4.2 Preparation of a wheat germ extract

It turns out that only in the Spring is wheat germ suitable for preparation of active extracts. This may have to do with a requirement for freshly harvested material. Anyway, write to General Mills, Minneapolis, MN in March or April and they will send a pound of fresh wheat germ from their Vallejo, CA plant. It needs to be "floated" in an organic solvent cocktail, and the dried floated material can be either stored in airtight tubes at -80øC or used immediately for preparation of an extract that can be stored as frozen aliquots at -80øC.

Flotation

Wear rubber gloves during all steps and do everything in a fume hood.

1. Mix 600 ml carbon tetrachloride and 240 ml cyclohexane until no more Schlieren mixing lines are visible. This ratio may have to be altered by the addition of relatively more carbon tet to obtain optimum separation of embryo from endosperm.
2. Stir in approximately 40 grams of WG into the above mixture.
3. Allow to settle for a few minutes until good separation is had.
4. Pour off floating wheat germ into Buchner funnel.
5. Allow this wheat germ to dry under fume hood for half an hour.
6. Filter the remaining sedimented wheat germ/carbon tet/cyclohexane to recover the solvent for reuse.
7. Repeat cycles of the above until you have prepared as much as you want. Weigh the air-dried material, pour into 15 or 50 ml falcon tubes, seal with parafilm and store at -80øC, essentially indefinitely.
8. It is a good idea to first optimize the carbon tet/cyclohex ratio on a smaller scale, i.e. with 150 ml carbon tet, 60 ml cyclohexane and 10 gram batches of WG. Once you have found a ratio that gives you about 20% of the total material floating, you are set to scale up.

4.2 Preparation of WG

Preparation of extract from floated material either fresh or stored at -80øC

1. Grind 6 grams in ice cold homogenization buffer in a baked, ice cold, small mortar and pestle as follows: add 3 ml and grind for 30 sec, add another 3 mls grind 60 sec, add 4 mls grind 1-2 min.
2. Transfer the paste (consistency of baby food) using a baked spatula into a 15 ml corex tube.
3. Spin 23 K X g for 10 min, remove supernate carefully and re-spin at 23 K X g for 10 min.
4. Apply the supernate to a G-25 centrifuge desalting column equilibrated in column buffer and desalt in the usual manner (pre-spin column at 1000 rpm; spin through sample at 1100 rpm). Re-spin desalted sample at 23 K X g rpm, for 15 minutes, aliquot and freeze in liquid N2 in 1 ml aliquots and store at -80øC.

IT IS EXTREMELY IMPORTANT TO WORK FAST AND USE EXCLUSIVELY BAKED

GLASSWARE AND WORK AT 4øC. The entire procedure should be finished within 1 hour.

Homogenization buffer

40mM Hepes pH 7.6	4mL 1M
100mM KAc	2.5mL 4M
1mM MgAc	0.1mL 1M
2mM CaCl2	0.2mL 1M
4mM DTT	0.4mL 1M
H2O	92.8 mL

Column buffer

40mM Hepes pH 7.6	4mL 1M
100mM KAc	2.5mL 4M
5mM MgAc	0.5mL 1M
4mM DTT	0.4mL 1M
H2O	92.6 mL