RESTRICTED

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REDUCTION OF SMOKE MUTAGENICITY BY LOWERING THE PROTEIN CONTENT OF TOBACCOS

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Proteins are universal constituents of living cells and the content in growing tobacco plants can be as much as 35% of lamina dry weight (1). Levels in cured tobacco are generally much lower as the concentration falls in the period of leaf maturation (see Figure 1) and again during curing. The content in cured tobacco is usually expressed as protein nitrogen: average values would be 0.9% for flue-cured and 1.5% for burley. Equivalent protein figures are 5.6% and 9.4% respectively. Protein nitrogen as determined on tobacco is, more accurately, acid-insoluble nitrogen - the extent to which it all represents protein is uncertain but is probably >90%.

Much recent interest in protein from tobacco has centred on its potential use as a nutrient of high value. For this purpose, studies have shown that to obtain good yields and extractability the tobacco must be harvested well before normal maturation in readiness for curing. As a result, leaf residue remaining after protein extraction has few of the attributes associated with quality tobaccos.

The residual protein content of cured tobacco is associated with several negative attributes of smoke composition and quality. Of particular concern is the claim by JTS that it is a major precursor of the compounds causing mutagenic activity in tobacco smoke condensate - the amino carbolines (2). Pyrolysis studies on individual amino acids have shown the formation of cyanide (3) and it is likely that it is also produced during the combustion of protein. Higher degrees of correlation have been found for total nitrogen than alkaloids, amino acids or nitrate with cyanide, benzo(α)pyrene, phenols and tar (4). The main extra factor in "total nitrogen" would be protein.

In view of the possible major significance of tobacco protein in relation to smoke biological activity, a programme of work is proposed with the objective of confirming its effects and establishing methods for its removal or reduction in tobacco. The initial phase of the programme would be divided into two projects with separate objectives.

1. To validate the claimed relationship between tobacco protein content and smoke mutagenic activity.

   This would involve the selection of tobaccos with different protein contents for cigarette manufacture to a common specification for subsequent evaluation by the Ames test.

2. To examine feasibility of reducing protein level, using known or experimental treatments.

It must be assumed that most known techniques for reducing protein concentrations or removing from tobacco will affect other components of the tobacco also. It would, therefore, be desirable to produce samples processed in different ways in order to confirm that the observed effect was due to change in protein concentration and not from other incidental effects.

Comparison of the following known treatments is proposed:

(a) Removal by precipitation from HLC liquid fraction or Cylproc treatment - dependent on samples of appropriate materials being produced from North America, comparison with their own controls.

(b) Proteolytic enzyme treatment applied to cured tobacco to produce approximately 50% reduction in protein N and increase in soluble N.

(c) Assessment of effects of sugars and acids as casing treatments, to interact with protein and possibly modify combustion of the protein.

Treatments (b) and (c) could be undertaken here and would yield tobaccos for cigarette manufacture and subsequent mutagenic activity assessment.

Evaluation of (a) would be dependent on samples of HLC being available from the USA, or on the progress of further work to increase the extraction rate with Cylproc treatments.

A further exploratory area which requires consideration is to examine selective extraction techniques which might be applied to cured tobaccos.

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3. Chotyk, O.T. & Schlotzhauer, W-S. 1973. Beit. Tabak 7, 165.

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Tobacco Protein and Smoke Mutagenic Activity : Proposed Outline

Programme of Work and its Interactions.

Phase 1.

Objective : To validate the hypothesis that mutagenic activity of smoke is directly related to the protein content of the tobaccos.

Activity : Examination of cigarettes made from tobaccos differing in protein content, using the Ames test to measure mutagenic activity.

Main staff involved : Dr. E.D. Massey, Dr. G.A. Few.

Timing : 1982 - 1983.

Phase 2.

Objective : To establish whether increases in proteins, amino acids or sugars, by addition to the blend, cause changes in smoke mutagenic activity.

Activity : Preparation and testing of a range of cigarettes with varying ratios of nitroganous and carbohydrate components obtained by selected additions to the blend tobaccos. Where necessary, isolation of tobaccos - specific compounds may be required in order to examine their effect.

Main staff involved : Dr. E.D. Massey, Dr. G.A. Few.

Timing : 1983.

Phase 3.

Objective : To establish, by laboratory scale trials, routes to processes for reducing the protein content of tobacco.

Activity : Examination of possible techniques for reducing the protein content of tobacco, including enzymes solvents and different processes such as HLC, Cylproc.

Main staff involved : A.N. Other + CONTRACT RESEARCH

Timing : 1983 - 84.

Phase 4.

Objective : To produce cigarettes using tobaccos reduced in protein content by one or more of the techniques identified in Phase 3.

Activity : Application of processes to larger quantities of tobacco for cigarette manufacture.

Main staff involved : A.N. Other + Process Staff.

Timing : Late 1983 - 84.

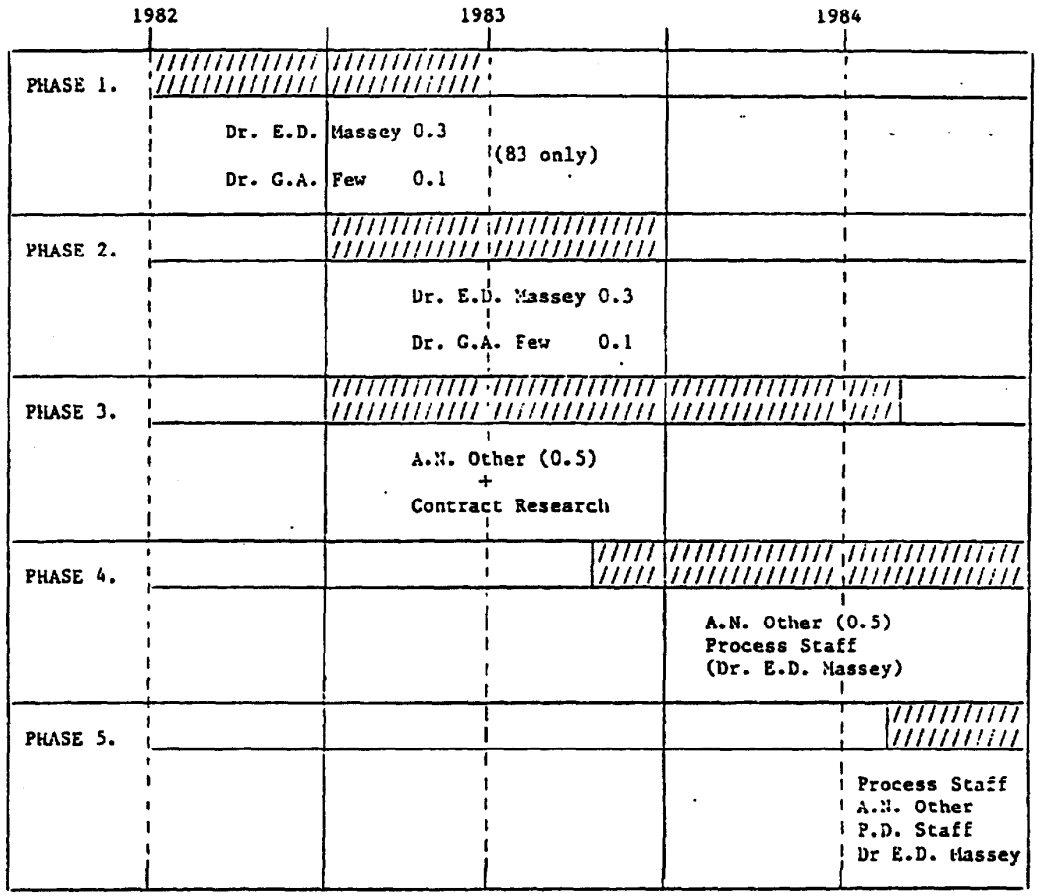
Phase 5.

Objective : To develop commercially acceptable cigarettes with reduced smoke mutagenic activity.

Main staff involved : Dr. E.D. Massey, A.N. Other, Process staff, Product Development staff.

Timing : Late '84 onwards dependent on progress of 3 and 4.

MAIN WORK ACTIVITIES AND PRINCIPAL RESEARCH INVOLVEMENTS.



FLOW DIAGRAM

Work Phases and Interactions, Ancillary/Support Service Requirements.

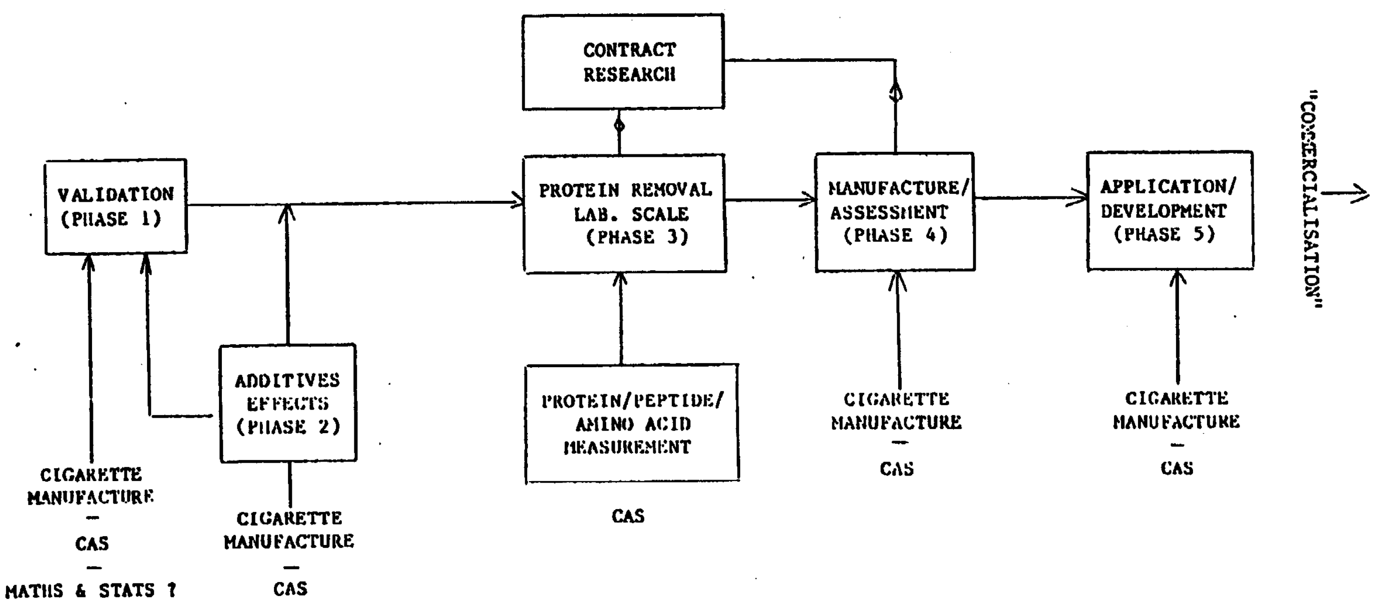
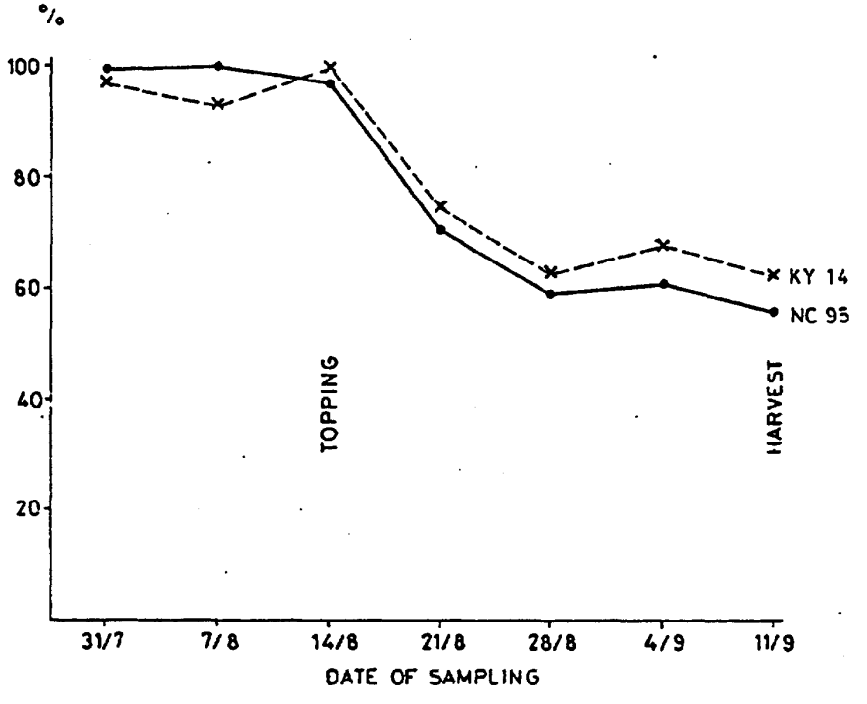


FIG. 1

RELATIVE PROTEIN CONTENT IN RELATION TO TIME OF GROWTH



DATA DERIVED FROM INFORMATION IN REF 1