ANNEXURE - I

1. DERTERMINATION OF MOISTURE

Principles: The loss in weight after drying the sample for 1 hour at 130^0C expressed in percentage is the moisture content.

Apparatus: Air oven, Lab. grinder, aluminium dishes mettler balance and desiccator.

Method: Weigh 2g of the powdered sample in a weighed aluminium dish and place in an air oven maintained at 130^0C for 1 hr. Cool in a desiccator to room temperature and report the loss in weight in percentage.

Calculation:

$$\text{Moisture (\%)} = \frac{A - B}{A - C} \times 100$$

Where A = wt. Of flour aluminium dish before drying

B = wt. Of flour + aluminium dish after drying

C = wt. Of aluminium dish.

Maximum permissible limit in flour

ISI . . . 13.0%

PFA . . . 14.0%

2. ESTIMATION OF GLUTEN

INTRODUCTION

The dough obtained by mixing wheat flour with water possesses the characteristics of plasticity and elasticity – characteristics of vital significances both during handling of the dough as well as in its end performance. The properties referred to above could be by the interaction of water insoluble proteins of wheat in the presence of water.

Principle

Gluten in a ample of flour could be estimated by washing the dough free of starch, sugars, water soluble proteins and other minor components. The wet cohesive mass obtained is referred to as wet gluten while the dried product obtained from it is referred to as dry gluten.

Method

Exactly 25g flour is kneaded with about 15-ml water to get a dough ball. The dough ball is allowed to remain immersed in water for one hour to ensure proper hydration after which, the starch is washed out by kneading gently in a gentle stream of water over a fine sieve or silk till the washed liquid is clear.

The gluten, which is cohesive, is pressed as dry as possible, and weighed. The wet gluten so obtained is dried at 100°C for 24 hr. and weighed again to get the value for dry gluten.

Calculation

Wet gluten (%) $\frac{A}{CC} \times 100$

Dry gluten (%) $\frac{B}{C} \times 100$

A = wt. Of wet gluten

where B = wt. Of dry gluten and C =wt. of flour.

3. DETERMINATION OF HAGBERG'S / FALLING NUMBER

Objectives: To determine the soundness of grain with respect/alphato germination and for an estimate of amylase enzyme.

Principle: The time in second required to stir and allow a viscometer stirrer to fall a fixed distance through a hot aqueous flour suspension being liquified by the enzyme in a standardised apparatus.

Apparatus: Grinder, balance and falling number apparatus.

Procedure: Grind 100g wheat in the lab grinder. Put on the heater of falling number apparatus and allow the water to come to boiling. Weight 7.0 g of sample and place in falling number tube. Add 25 ml of water. Insert rubber stopper and shake tube in upright position10 times and make sure all flour with viscometer stirrer. Place the tube in falling number water both and start the timer. The stirrer for 60 sec automatically stirs the flour suspension. The apparatus gives a buzzer and records time on the completion of the stirrer falling a fixed distance through the liquified gel. Calculate the falling number on 14% moisture basis.

Falling number is inversely proportional to alpha amylase activity of flour.

The normal values for the sound wheat vary from 200-350 and for the rain-affected wheat the falling number will be around 150 and less.

Where as liquification number (LN) $= \frac{6000}{FN - 50}$

is directly proportion to alpha – amylase activity of flour.

If FN = 300, LN = 24
FN = 150, LN = 60
FN = 80, LN = 200

4. PELSHENKE VALUE

Principle: wheat meal is made into a ball containing yeast solution, which will soon rise when placed in water. After a time, the dough ball commences to disintegrate and the total time when the first large piece of dough falls to the bottom of the beaker is related to strength.

Apparatus: 1. Controlled temperature cabinet maintained at 30^0C
2. 100 ml glass beakers.

Reagents: Yeast suspension made up daily by suspending 10g fresh compressed yeast in 100-ml water.

Method: 1. Weight 4g sample into 150ml low form beakers.
Mix 2.25-ml yeast suspension with meal using stirring rod.

1. Transfer resulting mass to palm of hand and knead into coherent round meal ball replace in beaker and cover with 80 ml water (30°C). note time of immersion and transfer beaker to constant temperature cabinet.

Note time when meal ball starts to disintegrate; record elapsed time in minutes between immersion and start of disintegration as time.

2. Time in minutes is “Test Number” or “Pelshenke value”.

5. DETERMINATION OF SEDIMENTATION VALUE

Principle: The volume of sediment, formed when flour is suspended in water and treated with lactic acid, consisting of swollen gluten and occulded starch is the sedimentation value.

Apparatus: Grinder, shaker and sieve (100 mesh)

Reagents: Isopropyl alcohol (99-100%)
Water containing 4 mg. Of bromophenol blue per litre. Lactic acid stock solution: dilute 250 ml 85% lactic acid to 1 litre with water. Reflux diluted acid 6hr. without loss of volume.

Lactic acid reagent: Mix thoroughly 180 ml lactic acid stock solution, 200 ml isoprophyl in water to make water 1 litre. Let stand 48 hr before using.

Procedure: Grind 100 g. of wheat in lab grinder. Sieve ground wheat using 100 mesh. Place 3.2 g of flour in 100-ml glass stoppered graduated cylinder.

Add 50 ml water containing bromophenol blue. Mix thoroughly flour and water by moving the cylinder horizontally 12 times. Start the timer and place the cylinder on shaker for 5 min. remove the cylinder end add 25 ml isopropyl alcohol – lactic acid reagent. Place the cylinder again on shaker for 5 min. and let stand exactly 5 min. at the end of 5 min read the volume in ml of sediment in cylinder. Calculate the value on 14% moisture basis. Sedimentation value is an index of quantity and quality of gluten.

Sedimentation value corrected to 14% moisture,

$$\text{= Sedimentation value un corrected} \quad X = \frac{100 - 14}{100 - \text{Original flour moisture}}$$

6. SDS – SEDIMENTATION TEST

Apparatus

1. Glass stoppered 100 ml graduated cylinders having distance of 160 mm Between zero mark at the bottom and 100 ml mark at the top.
2. Stop watch.

Reagents

1. Lactic acid solution: 3 ml of 88% Lactic acid is diluted (1:8 v/v) to 27 Ml with distilled water to make 1 litre.
2. Dissolve 20g SDS (Sodium Lauryl sulphate “Specially pure”) in distilled water to make 1 litre.
3. Add 20 ml of Reagent(1) to (2) (SDS – Lactic reagent)

Procedure

5 g of flour or 6 g whole-wheat flour is weighed and transferred to the 100-ml cylinder and 50-ml distilled water added. A stop clock set is going, and the material dispersed by rapid shaking for 15 sec. The contents were again shaken for 15 sec at 2 min and 4 min. Immediately after the last shake, 50 ml of the SDS – Lactic reagent was added and mixed in by inverting the cylinder four times before restarting the clock from zero time. Inversion (four times) was repeated at2,4 and 6 min before the clock was started once again from zero time. The contents of the cylinders were allowed to settle for 20 min (whole meals) or 40 min (flours) before the sedimentation volumes were read.

7. DETERMINATION OF ALCOHOLIC ACIDITY

Objective: Flours when stored for long, undergoes various types of deterioration, which in turn gives high values for alcoholic acidity, hence, alcoholic acidity is an index of deterioration of flour during storage.

Principle: Alcoholic acidity therefore refers to the combined acidity as we, get by, (1) hydrolysis of fats by lipases into free fatty acids, (2) hydrolysis of proteins into amino acids by proteins into amino acids by proteolytic enzymes. (3) acidity due to the presence of certain acids salts etc.

Method

Reagents

1. 90% alcohol.
   Dilute 700ml of 95% ethyl alcohol to 740 ml with distilled water.
2. 0.05 N. sodium hydroxide solution
   250 ml of 0.1 N NaOH diluted to alcohol.

Procedure

Weigh 5 g of sample into 100 ml conical stoppered flask and add 50 ml of 90 % ethyl alcohol: shake the contents of the flask for 1 hour and filter the alcoholic extract through No. 1 filter paper. tItrate 10 ml of alcoholic extract against 0.05 N NaOH using five drops of phenolphthalein as indicator. Calculate the percentage of alcoholic acidity as sulphuric acid.

A blank is also run using 10 ml of alcohol, in place of 10ml extract.

Calculation

Alcoholic Acidity (as H_2So_4)=$\frac{24.52AN}{W}$ per

N = Normality of standard sodium hydroxide solution and
W = weight in g of the material taken for the test.
A = volume in ml of the standard sodium hydroxide used in titration

8. ESTIMATION OF FAT ACIDITY (F. F.A)

Fat acidity is defined as the number of mg. Of potassium hydroxide required to neutralise the free fatty acids from 100 gm of grain and calculated to moisture free basis.

Reagents

1. Benzene
2. Alcohol 95%
3. 0.02N Potassium hydroxide
4. Phenolphthalein indicator

Determination

Place 20 g of the sample in a dry glass stoppered bottle and add 50 ml of benzene. The bottle is shaken for 1 hour and supernatent liquid is filtered through No. 1 filter paper. Exactly 5 ml of the filtrate is pipetted out into a dry conical flask and a few drops of the indicator is added. Then 5 ml of benzene and 10 ml of alcohol are added and then titrated against 0.02 N. KOH, till permanent pale pink colour is obtained. If the acidity is high, some turbidity is formed while titrating. In that case, an addition of 10 ml alcohol and 10 ml benzene clears the solution. Some time even more of the Benzene :Alcohol (1:1) solution may have to be added to clear the turbidity.

Calculation

F.F.A in mg. KOH = V X 56 mg.

Where V if the titration value in ml.

ANNEXURE-II

Moisture

Moisture content was estimated by heating the samples at 105^0C for 24 hours in an oven.

Volume expansion on cooking

In boiling test tube (cooking tubes) 20g of head rice was taken, to which 50 ml of distilled water was added, stirred with a glass rod to remove the air bubbles. The tubes were heated in a pressure cooker for 45 min at atmospheric pressure. After 45 min, the cooked rice sample was taken out and the volume of expansion was recorded.

Hydration at room temperature

The grains were soaked (~20g) in distilled water at room temperature. Samples were withdrawn at time intervals of 10, 15, 20, 30, 40 and 50 mins, 1 hr, 1.25 hr, 1.5 hrs, 1.75 hrs, 2 hrs and 24 hrs. Even initial samples before 10 mins are also withdrawn. Soaked rice was poured on to a strainer, and jerked gently and placed on a blotting paper. The surface moisture was removed carefully and moisture content of the grains was estimated.

Dehusking, Milling and powdering

Paddy samples were cleaned, dehusked and milled simultaneously in a McGill Miller of 1 kg capacity by using a load of 3 pounds and for a period of 1 min. The rice obtained was cleaned and powdered by using a SURABHI grinder. The powder obtained by this method generally passes through a 60-mesh sieve.

Amylose estimation

About 100 mg of powder on dry basis was weighed into a 100 ml conical flask. 1 ml alcohol was added, followed by 10 ml of 1 N sodium hydroxide and kept overnight. Next day the flask was placed in a boiling water bath, heated for 10 minutes and then cooled. The dispersion was transferred to a 100 ml volumetric flask and the volume was made-up with distilled water.

5 ml of this dispersion was transferred to a 100 ml volumetric flask, to which about 50 ml water was added, 1 ml of IN acetic acid was added, shaken well and 2 ml of 0.2% iodine in 2% potassium iodide was added. The volume was made up mixed well and kept aside for 20 to 25 min. Colour developed (blue) was read at 630 nm in a spectrophotometer, and the absorbance was noted down.

Preparation of standard amylose solution

About 100 mg (on d.b) of standard amylose was weighed and transferred to a 100 ml volumetric flask. 1 ml of distilled alcohol followed by 10 ml of 1 N sodium hydroxide was added. It was heated for about 2 – 3 min. on a water bath, the material goes into solution easily. It was cooled to room temperature. To this dispersion 3/4th volume of calculated quantity of I N hydrochloric acid was added and made up to the mark with distilled water. The dispersion was stored in a refrigerator.

One ml of this standard solution was pipetted out into a 100 ml volumetric flask, to which about 50 ml distilled water was added, mixed thoroughly by addition of 1 ml of IN acetic acid. Two ml of iodine of 0.2% concentration in 2% potassium. Iodide was added and made up to the mark and kept aside for 20-25 min. The colour was read at 630 nm, amylose equivalent / content was calculated using the following formula.

Amylose equivalent / content =

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Wt. of standard (db)}}{\text{Wt. of sample (db)}} \times 20$$

Swelling and solubility

About 500 mg of flour on dry basis was weighed and taken in a 100 ml beaker. To this about 25 ml distilled water was added. The dispersion was stirred at room temperature with a glass rod, placed in a water bath where different temperatures were maintained. The temperature were 60^0C, 70^0, 80^0, 90^0 and boiling water temperature. It was then heated for 30 min with occasional stirring. The beaker was removed and cooled to room temperature, and transferred thoroughly to a pre-weighed centrifuge tube and the contents were made to 25 g and this was centrifuged at 3000 rpm for 15 min.

The supernatant in the tube was transferred to a beaker and the residue in the centrifuge tube was weighed. From the supernatant 10ml was pipetted out to a pre-weighed petri dish. This dish was placed on a boiling water bath and evaporated to dryness, and heated in an oven at 105^0C for about 3 hours and then cooled in a dessicator and weighed. Weight of the solubles was noted down. Swelling and solubility was calculated from the following formula.

$$\text{Swelling power} = \frac{\text{Weight of swollen material (mg)}}{\text{Weight of the powder taken (mg) (d.b)} - \text{Weight of solubles (mg)}}$$

$$\text{Solubility (\%)} = \frac{\text{Weight of solubles x 2.5 x 100}}{\text{Weight of powder (mg, d.b)}}$$

Defatting of the rice sample for amylose estimation

Generally defatting is carried out by extracting the rice sample in powder form with 85% methanol, in a soxhlet apparatus for about 18 hours on a water bath. In another procedure about 20 ml of alkali dispersion was taken in a graduated and stoppered cylinder. To this petroleum ether was added and shaken for 20 min. It was allowed to settle down and ether at the top was pipetted out. Carbon tetrachloride was added and shaken well and allowed to settle down. From the top 5 ml of dispersion was pipetted out and colour was developed as before. After about 20 – 25 min, the colour was read at 630 nm in a Spectrophotometer.

Viscography by Rapid Visco Analyzer

3.5 g of the flour sample was weighed and transferred to the canister (aluminium container). To this calculated amount of water was added, taking into consideration 14% moisture of the sample, and total weight was made to become 28.5 g. The canister was placed in the instrument and functioning was started by heating at 50^0C. It was stirred for 1 minute and heated at the rate of 6^0 C per minute up to 95^0C and it was cooked at that temperature for 7 minutes and allowed to cool at the same rate as before. When the temperature came down to 50^0C, the experiment was stopped and the viscogram print out was taken. From the viscogram, peak viscosity (PV), hot paste viscosity (HPV) and cold paste viscosity (CPV) were noted down. Other derived parameters from these data, namely, break down = (PV - HPV), Set back = (CPV – PV), total set back = (CPV – HPV) were calculated.