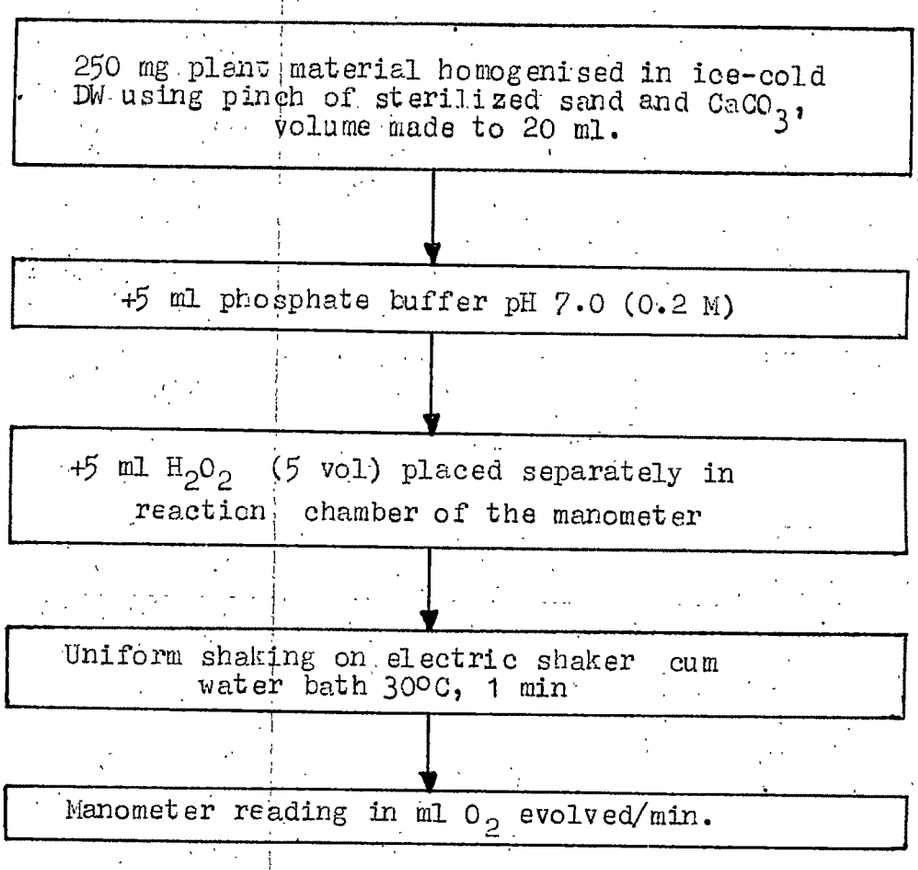
Fig. A.1 Flow Chart for Determination of Catalase Activity

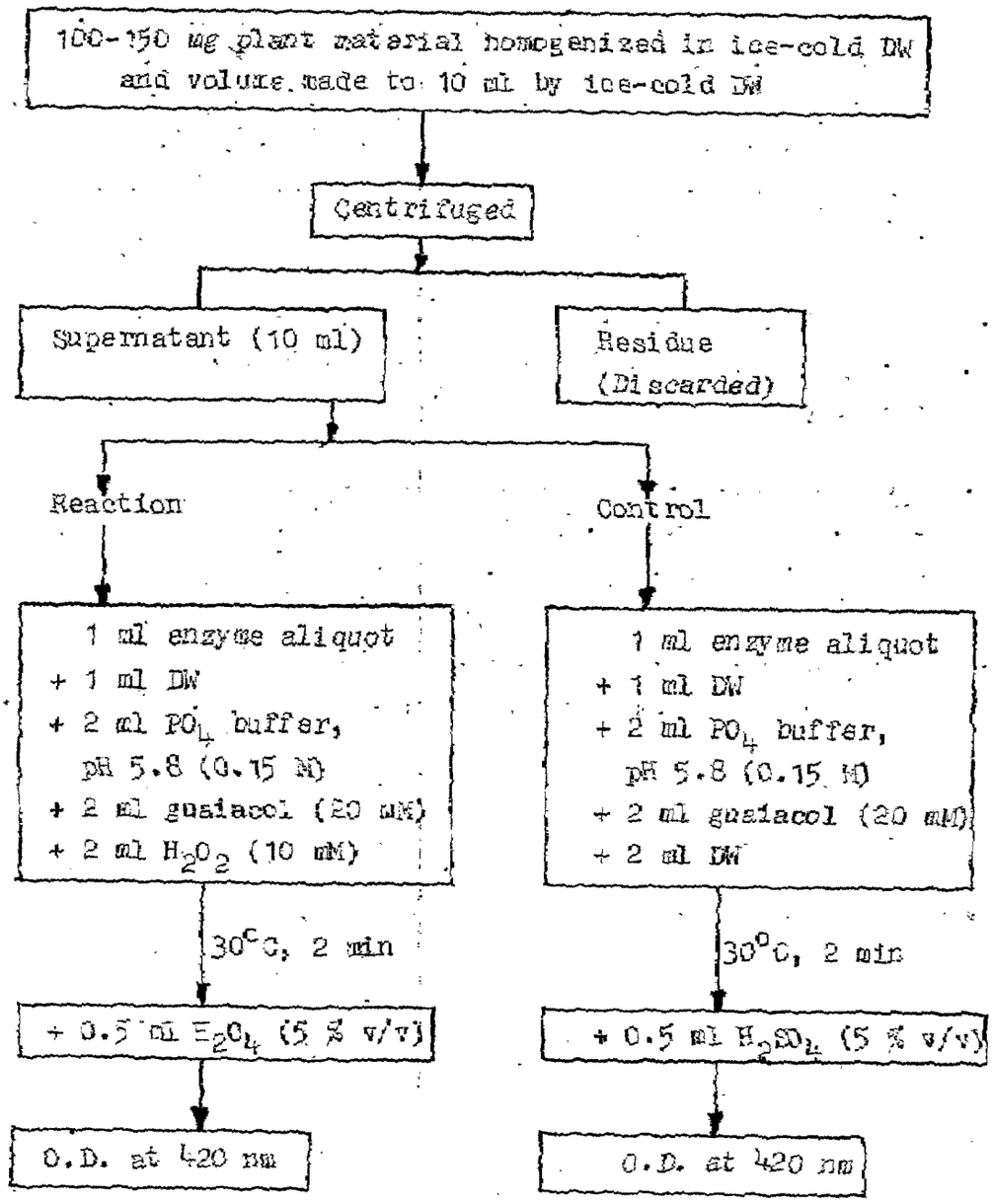



Preparation of Reagents Used

i. Phosphate buffer pH 7.0 (0.2 M) : A solution of 31.20 g of $NaH_2PO_4.2H_2O$ per litre and a solution of 28.39 g of $Na_2HPO_4$ per litre (or 71.7 g of $Na_2HPO_4.12H_2O$ per litre) was prepared. To make 100 ml of phosphate buffer pH 7.0; 19.5 ml of $NaH_2PO_4$ and 30.5 ml of $Na_2HPO_4$ wersolutions were added and volume made upto 100 ml by DW.

ii. $H_2O_2$, 5 vol : 5 ml of 100 v/v $H_2O_2$ (30 % $H_2O_2$ ) were added to 95 ml DW.

Fig. A.2 Flow Chart for Determination of Peroxidase Activity



The Preparation of Reagents Used

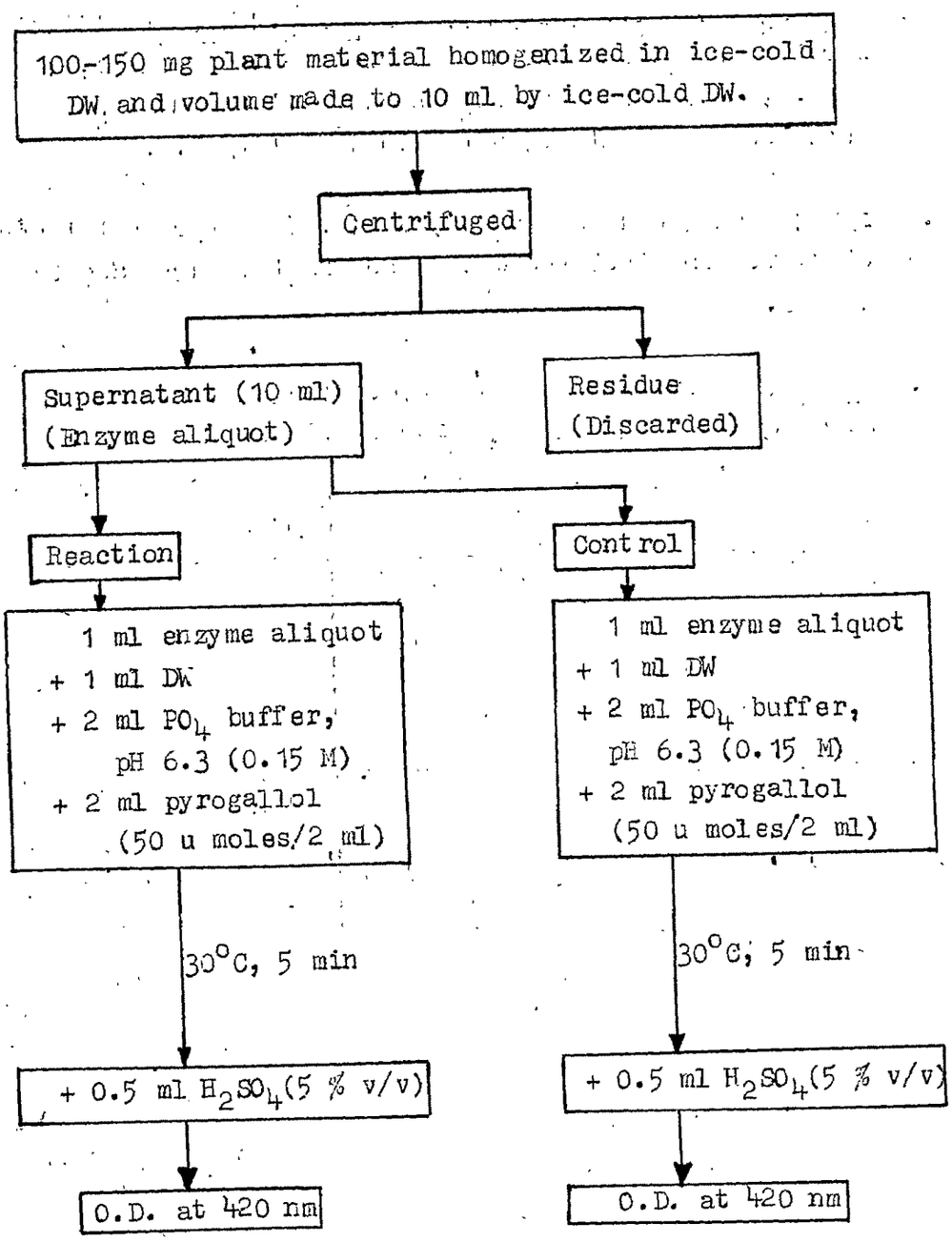
i. Phosphate buffer, pH 5.8 (0.15 M): A solution of 23.4 g of $NaH_2PO_4.2H_2O$ per litre and a solution of 21.29 g of $Na_2HPO_4$ per litre (or 53.77 g $Na_2HPO_4.12H_2O$ per litre). To make 100 ml of phosphate buffer, pH 5.8, 46.0 ml of $NaH_2PO_4$ and 4.0 ml of $Na_2HPO_4$ solutions were added and volume made upto 100 ml by DW.

ii. Guaiacol (20 mM): 0.22 ml of pure guaiacol added in 99.78 ml DW, shaken vigorously and stored in a brown bottle in fridge.

iii. $H_2O_2$ (10 mM): 0.11 ml of 100 v/v $H_2O_2$ (30 % $H_2O_2$) added to 99.89 ml DW (or 0.57 ml of 20 v/v $H_2O_2$ (6 % $H_2O_2$) were added to 99.43 ml DW).

iv. $H_2SO_4$ (5 % v/v) : 5 ml of concentrated sulphuric acid were added slowly, drop by drop, to 95 ml DW.

Fig. A.3 Flow Chart for Determination of Polyphenoloxidase Activity



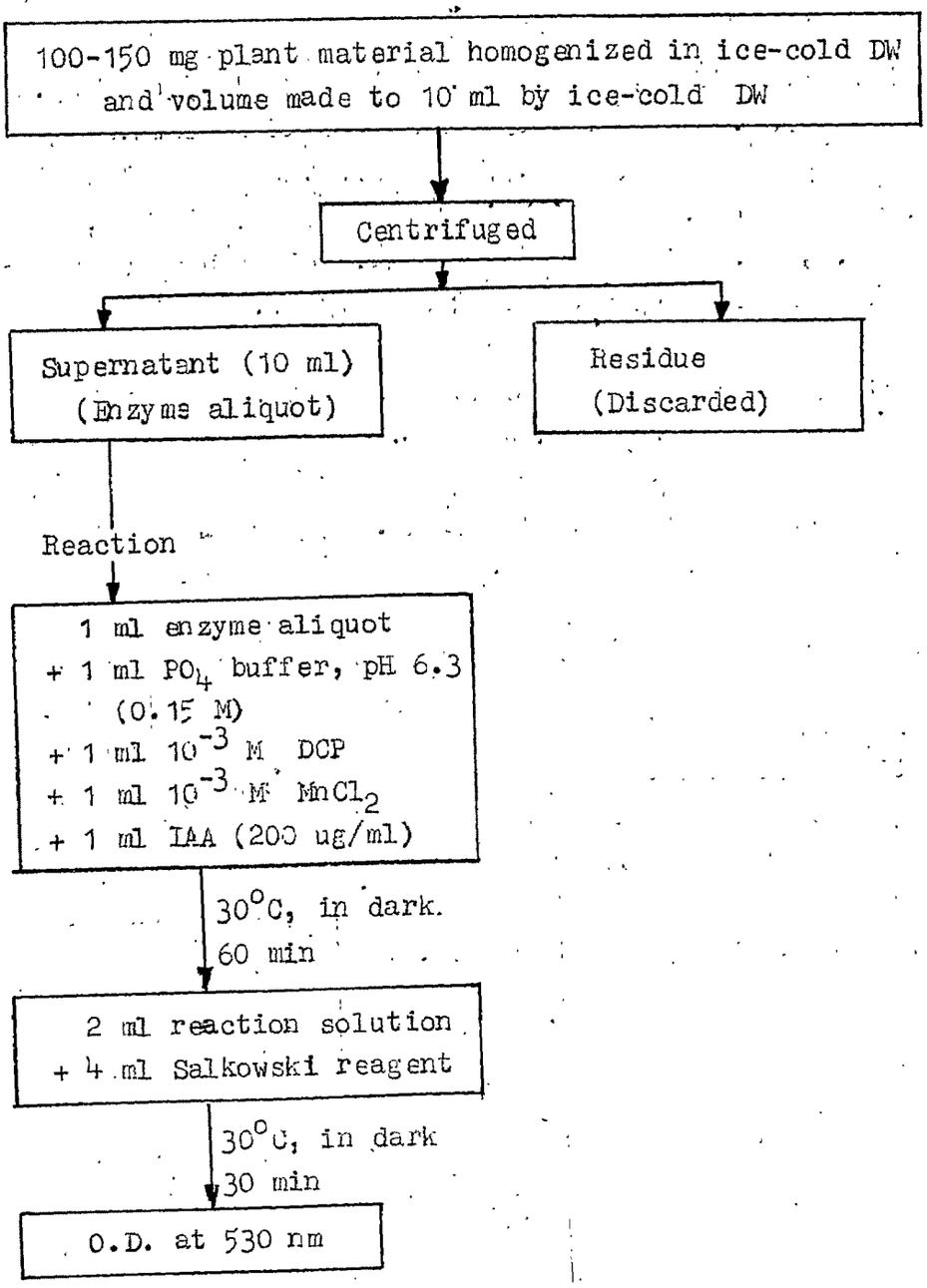
Preparation of Reagents Used

i. Phosphate buffer, pH 6.3 (0.15 M): A solution of 23.4 g $NaH_2PO_4 \cdot 2H_2O$ per litre and a solution of 21.29 g of $Na_2HPO_4$ per litre (or 53.77 g $Na_2HPO_4 \cdot 12H_2O$ per litre) were prepared. To make 100 ml of phosphate buffer, pH 6.3, 38.7 ml of $NaH_2PO_4$ and 11.2 ml of $Na_2HPO_4$ were added and volume made upto 100 ml by D.W.

ii. Pyragallol (50 u moles/2 ml) : 315.25 mg pyragallol was dissolved in distilled water and final volume made to 100 ml.

iii. $H_2SO_4$ (5 % v/v): 5 ml of concentrated sulphuric acid were added slowly drop by drop to 95 ml D.W.

Fig. A.4 Flow Chart for Determination of IAA-oxidase Activity




(In blank 1 ml DW was taken in place of 1 ml enzyme to estimate the O.D. due to 1 ml IAA (200 mg/ml) and in control 1 ml enzyme was taken as such but 1 ml DW was taken inp place of 1 ml IAA (200 ug/ml) to minus the O.D. due to endogenous IAA if any, in enzyme extract, from the reaction OD).

Preparation of Reagents Used

i. Phosphate buffer, pH 6.3 (0.15 m) : A solution of 23.4 g of $Na_2HPO_4.2H_2O$ per litre and a solution of 21.29 g of $Na_2HPO_4$ per litre (or 53.77 g $Na_2HPO_4.12H_2O$ per litre) were prepared. To make 100 ml of phosphate buffer, pH 6.3, 38.7 ml of $NaH_2PO_4$ and 11.2 ml $Na_2HPO_4$ were added and volume made upto 100 ml by DW.

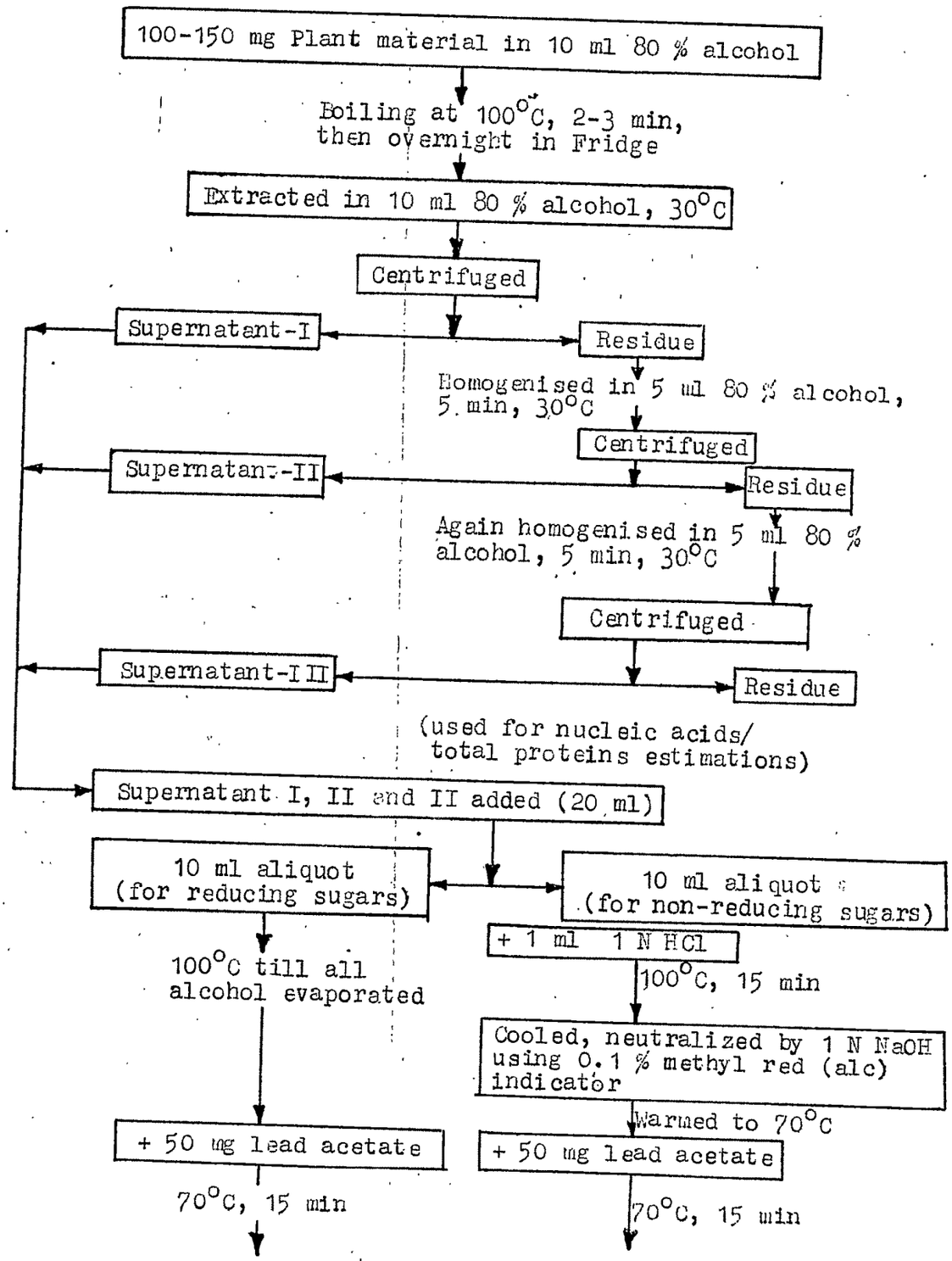
ii. 2-4, Dichlorophenol (DCP) ($10^{-3}$ M) : 16.30 mg of the DCP were dissolved in 100 ml DW.

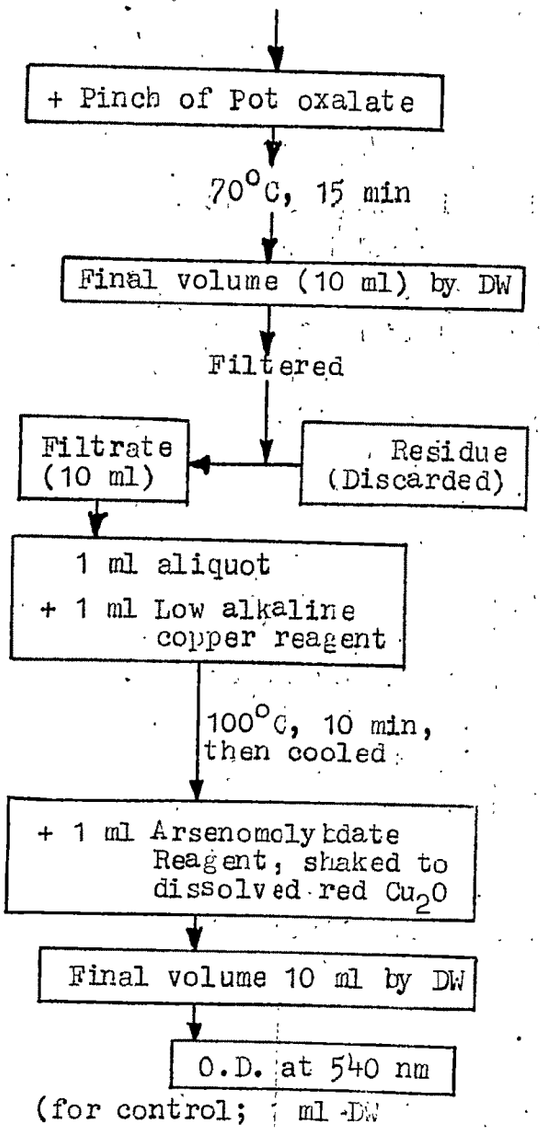
iii. Manganese chloride ($10^{-3}$ M) : 19.80 mg of $MnCl_2$ were dissolved in 100 ml of DW.

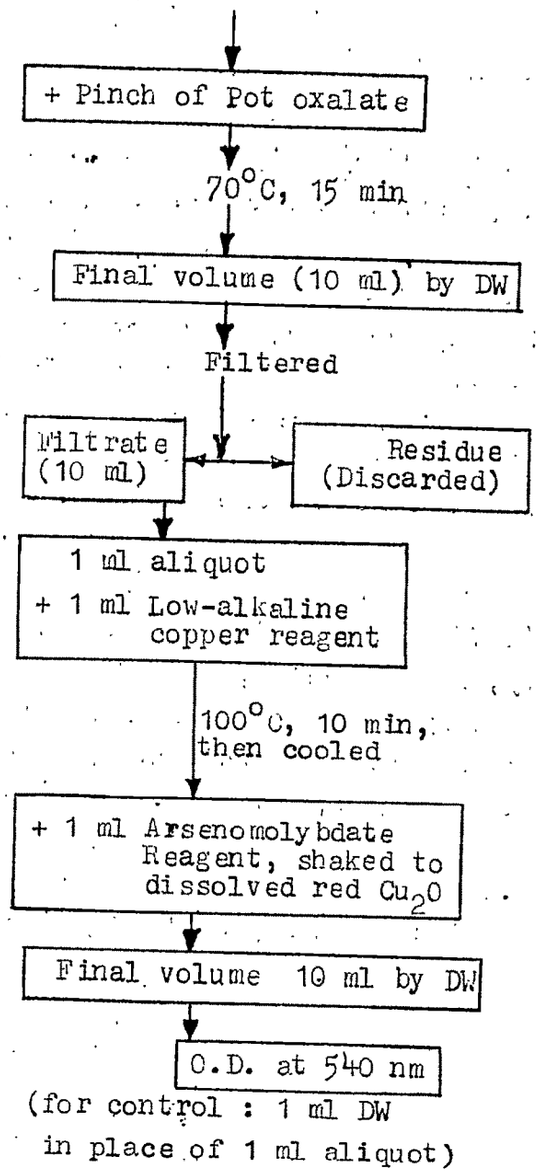
iv. Indole-3-acetic acid (IAA) (200 ug/ml) : 20 mg of IAA was first dissolved in 5-10 drops of 95 % ethyl alcohol and then the requisite volume of DW added with vigorous stirring to the alcoholic solution, i.e, later on total volume made to 100 ml with DW.

v. Salkowski Reagent : 2 ml of 0.5 M $FeCl_3$ were added to 100 ml of 35 % perchloric acid.

Fig. A.5 Flow Chart for Determination of Reducing and Non-reducing Sugars




+ Pinch of Pot oxalate
70°C, 15 min
Final volume (10 ml) by DW
Filtered
Filtrate (10 ml)
Residue (Discarded)
1 ml aliquot
+ 1 ml Low alkaline copper reagent
100°C, 10 min, then cooled
+ 1 ml Arsenomolybdate Reagent, shaked to dissolved red $Cu_2O$
Final volume 10 ml by DW
O.D. at 540 nm
(for control; 1 ml DW in place of 1 ml aliquot)


+ Pinch of Pot oxalate
70°C, 15 min
Final volume (10 ml) by DW
Filtered
Filtrate (10 ml)
Residue (Discarded)
1 ml aliquot
+ 1 ml Low-alkaline copper reagent
100°C, 10 min, then cooled
+ 1 ml Arsenomolybdate Reagent, shaked to dissolved red $Cu_2O$
Final volume 10 ml by DW
O.D. at 540 nm
(for control : 1 ml DW in place of 1 ml aliquot)

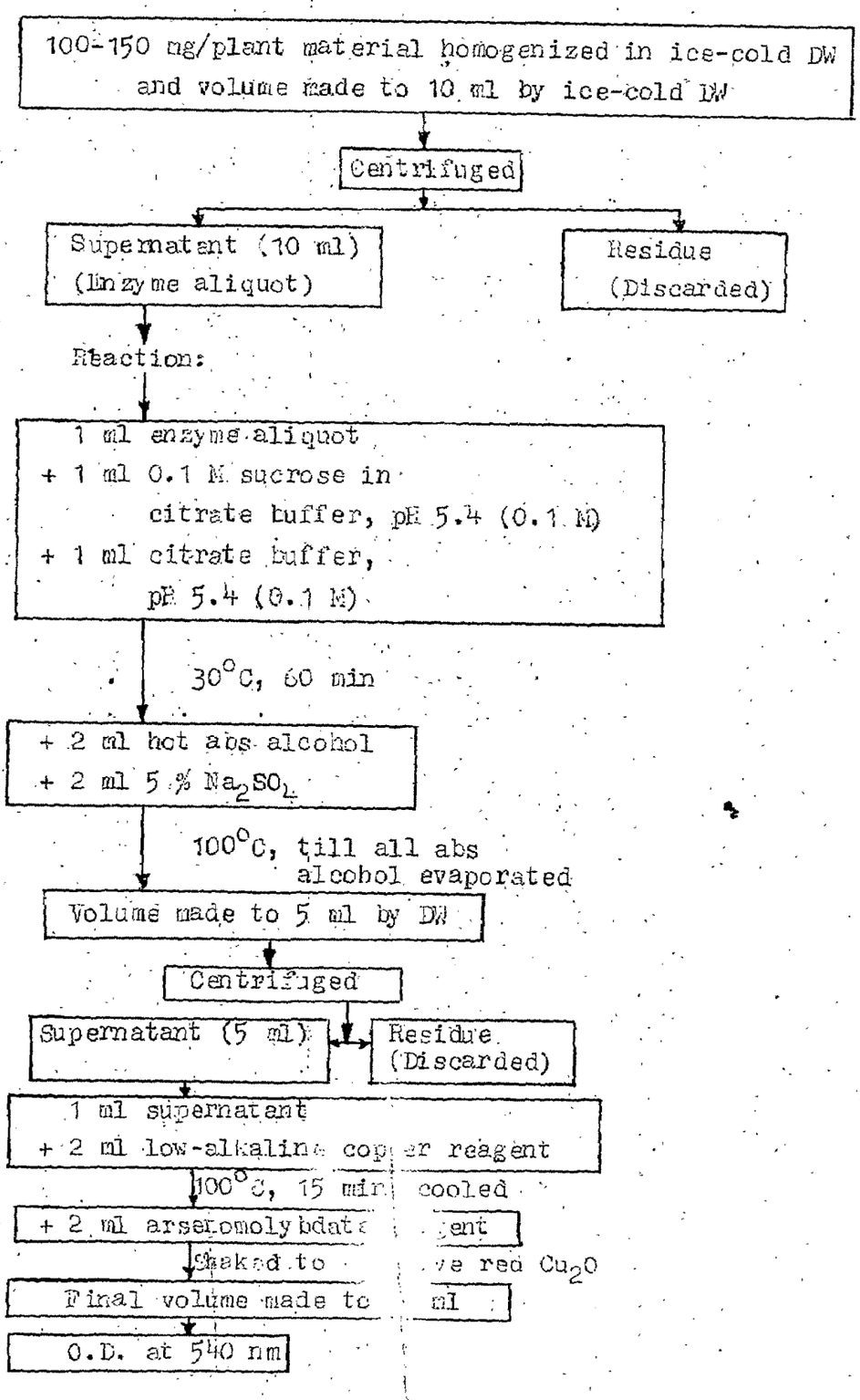
Preparation of Reagents Used :

i. Low alkaline copper reagent : Rochelle salt (12 g) and anhydrous sodium carbonate (24 g) are dissolved in about 250 ml of DW. A solution of 4 g of cupric sulphate pentahydrate in DW is added with stirring, followed by 16 g of sodium hydrogen carbonate. A solution of 150 g of anhydrous sodium sulphate in 500 ml of DW is boiled to expel air, then, the two solutions are combined and diluted to 1 litre. After 1 week of standing, the clear supernatant solution is used.

ii. Arsenomolybdate reagent : 25 g of ammonium molybdate in 450 ml of DW is added to 21 ml of conc. $H_2SO_4$ followed by, with stirring, 3 g of disodium hydrogen arsentate heptahydrate dissolved in 25 ml of DW. The mixed solution is incubated for 24 h at 37°C and stored in a glass-stoppered brown bottle.

iii. 0.1 % Methyl Red (alc) Indicator : 0.5 g of methyl red is dissolved in 300 ml of 95 % alcohol and diluted with 200 ml of DW.

Fig. 4.6 Flow Chart for Determination of Invertase Activity



(For control, 1 ml citrate buffer, pH 5.4 was taken in place of 1 ml 0.1 M sucrose in citrate buffer, pH 5.4 to deduct the amount of reducing sugars already present in 1 ml enzyme extract)

Preparation of Reagents Used

i. Citrate buffer, pH 5.4 (0.1 M) : A 0.1 M solution of citric acid (19.21 g per litre) and a 0.1 M solution of sodium citrate (29.41 g/litre) were prepared. To make 100 ml citrate buffer, pH 5.4, 16.0 ml of citric acid and 34.0 ml sodium citrate were added and volume made upto 100 ml DW.

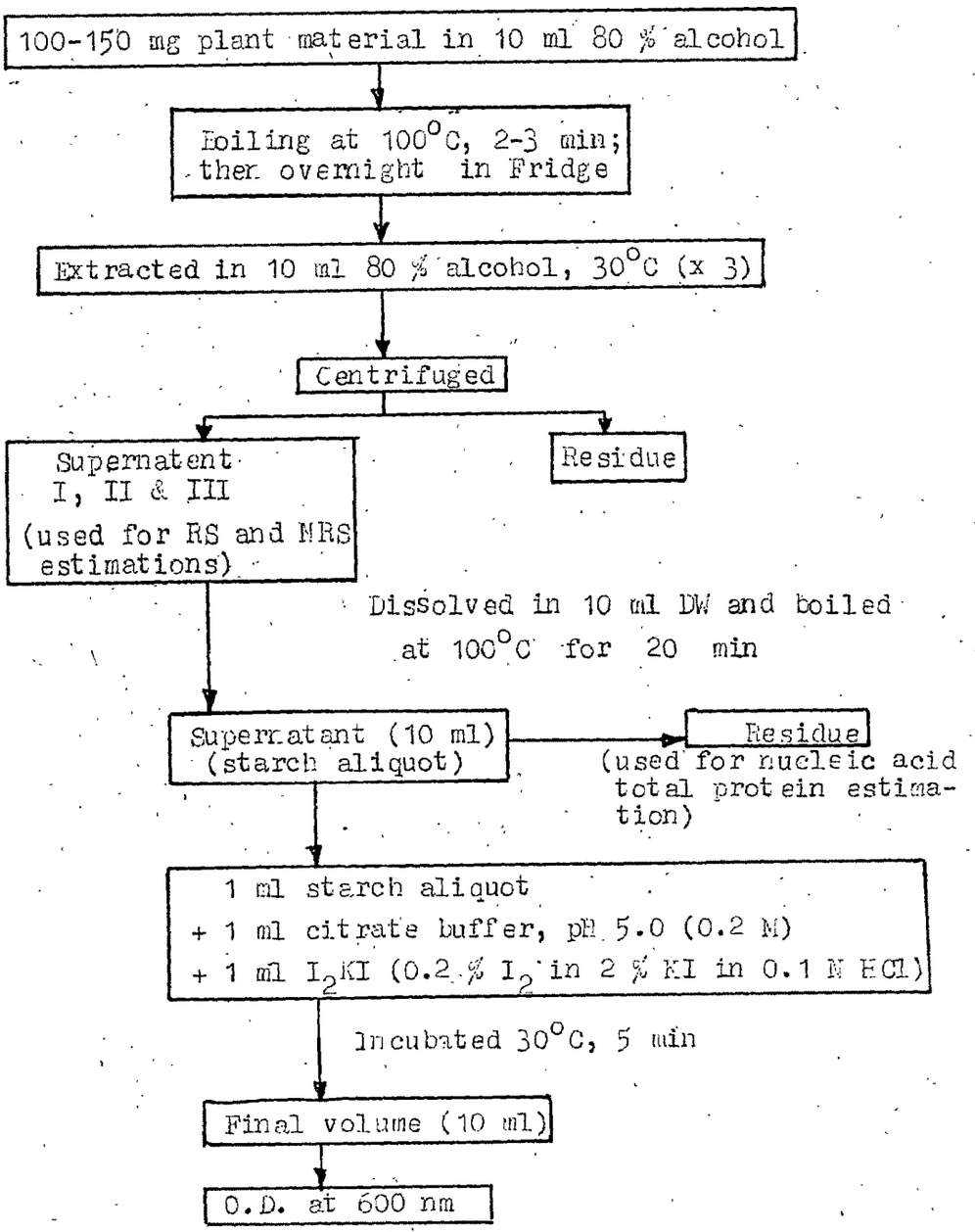
ii. 0.1 M Sucrose in citrate buffer, pH 5.4 (0.1 M) : 3.42 g of sucrose were dissolved in 90 ml of citrate buffer, pH 5.4 (0.1 M) and total volume made to 100 ml by citrate buffer, pH 5.4 (0.1 M).

iii. 5 % sodium sulphate : 5 g sodium sulphate dissolved in 100 ml DW.

iv. Low-alkaline copper reagent : as described earlier in methods of reducing and non-reducing sugars (Fig. ).

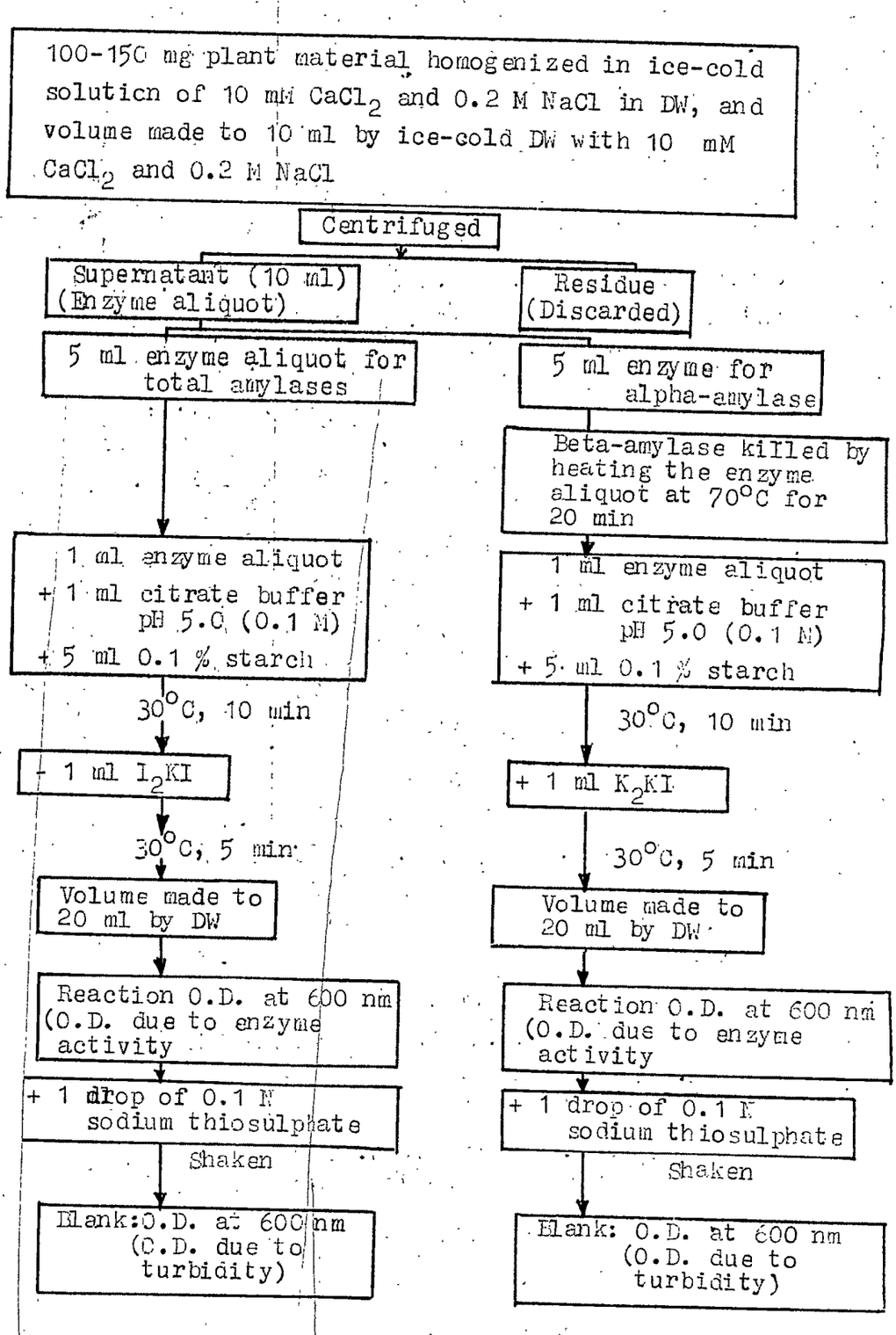
v. Arsenomolybdate reagent : as described earlier in methods of reducing and non-reducing sugars (Fig. ).

Fig. A.7 Flow Chart for Determination of Starch




(for control : 1 ml DW is taken in place of 1 ml starch aliquot)

Fig. A.8 Flow Chart for Determination of Alpha and Beta Amylase Activities



Control was prepared in the same way with 1 ml 10 mM $CaCl_2$ in 0.2 M NaCl solution in DW instead of 1 ml enzyme)

Preparation of Reagents Used

i. Citrate buffer, pH 5.0 (0.1 M) : Made up a 0.1 M solution of citric acid (19.21 g per litre) and a 0.1 M solution of sodium citrate (29.41 g per litre). To make 100 ml citrate buffer, pH 5.0, 20.5 ml of citric acid and 29.5 ml sodium citrate were added and volume made upto 100 ml with DW.

ii. 0.1 % starch : 100 mg of starch powder boiled for 2-3 minutes in 90 ml DW till the solution becomes 100 % transparent, cooled and volume made upto 100 ml by DW.

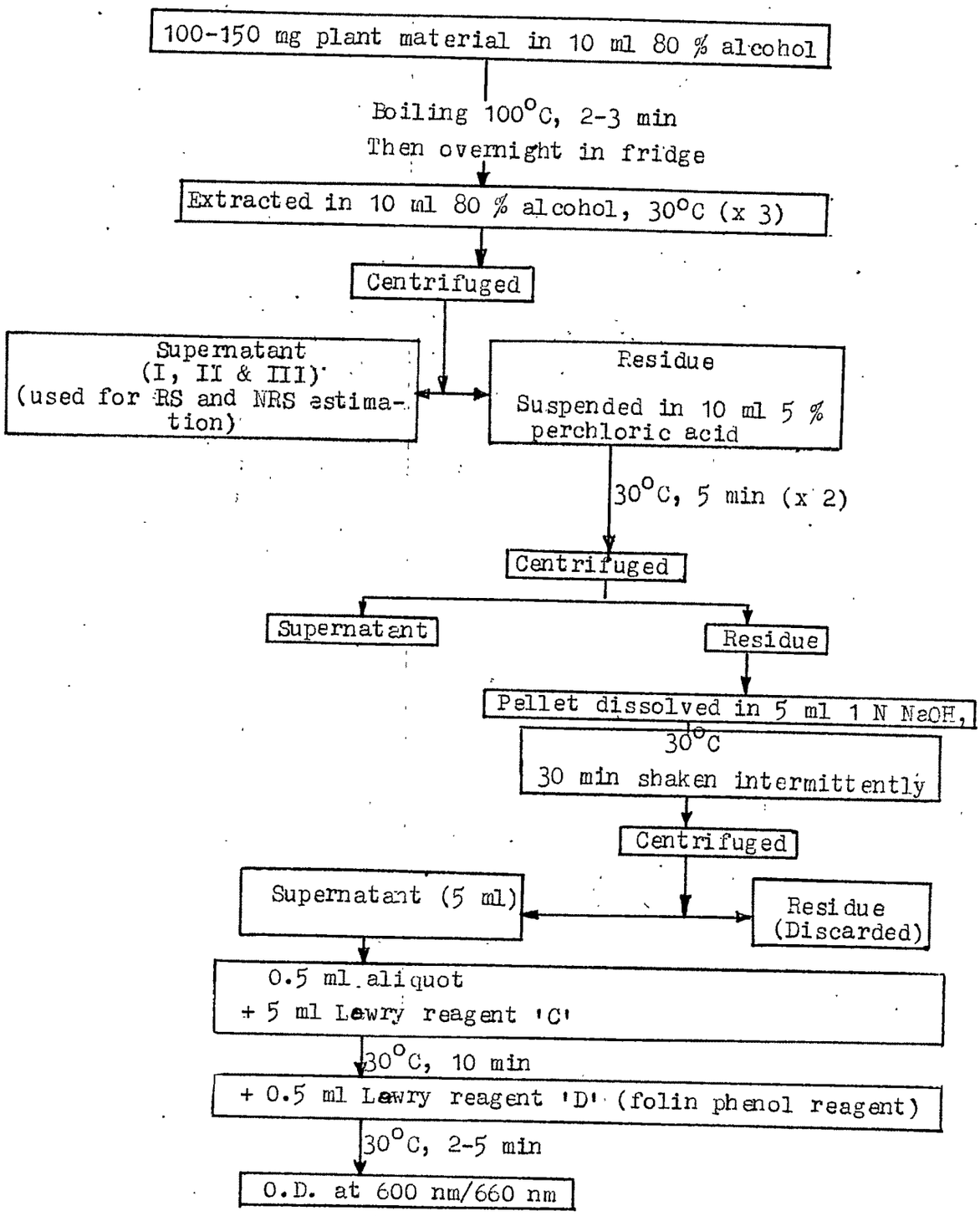
iii. $I_2KI$ reagent (0.2 % $I_2$ in 2 % KI in 0.1 N HCl) : 200 mg fresh iodine crystals dissolved in 2 % potassium iodide solution prepared in 0.1 N HCl (for 100 ml) of 0.1 N HCl, 0.88 ml conc HCl (35 % HCl) were dissolved in DW to make upto 100 ml volume).

iv. 0.1 N sodium thiosulphate : 2.48 g of sodium thiosulphate (MW - 248.18) were dissolved in 100 ml DW.

v. 10 m M $CaCl_2$ in 0.2 M NaCl : 11.69 NaCl and 1.11 g $CaCl_2$ were dissolved in DW and volume made to 1 litre.

355

Fig. A.9 Flow Chart for Determination of Total Proteins




(For control : 0.5 ml 1 N NaOH is taken in place of 0.5 ml protein aliquot)

Preparation of Reagents Used

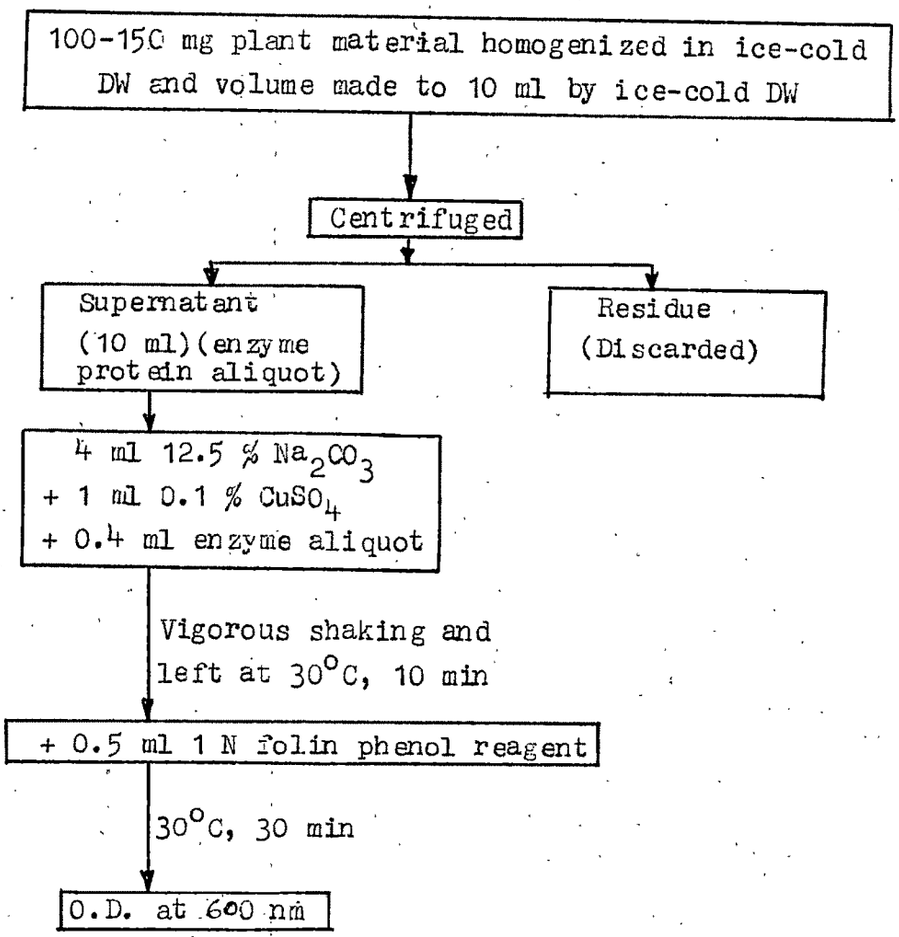
i. Lewry Reagent 'C' : Lewry Reagent 'A' (2 % $Na_2CO_3$ in 0.1 N NaOH) was prepared by adding 2 g $Na_2CO_3$ in 0.1 N NaOH and made to a volume 100 ml. (0.1 N NaOH was prepared by adding 0.4 g NaOH in 100 ml DW). Lewry Reagent 'B' (0.5 % $CuSO_4.5H_2O$ in 1 % sod. or pot. tartrate) was prepared by dissolving 500 mg $CuSO_4.5H_2O$ and 1 g sod. or pot. tartrate in distilled water andmade the volume to 100 ml. For Lewry Reagent 'C' 50 parts of Lewry Reagent 'A' and 1 part of Lewry Reagent 'B' were added.

ii. Lewry Reagent 'D' : 1 part of 3 N foline phenol Reagent Folin Ciocalteus Reagent = Phenol-indo-2,6-dichlorophenol) were added with 2 part of distilled water.

iii. 5 % Perchloric acid : 5 ml of 70 % perchloric acid were added to distilled water and final volume made to 70 ml by DW.

356

Fig. A.10 Flow Chart for Determination of Enzyme Proteins




(For Blank : 0.4 ml DW was taken in place 0.4 ml enzyme protein aliquot)

Preparation of Reagents Used

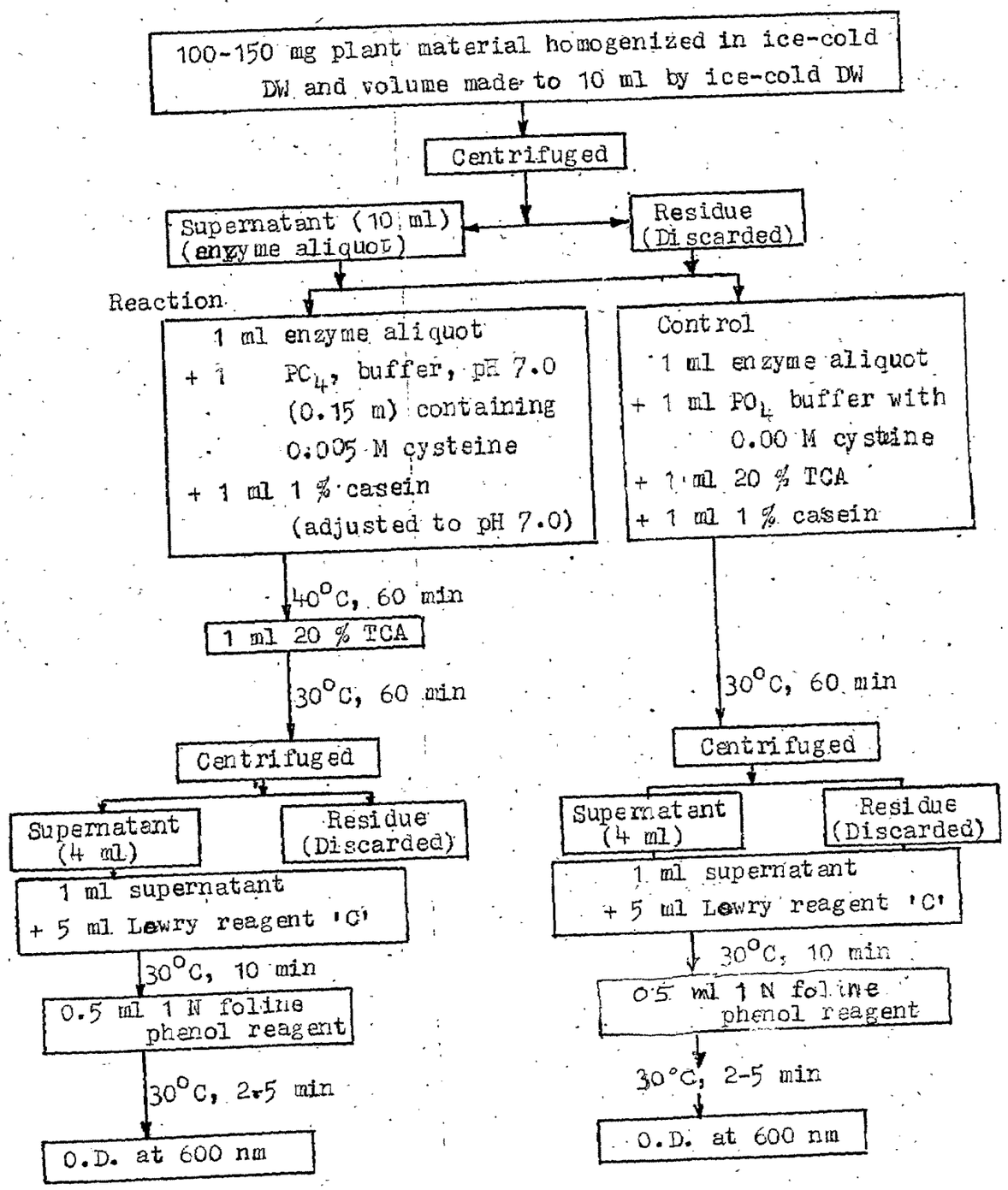
i. 12.5 % $Na_2CO_3$: 12.5 sodium carbonate dissolved in 100 ml DW.

ii. 0.1 % $CuSO_4$ : 100 mg of copper sulphate were dissolved in 100 ml DW.

iii. 1 N Folin phenol reagent : 1 part of folin phenol reagent was added to 2 parts of DW.

357

Fig. A.11 Flow chart for Determination of Protease Activity



Preparation of Reagents Used

i. Phosphate buffer, pH 7.0 (0.15 M) containing 0.005 M cysteine: A solution of 23.4 g of $NaH_2PO_4.2H_2O$ per litre and a solution of 21.29 g of $Na_2HPO_4$ per litre (or 53.77 g $Na_2HPO_4.12H_2O$ per litre) were prepared. To make 100 ml of phosphate buffer, pH 7.0, 19.5 ml of $NaH_2PO_4$ and 30.5 ml of $Na_2HPO_4$ were added and volume made up to 100 ml by DW. To prepare 0.005 M cysteine (MW 121.16) solution, 60.50 mg -cysteine/ 100 ml was added to the phosphate buffer or for .005 M -cysteine hydrochloride monohydrate (M.W. 175.64); 87.82 mg was added to 100 ml $PO_4$ buffer.

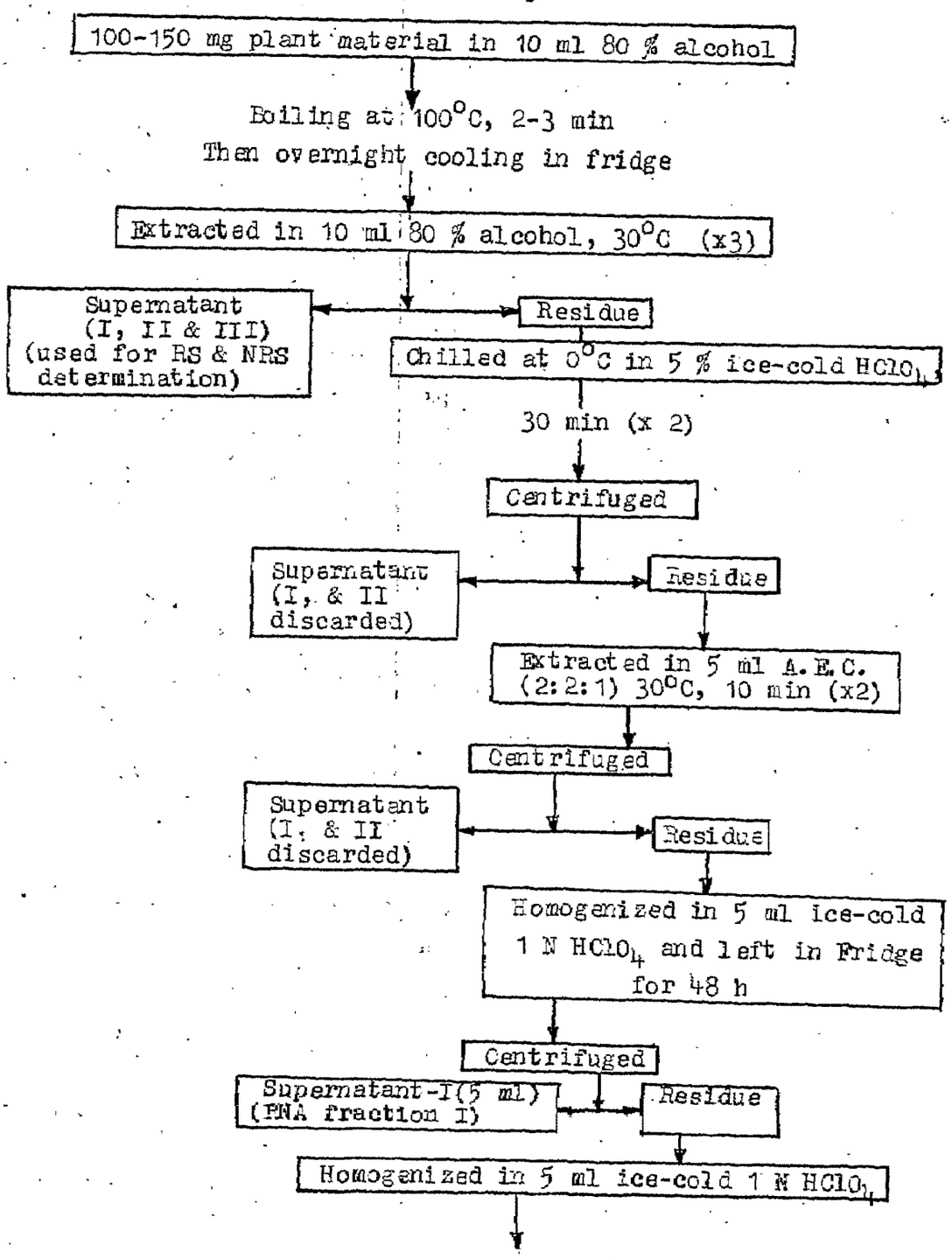
ii. 20 % TCA : 20 g of trichloroacetic acid dissolved in 100 ml DW.

iii. Lowry reagent A: 2 % $Na_2CO_3$ in 0.1 N NaOH Lawry reagent 'B' : 0.5 % $CuSO_4.5H_2O$ in 1 % Na or K-tartrate. Fresh Lowry reagent 'C' was prepared by adding 50 ml of 'A' and 1 ml of 'B'.

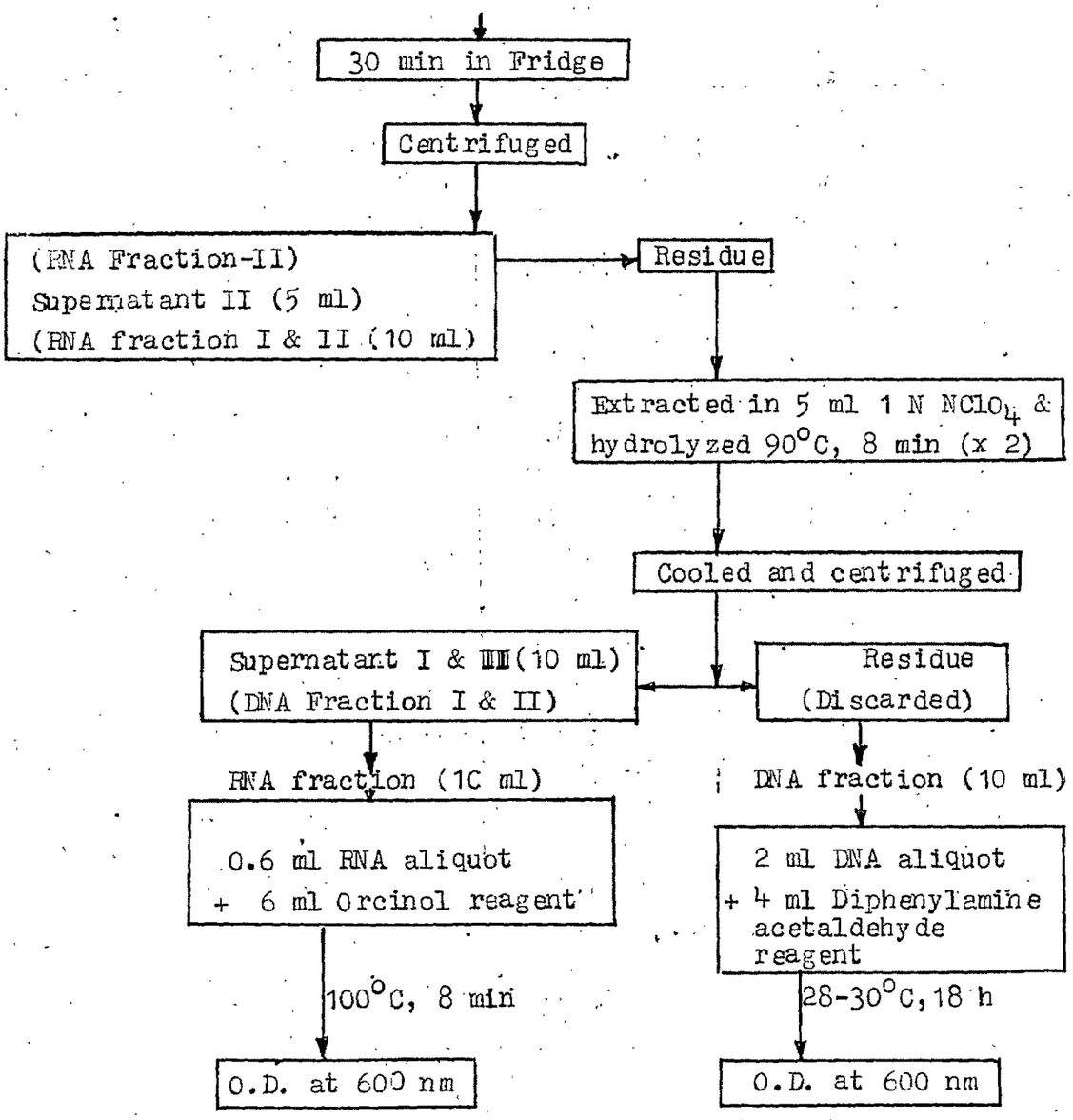
iv. 1 N folin phenol reagent (Lowry reagent 'D') : 1 part of folin phenol reagent + 2 parts of DW.

v. 1 % Casein : 1 gm cassin first dissolved in 2-3 ml 0.1 N NaOH, then diluted to 100 ml volume by phosphate buffer, pH 7.0.

Fig. A.12 Flow Chart for Determination of Nucleic Acid (RNA and DNA)




CONTD.


30 min in Fridge
Centrifuged
(RNA Fraction-II)
Supernatant II (5 ml)
(RNA fraction I & II (10 ml)
Residue
Extracted in 5 ml 1 N NClO4 & hydrolyzed 90°C, 8 min (x 2)
Cooled and centrifuged
Supernatant I & III (10 ml)
(DNA Fraction I & II)
Residue
(Discarded)
RNA fraction (10 ml)
DNA fraction (10 ml)
0.6 ml RNA aliquot
+ 6 ml Orcinol reagent
2 ml DNA aliquot
+ 4 ml Diphenylamine acetaldehyde reagent
100°C, 8 min
28-30°C, 18 h
O.D. at 600 nm
O.D. at 600 nm
(Control: 0.6 ml 1 N HClO4 in place of RNA aliquot)
(Control: 2 ml 1 N HClO4 in place of DNA aliquot)

Preparation of Reagents Used

i. Orcinol reagent (after Markham, 1955) : The fresh orcinol reagent was prepared by mixing 10 volume of 1 % orcinol with 40 ml volume of con. HCl and 1 volume of 10 % $FeCl_3 \cdot 3H_2O$.

ii. Diphenylamine acetaldehyde reagent (after Burton, 1956) : Diphenylamine reagent was prepared by dissolving 6 g diphenylamine in 460 ml acetic acid followed by addition of 6 ml conc $H_2SO_4$. 0.1 ml of 16 mg/ml acetaldehyde was added to each 20 ml of the diphenylamine reagent before use (16 mg/ml acetaldehyde is prepared by adding 0.2 ml pure acetaldehyde in 9.8 ml DW).

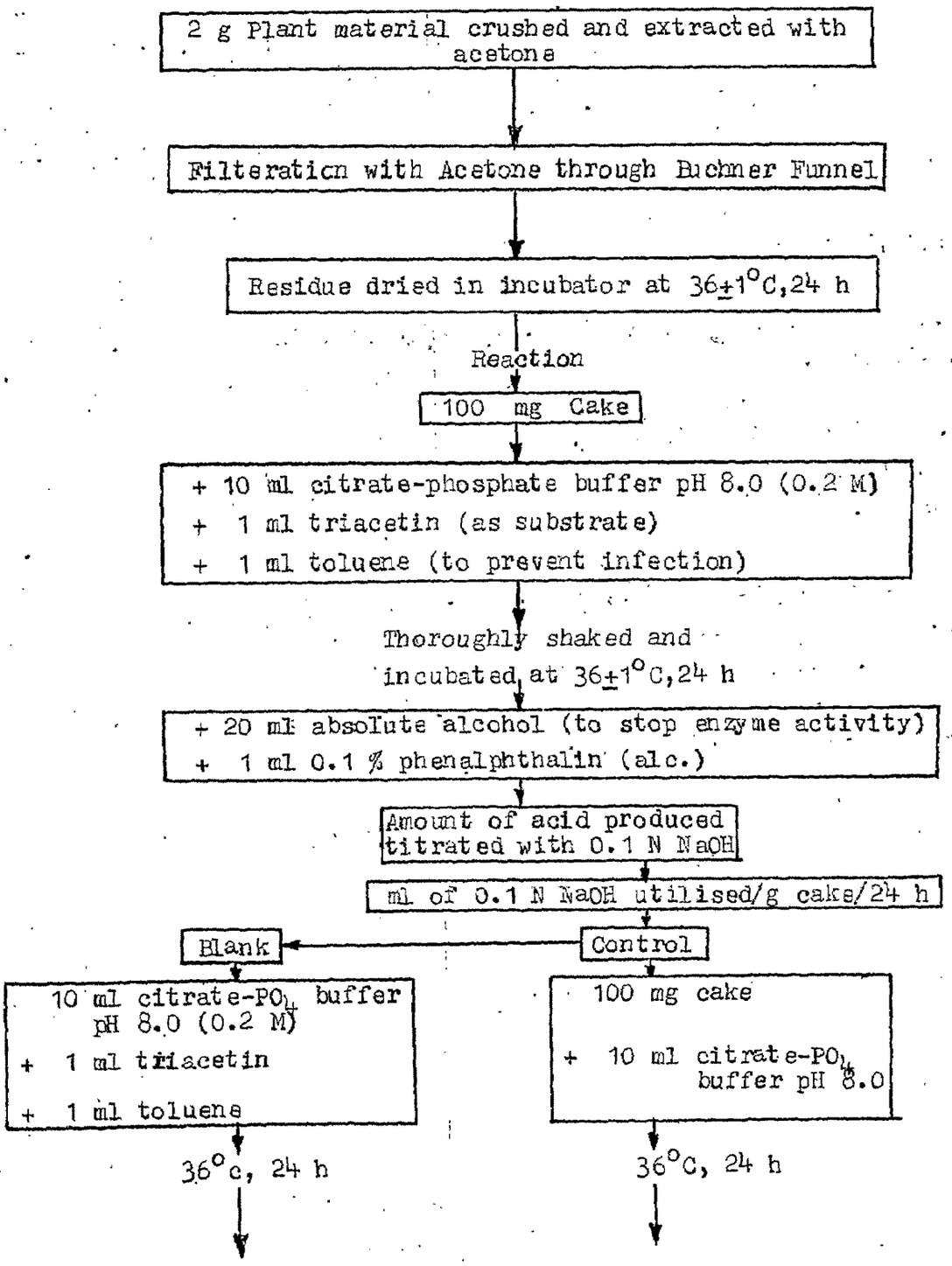
iii. 5 % Perchloric acid : 7.14 ml of 70 % perchloric acid is added with 92.9 ml DW (or 5 ml of 70 % perchloric acid taken and volume made to 70 ml by DW).

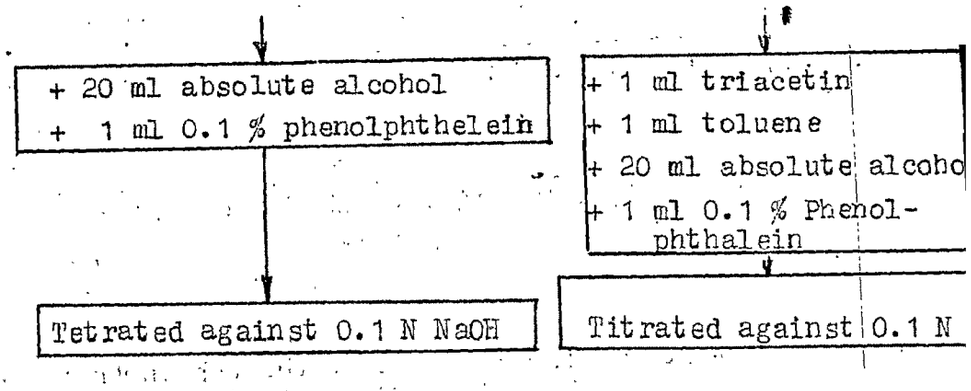
iv. 1 N perchloric acid : 11.1 ml of 60 % perchloric acid added with 89.9 ml DW or 9.0 ml of 70 % perchloric acid added with 91.0 ml DW.

v. Alcohol : Ether : Chloroform in 2:2:1 ratio.

360

Fig. A.13 Flow Chart for Determination of Lipase Activity






Preparation of Reagents Used

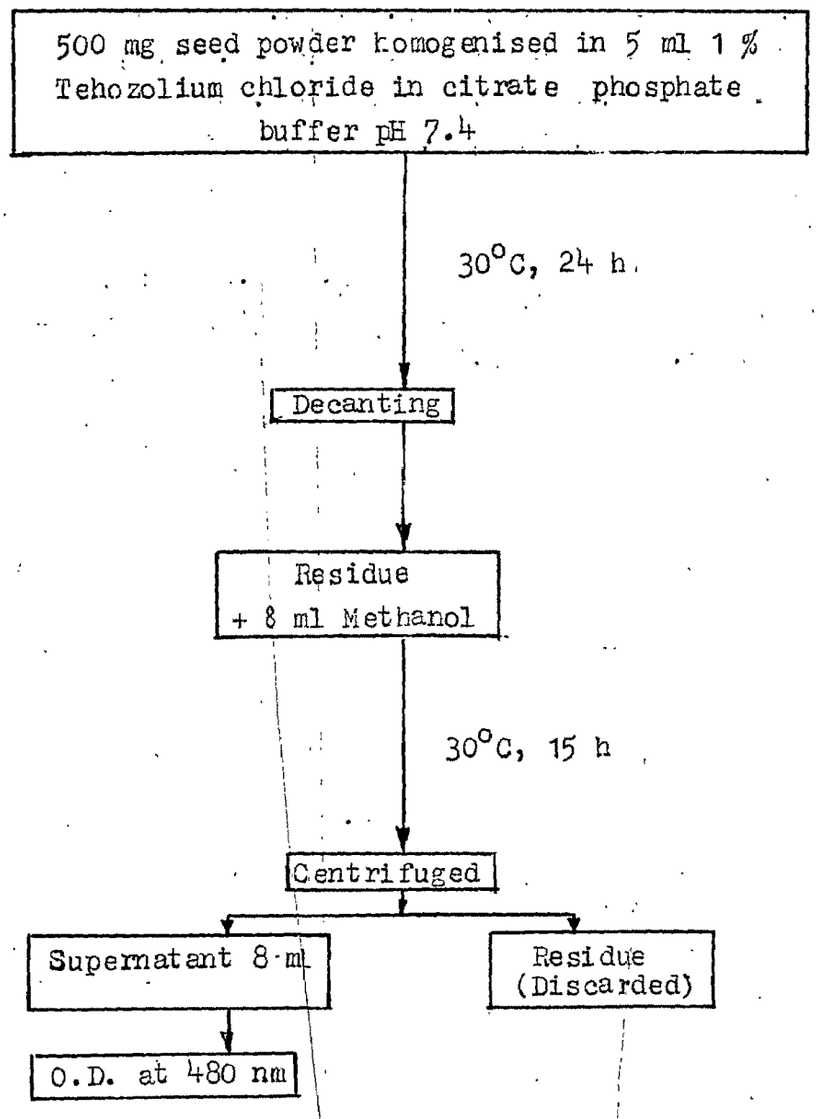
i. 0.1 N NaOH : 4 g NaOH (Mol wt. 40) dissolved in 1 litre DW.

ii. 0.1 % phenolphthalein (alc.) : 100 mg of phenolphthalein dissolved in 100 ml 95 % alcohol.

iii. Citrate-phosphate buffer, pH 8.0 (0.2 M) ; 97.25 ml of disodium hydrogen orthophosphate (Mol wt. 142), 0.2 M (2.83 g in 100 ml DW) added to 2.75 ml of citric acid (Mol. wt. 210), 0.1 M (2.10 g in 100 ml DW).

361

Fig. A.14 Flow Chart for Determination of Dehydrogenase Activity




(For Blank : Pure 8 ml methanol was taken)

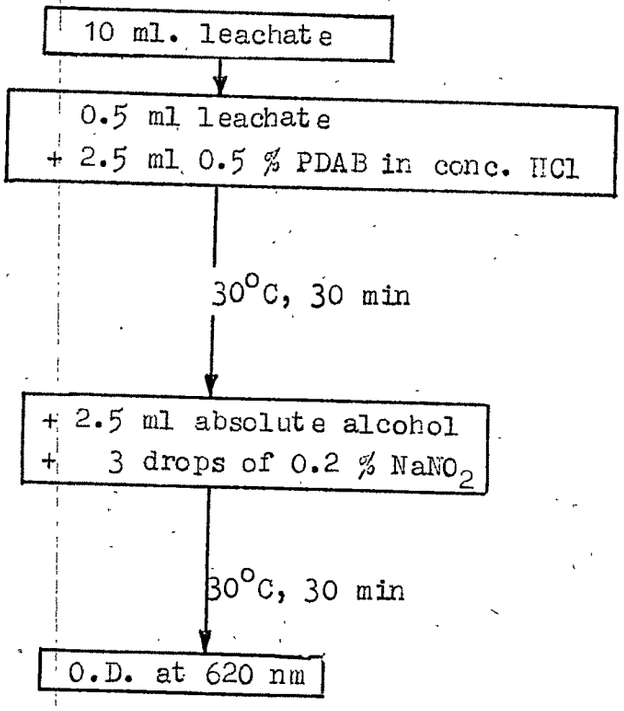
Preparation of Reagents Used

i. Citrate phosphate buffer, pH 7.4 : 91.85 ml of disodium hydrogen orthophosphate (Mol wt. 142), 0.2 M (2.83 g in 100 ml DW) added to 9.15 ml of citric acid (Mol wt. 210), 0.1 M (2.10 g in 100 ml DW).

ii. 1 % TTC in citrate-$PO_4$ buffer pH 7.4 (0.2 M) : 1 g Tetrazolium chloride dissolved in 100 ml of citrate-$PO_4$ buffer (0.2 M) pH 7.4

362

Fig. A.15 Flow Chart for Determination of Tryptophan




Preparation of Reagents Used

i. 0.5 % PDAB (Para-di-amino-benzaldehyde) in conc HCl : 50 mg of dye were dissolved in 10 ml conc HCl.

ii. 0.2 % sodium nitrite : 20 mg of sodium nitrite were dissolved in 10 ml DW.