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PROTOCOLS FOR EVALUATION OF WHEAT QUALITY

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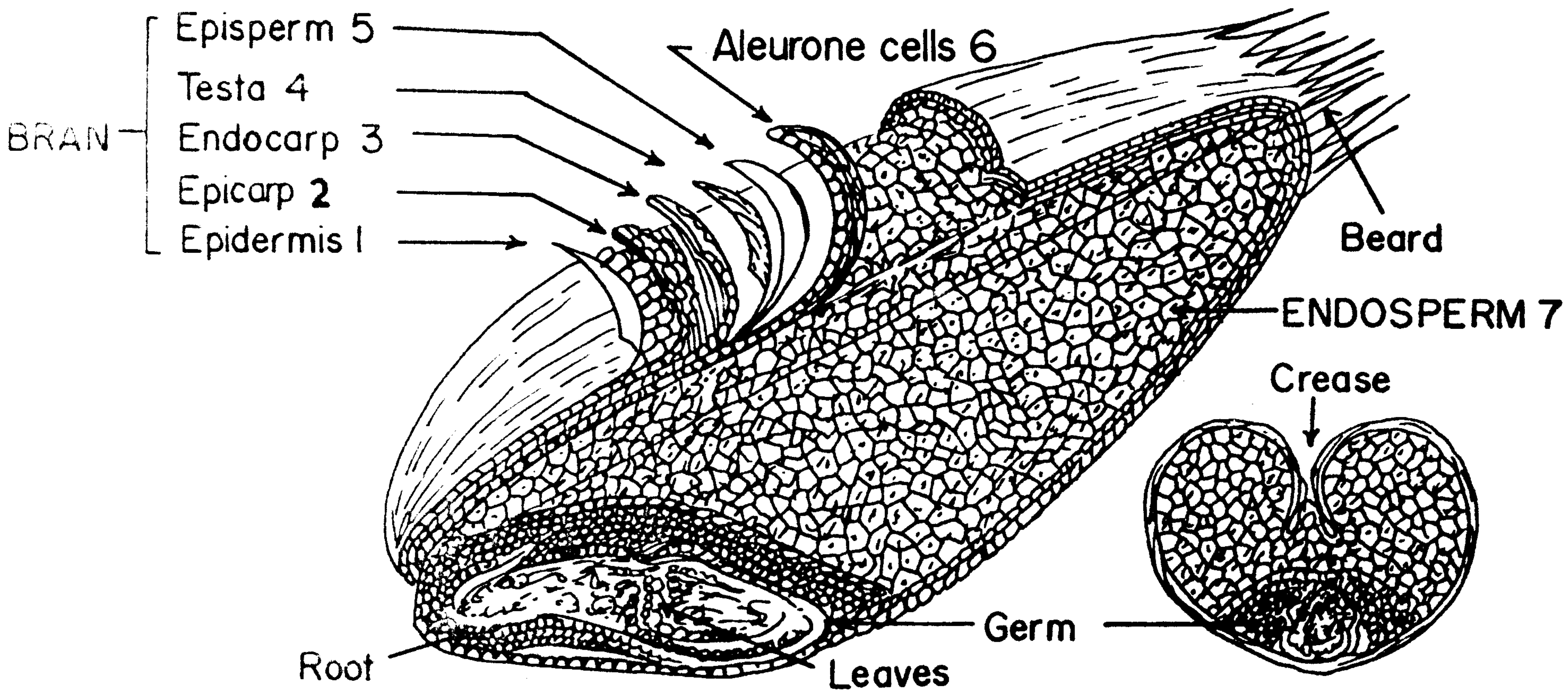
Structure of Wheat Kernel



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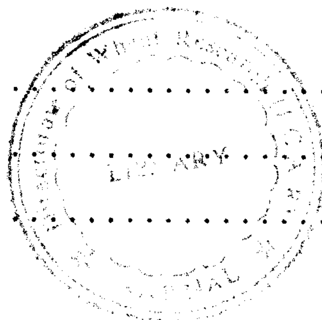

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FOREWORD

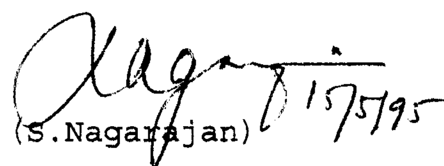
Wheat Quality is a very complex subject and to unfold its complexity, research work started way back in 1728. Since then lot many techniques, physico-chemical, chemical and rheological have been developed to understand the intricacies of the enigma why " poor quality wheats are poor and good quality wheats are good". Even after so much advancement, the ultimate resolutions to this problem comes from the baking test. However baking test can not be used extensively because of the paucity of material available with the wheat breeders in the early stages of varietal development. Research on wheat quality in Indian wheat programme remained confined mainly to assist the breeders in developing varieties with superior chapati making qualities. Sufficient information has also been documented classifying the established varieties for their end uses. but the wide spread use of electrophoresis technique which can help in the discrimination of large quantity of breeders material with half a grain, remained unexploited. Until 1991, probably, our wheat production was just sufficient to meet our food demand and had hardly any consideration for value addition and industrial usages. With the enforcement of the new trade related regimes under free trade, new opportunities have come in our way to expect buscuits and value added wheat products. Keeping alive to these aspirations and the opportunities for industrialization, the authors Dr. B.K. Misra & his team have done a com-

constant use in the breeding programmes world over.

The present compilation would prove a great help to all those who are engaged in wheat quality research and do not have easy access to the scattered information on various techniques published in the scientific literature.

Dr. B.k. Misra & his colleagues deserve appreciation for this timely useful compilation.

With all the best wishes.



(S.Nagarajan)

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INTRODUCTION

Concerted and dedicated efforts jointly put forth by scientists, farmers and planners successfully pulled out the country from the horrors of hunger which engulfed the entire nation in the middle of sixties. It was wheat crop which did the miracle through green revolution in early seventies. Since then we have never looked back and marching ahead majestically by breaking our own records of production. Wheat scientists not only have been successful in developing high yielding wheat varieties but also transformed the red coloured Mexican grain into lustrous golden colour, a luring choice of chapati consumers. Today, more than 95% of our wheat varieties are good to very good for chapati making properties. Our oozing food buffer stocks of 27 million tones where wheat alone contributes around 32% though have placed us in a commanding position in the over all world food scenario, but in no way warrant us to be complacent. It is because of the fact that our population growth is hardly showing any decreasing trend. While we shall have to continue to pursue our present production strategies, the eased situation on food front however permits us to take a little liberty to concomitantly drift our attention to earn profits from wheat. The present situation prompted the Union Govt. to allow export of wheat. Last year around 15000 tones of wheat at a total cost of approximately US$ 150 was exported to some Gulf countries. The export was well behind the projected target of 3

lakh tones. The reason was obviously the non-availability of sufficient quantity of export quality grain. In case we wish to enter the international grain trade and want to stay back, then we shall have to be quality conscious.

Concept of wheat quality is very complex as it is conceived in different ways by different people. A miller looks for a hard grain that can yield high amount of flour. Chapati consumer wants a variety with bold, lustrous and amber coloured grain. Baker's criteria show a wide variability in selection while pasta producers require altogether a different class of wheat (durum) which is hard with high yellow pigment and capable of making products with excellent cooking quality.

Research on Wheat Quality started way back in 1728 by an Italian Scientist-cum-Philosopher, Lacopo Bartolomco Beccari. Since then scientists have been engaged in resolving many of the intricacies attached with wheat quality but still they have a long way to go. It is because of the fact that till today baking and cooking tests are the final tests and all other tests only lead us to predict the quality of wheat genotypes. However, in a big breeding programme such tests can not be conducted on each and every material because of the intensive labour involved and the low quantity of the grain available in the early generation. Many efficient techniques are now available to screen thousands of samples right in their early stages of breeding.

The compilation of the standard methods was considered necessary to extend assistance to those institutions which do not have adequate library facilities.Every effort has been made to reproduce the protocol in its original form but in certain methods small modifications have been made. This has been done exclusively on our laboratory experience. To avoid any inconvenience to the researcher, reference with every method has been quoted to the maximum possible extent. Though large number of methods are available in the literature, only a few commonly used methods have been included. All these methods have been covered under three categories, e.g. physical, chemical and rheological methods.

PHYSICAL

METHODS

Of the several physical tests most of them are subjective, they are yet very crucial in providing a tentative quality status of the grain to the actual users.

Grain Appearance score (Max 10):

Three characters that include grain size, shape and colour are taken into consideration for scoring the grain appearance. Bold grains with attractive shape, amber golden colour and the lustre of the grain are major criteria for scoring. Grains with all these characters fetch higher price in the market. These characters help the purchaser of the grain in preliminary surveying of the market.

Vitreous kernel

Vitreousness is often correlated with hardness of the grain which in turn is a rough index of protein and gluten content in the grain. It is, therefore, considered that vitreous wheats produce more flour and semolina. The vitreousness in the grain is subjective and visually scored using 'x ray' film viewer. Video densitometer, a device commonly used for scanning of the gels, can also be used for the study of the vitreousness in the grain.

Damaged and infected kernel

Such type of grains adversely affect the overall quality of the end product. Besides poor recovery of flour, they tend to induce early staling in the product by shortening its shelf life. Damaged and infected grain has no market value except that it can be used as a feed.

Kernel Weight (1000 grain wt.)

Kernel weight is considered to be a function of kernel size and its density. While test weight determines the milling quality of all wheats, kernel weight is decisively superior in predicting the milling quality of hard grain. Both test weight and kernel weight pronounce for the same quality character i.e. milling quality but their relationship has not been conclusively studied. The electronic counter is used for counting 1000 grain weight in gm. Wide range of variability from 22-45 g have been recorded for bread wheat while for durums, the weight varies from 35-55 g.

Test Weight: (hectolitre wt./wt. per Unit Volume/bushel wt.)

Test weight usually determines the plumpness of the grain. Flour yield increases and flour ash decreases with the increase in test weight. A flour yield upto 70% and flour ash of 0.4% would be normal for a wheat with 75 kg hectolitre weight, whereas a flour yield of 62.5% and flour ash of 0.6% would be expected from a wheat with 62 kg hectolitre weight. Kernel size and shape are the two most important features which influence the over all test weight of the grain. Immature and shriveled wheat are usually low in test weight and give correspondingly poor yields of flour. A correlation co-efficient of + 0.75 between test weight and flour yield has been established by two separate group of workers. Instrument shown in Fig. 1 has been in use for a long time and still popular in most of the laboratories in the

world. Instrument (Fig 2) is the latest dual purpose electronic device for the estimation of moisture as well hectolitre weight. This latest device needs only 200 g of sample as compared to the old instrument where 800 gm is required.

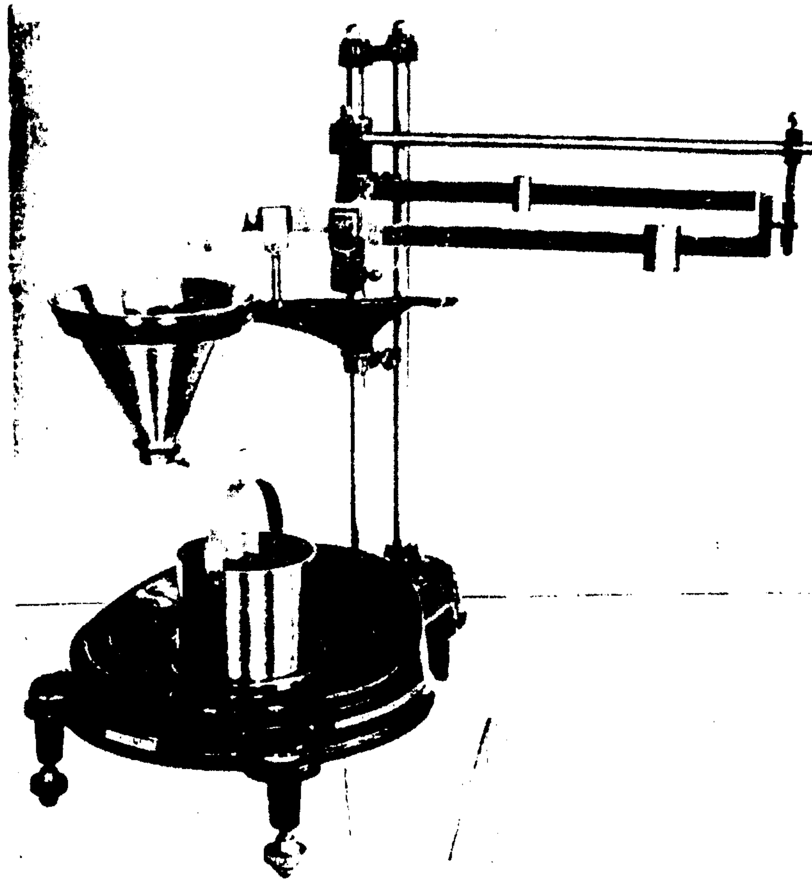
Initially, the test weight was expressed in lb per imperial bushel but most of the countries express it in terms of kg per hectolitre. For this lb per bu is multiplied by 1.247 to get kg per hectolitre.

At lower weights than 71 kg, the milling yield usually falls off rather rapidly with decreasing test weight. Immature wheats or wheat that is badly shriveled as a result of drought or disease is usually low in test weight and give correspondingly poor yield of flour.

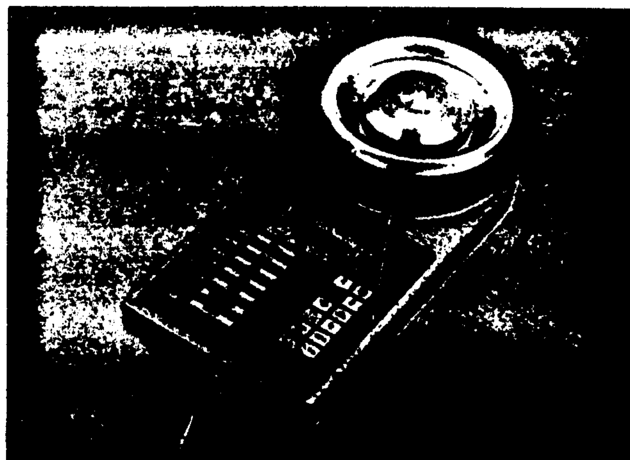
Procedure:

The following procedure has been found to be accurate in making weight per bushel tests and has been adopted as the standard method in the determination of test weight per bushel.

1. Make the test immediately after the sample has been brought to the laboratory, to prevent drying out of the grain with consequent change in its test weight per bushel.
2. Use 11/8 quarts of grain for making the test.
3. Fill the kettle from the hopper.



STANDARD APPARATUS FOR DETERMINING THE TEST
WEIGHT PER BUSHEL OF WHEAT AND OTHER GRAINS



AUTO ANALYSER FOR MOISTURE
AND HECTOLITRE WEIGHT

4. Make sure that -

   a. Opening at bottom of hopper is round and 11/4 inches in diameter.

   b. Bottom of opening in hopper is centered over the kettle and is exactly 2 inches above the top of kettle.

   c. The quart kettle has a capacity of exactly 67.2 cubic inches and the inside height of the quart kettle is 4 inches.

5. Do not jar the kettle before or during the stroking operation.

6. Use a stroker made of hardwood and that has smooth, half around edges.

7. Place the stroker on the edge of the kettle lightly without jarring the kettle.

8. Hold the stroker on the kettle so that the sides of the stroker are in vertical position.

9. Stroke the grain from the kettle with three full length zig- zag motion of the stroker. Each stroke covering one third the distance across the top of the test kettle and the stroker always lightly touching the kettle.

10. Use a beak which is both accurately graduated and sensitive.

11. Note the reading of the weight per bushel and multiply it by 1.247 to get hectoliter weight in kg.

Hardness

Hard grains when reduced to flour, their endosperm is cracked along the aleurone line & therefore yield more flour. On the contrary, most of the part of aleurone layer in soft wheats remains attached with endosperm which induces inconsistency in the flow of the flour while milling.

Water absorption is an important quality character because it has a direct bearing on the amount of bread produced. Crumb softness and shelf life are also influenced by the water absorption capability of the flour. Flour or semolina from hard grain absorb more water then extracted from soft grain. The reason being that hard grains when reduced to flour a large number of starch granules are damaged which subsequently absorb more water. On the contrary soft wheats, where the number of damaged granules is very low, absorb less quantity of water.

Several methods like pearling resistance, grinding resistance, particle size index, compression testing, penetrometer testing and cutting resistance have been described for testing the hardness of grain. Comparison of these methods was not found to be very satisfactory except that the results obtained by barbender hardness tester were well comparable with wheat hardness index (WHI) . The hardness was found to be highly influenced by narrow variation of moisture content. During last over one decade, Near Infrared Reflectance (NIR is in extensive use in U.K. for testing the hardness of grain.

Flour/semolina recovery

Non flour-producing substances like chaff, foreign material, shriveled grain etc. are removed before conditioning the 2 kg lot of grain. Recovery of flour from bread wheat and semolina from durum wheat is the heritable trait of the genotype. Several mills are available to test the milling quality of genotypes.

Yellow berry:

This is an important quality character of durum wheats which renders them unsuitable for pasta products. Uneven yellow spots or sometimes the whole grain becomes yellow because of the incidence of yellow berry . The central endosperm shows farinous (opacity) instead of vitreousness. Yellow berry has a negative effect on the quantity and quality of semolina and lowers the total protein content of the grain. The pasta products made out of yellow berry affected grains develop stickiness while cooking. Another adverse affect of yellow berry is the loss of yellow pigment in the grain.

Speck count:

Speck can be bran pieces, particles from black point or other foreign material visible in semolina. A common method used is to count the specks in 1.0 inch square with the aid of a magnifying glass. Specks affect the aesthetic quality as well weaken the spaghetti strains.

References:

1. Pomeranj,Y (1971), Wheat Chemistry and Technology Published by AACC

2. Heyne, E.G. (1987), Wheat and Wheat Improvement 2nd Ed.

3. Hook, S.C.W. Determination of wheat hardness-an evaluation of this aspect of wheat specification. FMBRA bulletin No.1 (1982) 12-23.

4. Norris, K.H., Hruschra, W.R.; Bean, M.M. and Slaughter, D.C. A definition of wheat hardness using near infrared reflectance spectroscopy. Cereal Foods World (1989) 34:696

CHEMICAL

METHODS

DETERMINATION OF MOISTURE

Equipment :

1. Oven
2. Analytical balance
3. Aluminium box

Procedure :

1. Weigh accurately about 5 g of wheat flour in an aluminium box having a close fitting lid.

2. Place the uncovered box with its lid in a well ventilated oven maintained at 100°C. At the end of five hours cool the box with the lid replaced to room temperature in a desiccator and weigh.

3. Calculate the loss in weight due to moisture and express as percentage.

Reference :

1. AACC, 46-12, 1983

TOTAL ASH CONTENT OF PLANT TISSUE

High ash content (>0.4%) in flour adversely affects the quality of end product.

Apparatus :

1. Porcelain or platinum crucible
2. Oven
3. Muffle furnace with temperature regulator
4. Desiccator
5. Analytical balance

Procedure

A. Flour, feeds and feed stuffs :

1. Weigh 3.5 gm of well mixed sample in a weighed porcelain or platinum crucible, dried at 100°C for 10 hours or overnight and weighed. This weight is the base.

2. Place the crucible in muffle furnace at not over 425°C, gradually increase temperature to 550°C for soft wheat flours and for feeds and feed stuffs, or 575°C to 590°C for hard wheat flours. Incinerate until light gray ash is obtained or to constant weight. Ash must not be allowed to fuse. Cool in desiccator, weigh soon after room temperature is attained.

3. If desired ash may be transferred to small counterpoised watch glass and weigh directly. To transfer ash invert dish. Usually ash will be transferred completely to watch glass by this procedure. If ash sticks, it can be removed with point of spatula.

Bread :

Use 3.5 gm of prepared sample and proceed as directed above. Report results on basis of fresh loaf or any desired moisture basis.

Note :

1. Nickel ash dishes are preferred by some to silica and porcelain. They must be thoroughly cleaned after each incineration.

2. For determination of ash by direct weighing, platinum ash dishes are preferable. If silica or porcelain ware is used, polish with line emery paper to maintain smooth glossy inner surface.

3. As flour ash is hydroscopic, do not cool more than about six samples in one desiccator in humid weather.

Microwave muffle furnace are now available for the estimation of ash content. It takes very less time as compared to AACC method.

Reference :

AACC 08-11 (1983)

CRUDE FIBRE

Apparatus :

1. Extracting apparatus with condenser to fit 600 ml tall form lipless beaker and hot plate adjustable to temperature that will bring 200 ml water at 25°C to rolling boil in 15 ± 2 minutes
2. Tall form lipless beakers, 600 ml.
3. Sintered (Gooch) crucibles.
4. Air oven maintained at 130° ± 2°C.
5. Electric muffle with rheostat or automatic control maintained at 500 to 600°C.
6. Desiccate with ignited CaO in it.
7. Filtering device; suction flask with suitable holder for crucible.
8. Apparatus designed to preheat acid, alkali and wash water, equipped with efficient condenser.
9. Filtering cloth (muslin cloth) of such character that no solid matter passes through when filtering is rapid.

Reagent :

1. Sulfuric acid solution containing 1.25g H_2SO_4 per 100ml.
2. Sodium hydroxide solution, containing 1.25 actual NaOH per 100ml, free or nearly so, from Na_2CO_3. Strength of acid and alkali solutions must be accurately checked.
3. Methyl alcohol 95%.
4. Petroleum ether.

Procedure :

1. Extract 2g dry matter with ethyl ether or petroleum ether or use flask. If material is difficult to wet, add 3 drops diluted. Add Antifoam agent.
2. Add 200ml boiling H_2SO_4 solution, immediately connect digestion flask to condenser and heat. (Contents of flask must come to boiling within one minute and boiling must continue briskly exactly 30 minutes. Rotate flask frequently until sample is thoroughly wetted. Take care to keep material from remaining on sides of flask out of contact with solution.
3. After 30 minutes remove flask, immediately filter through cloth and wash with boiling water until washings are no longer acid. Test with $BaCl_2$ solution.
4. Bring quantity of NaOH solution to boiling and keep at this temperature under reflux condenser until used. Wash charge back into flask with 200ml boiling NaOH solution using wash bottle marked to deliver 200 ml. Connect flask with reflux condenser and boil exactly 30 minutes timing boiling with alkali so that contents of different flasks reach boiling point ca. 3 minutes.
5. After 30 minutes remove flask and immediately filter through crucible or through filtering cloth in fluted funnel. If filtering cloth is used thoroughly wash residue with boiling water and transfer to crucible.

6. For material difficult to filter, after 30 minutes boiling remove funnel using vacuum and wash with hot 10% K_2SO_4 solution (K_2SO_4 solution may be added during filtering whenever filtration becomes difficult.

7. Return residue to digestion flask, thoroughly washing all residue from cloth with hot K_2SO_4 solution. Filter into crucible.

8. After thorough washing with boiling water, wash with Ca. 15 ml alcohol. Dry crucible and contents at 130°C to constant weight.

9. Cool in efficient desicator and weigh.

10. Ignite contents of crucible in electric muffle furnace until carbonaceous matter is consumed (ca. 20 minutes.).

11. Cool in desicator and weigh.

12. Report loss in weight as crude fibre.

Calculation :

$$\text{Crude fibre \%} = \frac{\text{loss in weight x 100}}{\text{weight of sample}}$$

DETERMINATION OF STARCH :

Apparatus :

1. Dried and ground sample.
2. 10 ml pipettes.
3. Test tubes.
4. 50 ml Beaker.
5. 100 ml volumetric flask.
6. Glass rod .
7. Whatman No.42 filter paper.
8. Clinical centrifuge.
9. Water bath with heater.
10. Oven.

Reagents :

Ethanol (80%)

Perchloric acid 9.2 N, 793 ml of 70% $HClO_4$ is diluted to 1 litre.

Perchloric acid 4.6 N, 397 ml of 70% $HClO_4$ is diluted to 1 litre.

Extraction procedure :

The dried sample is ground in a ball mill. 100 mg of this dried sample is transferred to a 15 ml centrifuge tube and 10 ml of 80% ethanol is added. A glass ball is placed on the top of the tube and the tube is kept on a water bath having 80-85°C. The material is centrifuged and decanted into a 50 ml beaker. This extraction is repeated 3 times and all extractions are evaporated

by keeping it on a water bath at 80-85°C till the volume of the alcohol is reduced to 3 ml. The volume is raised upto 25 ml with distilled water. The sugar is estimated in this extract.

The residue left in the centrifuge tube is dried in an oven at 80°C for the determination of starch.

To the centrifuge tube 2 ml distilled water is added. This tube is kept in boiling water bath for 15 minutes. 2 ml of 9.2 N $HClO_4$ is added while stirring the contents constantly. After stirring the contents for 15 minutes the suspension is made upto 10 ml and centrifuged. The supernatant liquid is collected and the residue is treated with 2.0 ml of 4.6 N $HClO_4$, stirred for 15 minutes and the volume is made upto 10 ml with distilled water. The content is centrifuged and then supernatants are combined and volume is made upto 50 ml with distilled water. This extract is used for starch determination.

Anthrone method for the determination of sugars:

The concentration of pentoses, hexoses, disaccharides including sucrose, lactose and maltose and hexuronic acids present either freely or alongwith polysaccharides can be estimated using this method. Anthrone, 10-keto-9,10-dihydroanthracene, a reducing product of anthroquinone reacts by condensing with carbohydrate furfural derivatives to produce a green colour in dilute and blue colour in concentrated solutions.

Apparatus :

1. Plant extract.
2. Test tubes.
3. 1 ml and 5 ml Pipettes.
4. Glass marbles. 5. Water bath with heater.
6. Cold water bath.
7. Colourimeter.

Reagents :

Anthrone reagents : Dissolve 2.0 gm of anthrone in 1 litre of concentrated H_2SO_4 Prepare fresh.

Method :

Pipette aliquotes of 1ml of the extract into test tubes. To each tube add 4ml of the Anthrone reagent allowing the reagent to run down the side of the test tubes. Place a glass marble on top of each tube to prevent loss of water by evaporation. Place the tube in a boiling water both for 10 minutes. Remove them and cool to room temperature in a water bath. Treat a reagent blank similarly. Measure the absorbance of the blue-green solution at 625 nm. Calculate the amount of sugars present in the extracts using a standard curve prepared from glucose.

Comment:

Alcohol interfears with colour development in the Anthrone-sugar reaction. Remove the alcohol by evaporation, if alcohol

extract is used. To remove the plant pigments which interferes with the colour development, pass the leaf extract through a column (1X 10cm) of magnesium oxide after diluting it to a known volume with water to reduce the alcohol content to less than 50%.

Evaporate the alcohol from the elute and use the extract for sugar determination. The extract should also be free from protein as a red colour is developed with tryptophan.

Phenol method for the determination of sugars:

2ml of sugar solution containing 1-70 µg of sugar is pipetted into a colourimetric tube and 0.05 ml of 80% phenol is added. 5 ml of concentrated H_2SO_4 is added rapidly. The stream of added acid being directed against the liquid surface rather than against the wall of the test tube in order to obtain good mixing. Tube is allowed to stand for 10 minutes, shaken and placed for 10-20 minutes in a water bath at 25-30°C before taking the readings. The colour is stable for several hours. The optical density is measured at 490 nm for hexoses and 480nm for pentoses and uronic acid (Dubios et al 1956).

Reference:

1. Dubios, M. Gilles, K.A.; Hamilton, J.K. Rebers, P.A. and Smith, F. (1956). Colourimetric method for sugar and related substances. Analytical Chemistry 28: 350-356.

2. Dubois, M.K., Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1951. A colorimetric method for the determination of sugars. Nature 168: 167.

REDUCING AND NONREDUCING SUGARS

Apparatus

1. Analytical balance.
2. Erlenmeyer flask, 100ml
3. Whatman No. 4 filter paper.
4. Pipettes, 5ml, 10ml.
5. Boiling water bath
6. Test tubes.
7. Microburette, 10ml.

Reagents:

1. Ethyl alcohol (95% by volume)
2. Acid buffer solution:Dissolve 3ml glacial acetic acid, 4.1g anhydrous sodium acetate and 4.5ml H_2SO_4 (sp.gr.1.84) and dilute to liter with water.
3. Sodium tungstate (12%):Dissolve 12.0g $Na_2WO_4.2H_2O$ and dilute to 100ml.
4. Alkaline ferricyanide solution (0.1N): Dissolve 33g pure dry $K_3Fe(CN)_6$ and 44g anhydrous Na_2CO_3 and dilute to 1 liter. To standardize add to 10ml of this solution, 25ml acetic acid salt solution and 1ml soluble starch-Kl solution and titrate with 0.1N thiosulphate. Exactly 10ml should be required to discharge blue colour completely.
5. Acetic acid salt solution. Dissolve completely 70g KCl and 40g $ZnSO_4.7H_2O$ in 750ml water,add slowly 200ml glacial acetic acid and dilute to 1 liter with water.

6. Soluble starch-potassium iodide solution.Suspend 2g soluble starch in small quantity of cold water and pour slowly in to boiling water with constant stirring. Cool thoroughly (or resulting mixture will be dark coloured).Add 50g Kl,dilute to 100ml and add one drop saturated NaOH solution.
7. Thiosulphate solution, 0.1N.

PROCEDURE:

Preparation of extract:

1. Introduce 5.675g flour into 100 or 125ml Erlenmeyer flask. Tip flask so that all flour is at one side and wet flour with 5ml alcohol.Then tip flask so that wet flour is at upper side and add 50ml acid buffer solution,keeping solution from coming in contact with flour until it has all been added to flask Then shake flask to bring flour into suspension. Add immediatly 2ml sodium tungstate solution and again mix thoroughly.
2. Filter at once (whatman no.4),discarding first 8-10 drops of filtrate.

Reducing sugars:

1. Pipette 5ml extract in to test tube (approx.50ml capacity length 20cm, diameter 2cm).
2. Add with pipette exactly 10 ml alkaline ferricyanide reagent mix and immerse test tube in vigorously boiling water

bath. Surface of liquid in test tube should be 3-4 cm below surface of boiling water. (Delay between filtering of extract and treatment in boiling water bath should not exceed 15-20 minutes Further delay may cause error due to sucrose hydrolysis in acid solution) Let test tube remain in boiling water bath exactly for 20 minutes.

3. Cool test tube and contents under running water and pour at once into 100 or 125ml E-flask. Rinse out test tube with 25ml acetic acid salt solution, adding risings to solution in Erlenmeyer-flask. Mix, add 1ml of soluble starch-Kl solution, mix thoroughly, and titrate with 0.1N thiosulphate to complete disappearance of blue colour. (A 10ml microburette is recommended for this titration.)

Nonreducing sugars:

1. Pipette 5ml filtered clarified flour extract into test tube (approximately 50ml capacity) and immerse for 15 minutes in vigorously boiling water bath.

2. Cool test tube and contents under running water and add exactly 10ml alkaline ferricyanide reagent. Carry out reduction and subsequent titration as described above for reducing sugars.

CALCULATIONS:

Reducing Sugars:

1. Calculate ml ferricyanide reduced by substracting ml thiosulphate required from thiosulphate equivalent of ferricyanide reagent .

2. Compute reducing sugar as mg maltose/10g flour by reference to Table (AACC).

Nonreducing Sugars:

Again calculate ml ferricyanide reduced, subtract from this value ml ferricyanide reduced in procedure for reducing sugars above(step 2), and express difference as mg sucrose per 10g flour by reference to Table (AACC).

NOTE:

Make following "blank" determination with each day's series of sugar detereminations to guard against changes in ferricyanide reagent and to correct for any reducing impurities in reagents:

(a) Combine 5ml alcohol(reagent 1), 50ml acid buffer (reagent 2), and 2ml sodium tungstate(reagent 3).

(b) To 5ml of this mixture (used in place of 5ml flour extract) add 10ml ferricyanide solution (reagent 4) and proceed as in determination of reducing sugars. It should require 10ml thiosulphate to discharge blue starch-iodine colour.

(c) If titration ("thiosulphate equivalent) falls within 10 ($\pm$0.05)ml reagent need not be discarded,but appropriate correction should be made in maltose calculations.

FERRICYANIDE-MALTOSE-SUCROSE CONVERSION TABLE

0.1N Ferricyanide reduced (ml)	Maltose per 100g flour (mg)	Sucroe per 100g flour (mg)	0.1N Ferricyanide reduced (ml)	Maltose per 100g flour (mg)	Sucrose per 100g flour (mg)
0.10	05	05	4.50	237	214
0.20	10	10	4.60	244	218
0.30	15	15	4.70	251	223
0.40	20	19	4.80	257	228
0.50	25	24	4.90	264	233
0.60	31	29	5.00	270	238
0.70	36	34	5.10	276	242
0.80	41	38	5.20	282	247
0.90	46	43	5.30	288	251
1.00	51	48	5.40	295	256
1.10	56	52	5.50	302	261
1.20	60	57	5.60	308	266
1.30	65	62	5.70	315	270
1.40	71	67	5.80	322	275
1.50	76	71	5.90	328	280
1.60	80	76	6.00.	334	285
1.70	85	81	6.10	341	290
1.80	90	86	6.20	347	294
1.90	96	91	6.30	353	299
2.00	101	95	6.40	360	304
2.10	106	100	6.50	367	309
2.20	111	104	6.60	373	313
2.30	116	109	6.70	379	318
2.40	121	114	6.80	385	323
2.50	126	119	6.90	392	328
2.60	130	123	7.00	398	333
2.70	135	128	7.10	406	337
2.80	140	133	7.20	412	342
2.90	145	138	7.30	418	347
3.00	151	143	7.40	425	352
3.10	156	148	7.50	431	357
3.20	161	152	7.60	438	357
3.30	166	157	7.70	445	362
3.40	171	161	7.80	451	367
3.50	176	166	7.90	458	372
3.60	182	171	8.00	465	377
3.70	188	176	8.10	472	382
3.80	195	181	8.20	478	387
3.90	201	185	8.30	485	392
4.00	207	190	8.40	492	397
4.10	213	195	8.50	499	402
4.20	218	200	8.60	505	407
4.30	225	204	8.70	512	---
4.40	231	209	8.80	519	---

Reference :

AACC Method 80-60 Page 1 of 2

DAMAGED STARCH

Definition:

This method determines the percentage of starch granules in flour or starch preparations which are susceptible to hydrolysis by alpha-amylase.

Apparatus:

1. Constant temperature water bath regulated at $30^{o}C \pm 0.1^{o}C$.
2. Micro-burette, 10ml capacity.
3. Corning test tube, 25 X 200 mm.
4. Boiling water bath and holder for large test tube.

Reagents

1. Acetate buffer. Dilute 4.1g anhydrous sodium acetate and 3ml glacial acetic acid to 1 liter with water; pH is 4.6-4.8.
2. Sulfuric acid solution. Add 100ml reagent-grade concentrated H_2SO_4 to ca. 700ml water; dilute to 1 liter. Final solution should be $3.68N \pm 0.05N$.
3. Sodium tungstate solution. Dissolve 12.0g $Na_2WO_4.2H_2O$ in water and dilute to 100ml.
4. Alpha-amylase solution.Dissolve a suitable fungal alpha-amylase preparation(counting 5000 SKB units per g) in reagent 1 in proportions of 1.0g enzyme preparation per 450ml buffer. Filter rapidly through coarse filter paper. This solution should be used within two hours.

Procedure:

1. Bring reagent 4 to 30°C in water bath. weigh 1g (14% moisture basis) of flour or starch sample into 125ml Erlenmeyer-flask and add 45ml of reagent 4. Obtain uniform suspension by use of glass rod and rubber policeman. Incubate in 30°C bath for exactly 15minutes from time of adding reagent 4.
2. At the end of 15 minutes add 3ml reagent 2 and 2ml reagent 3. Mix thoroughly, let stand for 2 minutes and filter through whatman no 4 or comparable filter paper, discarding first 8-10 drops of filtrate.
3. Immediately pipette 5ml of filtrate into 25 X 200 mm test tube and determine amount of reducing sugar present.
4. Determine reagent blank by carrying out steps 1 to 3, ommitting flour or starch sample.

Calculations:

1. Subtract mg maltose equivalent found in **procedure**, step 4, from that found in step 3.
2. Result of calculation 1 is multiplied by 1.65 to get the value of % damaged starch.

NOTE:

Suitable commercial preparations include Rhozyme 33 and Wallerstein Fungal Amylase Special.

Reference :

AACC Method 76-30A (1969) Page 1 of 2.

FALLING NUMBER DETERMINATION

Definition :

This method is based on the unique ability of alpha-amylase to liquefy a starch gel. Strength of the enzyme is measured by falling number, defined as time in second required to stir and allow stirrer to fall a measured distance through a hot aqueous flour gel undergoing liquefaction.

Scope :

Applicable to both meal and flour of wheat, rye, barley, other grains and malted cereals.

Apparatus :

1. Falling number apparatus, obtainable only from the manufacture :Falling Number AB, Norlandsgatan 16, Stockholm, Sweden.
2. Thermometer, having accuracy of $\pm$ 0.3 ^{o}C and calibrated in tenths of a degree.
3. Any mill which produces meal with particle size distribution as follows : >500 μ, 0-10 % ; >210 but <500μ, 25-40 % ; <210 μ, 75-50 %.
4. Automatic pipette : should deliver 25 + 0.3 ml.

Reagents :

1. Water bath temperature adjustment agents
2. Glycerol
3. Ethylene glycol
4. Isopropyl alcohol

Procedure :

Constant-temperature water bath :

Fill water bath to water-level control fixed at 1 inch below cover. Place rubber stopper containing thermometer in tube well and let remain until temperature reading becomes constant. If temperature of bath is between 98.0^{o}C and 99.8^{o}C, adjust it to 100°C with ethylene glycol or glycerol. Quantity required is shown in the table below.

Required Temperature Elevation ^{o}C	Quantity to be added	
	Ethylene Glycol % by volume	Glycerol % by volume
0.2	1.9	2.5
0.4	3.9	4.9
0.6	5.8	7.4
0.8	7.8	9.8
1.0	9.7	12.3
1.2	11.3	14.2
1.4	12.9	16.1
1.6	14.4	18.1
1.8	16.0	20.0
2.0	17.6	21.9

If bath temperature is below 98.0^{o}C, falling number determination cannot be made at 100.0^{o}C by temperature adjustment because of danger of boiling out contents of tube. Instead, falling number may be estimated as follows : Determine falling number at observed boiling point, e.g. 96.0^{o}C. Then adjust

temperature to 97.5^{o}C by adding 13.6% ethylene glycol and determine falling number again. Plot both values on graph paper in relation to temperature and extend slope of curve to 100.0^{o}C. Read falling number from graph at this point.

If temperature of boiling-water bath is above 100.0^{o}C, add 0.1% of isopropyl alcohol to water for each 0.1^{o}C of excess temperature. This will reduce boiling temperature to 100.0^{o}C. It is not necessary to make this adjustment if original boiling temperature is not higher than 100.2^{o}C. Observe thermometer immersion point, and if stem correction is applicable, use following formula :

$$\text{Stem correction} = Kn\ (T-t)$$

Where

K = 0.00016 for mercury:

n = number of degrees of mercury column above stoppered water bath:

T = temperature of water bath:

t = temperature of mercury above stopper (room temperature).

Preparation of meal :

Moisture content of grain should be within range of approximately 8-16%. Water should be added with less moisture content, and air- or vacuum-drying applied to grain with more moisture content.

Determination :

1. Weigh 7.0 gm (see note) of flour or meal meeting granular specification into dry falling number tube and tip to 45° angle. Add 25 ml water. Insert rubber stopper and shake tube in upright position 10 times (up and down), making sure all flour is suspended by upending.

2. Scrap down upper part of tube with viscometer-stirrer.

3. Remove stopper from bath well. Simultaneously start timer and place tube in bath, and lock into position, taking not more than 5 second.

4. Stir sample with viscometer-stirrer at rate of two strokes per second (down and up is one stroke), until clock reaches 60 seconds, for total of 110 strokes. Stop with stirrer in up position.

5. Record time in seconds.

6. Quickly remove test tube from bath and insert stopper or another empty test tube to prevent water evaporation.

7. Hold tube under running water and remove viscometer-stirrer. Starch gel is easily removed from tube by means of spatula with extended handle. Clean viscometer-stirrer thoroughly for next sample.

Calculation :

Report falling number on 14% moisture basis, using following formula :

$$\text{Falling Number (as-is)} \times \frac{100-14}{100-\text{moisture}(\%)\text{ of sample}} = \text{Falling Number (14\% moisture basis)}$$

Note :

If operator prefers to weigh sample on 14% moisture basis, following table may be used :

Weight of Sample Corrected for Moisture Content

Moisture Content (14 m.b.)	Weight of sample	Moisture Content (14% m.b.)	Weight of sample	Moisture Content (14% m.b.)	Weight of sample
%	gm	%	gm	%	gm
8.0	6.54	10.8	6.75	13.6	6.97
8.2	6.56	11.0	6.76	13.8	6.98
8.4	6.57	11.2	6.78	14.0	7.00
8.6	6.59	11.4	6.80	14.2	7.02
8.8	6.60	11.6	6.81	14.4	7.03
9.0	6.62	11.8	6.83	14.6	7.04
9.2	6.63	12.0	6.84	14.8	7.07
9.4	6.64	12.2	6.86	15.0	7.08
9.6	6.66	12.4	6.87	15.2	7.10
9.8	6.67	12.6	6.89	15.4	7.12
10.0	6.69	12.8	6.90	15.6	7.13
10.2	6.70	13.0	6.92	15.8	7.15
10.4	6.72	13.2	6.94	16.0	7.17
10.6	6.73	13.4	6.95	16.2	7.18

These days fully automatic instruments are available where most of the steps specified in the method are automatically covered.

References :

1. Hagberg, S. A rapid method for determining alpha-amylase activity. Cereal Chem. 37:218(1960).
2. Hagberg. S. Note on a simplified rapid method for determining alpha-amylase activity. Cereal Chem 38:202(1961)
3. Perten, H. Application of the falling number method for evaluating alpha-amylase activity. Cereal Chem 41: 127(1964).
4. Medcalf, D.G. Gilles, K.A., and Sibbitt, L.D. Detection of sprout damage in wheat. Northwest. Miller, Vol. 273, pp. 16-18, May, 1966.
5. Greenaway, W.T., and Neustadt, M.H. A summary report of estimation and control of experimental error in the falling number test. Cereal Sci. Today 12: 182(1967).
7. Greenaway, W.T., and Neustadt, M.H. Estimation and control of experimental error in the falling number test. U.S. Dept. Agr., Consumer and Marketing Service. Marketing Research Report No. 804(1967).

DEFATTING OF THE GROUND FLOUR

Lipids interfere in many of the methods for estimation of protein quantity and quality. Therefore their prior removal is necessary.

The lipid is removed by Soxhlet extraction using organic solvents like hexane or ethyl ether.

Apparatus :

1. Soxhlet extractor assembly
2. Absorbent cotton and
3. Vacuum desiccator

Reagent :

1. Hexane or Ethyl ether

Procedure :

1. Weigh 2 to 5 g sample depending on fat content and roll in a piece of filter paper and make into a sample packet. Put these packets of various samples into extractor flask of soxhlet apparatus, after placing some absorbent cotton at the bottom. Add hexane one and a half times the capacity of the extractor and extract lipids for a period of six hours at a condensation rate of five to six drops per second or for a period of 16 hours at two to three drops per second.

2. Remove excess of hexane by allowing to remain at room temperature and then store in a desiccator.
3. The resultant lipids are weighed and calculated on per cent basis

FAT ACIDITY

The fat acidity of grain is determined by extraction of the oil from the grain followed by titration of the free fatty acids present in the oil. For this determination, the oil is extracted from the grain with petroleum ether in a soxhlet extractor and the titration is carried out in a solution of benzene-alcohol with standard aqueous potassium hydroxide solution. A rapid method 2 of similar principle has been proposed in which the extract of oil is simplified by using a grinder-extractor. In both methods the fat acidity value is expressed as the no. of mg in potassium hydroxide required to neutralize the free fatty acids in 100g of the dry grain.

The new colourimetric method is based upon the reaction of fatty acids to form metallic soaps. An aqueous solution of cupric acetate when shaken with fatty acids dissolved in benzene reacts with the fatty acids to form benzene-soluble copper salts which impart a blue colour to the benzene solution. The reagent is sensitive enough to detect difference in the amounts of free fatty acids present in the oil from damaged grain. The difference in the smaller amounts of free fatty acids present in the sound grain, whose fat acidity values are generally below 20, are not so easily detected . The intensity of the colour in the benzene solution can be measured with an appropriate instrument. A comparative study was made of this colourimetric method with the usual procedure involving titration with standard potassium hydroxide solution.

Apparatus :

1. Grinder - extractor
2. Grain sample
3. Analytical balace
4. Test tubes
5. Filter paper
6. Fluted filter
7. Spectrophotometer

Reagents :

1. Benzene
2. Cupric acetate solution, 5%.

Procedure :

The percent transmittance of the solutions was measured with a Bausch & Lomb "Spectronic 20" spctrophotometer. As the fat acidity value increased, the intensity of the blue colour increased and the percent transmittance decreased. Fatty acid solution containing 10,20,30,50,80 and 100mg of fatty acid in 5ml benzene were treated with cupric acetate and transmittance curves from 350-700 µg were made. From these curves the wave length of 640 nm was chosen for measuring the percent transmittance of the copper soap solutions. At this wave length the transmittance of wheat and corn oil solutions in benzene is 98to 100 %.

A sample of grain weighing 40g is ground for 1 minute in a grinder-extractor. Then 50ml benzene are added to the ground sample and the oil is extracted by running the mill for 4 minutes The sample is filtered and 10ml of the filtrate measured into test tube containing 2ml of 5% cupric acetate solution. The tube is stoppered and shaken by inverting rapidly 50 times by hands. After the solution is separated into two layers, the top benzene layer is decanted through a fluted filter into a colourimeter tube and the percent transmittance read at 640 nm with a colourimeter or spectrophotometer. Benzene is used as blank for adjusting the instrument.

SEDIMENTATION TEST

This test is based on the fact that gluten protein absorbs water and swells considerably when treated with lactic acid in the presence of sodium dodecyl sulphate (SDS). The volume of sediment depends on the extent of swelling of gluten protein and correlated significantly (+0.7) with loaf volume.

Method I :

Apparatus :

1. Stop clock
2. Water bath
3. 100ml stoppered measuring cylinder.(These should have identical internal diameters and the distance between the 0 and 100ml graduations should be approximately 160mm).
4. 50ml measuring cylinder.

Reagents :

1. Pure sodium lauryl sulphate alternatively known as sodium dedocyl sulphate (S.D.S.)
2. 88% lactic acid (A.R.).

The required S.D.S./lactic acid reagent may be prepared by dissolving 20g S.D.S. in one litre of distilled water, to this 20ml of stock dilute lactic acid prepared by diluting one part by volume of 88% lactic acid with 8 part by volume of distilled water, is added and the reagent shaken or otherwise agitated until homogeneous.

Wholemeals:

These should be prepared by passing wheat through either a Tecator Cyclotec Mill fitted with a 0.5 mm screen.

Procedure:

The following method permits 4 determinations to be carried out at the same time, an additional 4 test may be started during the period in which first four sediment are setting.

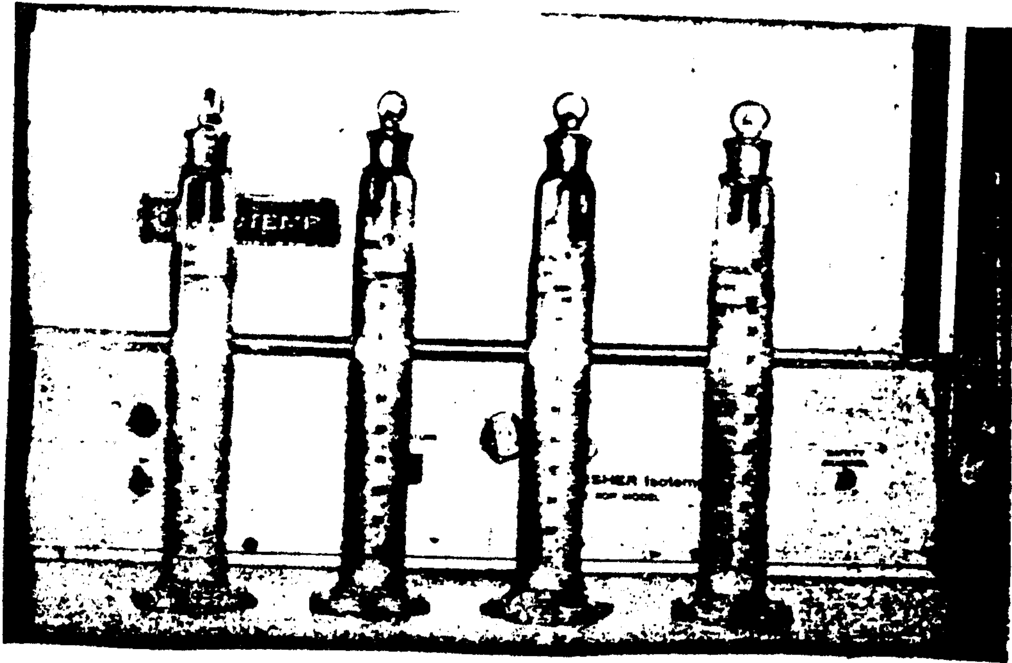
50ml of distilled water should be poured in each of the required number of 100ml cylinders prior to starting the test, similarly the required number of 50ml measuring cylinders should each be prefilled with 50ml S.D.S./lactic acid reagent.

Add 6g wholemeal (5g flour) to 50ml water (Cylinder 1) and start the stop clock. Shake rapidly for 15 seconds, keep the clock running continuously throughout the rest of the experiment. The times for commencement of the other operations are given, in minute, in the following table.

Cylinder no.	15 sec. shake in water	15 sec. shake in water	15 sec. shake in water 50ml S.D.S. invert 4X	Invert 4X	Invert 4X	Invert 4X	Read Sedimentation Volume	
							whole meal	Flour
1	0	2	4	6	8	10	30	50
2	0.5	2.5	4.5	6.5	8.5	10.5	30.5	50.5
3	1.0	3.0	5.0	7.0	9.0	11.0	31.0	51.0
4	1.5	3.5	5.5	7.5	9.5	11.5	31.5	51.5

Horizontal Through Distance

Sediment volume should be measured to the nearest ml.



SEDIMENTATION TEST

Method 2 :

Apparatus and reagents are same as in method I.

Procedure :

A 1 g of ground sample is placed into a standard clean glass test tube (155mm long, 16mm outer diameter, 14 mm inner diameter) containing 4ml distilled water. Shake for 2 seconds on vortex mixer. Keep for 5 minutes. Repeat again. After 5 minutes add 12ml SDS reagent. Invert 10 times. Note the sediment after 10 minutes.

Reference :

1. FMBRA bulletin 1977(6)Dec., 201-203
2. Dick, J.W. & Quick, J.S. (1983). Cereal Chemistry 60 : 315-318

ESTIMATION OF GLUTEN:

When water is mixed with wheat flour and the contents are kneaded, a cohesive mass of dough is formed. This mass on washing removes starch, bran and yields a viscoelastic gum like material known as gluten. Gluten is mainly composed of gliadin and glutenin proteins.

Apparatus :

1. Beaker
2. Glass rod
3. Sieve (100m)
4. Oven
5. Analytical balance

Reagents :

1. KI solution
2. Water

Procedure:

1. Take 10g of the given sample in a beaker.
2. Add to it 7 ml distilled water and make a dough with the help of glass rod for around 2 minutes.
3. After proper mixing,make a small ball of the dough and immerse it in a beaker containing water.
4. The ball is left for 30 minutes.

5. Place the beaker under tap water, put a sieve (100m) on it. Now the dough is washed gently under running water till a chewing gum type cohesive mass separates out Bran will settle over the sieve and starch will pass down to the beaker.

6. To ensure complete removal of bran, wash the extracted gluten with excess of water by streching inside the fingers.

7. To check whether whole of the starch is washed out or not, add KI to last extract. Absence of any violet colour indicates complete removal of starch.

8. Squeeze out the adhered water from the extracted gluten and weigh. This will be the weight of wet gluten. Dry at 105°C for 6 hours and weigh to get the yield of dry gluten.

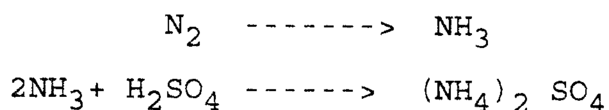
ESTIMATION OF NITROGEN PROTEIN CONTENT

Theory and principle

In the kjeldahl method of nitrogen estimation, the sample is digested in concentrated sulphuric acid. The acid acts as a dehydrating and oxidiging agent. Carbon in the sample is oxidised according to -

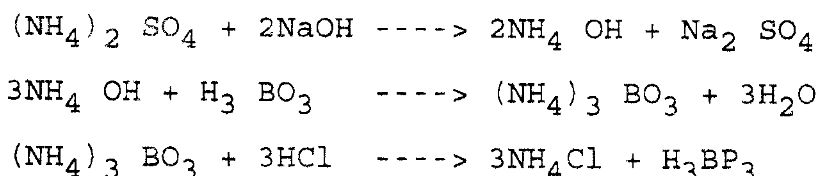
$$C + 2H_2SO_4 \text{ -------> } CO_2 + 2SO_2 + 2H_2O$$

The nitrogen of the sample is tansferred into ammonia. Carbon dioxide, water vapours and sulpher di-oxide escape out and the ammonia is held back as ammonium ions in the form of ammonium sulphate.



$$N_2 \text{ -------> } NH_3$$
$$2NH_3 + H_2SO_4 \text{ ------> } (NH_4)_2\ SO_4$$

Sodium Hydroxide is then added to the solution which transforms ammonium ions into ammonia, which is distilled off, absorbed in boric acid solution and titrated with standard hydrochloric acid.



$$(NH_4)_2\ SO_4 + 2NaOH \text{ ----> } 2NH_4\ OH + Na_2\ SO_4$$
$$3NH_4\ OH + H_3\ BO_3 \text{ ----> } (NH_4)_3\ BO_3 + 3H_2O$$
$$(NH_4)_3\ BO_3 + 3HCl \text{ ----> } 3NH_4Cl + H_3BP_3$$

Since the boiling point of sulfuric acid is not sufficiently high to oxidise organic substance quickly, potassium sulphate is added to raise its boiling point. Copper sulphate acts as a catalyst.

MICRO-KKJELDAHL'S METHOD:

Apparatus :

1. Micro-Kjeldahl digestion unit
2. Micro-kjeldahl distillation unit
3. Micro-kjeldahl digestion flasks
4. Conical flasks
5. Burrette and pipettes
6. Measuring cylinder

Reagents :

1. Concentrated Sulfuric acid.
2. Digestion mixture: 500 g of Na_2SO_4/K_2SO_4 (A.R.) + 10 g $CuSO_4$+ 10 g of selenium powder well mixed in a pestle and mortar and kept in a dry place.
3. Alkali - NaOH (40%) : Dissolve 400 g of NaOH in distilled water and make up volume to 1 litre.
4. Boric Acid (4%) : Dissolve 40 g of boric acid in distilled water and make up volume to 1 litre.
5. Mixed Indicator Solution: (a) Weigh 0.5g of bromo-ceresol green and dissolve in ethanol and makeup to 100ml. (b) weigh 0.1g of methyl red and dissolve in ethanol to make up to 100ml. Mix both (a) and (b).
6. 0.1N HCl acid: Dissolve 8.7 ml of concentrated HCl in 1 litre of distilled water .(Total volume should be one litre.)

PROCEDURE:

The wheat grain of a variety are ground in a laboratory flour mill. 0.5g of the powdered flour is weighed in a small piece of paper. The paper containing the weighed flour is folded and kept in a dry kjeldahl flask. 7.0g of digestion mixture and 20 ml of concentrated H_2SO_4 is added to it The flask is heated until frothing ceased and then simmered briskly. The solution becomes clear in 15 to 20 minutes. Heating is continued for 45 minutes. After cooling and diluting the contents,the solution is transferred to a distillation flask. Sodium hydroxide is added and the distillation flask is connected to a steam trap and a condenser. The condenser is adjusted such as it dipped bellow the surface of 100 ml of boric acid solution in a 500 ml conical flask. The contents of the flask are titrated with 0.1 N HCl using a few drops of mixed indicator. The methyl-red-bromo-cresol green is grey at the end point. The indicator shows a pink colour if the end point is exceeded. A reagent blank is also determined and its value is deducted from the titration. The amount of nitrogen that is determined by this method is multiplied by factor 5.7 for the determination of protein content.

Calculations:

Suppose the mass of the organic compound taken = Wg and V ml of N-normal acid be used by ammonia liberated from the kjedahlized substance.

$$\text{V ml of normal ammonia} = \frac{14 \times V \times N}{1000}$$

$$\text{\% of nitrogen} = \frac{14 \times V \times N}{1000 \times W} \times 100$$

$$= \frac{1.4 \times V \times N}{W}$$

KJEL-TEC METHOD:

Apparatus :

1. Digestor with built in thermostat
2. Stand for tubes
3. Set of digestion tubes
4. Heat shield
5. Exhaust Manifold with water aspirator (including stand and drip pan)
6. Stand for rapid cooling of tubes
7. Retainer plate
8. Scrubber Unit (optional)
9. Kjeltec Auto 1030 Analyzer including storage tanks for alkali, receiver solution, separate feed water and titrant.
10. Special checking thermometer.
11. Automatic Pipette, adjustable 10-30 ml.

Reagents

1. Sulphuric Acid, concentrated.
2. Kjeltabs, M-, Cu-, S- or Auto-type.

   M/3.5 (Hg)

   S/3.5 (Se)

   Cu/3.5 (Cu)
3. Hydrogen Peroxide 30-35%.
4. Alkali - use standard technical quality of Sodium Hydroxide Solution (35-40%).
5. Sodium Thiosulphate solution, (When mercury is used as catalyst).

   Mix 300 g of $Na_2S_2O_3 \times 5H_2O$ in a litre H_2O or 60 g in 1 litre of the alkali.

6. Boric Acid 1% with bromocresol green/methyl red indicator solution.

   Dissolve 100 gram of Boric Acid in 10 litre distilled or deionized water (1% solution).

   Add 100 ml bromocresol green solution (100 mg in 100 ml Methanol).

   Add 70 ml methyl red solution (100 mg in 100 ml Methanol).

   Add 5 ml 1 M (4%) NaOH. (The alkali is necessary to achieve a positive blank value).
7. Standard Acid solution, HCl

   Depending on sample size and nitrogen contents standard acid solutions ranging from a concentration of 0.05 to 0.5 molar may be used for titration.

PROCEDURE:

Weigh 0.5g of the sample. Add 10ml concentrated H_2SO_4 and 4.5g of catalyst mixture. Digest it at 450°C for 30-45 minutes till contents are clear (The colour of the solution will be blue when it is hot and white on cooling). After cooling add 75ml of distilled water and shake thoroughly till the contents are clear. Check before the start of distillation that the tubes connecting boric acid , alkaline and distilled water are in order. Distilled the digested content with the automatic kjeltec 1030. Protein value is automatically displayed on the monitor. the only manual operation is feeding the values as directed in the instruction manual.

Reference :

i. AACC 44-15A, 1983

2. Kjeltec Auto 1030.

ESTIMATION OF ACID-INSOLUBLE PROTEIN :

The amount of acid - insoluble proteins in the flour has been reported to be positively correlated with loaf volume. For maximum benefit to the wheat breeders, it is suggested that determinations of both acid insoluble and total protein would be necessary. From these values the proportion of residue protein in a flour may be determined.

Apparatus :

1. Analytical balance
2. Magnetic stirrer
3. Centrifuge
4. Kjeltec auto nitrogen analyser (alternatively micro-Kjeldahl method can be employed for the determination of protein).

Reagents :

1. 0.05 M Acetic acid
2. All the reagents required for protein analysis either by Kjeltec auto analyser or micro Kjeldahl method.

Procedure :

Flour (0.13 gm) is magnetically stirred in 0.05 M acetic acid (15 ml) for 45 minutes. After centrifuging the contents at 2000 rpm for 20 minutes, the entire centrifugate is subjected to protein determination either by Kjeltec auto analyser or micro Kjeldahl method.

The higher content of insoluble proteins predicts better loaf volume.

Reference :

1. Orth, R.A. and Bushuk, W.A. Comparative study of the proteins of wheats of diverse baking qualities. Cereal Chem. 49 (1972), 268-275.

2. Axford, D.W.E., McDermott, E.E. and Redman, D.G. Small scale tests of bread making quality. Milling Feed and Fertilizer (1978) May Issue, 18-22.

SEPARATION OF GLIADIN

Definition :

Polyacrylamide gel electrophoresis (PAGE) is a method of separating dissolved protein components on a polyacrylamide gel support medium according to their molecular size and electric charge. Gliadin proteins are the alcohol soluble proteins of wheat endosperm. The gliadin electrophoregram is the pattern of separated gliadin components at the completion of electrophoresis. After staining with a blue dye, gliadin components appear as bands in the gel.

Principle :

Extraction of the gliadin fraction of the wheat with an aquous ethanol solution and electrophoresis on polyacrylamide gel. Fixing and developing the gliadin bands using a specific dye solution. Destaining and photography of the gel, and evaluation of the pattern. Cultivers have band patterns (electrophoregrams) that are characteristic of the genotype and independent of growth conditions.

Scope :

This method is applicable to common or durum wheat samples in the form of whole grain, flour, farina or semolina. The sample can be as small as half a seed in cases where only one seed is available and it is needed for germination or planting.

Apparatus :

1. Electric sample grinder
2. Analytical balance
3. Vortex mixer
4. Table-top micro-centrifuge (10,000 X G maximum RCF) with 1.5 to 2.0 ml capacity centrifuge tubes.
5. pH meter
6. Two adjustable hand-held micro-pipettors, one of capacity 500 µl and one of capacity 10-20 µl.
7. Sample vials with capacity 2 ml.
8. Circulating constant temperature water bath regulated at 20 + ^{O}C, approximately 3 m of flexible tubing (8 mm inner diameter), and three tubing connectors (with male-female type connection).
9. Electrophoresis DC power supply with an output of 0-200 mA at 0-500 V.
10. Vertical slab-gel electrophoresis apparatus constructed of acrylic plastic (polymethylacrylate). The method can be used with any commercial apparatus that has an efficiently cooled gel chamber (for casting gels 1.5 to 3.0 mm thick and at least 150 mm long) and that can be filled rapidly and can accommodate at least 11 samples per gel.
11. Teflon slot former.

12. Electrophoresis electrode (two required) made of 150 mm of platinum wire (0.5 mm outer diameter) soldered to a male banana plug; the solder joint should be waterproofed with silicone sealant.
13. Electrophoresis lead wire (two required), approximately 1 mm in length, with a female banana jack at one end to attach to the electrode and a plug at the other end suitable for connection to the power supply.
14. Gel staining/destaining container and lid made of rigid polyethylene approximately 180 mm wide X 250 mm long X 45 mm deep. For convenience, the container should have a drain hole with plug near the base. Commercially available food storage containers are suitable.
15. Gel remover consisting of a thin sheet of rigid plastic or metal cut to fit the lower cooling chamber of the electrophoresis apparatus.
16. Variable speed shaker.
17. Clear glass plate 3 mm thick X 200 mm long.
18. Light box with visible light source (fluorescent) and a viewing surface at least 200 X 250 mm.
19. Photography copy stand.
20. Camera with close-up lens, loaded with technical pan film.
21. Photographic enlarger and 200 X 250 mm photographic paper.

Method-I (Lactate gel)

Reagents:

A. Acrylamide 32g
Bisacrylamide 1g } Water to make 100ml

B. Ferrous sulphate 0.032g : Water to make upto 100ml (This solution is always prepared fresh.)

C. Alumimnum lactate pH 3.1 (upper buffer)
Take 1g Aluminium lactate and dissolve in 100ml water. Adjust pH with lactic acid to pH 3.1 and then make up the volume to 700ml.

D. Lactic acid : pH 2.6 Approximately 7.0ml lactic acid in 4100ml water.

Gel Preparation:

For two gels

Reagent	Quantity	Comments
A	37.5 ml	Degas the contents and cool in deep freeze
B	15.0 ml	till it become solid.
Water	67.5 ml	Warm in hand until
Lactic acid (pure)	0.6 ml	crystals just melt.
Ascorbic acid	120.0 ml	Add H_2O_2 and pour the gel solution in pre cooled cassettes.
Hydrogen peroxide(0.6%)	0.3 ml	
Stain (0.5% aq. CBBR)	5.0 ml	

Fixer (12% TCA, for 30 min.) 5 ml dye in 250ml.

Destain 1-2 min. 50% methyl alcohol, 10% acetic acid followed by 6% acetic acid and 15% methyl alcohol (1-4 hour)

Extraction Procedure:

Weigh 25mg seed material. Take 4 µl/mg i.e. 0.1ml of ethanol 70%. Leave overnight. Centrifuge add in the supernatant, add 0.84 µl of fuscin dissolved in 60% glycerol.

Method II

Apparatus :

Same as in Method 1.

Reagent

Preparation of solution:

All solutions prepared using distilled water.

(a) **Stock solutions**

1) 0.94 M acetic acid/glycine buffer pH 3.1 for use as gel and tank buffer:

5.0 M acetic acid (188ml), diluted with water (appoximately 3 litre)and add glycine (approximately 30g) to adjust to pH 3.1. Make upto volume (5litre) with water to produce 5X concentrate stock solution.

2) Acrylamide solution:

Acrylamide (300g)and N, N-methylenebisacrylamide (15g) dissolved in 0.94 M acetic acid/glycine buffer, made upto 1 litre with stock buffer, filtered and store in the dark at 4°C to produce a 5X concentrate stock solution.(NB: Both acrylamide and N,N-methylenebisacryla-

mide carry hazard warnings and therefore, should be handled with extreme care in the dry or unpolymerized state.

3) Trichloroacetic acid (gliadin protein precipitant):
Trichloacetic acid (960g) dissolved in water and made upto volume (2 litre).

4) PAGE blue G-90 (gliadin protein stain):
PAGE blue G-90 (0.25g) made upto 1 litre with water.

(b) Working Solution:

The following solutions are prepared fresh as required:

1) 6% urea (gliadin extracting solution):
Urea (6g) dissolved in water and made upto 100ml.

2) Pyronine-G (tracker dye):
Pyronine-G (4ml) dissolved in 6% urea solution (1ml) with thorough mixing.

3) Ferruos Sulphate:
90 mg ferrous sulphate (heptahydrate) dissolved in water (100ml).

4) Hydrogen peroxide (gel polymerization catalyst):
Hydrogen peroxide (100ml volumes) in water (0.7% v/v).

Gliadin extraction:

Single kernel is placed in the fold of a filter paper on the steel block and crushed with a hammer. The resulting wholemeal is transferred to a sealable plastic centrifuge tube and extracted with 6% urea (100-130 µl) dependent on grain size for 1 hour at room temperature,with occasional vortex mixing. 5 ul of pyronine-G solution are then added to each tube before centrifugation at 11750 X g for 3 minutes Clarified gliadin extracts are then ready for loading onto polyacrylamide gels.

Gel recipe and preparation:

To prepare 200ml of gel solution (sufficient for two 180 X 140 X 2.7 mm gels) the following quantities are used:

Stock acrylamide	40 ml
Ferrous sulphate solution	10 ml
L-ascorbic acid	0.2g/25 ml water
Water	125 ml

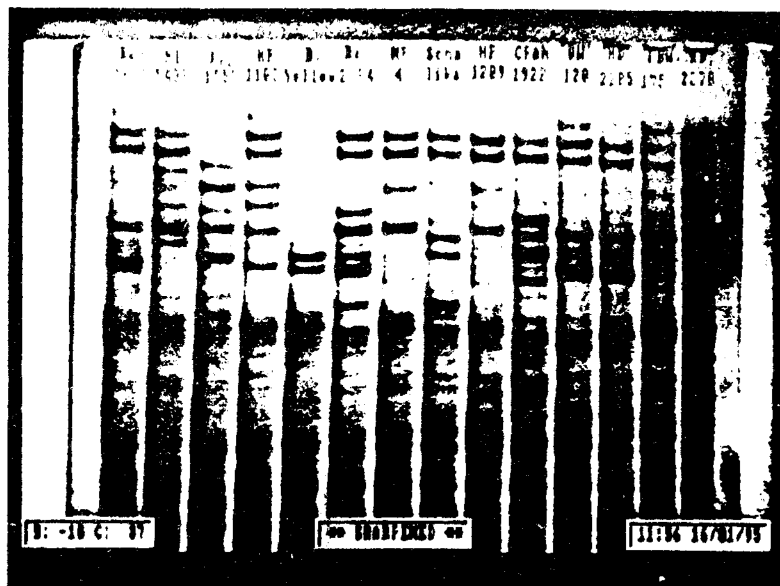
The solution is cooled to 1°C in a bath of solid CO_2 methylated spirit, 0.4 ml hydrogen peroxide (0.7%) added to initiate gel polymerization, and then poured,without delay, into the casting box to cover the gel cassettes. The box is tapped gently to expel any trapped air and the well formers placed in position in the cassettes. Polymerization takes approximately 4 minutes and gels can be used 30 minutes later. Prepared gels may be stored in a humid atmosphere at 4°C upto one week if not required for immediate use.

Electrophoresis Condition:

The electrophoresis tank is coupled to the power supply and buffer circulated in the lower buffer vessel only during the initial stages of electrophoresis (3 minutes). Electrophoresis is then allowed to proceed, with full buffer circulation, at a constant voltage (400V) for a total of 130 minutes,which is equivalent to 1.5 times the time taken for the tracker dye band to migrate to the end of the gel. The tank buffer temperature is controlled at 20 $\pm$ 2°C by adjusting the passage of water through the cooling coils in the base of the buffer tank.

Gel Staining:

Gel is removed from the electrophoresis unit, the upper glass plate taken off,and placed in a solution trichloroacetic acid/water/PAGE blue G-90 (19:57:4) in a sealed polyethylene tray. Staining is allowed to continue overnight at ambient temperature. No destaining is required, but, due to the colloidal nature of the stain, gel benefits from a short rinse in water combined with gentle brushing of the gel surface, particularly when required for photography.



ELECTROPHORETIC BANDING PATTERN OF GLIADIN PROTEINS IN INDIAN WHEATS

QUALITATIVE SDS- PAGE ELECTROPHORESIS OF GLUTEN PROTEINS:

Apparatus :

Same as for Glidin

Reagents :

Stock solution	Quantity	Volume	Amount used (2 gels)
Running gel (12% acrylamide)			
1. 3 M Tris buffer pH 8.8	36.33g	100ml	10.0 ml
2. 60% (w/v) Acrylamide	60.00g .		16.0 ml
+ 0.9% (w/v) Bis-acrylamide	0.90g .	100ml	
3. Distilled water			46.4 ml
4. 10% S.D.S.	10.00g	100ml	0.8 ml
5. 0.8% Ammonium persulphate	0.80g	100ml	6.8 ml
6. TEMED (Ready to use)	0.04ml		0.04ml
Stacking gel			
1. 0.5 M Tris buffer pH 6.8	6.05g	100ml	4.0 ml
2. 25% (w/v) Acrylamide	25.00g .		2.2 ml
+3.5% (w/v) Bis-acrylamide	3.50g .	100ml	
3. Distilled water			8.0 ml
4. 10% S.D.S.	10.00g	100ml	0.1 ml
5. 0.8% Ammonium persulphate	0.80g	100ml	2.0 ml
6. TEMED (Ready to use)	0.02ml		0.02ml
For Extraction			
1. 0.062 M Tris,pH 6.8	0.75g .		
2. 2% S.D.S.	2.00g .	100ml	0.4 ml
3. 5% (v/v)Mercaptoethanol	5.00g .		
Running buffer			
1. Tris	12.12g .		
2. Glycine	56.80g .	4000ml	4000 ml
3. S.D.S.	4.00g .		
Stain			
1. 0.10 % Coomessie brilliant blue R 250	2.00g .		250 ml
2. 40 % Methanol	800.00ml.	2000ml	or
3. 10 % T.C.A.	200.00g .		as per need

NOTE: It is important to make up the ammonium persulphate fresh and not to add it, nor the TEMED, until just before pouring the gels as they initiate the polymerization.

Tris: Hydroxymethyl amino methane (molecular weight 121.12) .

TEMED: N,N,N,N'tetramethylethylene diamine.

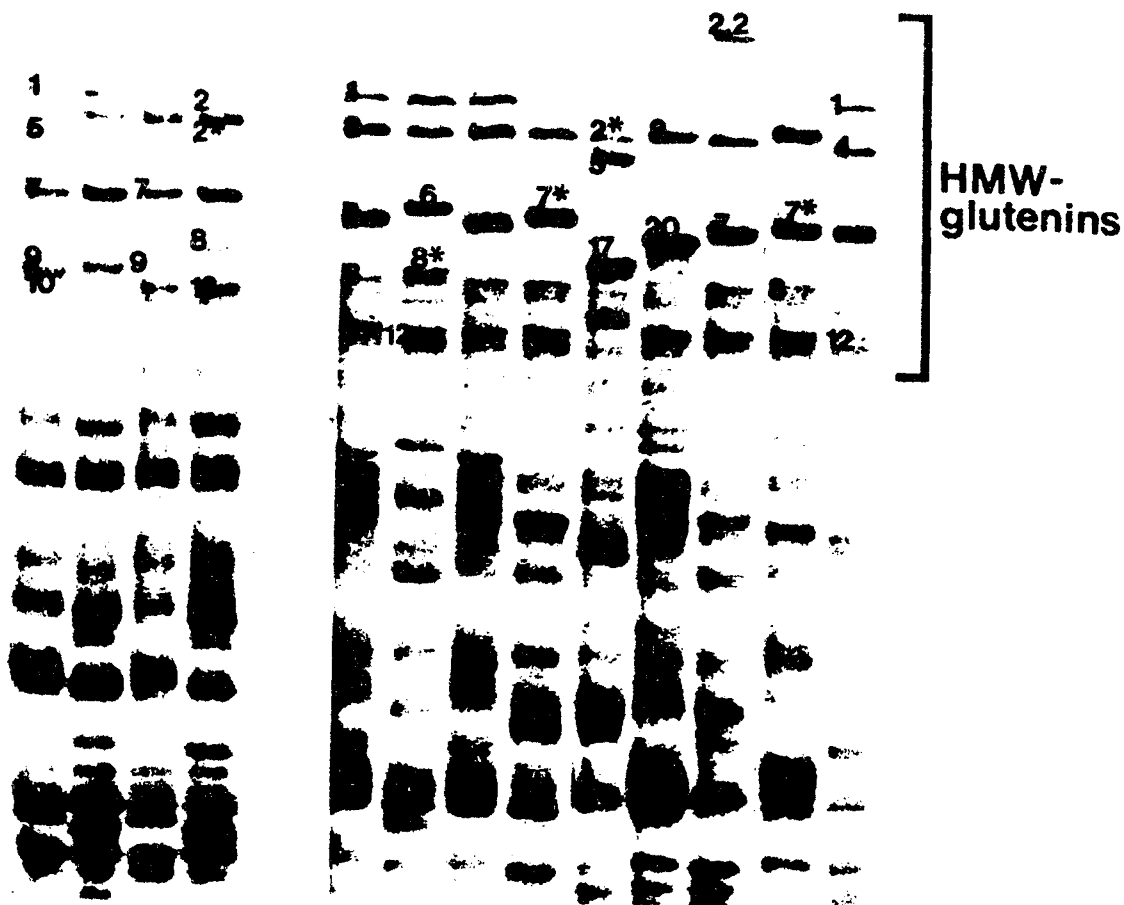
Bis acrylamide: N,N'-methylene bisacrylamide

Procedure :

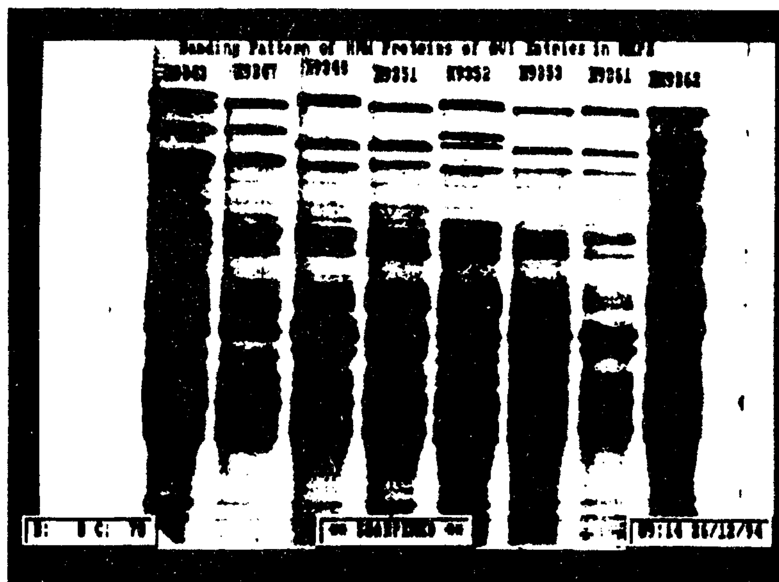
The procedure is modified from a method reported by Fullington et.al. (1983) so as to give good separation of the gluten proteins rather than total proteins. Total protein is extracted from a 30 mg flour sample with 1ml of 2% (w/v) SDS plus 5% (v/v) 2-Mercaptoethanol in a 0.062 M tris buffer adjusted to pH 6.8 with concentrated HCl. A small amount of bromophenol blue is added as a tracker dye. This mixture is shaken vigorously before being placed in a kerry sonicator for 30 minutes. The sample is then shaken again and left at room temperature for 30 minutes before 0.08 ml of 50% (v/v) glycerol solution is added. The sample is then centrifuged and the supernatant is stored at $20^{o}C$.

The electrophoresis unit used is a vertical slab gel apparatus which allowed two gels to be run simultaneously. Plate dimensions are 200 X 160 mm and these are separated with 1.4 mm spacers. Immediately after pouring the running gel, a small amount of water saturated butanol is also added which floats on top of the gel and helps smooth the upper surface. After the

polymerisation of gels the butanol is washed off by rinsing with deionised water,the stacking gel is then poured and a comb producing 15 slots is inserted. The comb is removed after 30 minutes and 0.02 ml of sample was loaded into each slot. The apparatus is then placed in an electrophoresis tank containing inrunning buffer 12.12g tris, 56.8g of glycine and 4g of SDS 4 litre of distilled water. Initially a 40 mA current is exerted with a change power supply but this is increased to 60 mA after the first hour. The gel is then run until 45 minutes after the tracker dye has disappeared from the bottom of the gel. Gels are stained for 24 hours with 0.02% coomassie brilliant blue R 250 in 20% methanol solution containing 10% TCA. Gels are destained with 10% TCA for 24 hours. The tracks are then studied with UVP-vidiodensiometer.



STANDARD HMW GLUTENIN SUBUNITS



ELECTROPHORETIC BANDING PATTERN OF GLUTENIN PROTEINS IN INDIAN WHEATS

LYSINE DETERMINATION

The lysine being a limiting amino acid in most of the cereals has a great nutritional importance. Usually the contents of lysine in wheat is around 2 % which makes wheat grain an imbalanced food for human nutrition. Efforts made in improving its level in genotypes have not yielded appreciable results.

The colorimetric method designed by Tsai (Purdue University) and modified by Villegas (CIMMYT) is recommended for the determination of lysine.

Apparatus :

1. Centrifuge
2. Oven
3. Test tube
4. Pipette
5. Analytical Balance
6. Vials
7. Spectrophotometer
8. Centrifuge tubes

Reagents:

1. Papain solution, 4mg of papain per ml of phosphate buffer 0.03 M, pH 7.4.
2. Carbonate buffer 0.05 M ,pH 9.0.
3. Borate buffer 0.05 M, pH 9.0.

4. Copper sulphate suspension.

Solution A: 2.8g of $CuCl_2.2H_2O$ are dissolved in 100ml distilled water.

Solution B: 13.6g of $Na_3PO_4.12H_2O$ are dissolved in 200 ml distilled water.

Pour mixture A into B with swirling, centrifuge to bring out the precipitate and discard the supernatant. The pellet is then resuspended 3 times in 15ml of borate buffer pH 9, centrifuging after each suspension. After the third washing resuspend the pellet in 80ml of the borate buffer. The reagent can be used for one week.

5. 2-Chloro-3,5-dinitropyridine solution. Prepare just prior to its use. A solution containing 30mg of 2-chloro-3,5-dinitropyridine per ml of methanol.

6. HCl solution 1.2 N.

7. Mixture of amino acids:

Cystine	20 mg	Phenylalanine	40 mg
Methionine	20 mg	Valine	40 mg
Histidine	30 mg	Arginine	50 mg
Alanine	30 mg	Serine	50 mg
Isoleucine	30 mg	Aspartic acid	60 mg
Threonine	30 mg	Glutamic acid	300 mg
Tyrosine	30 mg	Leucine	80 mg
Glycine	40 mg	Proline	80 mg

Weigh 100mg of the amino acid mixture and dissolve in 10ml carbonate buffer.

Procedure:

1. Weigh 100mg of finely defatted sample in a vial and add 5 ml of papain solution.Be sure to wet all the sample and shake it at least twice in the first hour of incubation. Carry on blanks with papain solution.

2. Incubate overnight at 65°C. Remove the hydrolysates from incubation oven and shake, then allow to settle and to adjust to room temperature by which time the supernatant should be clear, or centrifuge (One aliquot of 1ml from this hydrolysate can be used for tryptophan determination).

3. Pipette 1ml aliquot into a centrifuge tube and add 0.5ml of carbonate buffer and 0.5ml of the copper phosphate suspension.

4. Shake the mixture for 5 minutes and centrifuge.

5. Pipette 1ml aliquot of the supernatant into a large test tube and add 0.1ml of 2-chloro-3,5-dinitropyridine-methanol solution and shake well.

6. Allow the mixture to stand for 2 hours at room temperature. (shake every 30 minutes)

7. Add 5 ml of HCl 1.2 N and shake well.

8. Add 5 ml of ethylacetate, stopper tubes, mix well inverting the tubes at least 10 times, then extract the upper phase using a syringe adapted with a polyethylene tube. This step must be performed 3 times.

9. Transfer the aquous phase to calibrated tubes and read in the spectronic-20 at 390 nm against the blanks.

10. Calculate lysine content of the samples by comparing with a standard curve and report on a protein basis.

Standard Curve :

1. Prepare a standard curve in a range of 0 to 200 µg of lysine per ml.

2. Stock solution of lysine: 62.5mg of lysine-monohydrochloride in 20ml of carbonate buffer (2,500 µg lysine per ml).

3. Dilute with carbonate buffer the stock solution of lysine to 0,250,500,750,1000 µg lysine/ml.

4. From each one of these solution,take 1ml and add 4ml papain solution (5mg papain/ml of phosphate buffer).

5. Pipette 1ml of each solution into centrifuge tube,add 0.5ml of the amino acid mixture solution, and 0.5ml of copper phosphate suspension. Continue with procedure on step (4).

DETERMINATION OF β-CAROTENE CONTENT IN DURUM WHEATS

Yellow colour in durums imparts attractive appearance to the pasta products and therefore majority of the pasta consumers prefer the yellow pigment. Xanthophills and specially β-carotene contributes to the colour production in the semolina. High lipoxygenase activity has been described to be responsible for an appreciable loss to β-carotene. Linoleic acid acts as a source of substrate for the enzyme for linoleic acid hydro peroxides which ultimately oxidize the pigment. β-carotene acts as a preservative. Durum endosperm contains twice the concentration of β-carotene than that of aestivum. Since β-carotene is highly susceptible to oxidation precaution has to be taken for its determination.

Method I :

Apparatus :

1. Analytical balance
2. Whatman No. 1 filter paper
3. Volumetric flasks, 50ml, 100ml and 250ml
4. Spectrophotometer
5. Erlenmayer flask

Reagents :

1. Water saturated n-butanol: Prepare a solution of n-butanol and water in a proportion of 6:2 (v/v), and shake vigorously. Use the clear upper layer after separation of the phases.

2. Diethyl Ether

3. Synthetic β-Carotene, crystalline.

preparation of extract :

Weigh 10g of sample (Whole meal/flour/semolina) and disperse it in 50ml of water-saturated n-butanol to give a homogeneous suspension. Shake gently and allow it to stand overnight (16hours) at room temperature under dark.Shake and filter completely through the filter paper (Whatman No. 1) into a 100ml volumetric flask.

Determination :

Measure the optical density of the clear filtrate at 440 nm as absorbance, using the spectrophotometer.Use unfiltered water-saturated n-butanol as blank .Evaluation of the contents is based on a β-carotene calibration curve (related to the β-carotene content in a 10ml solution).

Preparation of standard solution of β-carotene :

In a 100 ml volumetric flask, weigh 0.025g of β-carotene.Dissolve it in diethyl ether and make up to the mark with diethylether.20ml of this homogeneous solution (=5mg β-carotene) is pipetted into a 250 ml volumetric flask.Make upto the mark with water-saturated n-butanol.Take 25ml of this solution and place in a 100 ml volumetric flask make-up with water-saturated n-butanol.

This standard solution has the following concentration:

1ml = 0.005 mg = 5ug β-carotene

Preparation of calibration curve :

Prepare suitable dilutions of the standard solution with water-saturated n-butanol in calibrated 10ml volumetric flasks (e.g.from 0.5ml to 3ml of standard solution in 10ml). Measure the absorbance, A, of each dilution and establish a calibration curve (β-carotene in 10ml of solution as a function of absorbance).

Calculation :

The yellow pigment content, Wp, Expressed as milligrams of β-carotene in 100g dry matter, is equal to

$$Wp = \frac{a . 5}{100 - H}$$

Where, a = is the β-carotene content of a 10ml extract (equivalent to 2g of the test sample), in mgs

H = is the moisture content of the test sample, expressed as percentage by mass.

AACC Method (1962) :

This method can be used for screening a large number of samples for their β-carotene content.

Apparatus :

Same as in method I

Reagent :

Saturated n-butanol (as described in method I).

PROCEDURE :

Preparation of extract :

Weigh 8g semolina or flour into 150ml glass stoppered Erlenmeyer flask and add 40ml water-saturated n-butanol. Shake contents for 1 minute and let stand for 16 hours. Shake and filter through Whatman no. 1 into 50ml volumetric flask.

Measure transmission of extract in colourimeter using 440 nm and employing standard containing reagent water saturated n-butanol. To obtain the values for carotene content (ppm) the transmission reading of unknown sample is put in the equation

$$= 0.174 + 16.57 L$$

L = apparent density (transmission value)

As described earlier yellow pigment is essentially a preferable feature of durum wheats. Range of β-carotene is generally 4 to 8 ppm but durums with less then 5ppm of β-carotene are not acceptable in the international market. Some countries offer little preference to yellow pigment.

Reference :

AACC method 14-50 (1962)

RHEOLOGICAL

METHODS

MIXOGRAPH

The mixograph was originally designed by Swanson and Working in 1933 to provide a method of measuring quality of gluten structure. The device measures the rate of dough development,the maximum resistance of dough to mixing,and the duration of resistance to mechanical overmixing. The mixograph available earlier used to require large quantity of flour for testing the above parameters. However, a 2g mixograph is now available to test early generation material.

The mixing action of the mixograph is provided by four vertical planetary pins revolving about three stationary pins in the bottom of the bowl. The mixing action can be described as a pull, fold and repull action which is much more severe than is produced by the farinograph. As a result, the main advantage of the mixograph is the speed with which a test can be conducted. As the dough is developed, an increasing force is required to force the revolving pin through the dough. This increasing force is measured as a tendency to rotate the bowl which is mounted on the center of a lever system. The resistance to rotation is provided by a tension spring having twelve possible tension setting which allow adjustment of the tension to a level suitable for the type of flour being tested. Position 8 to 11 are most commonly used. Higher setting are used for comparisons between doughs of strong flour and lower setting for weak flours.

According to the first systematic and statistical study relating mixograph characteristics to baking results. Peak height,width and weakening angle were positively correlated with protein content and loaf volume. The authors felt that mixogram characteristics tended to reflect baking strength of a flour, mainly because of the high correlation between loaf volume and protein, and the correlation between protein and mixogram characteristics.The angle between ascending and descending portions of the curve tended to decrease with increasing protein but not in a linear fashion. The relationship of mixing time and of development angle with protein content was curvilinear.

The characteristics of the mixograph curve are the result of the changing plastic,elastic and viscous properties of the dough during mixing. During the initial ascent of the curve, water is being brought into contact and absorbed by the protein and starch, and because of the folding and stretching action of the mixing pins, the dough begins to be developed.As the dough develops, the force required to move the pins through the dough increases to a maximum plasticity or maximum mobility. This point correspond to the top of the curve. Beyond this point mechanical degradation of the dough causes an increase in mobility resulting in the curve sloping downward and tailing off.

Many attempts have been made to assign numerical values to various parameters of mixograph curves and relate to these measurements to quality factors. Some of these measurements are shown in figure and are identified as follows:

Peak time:

The time required for the peak to reach maximum height is similar to the dough development time of the farinograph (line DC). An increase in peak time is associated with an increase in mixing time required for development of the dough.

Area under the curve:

This area is enclosed by the base line and a line drawn through the center of the curve until either a specified time has elapsed since the mixograph is started for a specified time has elapsed since the curve peak is attained. The larger the area the stronger the flour and the greater its tolerance to overmixing.

Peak height:

The height from the base line to the center of the curve at maximum plasticity is the peak height (line CH). The measurement provide information about flour strength and absorption.

Height of a curve at a specified time after peak or start of mixing:

The height of the curve at a specified time is similar to the farinograph "tolerance index" or "drop off" values. Higher values indicate a flour which is more tolerant to mixing.

Angle between ascending and descending portion of the curve at peak:

This angle is obtained by drawing a line from the center of the curve at its peak down the center of the curve in both directions (angle 1). A large angle is associated with a more tolerant flour.

Weakening angle:

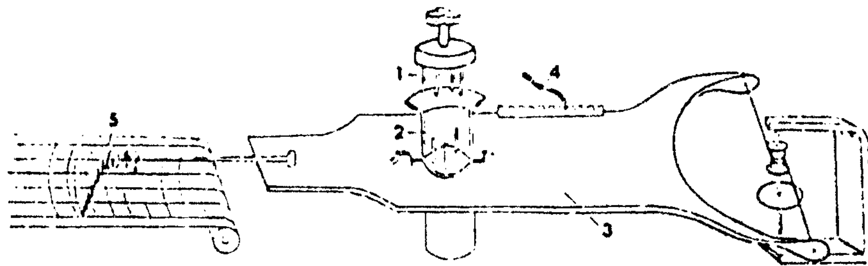
This angle is formed by drawing a line from the center of the curve at its peak down the descending portion of the curve and a line horizontal to the base line through the center of the curve at its maximum height (angle 2).The size of the angle is inversely related to mixing tolerance.

Development angle:

This angle is formed by a line drawn horizontal to the base line through the center of the curve at its maximum height and a line drawn through the center of ascending portion of the curve (angle 3).

The shape of the mixograph curves vary according to wheat class and variety as well as the environmental conditions under which the wheat is grown. Figure shows the variability in mixogram characteristics between five different classes of wheat.

Development angle and mixing time are moderately and negatively correlated.Area is not significantly correlated with loaf volume. It has been concluded that the most important use of

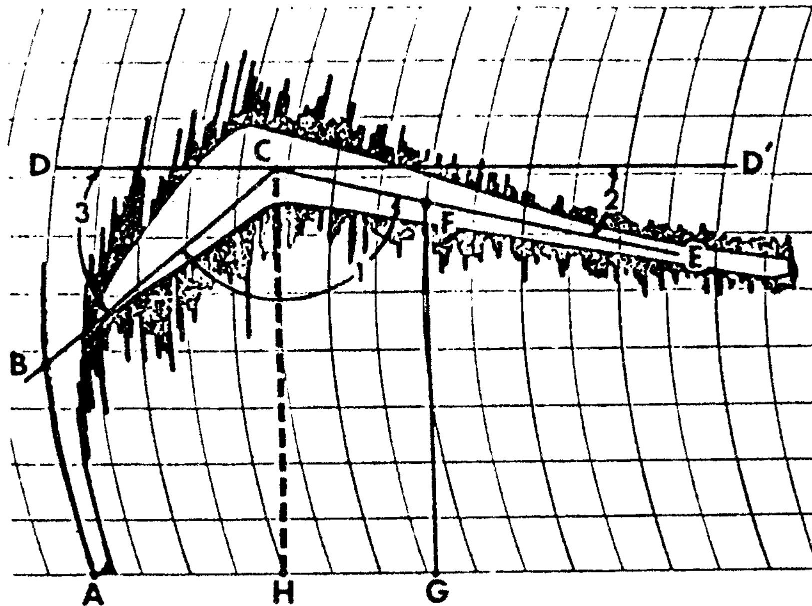


THE BASIC PARTS OF THE MIXOGRAPH

1. MIXING PINS   2. MIXING BOWL

3. SWIVEL BASE OR LEVER SYSTEM

4. TENSION SPRING   5. KYMOGRAPH



MIXOGRAM MEASUREMENTS

the mixograph is to furnish information which supplements baking data, for example, mixing requirements,mixing tolerance and varietal pattern.

Although the mixograph is extensively used, its value is limited because of several factors. These factors relate to the fact that the mixograph is not standardized instrument. Under uniform conditions in any one laboratory, a usable level of replication can be obtained. However in interlaboratory collaborative work the variability is high. A portion of the problem is that although there is an AACC method for use of the mixograph, the method contains only a general description of the instrument's use. For example, varied absorptions are used. Bread flour are often analyse at an absorption considered optimum for a bread dough as determined subjectively by manual manipulation of the dough or subjectively by other physical tests such as protein content.Some operators have used a fixed absorption; e.g., 66% absorption at a 14% moisture basis. Others use a fixed absorption on an "as is" basis. addition to operational techniques other factors such as operator,mixer speed operator,temperature,spring tension setting,spring variability,and atmospheric pressure have substantial effects on the curves obtained and must be considered.

One use of the mixograph, which has appeared often in the literature, is its use to evaluate wheat quality based on the curves obtained from ground whole wheat. The purpose of using wheat meal instead of flour is to avoid the time and expense

involved in preparing a refined flour as well as allowing evaluation of small wheat samples, such as early generation breeders' samples.

In conclusion, both the mixograph and farinograph provide information concerning the quality of wheat in relation to mixing characteristics. The information derived, however, must be supplemented with other quality tests including the baking test in order to establish overall quality of the wheat sample.

Reference :

Rheology of wheat products. Edited by H. Fondi. Published by AACC. p 37-49.

THE ALVEOGRAPH FLOUR TEST WITH THE RCV 4:

Principle:

The CHOPIN alveograph measures the baking force of wheat (W) as well as the extensibility (G,L)and strength (P), through the biaxial deformation of a dough sample.

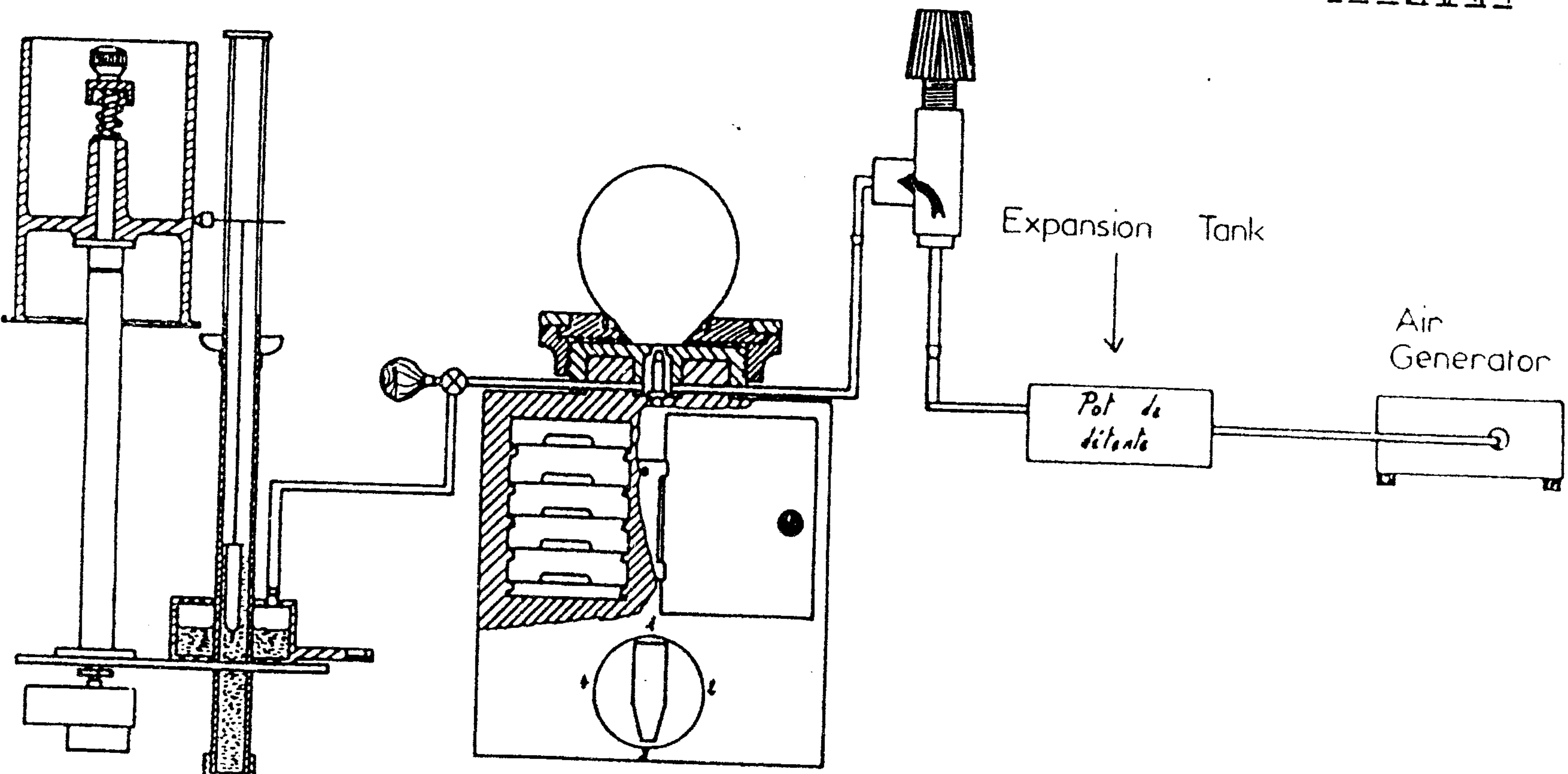
These various parameters W,P,L,P/L are recorded and calculated automatically by the calculator unit of the RVC 4.The relaxometer unit of the RVC 4 measure the relaxation characteristics of the doughs under pressure. This new measurement completes the classic data provided by the alveograph. The technological quality of wheats tested can thereby be better characterized.

The dough sample, obtained according to the alveographic procedure is deformed under a constant flow of air pressure.The air intake is stopped by an electrovalve after the inflation of the volume Vo. The drop in pressure due to the weakening of the film of dough under pressure Po is measured within the bubble using a pressure pick-up.The relaxation time is the time it takes the pressure to drop from Po to a pre-selected pressure value. It is the measurement of the time of half-relaxation from Po to Po/2 after inflation of a fixed volume of air Vo = 100 ml. Graph of pressure over time (alveogrammes & relaxogrammes) may be obtained by adding a recording table. The data obtained may be recorded and stocked on a printer.

- PNEUMATIC CIRCUIT ALVEOGRAPH AND MANOMETER

ALVEOGRAPHE MA 82

DIAGRAM



CHOPIN
20 AVENUE MARCELIN BERTHELOT
92 VILLENEUVE LA GARENNE

Apparatus :

1. Alveograph
2. Burette
3. Balance
4. Timer
5. Planimeter

Reagents :

1. Sodium chloride solution :

   Dissolve 25 g of sodium chloride in distilled water and make upto 1000 ml.

2. Refined vegetable oil low in polyunsaturates, with an acid value less than 0.4, such as olive oil or groundnut oil.Store in a dark place in a stoppered container and replace regularly (every 3 months).

Calibrating the air generator (92 mm water):

Progamme the RCV 4 as follows:

Circuit on 1/2 hour before use. Display of 8888.

Alveo mode,

[Ent] display of pressure -----> [Zero] display of 0000 -----> [Ent] -----> Put the handle 41 in position 3 -----> Press on the [Start] button of the RVC 4 -----> Wait 30 seconds for the pressure to be stabilized in the pneumatic circuit.The value of stabilized pressure display must be 92 mm. If this is not the case,act on the potentiometer of the air generator 46 -----> Perform this calibration slowly so the pressure in the circuit can stabilize. On the recording table the pen must climb from 0 to 92 mm .

calibrating the flowmeter (60 mm water)

Proceed in exactly the same way as for the calibration of the air generator above,using the start and stop buttons of the RVC4.Calibrate the needle tap of the flowmeter 38 to obtain a pressure of 60 mm water displayed on dial 2. Put the handle 41 back into position 1.Press the stop button of the RVC4. Your alveograph in now ready for the test.

Experimental conditions

Preparing the sample:

Mix and homogenize the sample before the test by passing it through a very large-mesh sieve,or even better,by means of a mechanical device designed to obtain perfect homogeneity such as the CHOPIN MR 2L ROTARY MIXER.

Determining the water content:

Use the official method (Standard reference ISO V03-701 AFNOR or ICC n° 110) drying one and one-half hours at 130°C under the condition defined by this method. The care and the precision with which the determination is carried out greatly affect the value of the alveograph test results.

The use of rapid moisture testers, of whatever sort, is possible only to the extent that they do not deviate more than = 0.2% (absolute value) from the determination obtained using the official method.

Controlling the temperature of the apparatus and the ambient area:

The temperature of the mixer and the alveograph is automatically maintained at 24°C and 25°C respectively. Verify the temperature of the thermometers before and during the use of the apparatus.

The apparatus must be used in a room where the temperature is maintained in between 18 to 22°C and where the air is neither too dry nor to damp (relative humidity level between 55 to 70 %). The temperature of the flour and salt water must be around 20°C. There should be no sunlight shining directly on the alveograph or the mixer,nor should there be any significant drafts during the tests (as would be the case with an apparatus placed in front of the window).

Test sample: Weigh out 250g of flour.

Calculating the quantity of salt water to add to the flour:

The salt water is a solution of 2.5% NaCl in distilled water (verify that the water is not abnormally acid) or in permuted water.Weigh the sodium chloride or pure fine table salt and pour this into a gauged 1000ml flask. Then fill with water until this volume is reached. The quantity of salt water to be used is indicated by the special burette directly graduated according to the percentage of moisture content in the flour (Table).

WATER ADDITION TABLE (ICC)

FOR MIXING PRIOR TO ALVEOGRAPH :

Moisture Content %	Amount To Add ml	Moisture Content %	Amount To Add ml	Moisture Content %	Amount To Add ml
8.0	156.1	12.0	138.3	16.0	120.6
8.2	155.2	12.2	137.5	16.2	119.7
8.4	154.4	12.4	136.6	16.4	118.8
8.6	153.5	12.6	135.7	16.6	117.9
8.8	152.6	12.8	134.8	16.8	117.0
9.0	151.7	13.0	133.9	17.0	116.1
9.2	150.8	13.2	133.0	17.2	115.2
9.4	149.9	13.4	132.1	17.4	114.3
9.6	149.0	13.6	131.2	17.6	113.4
9.8	148.1	13.8	130.3	17.8	112.5
10.0	147.2	14.0	129.4	18.0	111.7
10.2	146.3	14.2	128.6	18.2	110.8
10.4	145.5	14.4	127.7	18.4	109.9
10.6	144.6	14.6	126.8	18.6	109.0
10.8	143.7	14.8	125.9	18.8	108.1
11.0	142.8	15.0	125.0	19.0	107.2
11.2	141.9	15.2	124.1	19.2	106.3
11.4	141.0	15.4	123.2	19.4	105.4
11.6	140.1	15.6	122.3	19.6	104.5
11.8	139.2	15.8	121.4	19.8	103.7

Mixing the dough :

Pour 250g of flour into the mixer. Put on the lid and secure it with the two tightening screws. Start the motor and chronometer simultaneously. Pour through the hole of the lid the proper amount of salt water (in about 20 seconds). **Allow the dough to form for 1 minute.** At the end of first minute,stop the motor,remove the lid and scrape off with a spatula any flour sticking to the lid or to the corners so that the entire amount undergoes hydration.**The operator has one minute to do this and to replace the lid.** At the end of the second minute, start up the motor again. Let the mixing continue for 6 minutes. At the end of 8 minutes, stop the motor and proceed with extrusion.

Extrusion: (Forming and relaxing the dough patties)

Preparation ahead of time:

1. Pure peanut oil,
2. The two rolling frames composed of two rails 21 which rest freely on the glass plate 14,assembled by two rivets to the metal holder 48.
3. Oil each plate as well as the glass plate with 2 measures of oil and spread a continuous film over the surfaces. Oil as well,with one measure,the extrusion plate 7 from point a to a' and slide it between the screws K and K'.

Extrusion procedure:

Disengage the extrusion aperture by raising the register F held in the upper position by a knurled knob. Make sure that the register F is in the right position at the end of the test. Reverse the rotation of the kneader.The dough extrudes in the form of a strip.Cut off and discard the leading end of this strip (approx. 2 cm). When the dough reaches the level of the small indented notches ee',rapidly cut with a knife,pull out the extrusion plate and slide the dough on to the glass plate which has been previously oiled. Extrude successively the five pieces of dough without stopping the motor,the previously oiled plate 7 having been put back in place each time. The first four pieces of doughs are arranged 2 by 2, the extrusion direction corresponding to the large axis of the rolling table. The 5 pieces of dough is left on the extrusion plate. Stop the motor. Roll the 5 pieces of dough (2+2+1)by means of the previously oiled roller (Two measures) which the user will slide on the rails 12 times in a row (six complete movements back-and-forth).Cut with a clean movement the piece of dough into a dough patty with the circular die. Cut away the surplus dough. Bring the circular die containing the dough to the dough-relax plate into which the dough patty is to be placed. If the dough patty sticks to the sides of the circular die, free it by tapping the table with the heel of the hand (do not touch with the fingers).If the dough patty sticks to the glass,lift it up slightly and slide the dough-relax plate under it. Immediately place each used dough-

relax plate in the isothermal tempering co- apartment (25°C) of the alveograph. Proceed in the order of extrusion the first dough patty being placed on the top. Role and cut out the 5th dough patty.

During the rest period (20 minutes) of the patties,calibrate the alveograph and the RCV 4 in the same fashion. Perform the following sequences after turning on the circuit of the RVC 4 one-half hour in advance.

RCV 4 in mode A.

{Ave] -----> [Clear] to erase the memory of the RCV4 -----> [Ent] -----> [Zero] return the pressure pick-up to zero -----> [Code] put in 4 digit -----> [Ent]-----> [date] if applicable [Ent] -----> [T.ins] 2.88 -----> [Ent] -----> [Mult] 0.5 -----> [Ent] -----> [Relaxo] in mode R -----> [Clear] display of "Pret" ; the RCV 4 is ready to record the measurements.

Alveograph test on rested dough patties:

The preceding step having been carried out normally,this test begins exactly 28 minutes after starting the mixing.

Forming the test patty:

Turn switch 41 to position 1. Control the thickness of the test dough patties between the fixed plate 1 and the press by performing the following operations -

Raise the upper press 2 by turning twice to loosen it.

Remove the ring 8 and the plug 7 (important: turn over the plug 7 so that it lies on the ring 8 flat on the table,so that it does not spin off onto the floor). Oil the fixed plate 1 (with one drop)and spread the oil without touching the beveled edge. Also oil inner side of plug 7. Slide the first dough patty onto the fixed plate and centre it. **Push only the edge of the dough patty to center it.NEVER TAP THE REST PLATE ON THE REVELED EDGE OF THE PRESS TO HELP SLIDE ON THE DOUGH PATTY:** Put back the plug 7 by turning it slightly on its seating and tighten it by the ring 8 until it lines up with the red reference points. Flatten the dough patty by slowly lowering the press.Remove the plug 7 to free the dough patty.

Warning:

The switch 41 passes directly from position 1 to position 3..The patty is automatically detached with the RCV 4. The rubber bulb is unnecessary.The handle of the tap 17 must remain in position B' through out the tests.

Developing the dough patty:

Turn switch 41 to position 3.Iposition 3 the air generation starts. Press the [Start] button on the RCV 4. The patty is automatically detached and inflated . Measurements are provided by the RCV 4.The recording graph starts as soon as the automatic detatchment.Carefully observe the bubble and as soon as a rupture appear in the membrane,put the switch 41 in position 1 to stop

the air intake. The RCV 4 records the rupture of the bubble and flashes ("End") on the display. The calculations of P,L,P/L and W of this curve are the performed and memorized.Press the (stop) button of the RCV 4.The presentation of the recording graph will be interrupted at once;the printer will then begin recording the values obtained.

Continuing the tests:

After you have pushed the stop button,the RCV 4 is ready for a new test.

Prepare the alveograph for this new test:

Switch 41 in position 1,press in upper position, dough from the previous test removed. Proceed successively to test the four other patties in the same manner as above .Five curves are thus obtained on the recording graph,and each value of P,L,P/L and W is stored in the memory of the RCV 4.

Expressing the results:

Between the tests it is possible to call up the value calculated for the last curve by pressing the following buttons.

```
[Stop] -----> [Scan] -----> [Scan] -----> [Scan] -----> [Scan]
                P             L            P/L            W
```

The tests resume simply by pressing on the [Start] button according to the above procedure. At the end of the tests, call up the results for P,L,P/L and W of each of the curves.The curves are automatically numbered from 1 to 5 (the capacity of the memory is five curves).

The final result of the alveographic test is the average of the curves selected. The printer records the averages as soon as you press the [Ave] button of the RCV 4.To obtain the curves, press the [Ent] button directly after obtaining the averages.

MAXIMAL OVER PRESSURE P:

The average of the maximal ordinates measured in millimeters and multiplied by 1.1 represents the value of the maximal overpressure P which is in relation to the resistance of the dough to deformation.

AVERAGE ABSCISSA AT THE RUPTURE L :

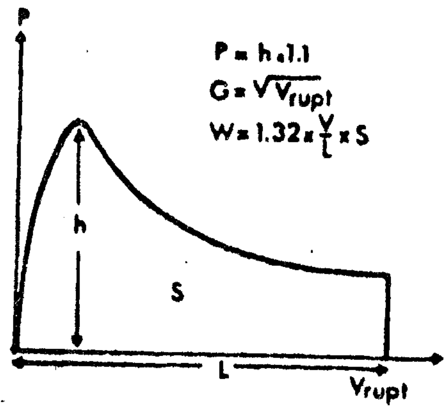
The abscissa at the rupture point of each curve is measured in millimeters on the zero line starting from the origin of the curve up until the point corresponding with the clear drop in pressure due to the rupture of the bubble. The average of the abscissas at the rupture of the curves presents the length L.

P/L RATIO:

This ratio is conventionally called the curve configuration ratio.

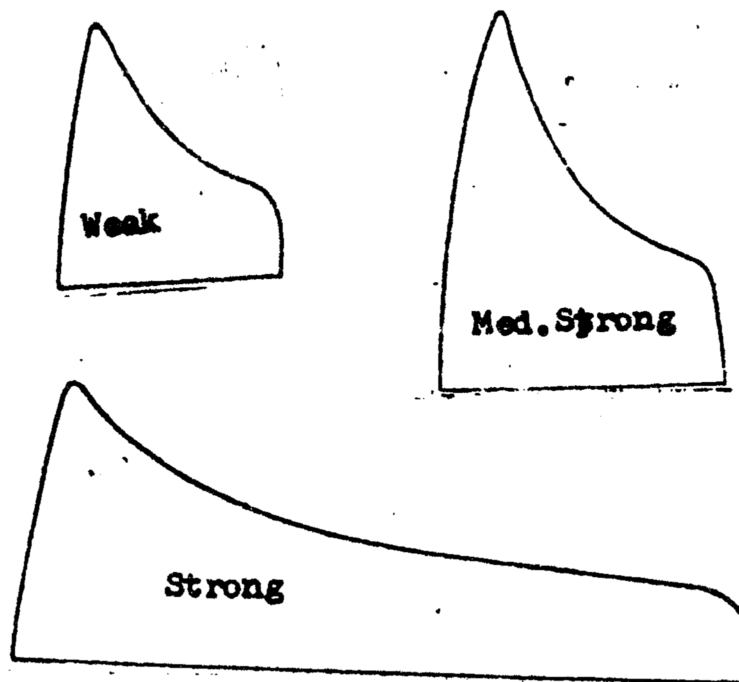
DEFORMATION WORK W :

The deformation action of the dough,represented by the symbol W and expressed in 10^{-4} joules, is calculated automatically by the RCV 4 after the area of the curve has been integrated.



A REPRESENTATIVE ALVEOGRAM.

P = OVERPRESSURE (MM).
L = ABSCISSA AT RUPTURE (MM).
G = SWELLING INDEX (ML).
V = VOLUME OF AIR (ML).
W = DEFORMATION ENERGY (10^{-4} X J).
H = MAXIMUM HEIGHT (MM).
S = AREA UNDER THE CURVE (CM^2).



DIFFERENT TYPES OF ALVEOGRAMS

CALCULATION REFERENCE:

$$W = 1.32 \times \frac{V}{L} \times S$$

where,

V = volume of air, in cubic millimeter,

L = average abscissa at the rupture, in millimeter,

S = surface of the curve, in square centimeter.

1.32 = is a coefficient linking various factors (i.e. the curve ordinate and pressure corrospondance, coefficient of pressure gauge, average mass of the test piece, coefficient of correlation between first generation apparatus and current apparatus).

Relaxation Test:

For this, the experimental details are same as described before. It is possible between test to call up the value calculated for the last curve, by the following sequence:

[Stop] ----->	[Scan] ----->	[Scan] ----->	[Scan] ----->	[Scan]
	Pmax	Po	PO/Pmax	T.Relax

The test are continued simply by pressing on the [Start] button according to above procedure. At the end of the tests, view the result for Pmax, Po, Po/Pmax and T. relax for each of the curves. The curves are automatically numbered from 1 to 5 (the capacity of the memory is nine curves).

MAXIMAL OVERPRESSURE Pmax :

The average of the maximal ordinates measured in millimeters of water represents the value of the maximal overpressure Pmax which is related to the resistance of the dough to deformation.

$$Pmax = P \text{ alveogramme}$$

PRESSURE AT 100 ml: Po (or other volumes)

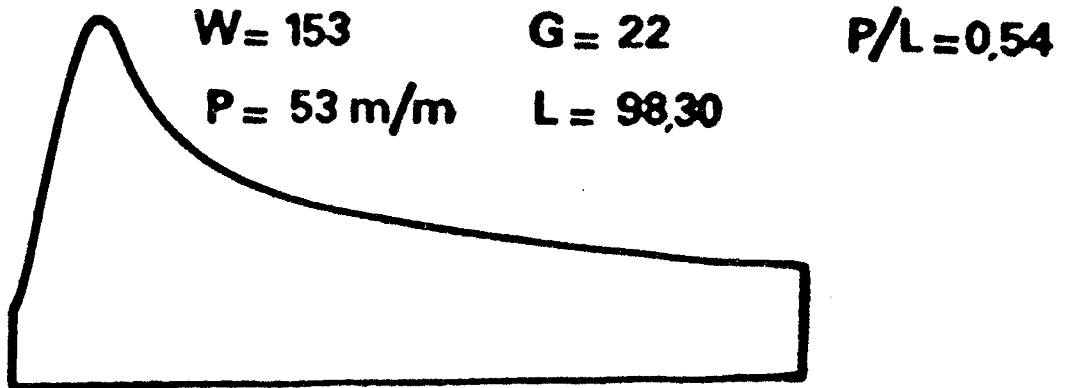
The average of the pressures at 100 ml volume of air blown into the bubble represents the value of Po.

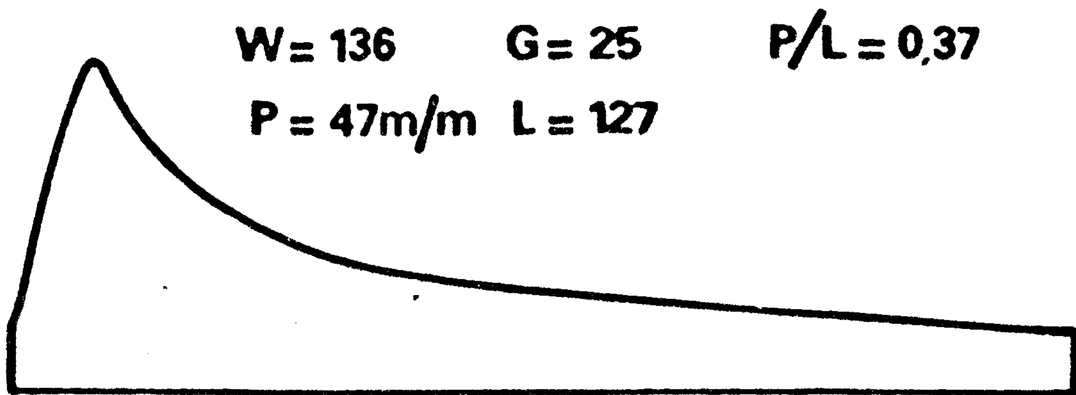
Po/Pmax RATIO:

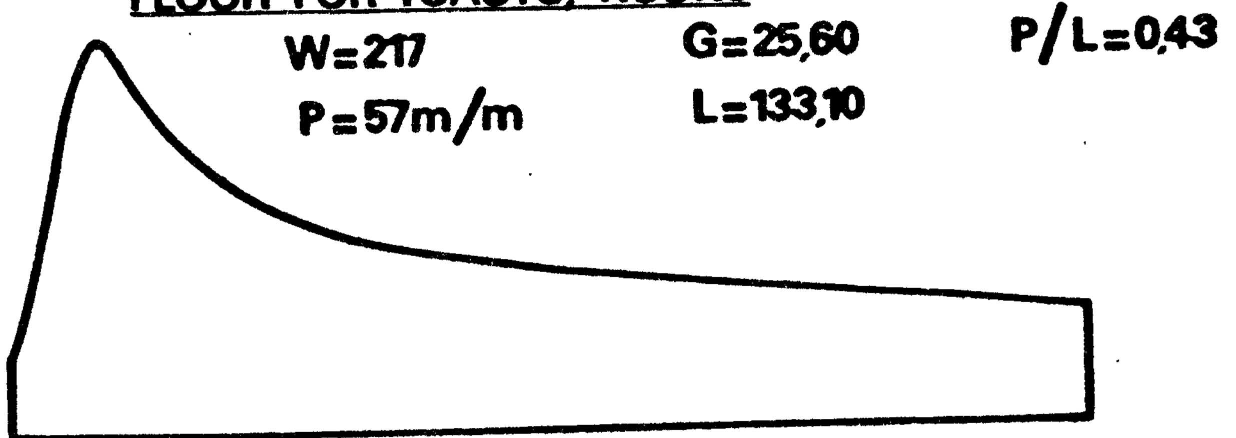
Average of the Po/Pmax ratio of the each curve .

TIME OF 1/2 RELAXATION T.relax:

Average of the time taken for the pressure within the bubble to drop from Po to Po/2. This time is expressed in seconds, to the 1/100 sec.

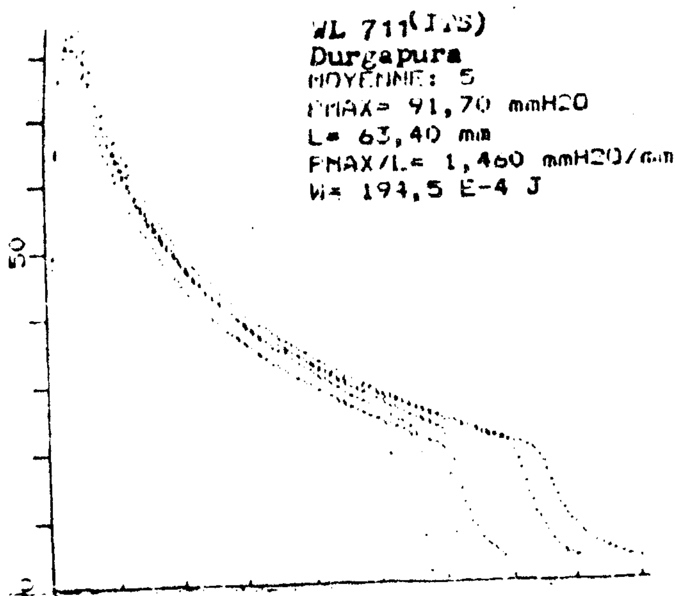


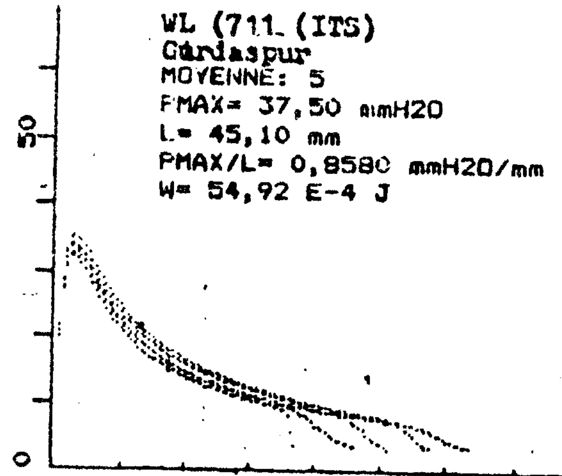


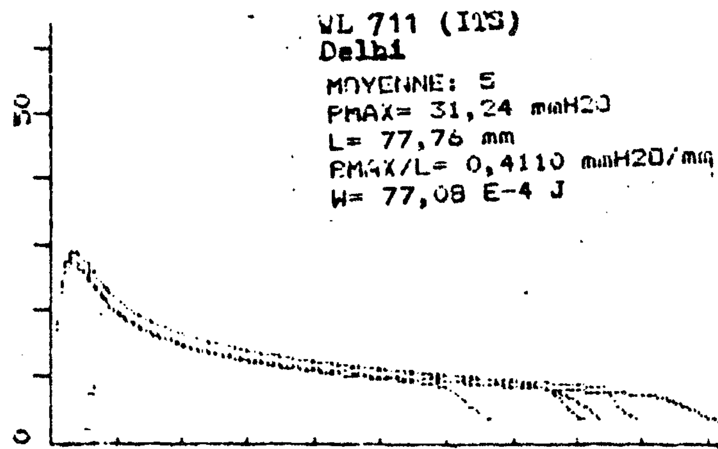


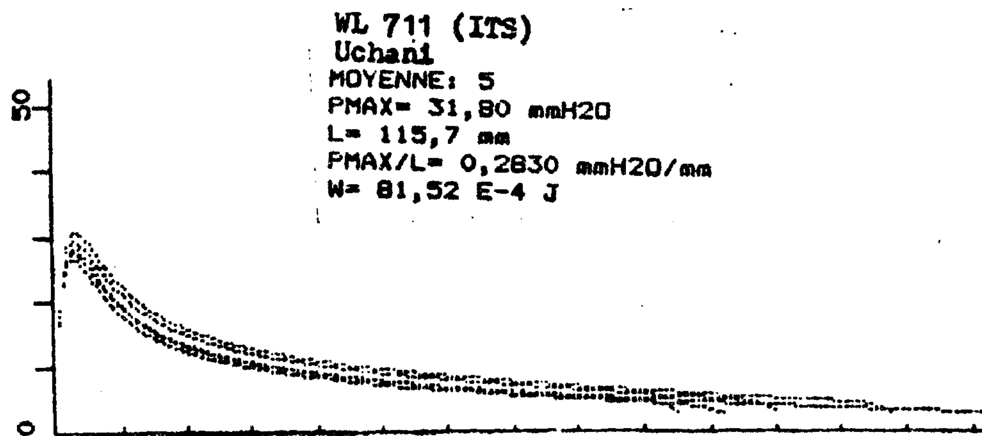
ALVEOGRAMS OF DIFFERENT

TYPES OF FLOURS









EFFECT OF LOCATION ON ALVEOGRAMS

CONVERSION TABLE OF LENGTH L INTO SWELLING G

(ACCORDING TO THE FORMULA : G = 2.22 L

L	G	L	G	L	G	L	G
45.70	15.00	81.20	20.00	126.90	25.00	182.80	30.00
46.30	15.10	82.00	20.10	127.90	25.10	184.00	30.10
46.90	15.20	82.90	20.20	129.00	25.20	185.20	30.20
47.50	15.30	83.70	20.30	130.00	25.30	186.40	30.30
48.20	15.40	84.50	20.40	131.00	25.40	187.70	30.40
48.40	15.50	85.30	20.50	132.00	25.50	188.90	30.50
49.40	15.60	86.20	20.60	133.10	25.60	190.13	30.60
50.00	15.70	87.00	20.70	134.10	25.70	191.40	30.70
50.70	15.80	87.90	20.80	135.20	25.80	192.60	30.80
51.30	15.90	88.70	20.90	136.20	25.90	193.90	30.90
52.00	16.00	89.60	21.00	137.30	26.00		
52.60	16.10	90.40	21.10	138.30	26.10		
53.20	16.20	91.30	21.20	139.40	26.20		
54.00	16.30	92.10	21.30	140.50	26.30		
54.60	16.40	93.00	21.40	141.50	26.40		
55.30	16.50	93.90	21.50	142.60	26.50		
56.00	16.60	94.70	21.60	143.70	26.60		
56.60	16.70	95.60	21.70	144.90	26.70		
57.30	16.80	96.50	21.80	145.80	26.80		
58.00	16.90	97.40	21.90	147.00	26.90		
58.70	17.00	98.30	22.00	148.00	27.00		
59.40	17.10	99.20	22.10	149.10	27.10		
60.10	17.20	100.10	22.20	150.20	27.20		

60.80	17.30	101.00	22.30	151.30	27.30
61.50	17.40	101.90	22.40	152.40	27.40
62.20	17.50	102.80	22.50	153.60	27.50
62.90	17.60	103.70	22.60	154.70	27.60
63.60	17.70	104.60	22.70	155.80	27.70
64.30	17.80	105.60	22.80	156.90	27.80
65.10	17.90	106.50	22.90	158.10	27.90
65.80	18.00	107.40	23.00	159.20	28.00
66.50	18.10	108.40	23.10	160.30	28.10
67.30	18.20	109.30	23.20	161.50	28.20
68.00	18.30	110.20	23.30	162.60	28.30
68.70	18.40	111.20	23.40	163.80	28.40
69.50	18.50	112.10	23.50	164.90	28.50
70.30	18.60	113.10	23.60	166.10	28.60
71.00	18.70	114.10	23.70	167.30	28.70
71.80	18.80	115.00	23.80	168.40	28.80
72.50	18.90	116.00	23.90	169.60	28.90
73.30	19.00	117.00	24.00	170.80	29.00
74.10	19.10	117.90	24.10	172.00	29.10
74.90	19.20	118.90	24.20	173.10	29.20
75.60	19.30	119.90	24.30	174.30	29.30
76.40	19.40	120.90	24.40	175.50	29.40
77.20	19.50	121.90	24.50	176.70	29.50
78.00	19.60	122.90	24.60	177.90	29.60
78.80	19.70	123.90	24.70	179.10	29.70
79.60	19.80	124.90	24.80	180.30	29.80
80.40	19.90	125.90	24.90	181.50	29.90

PREPARATION AND EVALUATION OF END PRODUCTS

TESTING OF VARIETIES FOR BREAD MAKING PROPERTIES:

The following standard recipe is generally used for testing the wheat varieties for their bread making qualities.

Appratus :

1. Analytical balance
2. Dough mixer
3. Fermentation chamber
4. Baking moulds
5. Proofing chamber
6. Rotatory oven
7. Loaf volumeter
8. Knife

Reagents :

Media	100g
Water	60ml
Sugar	5.0 g
Yeast	2.0 g
Salt	2.0 g
Shortening Ghee (Saturated fat)	3.0 g

Procedure:

1. All the above mentioned ingredients except ghee are mixed and kneaded together in a dough mixer for 1 minute to form a dough.

2. The dough is left for 1 hour 40 minutes at 30-32°C for fermentation.

3. After fermentation, the dough is once again kneaded with ghee in the dough mixer for 40 seconds and moulded in desired shape.

4. The baking moulds are placed inside the proofing chamber which is maintained at 35.5°C with 92% humidity.

5. The loaves are left for 50 minutes.

6. At the above temperature amylase enzyme become more active which leads to formation of maltose,the main food source for yeast enzymes. Evolution of CO_2 leads to steady increase in loaf volume.

7. After proofing, the loaves are baked in a rotatory oven maintained at 220°C for 25 minutes. Volume of the backed loaves is immediately measured by the loaf volumeter. The final evaluation of bread is done after 18 hours, taking its loaf score consideration.

LOAF SCORE :

The following properties are then assessed and used in combination to determine 1 - 10 scores for suitability for bread making.

(a) Volume: measurement are made by displacement of rape seed. High volumes are preferred.

(b) External appearance: observations are made of general appearance and crust colour.A good loaf should have an attractive golden brown crust, good shape and freedom from torn crust.

(c) Crumb texture:the crumb texture of the surface exposed by cutting the loaf is assessed by stroking with fingers. Silkiness is preferred to roughness.

(d) Crumb cell structure: ideally the cell should be thin walled,elliptical in shape and of uniform size which should be neither too large nor too small.

(e) Crumb resilience: this is assessed by pressing the cut surface of the loaf with the fingers. A soft crumb which exhibits elastic recovery after depression is desirable.

Reference :

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FORMULA FOR EVALUATION OF BREAD

1. Volume weightage

Vol. Less than	Wt.
400	4.00
400	5.00
405	5.25
410	5.50
415	5.75
420	6.00
425	6.25
430	6.50
435	6.75
440	7.00
445	7.25
450	7.50
455	7.75
460	8.00
465	8.25
470	8.50
475	8.75
480	9.00
485	9.25
490	9.50
495	9.75
500	10.00
505	10.25
510	10.50
515	10.75
520	11.00
525	11.25
530	11.50
535	11.75
540	12.00
545	12.25
550	12.50
555	12.75
560	13.00
565	13.25
570	13.50
575	13.75
580	14.00
585	14.25
590	14.50
595	14.75
600	15.00

	Score
2. Stickiness	
Non-sticky	2.0
Partial	1.0
Sticky	0.0
3. Appearance	
Excellent	2.0
Good	1.5
Fair	1.0
Non-Attractive	0.0
4. Crust colour	
Excellent	2.0
Good	1.5
Fair	i.0
Non-attractive	0.0
5. Crumb colour	
Excellent	3.0
Good	2.0
Fair	1.0
Non-attractive	0.0
6. Texture	
Excellent	4.0
Good	3.0
Fair	2.0
Nonattractive	0.0
7. Taste and Aroma	
Excellent	2.0
Good	1.5
Fair	1.5
Bad	0.0

Total = 30
Score to be reduced to maximum 10

COOKING CHARACTERISTICS OF MACARONI

Apparatus

1. Oil or Prestone constant-temperature bath adjusted to maintain cooking water temperature at 95.5°C-96.0°C. With mineral oil, bath should be held at 105°C-106°C; with undiluted Prestone, temperature of 101.0°C-101.5°C is required.
2. Tall-form lipless beakers, 500-ml.
3. Buchner funnels or tared weighing basket of nichrome gauze, approximately 3 inches high and 3 inches diameter.
4. Recording tenderness tester (see ref.1).
5. Volumeter, consisting of 50-ml. Erlenmayer-flask fitted with ground-glass joint and measuring tube graduated from 0 to 10 ml. in 0.05 ml.

Procedure

Tenderness of cooked product:

1. Place 500-ml tall-form lipless beakers in bath and add 250 ml. water which has been previously heated to 95°C, cover beaker with watch-glass,and let stand until water temperature reaches 95.5°C-96.0°C.

2. Introduce 25 g sample of macaroni previously broken into approximately 1-to 4-inches lengths (or use elbow macaroni) and stir thoroughly with glass rod.

3. Cook for 30 minutes with brief stirring at 10-minutes intervals.

4. Remove beaker, drain macaroni in tared weighing basket for 2 minutes, weigh, wash three times with cold water, and store under water until ready for testing for tenderness. Retain original water in which macaroni was cooked, for disintegration test.

5. For measurement of tenderness, select samples at random and withdraw from water by gripping ends with forceps.

6. Absorb surface moisture from each strand by placing upon filter paper.

7. Locate strand under plunger of tenderness tester and lower plunger until it rests freely upon sample.

8. Apply load at constant rate of approximately 12 g. per second until recorded curve shows definite evidence that "break" or "yield" point has been passed.

9. Make five such tests. In most cases, all five records can made upon a single chart.

10. Evaluate graphic records obtained, and calculate single-figure tenderness score from following values:

a) Time to "break"-time in second from beginning of application of load until the "break" or "yield" point is reached.

b) Time in second from beginning of application of load until sample has been compressed to arbitrary thickness (selected to fall somewhat below "break" point in majority of samples). For macaroni 3/16 inches in diameter, value of 0.115 inches is suggested.

c) The smaller angle made between a prolongation of liner portion of curve and upper horizontal line of chart.

d) Ratio of a to b.

11. Tenderness score is computed by formula:

    Score = time to break (a) + angle (c) + 10 X ratio (d).

12. Values obtained will be comparable only when material of similar size and section is tested, since any variation in arbitrary thickness selected for value b will affect absolute magnitude of results.

Volume increase on cooking:

1. Fill volumeter to zero mark with high-boiling petroleum naphtha or kerosene.

2. Introduce 10g. uncooked macaroni, broken into small pieces, through top of tube, tap apparatus gently to dislodge air bubbles, and read increase in volume. Multiply results by 10 and record as dry volume per 100 g.

3. In similar manner, determine volume of cooked macaroni and calculate increase in volume.

Water absorption during cooking:

Determine increase in weight upon cooking and express as %.

Resistance to disintegration (residue):

1. Evaporate drainings from sample cooked as directed under "Tenderness of cooked sample," above, to dryness on steam bath in weighed beaker or dish, dry at 130°C for 1 hour and weigh.

2. If presence of added salt is indicated, residue must be ashed, chlorides determined, and correction made.

Note

It has been shown (ref. 1) that a very close relationship exists between wet weight and volume, and volume can therefore be calculated by formula:

Volume of cooked macaroni = (1.0085 x wet weight of cooked sample)-8.81

References

1. Binnington, D.S., Johannson, H., and Geddes, W.F. Quantitative methods for evaluating the quality of macaroni products. Cereal Chem. 15: 149 (1939).

2. Giorn. risicoltura 25: 251(1935).

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