Quality evaluation of monosomic and chromosome substitution lines of common wheat, Triticum aestivum, L.
by Charles William Green

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Agronomy
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Abstract:
Three sets of substitution lines of the wheat variety Chinese Spring with chromosomes from the donor varieties Thatcher, Hope and Timstein were evaluated for quality to determine which chromosomes carried genes governing bread wheat quality. Chromosomes Hope 2A, 3A, 7B and 2D, Thatcher 4A, 2B and 2D and Timstein 1D, 2D and 6D all appeared to carry genes governing gluten strength. Milling characteristics appeared to be carried on chromosomes Hope 4A 3B, 3D and 5D, Thatcher 3B, 3D and 7D and Timstein 3B, 3D, 5D and 6D,. There was a direct relationship between the percent water absorption of the flour and the percent of damaged starch.

Starch gel electrophoresis of ten of the chromosome substitution lines and the Chinese'Spring and Thatcher parents was made. The electrophoretic patterns for the substitution lines were the same as for Chinese Spring except for Hope 2A, which showed a slightly different pattern. The Thatcher parent had a distinctively different pattern from Chinese Spring.

Several monosomic and disomic lines of Kharkof MC-22 and Kharkof MC-22 x Itana lines were also evaluated for quality. The most striking example of the monosomic effect on quality was shown by chromosome ID. Lines monosomic for 1D exhibited abnormal gluten characteristics. This indicates that chromosome 1D carries genes that govern baking quality which cannot operate effectively in the monosomic condition.

Starch gel electrophoresis showed the same electrophoretic patterns for the monosomic 1D and disomic 1D populations.

Each variety of wheat appears to have a distinctive electrophoretic pattern, but there does: not appear to be any association between electrophoretic pattern and quality.

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by

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A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

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ABSTRACT

Three sets of substitution lines of the wheat variety Chinese Spring with chromosomes from the donor varieties Thatcher, Hope and Timstein were evaluated for quality to determine which chromosomes carried genes governing bread wheat quality. Chromosomes Hope 2A, 3A, 7B and 2D, Thatcher 4A, 2B and 2D and Timstein 1D, 2D and 6D all appeared to carry genes governing gluten strength. Milling characteristics appeared to be carried on chromosomes Hope 4A, 3B, 3D and 5D, Thatcher 3B, 3D and 7D and Timstein 3B, 3D, 5D and 6D. There was a direct relationship between the percent water absorption of the flour and the percent of damaged starch.

Starch gel electrophoresis of ten of the chromosome substitution lines and the Chinese Spring and Thatcher parents was made. The electrophoretic patterns for the substitution lines were the same as for Chinese Spring except for Hope 2A, which showed a slightly different pattern. The Thatcher parent had a distinctively different pattern from Chinese Spring.

Several monosomic and disomic lines of Kharkof MC-22 and Kharkof MC-22 x Itana lines were also evaluated for quality. The most striking example of the monosomic effect on quality was shown by chromosome 1D. Lines monosomic for 1D exhibited abnormal gluten characteristics. This indicates that chromosome 1D carries genes that govern baking quality which cannot operate effectively in the monosomic condition.

Starch gel electrophoresis showed the same electrophoretic patterns for the monosomic 1D and disomic 1D populations.

Each variety of wheat appears to have a distinctive electrophoretic pattern, but there does not appear to be any association between electrophoretic pattern and quality.

INTRODUCTION

Wheat varieties fall into several different classes, depending on morphological differences and potential market use. Hard red wheats are used primarily for bread production, while the chief use of white wheats is for pastry products. A good quality hard red wheat variety must have suitable milling properties, which include normal bolting, or sifting properties, and a high yield of flour. A bread flour of good quality has a high water absorption capacity, a medium to medium-long mixing requirement, satisfactory mixing tolerance, good loaf volume potentialities, and should yield a loaf having good internal crumb grain and texture with acceptable color.

The protein complex known as gluten is one of the most important components of bread wheat flour. The ability of a dough to expand and provide a large loaf is due to the unique properties of gluten. The variation in quality between different bread wheat flours of the world lies mainly in the quantity and quality of gluten they contain.

Breeding for bread wheat quality has been an objective in most wheat breeding programs for many years. If wheat breeders knew which chromosome or chromosomes carried the genes governing quality, their task would be simplified. With the development of monosomic (2n-1) and nullisomic (2n-2) lines in hexaploid wheat a new genetic tool became available for determining which chromosomes carry genes for a particular inherited character. For example, quality differences of the various monosomic lines should indicate which chromosomes carry the desired germ plasm. If a particular

monosomic or nullisomic line differs from the normal, one can conclude that the missing chromosome carries a gene or genes which affect quality.

Monosomics and nullisomics can also be used by wheat breeders to transfer a particular chromosome pair from one wheat variety to another. With a complete set of these substitution lines, (21 lines each differing by one pair of chromosomes from a donor variety) the contribution of each donor variety chromosome to a particular character can be determined. By evaluating a given series of substitution lines for quality, one should be able to determine which donor variety chromosomes carry genes influencing quality by noting those lines that differ from the normal.

The objectives of this investigation were to locate the chromosome or chromosomes in bread wheat which carry genes that influence flour quality and to determine the biochemical basis for quality differences found in wheat. Quality evaluations of monosomic and chromosome substitution lines and a study of the protein from the flour of these wheats were made.

REVIEW OF LITERATURE

I. Wheat Proteins

Wheat flour consists of approximately 8-13% protein, 13-15% moisture, 65-70% starch, 0.8-1.5% fat, 1.5-2.0% sugar and 0.3-0.6% ash (12). The distinctive place of wheat flour in the economics of the world can be attributed chiefly to the unique properties of the proteins of wheat as compared to those of other cereal grains. For this reason, the wheat proteins have long been of great interest to cereal chemists and others associated with the cereal industries (1).

Protein is the name given to a class of organic compounds, each member of which consists of various amino acids linked together. An amino acid is an organic acid containing both amino (NH_2) and carboxylic (COOH) groups. There are 18 known amino acids in flour proteins, so one can see that the number of different combinations, that is, the number of different protein molecules that are possible is almost infinite (6). The molecular weights of flour proteins are known to vary from about 40,000 to 2 or 3 million (13). Wheat flour protein consists of approximately 50-55% carbon, 6.5-7.5% hydrogen, 15-19% nitrogen, 22-27% oxygen and 0-3.5% sulphur (12).

The earliest investigation of wheat proteins was the classic work of Osborne (21). His classification of wheat flour proteins on the basis of solubility characteristics has been the basis for much of the subsequent research in this area. He concluded that wheat proteins were comprised of five fractions: 1) a water soluble, heat-coagulable albumin; 2) a globulin, soluble in dilute salt solution; 3) an ill-defined proteose;

4) gliadin, soluble in 70% ethanol; and 5) glutenin, soluble in dilute acid and base. The latter two (gliadin and glutenin) make up the water insoluble gluten which is considered to be the protein complex responsible for the unique properties of wheat flour. Gluten is the viscid substance that gives adhesiveness to dough. This unique property of wheat flour makes possible the production of a "risen" loaf of bread.

Gliadin and glutenin, when hydrated, are quite different in physical properties (6). Glutenin is similar to gluten, although it is tougher and more rubbery and does not stretch as easily. On the other hand, gliadin is syrupy and flows readily. The properties of these two fractions are blended together in the whole gluten. The molecular weights of the proteins in these two fractions are known to be quite different; 40,000-50,000 for gliadin and 2-3 million for glutenin (13).

It has been well established by the work of many investigators that flour from different varieties of wheat yield gluten with varying physical properties, as does flour from the same wheat variety grown under various environmental conditions. Different types of equipment are available for measuring extensibility, mixing characteristics, plasticity, and elasticity of gluten. The problem has been to explain differences in terms of chemical or physical structure of the gluten proteins (1).

Over the years, numerous attempts have been made to fractionate gluten and obtain preparations of pure, individual protein components. A continuing effort has been made to fractionate gluten into its protein constituents in terms of electrophoretic behavior. Laws and France (17) studied the electrophoretic patterns of wheat gluten by moving boundary

electrophoresis. They showed the presence of more than one component, together with evidence of component interaction which produced asymmetry in the patterns. Jones, _et al_. (15), also using moving boundary electrophoresis, observed at least four major components and one minor component in gluten. Their work also produced asymmetric patterns indicating component interaction.

In recent years gel electrophoresis (28) has been used in the study of flour proteins. This system was developed by chemists working with the proteins of blood plasma, and it has been adapted to the study of flour proteins. Since protein molecules are electrically charged, they will move through the pores of a gel at different rates depending on their relative electrical charge and molecular size. Starch and acrylamide are the gel media commonly employed for electrophoresis of flour proteins.

A typical starch gel electrophoresis pattern with wheat gluten shows from about eight to 32 fairly distinct bands, depending on the electrophoretic conditions employed and the wheat sample used. In addition, there is a heavy band at the bottom of the pattern. This heavy band is the starting point at which the protein solution was inserted into the gel. The protein in this band moves very little, if at all. Dimler (6) indicates that all of the protein of the gliadin moves into the gel, whereas none of the glutenin protein moves. Failure of the glutenin protein to move into the gel during starch gel electrophoresis is due to its heavy molecular weight (13). The glutenin molecules simply are too large to move through the extremely small pores of the starch gel.

Several investigators (8, 10, 14, 35) have used starch gel electrophoresis with varying degrees of success. Graham (10) found differences in the protein components of different wheat flours and she observed that the greatest differences occurred among the slow-moving protein components. Alton and Ewart (8) observed that the electrophoretic patterns of 8 different wheat varieties showed significant differences and concluded that, "in a single run, a 'fingerprint' could be obtained for most, if not all, of the flour proteins." Cluskey, et al. (4), Coulson and Sim (5) and others have also observed differences in electrophoretic composition of gluten from different varieties of wheat.

Recently, some investigators have been switching from starch gel to acrylamide gel (24). Acrylamide gel has the advantage of being transparent allowing it to be scanned with a densitometer, thus providing quantitative measurements.

Some investigators have tried to explain the differences between wheats in terms of their amino acid content. Pence, et al. (22) determined the amino acid content of the gluten from 17 wheat flours representing a complete range of types and varieties. The composition of the gluten was essentially uniform, despite the wide range in type and source of the glutens and the wide range in protein content and baking characteristics of the flours. Similar comparisons on a limited number of wheats are reported by Hepburn, et al. (11), who found no differences in the amino acid content of the proteins of two hard red spring and two hard red winter wheats. Simmonds (27), however, did observe small, but significant differences in the amino acid content of six Australian wheats.

The sulfur-containing amino acids, methionine, cysteine and cystine, have been of particular interest since they are believed to be involved in intra- and inter-molecular bonds which may determine physical properties of the gluten or dough (29, 32). The importance of the disulfide linkage to wheat gluten properties has been known for many years. Splitting of this linkage by adding a reducing agent to the dough will immediately destroy its elastic properties (6).

II. Bread Wheat Quality Inheritance

The proof that wheat quality characteristics are inherited is not lacking, but the determination of the number of genes involved is still a subject of considerable investigation. The early inheritance studies were conducted by making the appropriate crosses and then evaluating the segregating generations. Most of these studies suggested multiple gene inheritance, but the exact inheritance patterns were not determined.[1/]

The fact that wheat quality is strongly influenced by environment has made the determination of its inheritance difficult. This is compounded by the lack of accurate quality tests for evaluating small amounts of early generation material. If later generation material is used for complete milling and baking tests, the plant breeder is faced with the problem

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1/ Welsh, James R. A Monosomic Inheritance Study to Locate Genetic Factors for Protein Quality and Morphological Characters in Hard Red Winter Wheat. Doctor of Philosophy Thesis, Montana State College, 1963.

of propagating a large number of lines to insure the complete testing of all genotypes. These facts, combined with year-to-year variation due to environment, have presented serious problems in accurately determining bread wheat quality.

Recently Welsh and Hehn (34) and Mattern, et al. (19, 20) approached quality inheritance studies by use of monosomic and chromosome substitution lines, respectively. Welsh and Hehn (34) crossed monosomic lines of Kharkof MC-22, a relatively weak flour variety, with normal Itana, a strong flour quality variety. The monosomic Kharkof MC-22 lines were used as the females and the normal Itana lines as the pollen source. F_2 populations resulting from selfed monosomic and disomic F_1 plants were evaluated for quality by the use of the wheat meal fermentation time test and the farinograph test. It was found that the monosomic 1D lines exhibited extremely weak flour characteristics as determined by these two tests. Populations disomic for chromosome 1D reacted normally under both tests. This was considered evidence that chromosome 1D is extremely important in the determination of bread flour quality characteristics.

Mattern, et al. (19, 20) made a preliminary quality report on the effect of substituting Cheyenne chromosomes into the Chinese Spring background. These substitution lines were produced by the method outlined by Sears (26). Following the fourth backcross in seventeen of the lines, seed was increased for quality evaluation. The four remaining lines, 2A, 7A, 2B and 2D, had not yet reached the fourth backcross so they were not evaluated. Milling yield, flour particle size, protein content, flour ash, dough mixing characteristics, and baking properties were determined on the

seventeen lines. Substitution line 5D was high in flour yield and line 6D was low in flour yield, indicating that chromosomes 5D and 6D carry genes that are important in determining milling yield. Chromosomes 4B, 7B, and 5D appeared to strengthen the dough mixing characteristics, whereas chromosome 1D appeared to weaken these characteristics as determined by the mixograph curves. Major factors influencing baking characteristics appeared to be located on chromosomes 4B, 7B and 1D. Chromosome 5B appeared to be important in determining flour particle size.

Kuspira and Unrau (16) studied the effect of substituting Thatcher chromosomes into a Chinese Spring background on several characteristics. Protein analysis of the grain from 19 Thatcher substitution lines suggested that five chromosomes, 5A, 5B, 7B, 3D and 4D, induced a significant increase in protein over Chinese Spring. It was proposed that at least five genes or sets of genes were responsible for protein increases in this set of substitution lines.

MATERIALS AND METHODS

Two types of plant materials, chromosome substitution lines and monosomic lines, were used for quality evaluation in this study.

I. Chromosome Substitution Lines

Three sets of substitution lines in the spring wheat variety Chinese Spring, with substituted chromosomes from donor varieties Hope, Thatcher and Timstein, were obtained in the fall of 1963 from Dr. John Kuspira, University of Alberta, Edmonton, Alberta, Canada. These substitution lines were produced from crosses of nullisomic (2n-2) Chinese Spring lines with the donor variety as suggested by Sears (26). The procedure can be described as follows:

1. Nullisomic Chinese Spring ♀ x donor variety ♂.
2. Backcross monosomic F_1 ♂ to nullisomic Chinese Spring ♀.
3. Repeat step 2 until the BC_5 generation is reached.
4. Self BC_5 monosomic plants and select disomic offspring.

Identification of monosomic, nullisomic and disomic plants was made by cytological observations.

Line 1A was missing in all three substitution series. Thatcher 1D and Timstein 7A substitution lines were also missing.

The substitution lines were grown in a randomized block design with four replications at Bozeman, Montana in 1964. The Thatcher parent and normal Chinese Spring parent were also included in the experiment. However, the Hope and Timstein parents were not included due to insufficient seed stocks of these two varieties.

During the summer of 1965, 200 grams of seed from each replicate of these lines were milled at the Western Wheat Quality Laboratory, Pullman, Washington. The milling system was a modification of the procedure developed at the Hard Red Wheat Quality Laboratory, Manhattan, Kansas (9). Brabender Quadrumat Sr. laboratory mill components, 8 inch Tyler testing sieves, and Strand sedimentation sieve shakers were employed. Breaking was accomplished with a Quadrumat Sr. laboratory break head mill with the sifter reel removed.

Middlings were reduced with a Quadrumat Sr. reduction head equipped with a vibratory feeder. The first three rolls of the reduction head were original equipment. A smooth roll was substituted in position 4 and adjusted as close to roll 3 as possible without touching. The mill and flow diagram are pictured in Figures 1 and 2, respectively.

All samples were tempered to 15% moisture for 18 hours with distilled water containing a wetting agent (0.1% Aerosol OT). Weights of tempered wheat varied from 198.5 g. to 204.6 g.

Break stock remaining on a 32 mesh Tyler sieve after 1 minute of sifting was weighed as bran. Middlings remaining on a 100 mesh Tyler sieve after 3 minutes of sifting were passed through the reduction head. Reduction stock remaining on a 100 mesh Tyler sieve after 3 minutes of sifting was weighed as shorts. Break and reduction flours passing through the 100 mesh Tyler sieves were combined as total flour. The flour was blended prior to analytical and farinograph tests. Flour yield calculations were based on weight of wheat milled.

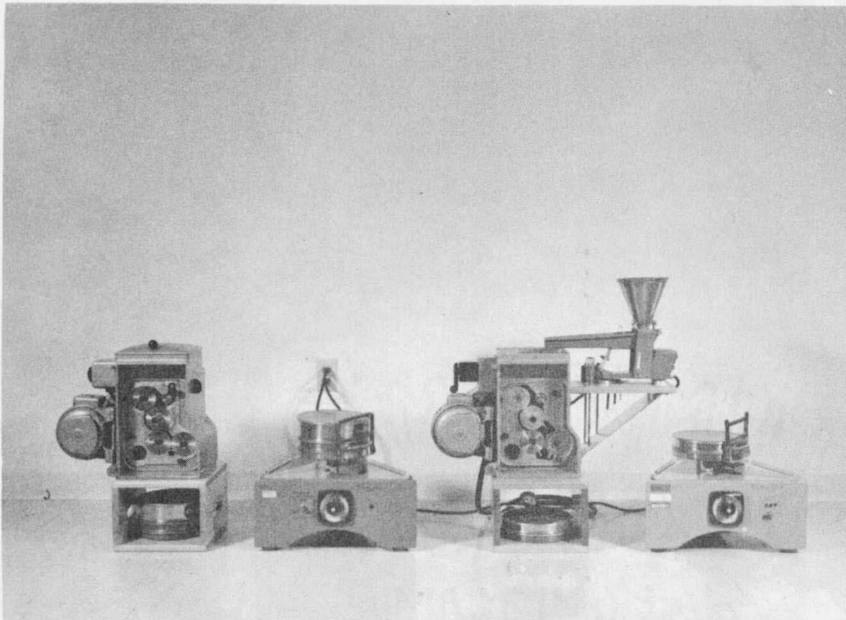

Figure 1. Brabender Quadrumat Sr. milling equipment as modified at Pullman, Washington.

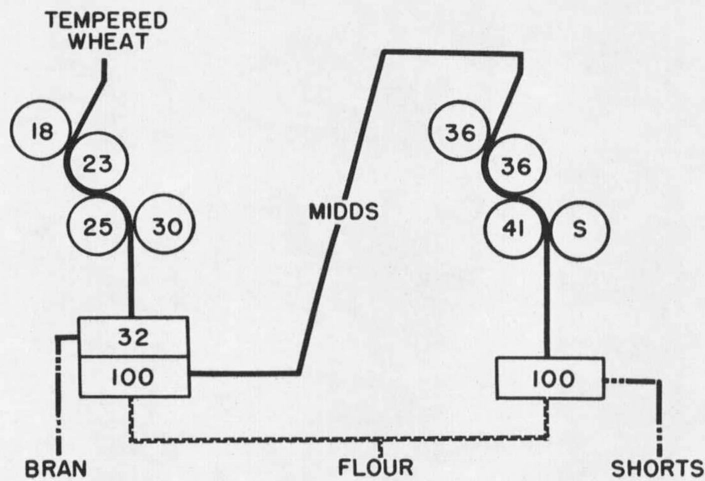

Figure 2. Flow diagram for Brabender Quadrumat Sr. milling system as modified at Pullman, Washington.

When sample weights, feed rates, roll settings and sifting times are all held constant, differences in milling performance are reflected by differences in total flour yield.

Starch damage was obtained by a method similar to that outlined by Donelson and Yamazaki (7). The reagents used were prepared as follows:

1. Buffer solution: Dissolve 3 ml. of glacial acetic acid in 4.1 g. of anhydrous sodium acetate and make up to 1 liter with water.
2. Sulfuric Acid, 3.58 N: Dilute 10 ml. of concentrated H_2SO_4 to 100 ml. with water.
3. Sodium tungstate solution, 12%: Dissolve 12.0 g. $Na_2WO_4 \cdot 2H_2O$ in water and dilute to 100 ml.
4. Alkali ferricyanide reagent, 0.1 N: Dissolve 33 g. pure, dry $K_3Fe(CN)_6$ and 44 g. anhydrous Na_2CO_3 in water and dilute to 1 liter. To standardize, add to 10 ml. of the solution 25 ml. acetic acid-salt solution (reagent 5) and 1 ml. soluble starch-KI solution (reagent 6) and titrate with 0.1 N thiosulfate. Exactly 10 ml. should be required to discharge the blue color completely.
5. Acetic acid-salt solution: Dissolve completely 70 g. KCl and 40 g. $ZnSO_4 \cdot 7H_2O$ in 750 ml. water, add slowly 200 ml. glacial acetic acid, and dilute to 1 liter.
6. Soluble starch-KI solution: Suspend 2 g. soluble starch in a small quantity of cold water and pour slowly into boiling water with constant stirring. Cool thoroughly, add 50 g. KI, dilute to 100 ml. and add 1 drop of saturated NaOH solution.

7. Thiosulfate solution, 0.1 N: Dissolve 24.82 g. $Na_2SO_3 \cdot 5H_2O$ and 3.8 g. of borax in water and dilute to 1 liter.

Starch damage was determined as follows: Place 2 g. of flour in a 50 ml. Erlenmeyer flask, add 0.2 g. Rhozyme 33 and 18.5 ml. of buffer solution (reagent 1) and mix thoroughly. Place immediately in a water bath set at 30° C. for 15 minutes. Remove from the water bath and add 0.8 ml. of 10% H_2SO_4 (reagent 2) and 0.8 ml. of sodium tungstate solution (reagent 3) and mix. Let stand 2 minutes and filter (No. 4 Whatman or equivalent), discarding the first 8-10 drops. Pipet 5 ml. of the extract into a 125 ml. Erlenmeyer flask, add 10 ml. of alkali ferricyanide solution (reagent 4), mix and immerse flask in boiling water for 20 minutes. Remove from boiling water, cool under running water and add 25 ml. of acetic acid-salt solution (reagent 5) and 1 ml. of starch-KI solution (reagent 6) and mix. Titrate with 0.1 N thiosulfate solution (reagent 7) and record the ml. of thiosulfate used to completely discharge the blue color. The milliliters of thiosulfate used were then converted to maltose values with the aid of the conversion table of Sandstedt (25) which is reproduced in Cereal Laboratory Methods (2). The maltose values were then converted to percent damaged starch by multiplying by the conversion factor 1.64 and dividing by 100.

The farinograph data (absorption, peak, stability and valorimeter) were obtained by the constant flour weight procedure (2) using a Brabender Farinograph equipped with a 50 g. mixing bowl. Protein content was obtained by the standard macro-Kjeldahl method.

The flour used for sedimentation was obtained by grinding approximately 50 g. of untempered wheat (approximately 10% moisture) with a Quadrumat Jr. mill with the sifter reel removed. The ground wheat was sifted over a 100 mesh Tyler sieve equipped with a bottom pan and shaken mechanically for 90 seconds. The flour was tested for sedimentation test by the standard method (2).

An analysis of variance and the least significant difference were determined on all data obtained.

Starch gel electrophoresis was run on the proteins of several of the substitution lines that differed in quality from the Chinese Spring check. The electrophoresis apparatus and procedure were similar to those described by Cluskey (3). The apparatus was composed of essentially three parts: a tray or trough which contains the gel medium, buffer chambers containing an electrode system and a constant voltage source (Figure 3). Dimensions of the tray and buffer chamber apparatus were 24 cm. by 19 cm. The gel was 15 cm^2 and 7 mm thick and came in direct contact with the buffer in each well.

Aluminum lactate-lactic acid containing 3 M urea was used as the buffer. It was prepared by dissolving 2.45 g. of aluminum lactate and 360 g. of urea in distilled water diluted to two liters. The pH was adjusted to 3.1 with lactic acid.

The protein was extracted from the flour in the following manner: Ten grams of flour was defatted by mixing with 20 ml. of n-butanol and filtering. The extraction was repeated three times. After the last filtration the residue was transferred to a beaker and allowed to air