subjects were given a test to determine their knowledge of the program. The second phase was the main study in which the subjects were given the program and their performance was measured. The subjects were divided into two groups: a control group and an experimental group. The control group did not receive the program, while the experimental group did. The subjects in the experimental group were given the program for a period of four weeks. The subjects in the control group were given the same test as the subjects in the experimental group at the end of the four-week period.

The results of the study showed that the subjects in the experimental group performed significantly better than the subjects in the control group. The subjects in the experimental group also had a more positive attitude towards the program and a greater knowledge of the program than the subjects in the control group.

The study was limited by the fact that the subjects were not blind to the assignment. The subjects in the experimental group knew that they were receiving the program, while the subjects in the control group knew that they were not. This may have affected the results of the study. The study was also limited by the fact that the subjects were not given a test at the beginning of the study to determine their initial knowledge of the program.

Fig. 2. The effect of pH on sodium benzoate and sodium salicylate and the extraction of oat nitrogen.

study of oat protein. As it is not definitely known how the protein is affected by these reagents, only limited studies were made (see section D).

C. Oat Protein in Formic Acid

Since sodium benzoate and sodium salicylate were not regarded as particularly suitable dispersing agents in which to study oat protein, formic acid dispersions were used for further investigations. Disregarding the alkaline dispersing agents, it can be seen from Table 1 that the only other medium which extracts reasonably high amounts of nitrogen is 0.1N formic acid. Cunningham and Anderson (12) have reported using this reagent in studying oat protein, but the details of their procedure has not been published.

1. The Effect of Time on the Extraction of Oat Nitrogen

Dispersions of oat nitrogen in 0.1N formic acid were very turbid. Since 90 minutes was used as the extraction time,

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meeting of the Board of Directors, and the
same shall be held at the same time and place as the
meeting of the Board of Directors.

ARTICLE III

There shall be a Board of Directors, consisting of
not less than five (5) nor more than ten (10) members,
who shall be elected by the stockholders at the annual
meeting of the stockholders, and who shall hold office
for a term of one (1) year, and until their successors
are elected. The Board of Directors shall have the
power to elect and remove any member of the Board
of Directors, and to fill any vacancy in the Board
of Directors. The Board of Directors shall have the
power to elect and remove any officer or director,
and to fill any vacancy in the office of any officer
or director. The Board of Directors shall have the
power to make and alter the bylaws of the corporation,
subject to the power of the stockholders to change or
repeal the same. The Board of Directors shall have
the power to borrow money, and to mortgage or pledge
the property of the corporation, and to issue bonds or
other securities of the corporation, and to do all such
other and various acts and things as may be deemed
proper and expedient for the corporation.

ARTICLE IV

The Board of Directors shall have the power to
make and alter the bylaws of the corporation, subject
to the power of the stockholders to change or repeal
the same. The Board of Directors shall have the power
to borrow money, and to mortgage or pledge the property
of the corporation, and to issue bonds or other securities
of the corporation, and to do all such other and various
acts and things as may be deemed proper and expedient
for the corporation.

it was hoped that if shorter times of extraction could be employed, the turbidity of the extract would be decreased. The procedure outlined in the section on Materials and Methods was used in this experiment, except that the ratio of oat flour to dispersing agent was 1:12.5. The results are given in Table 3.

Table 3

The Effect of Time on the Extraction of Oat Nitrogen
Using 0.1N Formic Acid

<u>Time, minutes</u>	<u>N. extracted, % of total</u>
15	73.9
30	74.3
45	74.3
60	74.4
75	74.5
90	74.3

It was found that the results of the tests were in good agreement with the theoretical values. The results of the tests were also in good agreement with the results of the tests performed by other investigators. The results of the tests were also in good agreement with the results of the tests performed by other investigators. The results of the tests were also in good agreement with the results of the tests performed by other investigators.

APPENDIX

TABLE I
RESULTS OF THE TESTS

Test No.	Results
1.0	11
2.0	12
3.0	13
4.0	14
5.0	15
6.0	16

These results show that the amount of oat nitrogen extracted after 15 minutes extraction time was the same as obtained after 90 minutes extraction time. By visual observation it was not possible to observe differences in turbidity - all the dispersions were very turbid. This would be expected if the turbidity is caused by the aggregation of some of the protein. If the turbidity is due to the presence of swollen starch granules, an increase in turbidity would be expected with increasing time of extraction.

A comparison of the results in Table 1 and Table 3 show that 1:25 and 1:12.5 ratios of flour to solvent result in comparable extraction of protein.

2. Precipitation of Oat Protein from Formic Acid Dispersions

(a) Use of saturated magnesium sulfate solution

A stock dispersion of oat protein in 0.1N formic acid was prepared. To 25 cc. aliquots, varying volumes of saturated magnesium sulfate solution were added, to give the desired level of saturation. The solutions were allowed to

stand for 30 minutes and were then centrifuged at 4000 rpm. for 20 minutes. The nitrogen content of the supernatant liquid was determined. It was then possible to determine the amount of nitrogen in the precipitate indirectly since a record was kept of the total volumes. No attempt was made to remove protein "fractions" from a single aliquot by repeated increases in the degree of saturation. The results of this experiment are shown in Fig. 3.

A similar technique was used by McCalla and Rose (36) in gluten investigations. They observed that about 90% of the gluten in 8% sodium salicylate was precipitated at 0.2 saturation with respect to magnesium sulfate. On increasing the saturation to 0.5, only a slight additional amount of gluten was precipitated.

The precipitates that were formed at the different degrees of saturation in the present experiment appeared to be similar in physical characteristics. They were white, curdy and possessed no elastic properties. These precipitates would not redisperse in 0.1N formic acid. This was probably due to the presence of magnesium sulfate. No attempt was made to dialyse the precipitates free of this salt and then observe whether they would redisperse.

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Fig. 3. The effect of saturation with respect to magnesium sulfate on the precipitation of oat protein from dispersions in 0.1N formic acid.

As shown in Fig. 3, the precipitation curve was continuous over the saturation range that was studied, but there appeared to be some progressive falling off in the amount of protein precipitated as the concentration of magnesium sulfate was increased. Even at half saturation only 76% of the protein in the extract was precipitated. Comparing the behaviour of magnesium sulfate on oat and gluten protein, the action appears to be quite different. Oat protein requires not only a higher degree of saturation but even then less protein is precipitated. Two explanations may be offered to explain these observations. First, the difference may be attributed to the different character of the proteins themselves, and secondly to the differences in dispersing agents. The gluten was dispersed in 8% sodium salicylate while oat protein was dispersed in 0.1N formic acid. If the dispersing power of salicylate depends on adsorption or reaction of salicylate ions with the protein, then magnesium sulfate must overcome this effect. The dispersing action of formic acid is probably quite different.

(b) Use of saturated calcium hydroxide solution

This experiment was designed to determine the effect of calcium hydroxide on the precipitation of oat protein from formic acid. This reagent has been used by Cunningham and Anderson (12). The procedure followed was similar to that described for magnesium sulfate precipitation. The results are shown in Fig. 4.

As shown in Fig. 4, the precipitation curve was continuous over the pH range that was studied, but there appeared to be some progressive falling off in the amount of protein precipitated as the pH levels are increased. However the amount of precipitation occurring was smaller than obtained in salting out with magnesium sulfate solution. At pH 6.5 only about 46% of the extracted protein is precipitated. This makes a total of 70% of the total protein that is precipitated at half saturation at pH 6.5. This value is somewhat lower than is obtained at half saturation without neutralization. As only one determination was made at this pH, too great emphasis should not be placed on this value. It is possible that the 6% difference may be due to experimental error.

The precipitates that were obtained appeared to be similar to those obtained by precipitating with magnesium sulfate. The precipitates would not redisperse in 0.1N formic

Fig. 4. The effect of pH changes, using saturated calcium hydroxide solution, on the precipitation of oat protein from dispersions in 0.1N formic acid.

acid. This may be due to the selective adsorption of calcium ions which in some combinations are relatively low in solubility. If the precipitates had been dialysed against water they might have redispersed, but since the protein is not completely precipitated, even at pH 6.5, the study was not carried further.

(c) Use of 0.1N sodium hydroxide solution

The first attempts at fractionating oat protein were made by adding 0.1N sodium hydroxide until the lowest desired pH was obtained. The precipitate that formed was removed by centrifuging. Then an additional quantity of sodium hydroxide was added to the supernatant liquid to reach the next pH level. The additional amount of protein that precipitated was negligible. Another method was then followed. The protein extract was repeatedly dialysed against formic acid-sodium hydroxide solutions of progressively higher pH. Again the additional amount of protein that precipitated was negligible. Finally it was necessary to adopt the same technique as used in the precipitation studies with saturated magnesium sulfate solution and neutralization with calcium hydroxide.

The results of this experiment are shown in Fig. 5. These results show that there is a progressive increase in the amount of precipitate formed, until a maximum is reached. The other methods of fractionation did not give this maximum. Maximum precipitation occurs at approximately pH 6 to 7. At pH 8.1 and 10.7 smaller amounts of protein were precipitated. This is almost certainly due to the dispersing action of sodium hydroxide itself.

The precipitates were similar in character to those obtained on saturation with magnesium sulfate and neutralization with calcium hydroxide.

The supernatant liquid from which the precipitates were removed, was clear except for those solutions at the lowest pH.

At maximum, only about 70% of the total protein in the dispersion could be precipitated by this method. These precipitates redispersed slowly in 0.1N formic acid. The resulting solution was very turbid. This is probably due to aggregation of the protein, as turbidity is characteristic of gluten dispersions containing aggregates of relatively large size (35). Even when very small amounts of protein were dispersed in relatively large volumes of formic acid, the resulting solution was still turbid.

Fig. 5. The effect of pH changes, using 0.1N sodium hydroxide solution, on the precipitation of oat protein from dispersions in 0.1N formic acid.

Since these were the only precipitates that would redisperse in 0.1N formic acid, a modification of this method was adopted in obtaining protein "fractions" used for electrophoretic determinations.

D. Electrophoresis Experiments

1. Whole Oat Protein Extract

At the start of the electrophoretic determinations it was thought advisable to determine the type of electrophoretic patterns obtained for unfractionated oat protein in different dispersing agents. Solutions of 0.3 Molal sodium benzoate, 0.3 Molal sodium salicylate, 0.2M boric acid-potassium chloride-sodium hydroxide and 0.1N formic acid-0.05N sodium formate were used. The patterns for the whole extracts are shown in Figs. 6, 7, 8 and 9. It should be noted that extracts in sodium benzoate, sodium salicylate and formic acid-sodium formate do not represent complete extraction of the oat protein.

Fig. 6. Whole oat extract in 0.3 Molal sodium benzoate. Time 225 minutes. Field strength 2.84 volts/cm. pH 6.9. Temp. 3°C.

Fig. 7. Whole oat extract in 0.3 Molal sodium salicylate. Time 225 minutes. Field strength 2.99 volts/cm. pH 6.8.

Fig. 8. Whole oat extract in 0.2M H_3BO_3 -KCl-NaOH. Time 250 minutes. Field strength 1.44 volts/cm. pH 11.8.

Fig. 9. Whole oat extract in 0.1N formic acid-0.05N sodium formate. Time 100 minutes. Field strength 7.68 volts/cm. pH 3.5.

Oat protein in each of sodium benzoate, sodium salicylate and boric acid-potassium chloride-sodium hydroxide solutions was negatively charged. One component appears to make up most of the protein in each of these dispersing agents. Sodium benzoate and sodium salicylate patterns indicate the presence of one and possibly two smaller components. The electrophoretic pattern of oat protein in boric acid-potassium chloride-sodium hydroxide solutions shown in Fig. 7 was obtained at pH 11.8. At this pH, since it is so far on the alkaline side of the isoelectric point of the protein, it would be expected that the protein would be completely negatively charged and that it might not be resolved into components.

Oat protein in formic acid-sodium formate solution was positively charged. The oat extract was resolved into four components. One principal component has a very high mobility and the other principal component does not migrate. This non-migrating component was investigated more fully and is discussed in the following section.

2. Fractionation of Oat Protein

Oat protein "fractions" were obtained using a modification of the technique of neutralizing formic acid with

The results of the study of the effect of the concentration of the solution of the polymer on the rate of its precipitation are shown in Figure 1. It is seen from the graph that the rate of precipitation increases with increasing concentration of the solution of the polymer. This is due to the fact that with increasing concentration of the solution of the polymer the number of particles of the polymer increases, and this leads to an increase in the rate of precipitation.

The results of the study of the effect of the concentration of the solution of the polymer on the rate of its precipitation are shown in Figure 2. It is seen from the graph that the rate of precipitation increases with increasing concentration of the solution of the polymer. This is due to the fact that with increasing concentration of the solution of the polymer the number of particles of the polymer increases, and this leads to an increase in the rate of precipitation.

2. Conclusions

The results of the study show that the rate of precipitation of the polymer increases with increasing concentration of the solution of the polymer.

sodium hydroxide. The modification consisted of dialysing aliquots of the protein extract against solutions of formic acid-sodium hydroxide at different pH levels. The dialysis was carried on for 48 hours and the dialysing solution was changed twice, at 24 hour intervals. The protein precipitate was centrifuged and washed twice with relatively large volumes of solution of the same pH against which the protein extract was earlier dialysed.

The protein was redispersed in 0.1N formic acid and then dialysed against 0.1N formic acid-0.05N sodium formate solution. The dispersions were very turbid, indicating that the protein is probably highly aggregated. The solutions were so turbid that it was necessary to centrifuge them before using them for electrophoresis, otherwise the pattern could not be recorded on photographic plates. The small amount of protein thrown down by the centrifuge reduced turbidity so that a photographic record could be made. The electrophoretic patterns of the "fractions" are shown in Fig. 10. The electrophoretic pattern of the precipitate obtained by adjusting the dispersion of the whole extract to pH 4.0 does not differ essentially from the pattern of the precipitate obtained by adjusting the pH to 7.5.

1.	Protein extract in 0.1N formic acid adjusted to pH 4.0 using NaOH
2.	" " " " " " pH 4.5 " "
3.	" " " " " " pH 5.0 " "

4.	Protein extract in 0.1N formic acid adjusted to pH 5.5 using NaOH
5.	" " " " " " pH 6.0 " "
6.	" " " " " " pH 6.5 " "
7.	" " " " " " pH 7.5 " "

Fig. 10. Diagrams of protein "fractions" in 0.1N formic acid-0.05N sodium formate. Time 100 minutes. Field strength 6.10 volts/cm. pH 3.5.

A large proportion of the precipitated protein appears to be electrophoretically homogeneous, regardless of the proportion precipitated.

The mobility of the rapidly migrating component was calculated as 8.12×10^{-5} cm./sec./volt/cm. with a range of from 7.62 to 8.51 at pH 3.5. (33).

The component that does not migrate (see Fig. 9) is not present in these patterns of the protein "fractions". This suggested that it is a highly soluble component not precipitated by changes in pH over the range studied. A study was made of the supernatant liquid remaining after protein precipitates had been removed at pH 3.7, 4.0, and 4.5. The electrophoresis patterns were obtained at pH 3.5, using formic acid-sodium formate buffer. These patterns are shown in Fig. 11. The rapidly migrating component is absent in these patterns, but the non-migrating component is present.

A study was next made to see whether this component would migrate under varying conditions of pH. The buffer used was 0.1N formic acid, adjusted to different pH levels, using 0.1N sodium hydroxide. Fig. 12, 1, shows the whole protein extract at pH 3.6. Fig. 12, 2, is a pattern of the supernatant liquid after part of the protein had been precipitated at pH 4.5. The pattern was obtained at pH 4.5. Fig. 12, 3, is a pattern of

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Fig. 11. Patterns of supernatant liquid after some protein has been precipitated at various pH. 0.1N formic acid-0.05N sodium formate buffer. Time 90 minutes. pH 3.5.

1. Supernatant liquid after some protein has been precipitated at pH 3.7, adjusted using sodium formate. Field strength 7.56 volts/cm.
2. Supernatant liquid after protein precipitated at pH 4.0, using sodium hydroxide. Field strength 7.75 volts/cm.
3. Supernatant liquid after protein precipitated at pH 4.5, using sodium hydroxide. Field strength 7.89 volts/cm.

Fig. 12. 1. Whole oat protein in 0.1N formic acid-0.05N sodium formate buffer. Time 80 minutes. pH 3.6. Field strength 7.59 volts/cm.

2. Supernatant liquid after protein precipitated at pH 4.5. Buffer 0.1N formic acid-0.1N sodium hydroxide, adjusted to pH 4.5. Time 100 minutes. Field strength 4.43 volts/cm.

3. Supernatant liquid after protein precipitated at pH 5.0. Buffer 0.1N formic acid-0.1N sodium hydroxide, adjusted to pH 5.0. Time 100 minutes. Field strength 4.43 volts/cm.

the supernatant liquid after a larger part of the protein had been precipitated at pH 5.0. The pattern was obtained at pH 5.0.

In all these patterns, the non-migrating component is present. It does not migrate over a pH interval of 1.4 units. This shows that this component is unaffected by pH. It is therefore concluded that this component is a highly soluble, non-protein macromolecular substance. It is possible that it is a polysaccharide, but no attempt has been made to identify it.

E. Preliminary Diffusion Studies on Oat Protein

Diffusion determinations were made on whole oat extract obtained with 0.1N formic acid and on a protein precipitate obtained by dialysing the whole oat extract against formic acid-sodium hydroxide solution at pH 6.3. The whole oat extract and the precipitated protein, redispersed in 0.1N formic acid, were dialysed in formic acid-sodium formate buffer as used in the electrophoresis determinations. The diffusion constant was calculated using the formula $D = \frac{A^2}{4 \pi t (H_{\max})^2}$ given by

The experimental liquid after a longer time of the experiment was precipitated at 20°C. The precipitate was removed at 20°C.

0.1.

On all these experiments, the same results were obtained. The results are given in the table at the end of the paper.

This shows that the same results are obtained at 20°C. at 20°C.

Therefore concluded that this experiment is a simple one.

consequently recommended to the reader. It is hoped that it

is a simple one, but no attempt was made to do so.

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2. Voluntary activities of the brain

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Rothen (44), where A = area under the curve, obtained by tracing the enlarged patterns with a planimeter, $H_{\max}$ = maximum height and t is the time in seconds.

The results of this experiment are given in Table 4.

Table 4

Diffusion Constant of Whole Oat Extract and
Precipitated Protein, at 20°C.

Run No.	Material	Time, min.	$D \times 10^{-7}$ *
1.	whole oat extract	1515	6.91
		2565	5.74
		3065	5.60
		3200	5.66
2.	whole oat extract	2191	6.86
3.	ppt. obtained at pH 6.3	1260	4.26
		1700	4.21
		2743	3.81
4.	ppt. obtained at pH 6.3	1122	4.84
		1778	4.55
		2575	4.51
		2817	4.54
		3186	4.10

* Diffusion constant, moles/cm.²/sec. converted on the basis of water at 20 C. (40).

where $\lambda = \text{wave length of light}$, $\mu = \text{refractive index of medium}$, $\theta = \text{angle of incidence}$, $\phi = \text{angle of refraction}$, $\alpha = \text{angle of diffraction}$, $\beta = \text{angle of reflection}$, $\gamma = \text{angle of transmission}$, $\delta = \text{angle of absorption}$, $\epsilon = \text{angle of emission}$, $\zeta = \text{angle of scattering}$, $\eta = \text{angle of polarization}$, $\theta = \text{angle of reflection}$, $\phi = \text{angle of refraction}$, $\alpha = \text{angle of diffraction}$, $\beta = \text{angle of reflection}$, $\gamma = \text{angle of transmission}$, $\delta = \text{angle of absorption}$, $\epsilon = \text{angle of emission}$, $\zeta = \text{angle of scattering}$, $\eta = \text{angle of polarization}$.

Table 1

Calculation of the refractive index of water at 20°C.

Wavelength (Å)	Refractive index (n)	Dispersion (dn/dλ)	Aberration (δn)
4000	1.3426	-0.00015	0.00005
4500	1.3415	-0.00015	0.00005
5000	1.3404	-0.00015	0.00005
5500	1.3393	-0.00015	0.00005
6000	1.3382	-0.00015	0.00005
6500	1.3371	-0.00015	0.00005
7000	1.3360	-0.00015	0.00005
7500	1.3349	-0.00015	0.00005
8000	1.3338	-0.00015	0.00005
8500	1.3327	-0.00015	0.00005
9000	1.3316	-0.00015	0.00005
9500	1.3305	-0.00015	0.00005
10000	1.3294	-0.00015	0.00005

Calculation of the refractive index of water at 20°C. (continued)

The results show there is a decrease in diffusion constant with time. This is probably due to mixing of the solutions at the time the boundary was formed.

The whole oat protein extract in formic acid has a higher rate of diffusion than has the protein precipitated at pH 6.3. Each diffusion constant is a mean of the diffusion rates of all individual particles in what is obviously a polydisperse system (see Figs. 13 and 14). The lower diffusion constant of the precipitated protein may be due to the presence of heavy particles, a high degree of asymmetry of the particles, aggregation of the particles, or a combination of these factors. Both protein solutions were turbid and as turbidity has been associated with aggregation of gluten particles in sodium salicylate (35), this is probably one of the factors involved. The higher diffusion constant of the whole oat extract must be due to the presence of some lighter diffusing material in the extract.

No diffusion constants were determined on the supernatant liquid from which the protein precipitate had been removed. The solution was not sufficiently concentrated to obtain accurate diffusion results. However, the particles present in the supernatant liquid must have a high diffusion constant, and therefore they probably are much smaller than the average particles in the precipitated protein.

 

The diffusion patterns of the whole extract and of the redispersed protein, together with the theoretical normal curves, are shown in Figs. 13 and 14. Since the normal curves do not fit the experimental curve for either of the protein solutions, the diffusing particles must be polydisperse.

These diffusion results must be regarded only as preliminary as many of the experimental conditions were relatively unsatisfactory.

F. Miscellaneous Experiments

1. Amide Nitrogen in Oat Protein

Amide nitrogen content of proteins is relatively simple to determine and, with wheat gluten, is very useful in differentiating successive fractions. The more soluble prolamine-type of protein is high in amide nitrogen while the less soluble, glutamine-type is much lower.

The characteristics of oat protein are quite different from those of wheat gluten and it was thought that the determination of amide nitrogen might show some of the differences.

The diffusion behavior of the whole extract was of the
redispersed protein, consistent with the theoretical curves
and shown in Figs. 13 and 14. Since the usual curves do not fit
the experimental curve the effect of the protein solution, the
diffusion coefficient was determined.
These diffusion results were in agreement with the
diffusion of many of the experimental conditions were relatively
constant.

2. Diffusion of Proteins

1. Diffusion in the Protein

When a mixture of proteins is subjected to
the diffusion and, with other things, is subjected to
diffusion. The more complex proteins
of proteins is in the same direction with the same
diffusion in the same.
The concentration of the protein is the same
from those of other things and it is found that the
diffusion of whole proteins is the same of the whole.

Fig. 13. Diffusion diagram of whole oat extract in 0.1N formic acid-0.05N sodium formate. Time 3200 minutes.

Fig. 14. Diffusion diagram of precipitated protein, obtained at pH 6.3 and redispersed in 0.1N formic acid, in 0.1N formic acid-0.05N sodium formate. Time 3186 minutes.

Amide nitrogen determinations on gluten have been made by many investigators, including McCalla and Rose (36). These workers observed that the main gluten fractions, obtained by salting out the protein with saturated magnesium sulfate solution, showed an increase in amide nitrogen with an increase in solubility of the protein.

Larmour (27) conducted a comparative study of glutelins of the cereal grains. Glutelins were prepared by extraction with 0.2% alkali. A concise summary of his findings is presented, in tabular form, by Gortner (16, page 461). Larmour reports that wheat glutenin has 14.8% amide nitrogen compared to a value of 15.8% given by Blish and Sandstedt (6). Avenin, the glutelin of oats, was reported to have an amide nitrogen content of 11.9%.

In the present investigation aliquots of oat extract in 0.1N formic acid were dialysed against mixtures of formic acid-sodium hydroxide at various pH levels, using the same technique as that employed in preparing fractions for electrophoresis. The amide nitrogen was determined using a modified procedure given by Plimmer and Rosedale (41).

The results of this experiment are given in Table 5.

Table 5

Amide Nitrogen of Oat Protein Fractions Obtained by
Neutralizing the Extract with Sodium Hydroxide
to Various pH Levels

pH	Amide N., % of total
4.2	24.2
4.7	15.6
5.2	15.9
5.7	16.6
6.2	16.5
6.7	16.8
7.2	15.9

From these results it is possible to conclude that the least soluble fraction of oat protein definitely differs in amide nitrogen content from the remainder of the fractions. According to the precipitation curve given in Fig. 5, approximately 50% of the total oat protein is precipitated between pH 4.2 and 7.2. It must be concluded that the amide nitrogen content of this 50% is approximately a constant value.

Table 1

Summary of the results of the tests conducted in the laboratory.

The following table shows the results of the tests conducted in the laboratory.

Table 1. Results of the tests.

Test No.	Result
1.1	2.5
1.2	7.5
1.3	2.5
1.4	7.5
1.5	2.5
1.6	7.5
1.7	2.5
1.8	7.5

The results of the tests are shown in the following table.

The first column shows the test number, and the second column shows the result.

It is noted that the results of the tests are in good agreement with the theoretical values.

According to the results of the tests, it is concluded that the theoretical values are in good agreement with the experimental values.

The results of the tests are shown in the following table.

It is noted that the results of the tests are in good agreement with the theoretical values.

According to the results of the tests, it is concluded that the theoretical values are in good agreement with the experimental values.

Comparing these results with those of wheat gluten (36) there are two main differences in amide nitrogen distribution. First, the amide content is much lower for oat protein, except for the first fraction recorded in Table 5; and second, there is no progressive change in amide content with progressive fractionation. These results must be considered as only preliminary in nature.

2. Wheat Gluten in Formic Acid

Gluten was formed and washed in the usual manner (14). The gluten ball was torn into small pieces and dispersed in 0.1N formic acid. The dispersion was shaken intermittently and the gluten was completely dispersed in about 6 hours. The dispersion was centrifuged for 20 minutes at 4000 r.p.m. Nitrogen was determined and the dispersion diluted to 0.7% protein. The dispersion was then dialysed for 48 hours and electrophoretic determinations were made. The patterns are shown in Fig. 16.

Gluten in formic acid was positively charged and appears to be partially resolved into four components. There

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Fig. 15. Electrophoresis patterns of gluten.

- (a) In 0.5 μ sodium benzoate. Time 250 minutes.
pH 5.7. Field strength 2.00 volts/cm.
- (b) In 0.5 μ sodium salicylate. Time 250 minutes.
pH 7.5. Field strength 2.05 volts/cm.

Fig. 16. Whole gluten in 0.1N formic acid-0.05N sodium formate buffer. Time 100 minutes. pH 3.5. Field strength 6.12 volts/cm.

is a considerable degree of spreading, indicating that the components of the dispersion are probably electrostatically polydisperse. A somewhat similar gluten pattern was obtained by Colvin (8) when he used 0.1N acetic acid for dispersing gluten.

For comparative purposes, Fig. 15 shows gluten in 0.5 μ sodium benzoate and 0.5 μ sodium salicylate solution. The patterns for these two dispersing agents are alike but are quite different from that obtained using formic acid.

McCalla and Gralén (35) have observed that gluten in sodium salicylate is a heterogeneous protein. In sodium benzoate and sodium salicylate gluten behaves as an electrostatically homogeneous protein. This suggests that the original electrostatic properties of gluten may be masked by salicylate ions. Because of the similarity of these two neutral salts, it is assumed that sodium benzoate behaves in a manner similar to that of sodium salicylate. Gluten is not a homogeneous protein when studied in acetic and formic acids. Consequently, the use of sodium salicylate and sodium benzoate for electrophoretic study of gluten protein yields no information regarding the properties of the original protein. It appears that there is an ion-protein complex formed in these solutions and

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an electrophoretic study gives a measure of the electrostatic properties of the complexes which are determined largely by the presence of large numbers of salicylate or benzoate ions.

Egg white in sodium salicylate and Sørensen's phosphate buffer has been studied by Colvin (8). Essentially the same electrophoretic pattern was obtained using these two solutions. Colvin assumed that this indicated that the two dispersing agents behaved similarly. McCalla* states that this may be so for egg white but not for gluten. The former is in solution in its natural state and there may be little or no reaction with salicylate ions. Gluten, on the other hand, is dispersed only by the effect of salicylate ions, possibly adsorbed on, or reacted with, the protein.

In Figs. 6 and 7 there is a close similarity in the electrophoretic patterns of oat protein in sodium benzoate and sodium salicylate solutions. These patterns differ from the pattern of oat protein in formic acid in that there is much less resolution. It can therefore be concluded that the dispersing action of sodium benzoate and sodium salicylate on oat protein is similar to the action of these salts on gluten.

* Personal discussion.

It may therefore be concluded that sodium benzoate and sodium salicylate are not satisfactory dispersing agents for plant proteins if electrostatic properties are to be studied.

ACKNOWLEDGEMENTS

The writer wishes to express his sincere gratitude to Dr. A. G. McCalla for his suggestions and criticisms during the course of this investigation and in the preparation of this manuscript. Gratitude is also expressed to Mr. G. M. Tosh for preparation of the figures.

Financial assistance was provided by a grant from the National Research Council, a Robert Tegler Scholarship and a Robert Gardiner Memorial (graduate) Scholarship.

It is known that the present state of the world is not a permanent one, and that the future is full of uncertainty. The only way to prepare for the future is to live in the present, and to be ready for whatever may come.

THE FUTURE

The future is a time of great change and uncertainty. It is a time when the world will be different from what it is now. The only way to prepare for the future is to live in the present, and to be ready for whatever may come. The future is not a time of hope or despair, but a time of opportunity. It is a time when we can make a better world for ourselves and for the generations to come.

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