Six-month report to programme management committee
(Food Link)

September 2003

FQS-Link project

Project Number: FQS12

Project Title: Rapid Analytical Systems for Raw Produce Quality and Safety Attributes

Project start 01-02-2001
Project end 31-01-2004

Elapsed time 30 months

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1. Project objectives

To investigate the scientific and technological issues in the implementation of rapid sensor instrumentation for organophosphorus (OP) residues in raw food products, as a model system for the further development and extension to other analytes.

2. Project progress

Current progress is shown on the attached Gantt chart. This chart shows completed work and progress compared with the original project plan. Overall, 89% of the project work is complete with 87% of the project time elapsed.

3. Milestones for six month period

No milestones were due for completion during this six month period.

4. Research report

Workpackage 1(Acetylcholinesterase enzymes)

Task 1.5 is active to the end of the project. On going experiments have shown that the enzymes are stable when immobilised to the carbon electrodes. The buffer system used appears to have an effect on the stability of the different mutant enzymes. Despite this fact, for simplicity, a phosphate buffer system will be used for all the electrodes.

Workpackage 2 (OP extraction)

The extraction system is now characterized with an incorporated oxidation step (Task 2.3). Some minor modifications are being assessed to reduce the reaction time from five to ten minutes to a two minute reaction. This oxidation method uses a gentle reaction which does not for the oxidize one of the target pesticides, Pirimiphos-methyl. Studies showed that the oxidation method caused inhibition of the enzyme on the electrodes. To prevent this, excess sodium hypochlorite would have to be removed and the concentration of alcohol controlled. Studies have also shown that components from the grain extract protected the enzymes on the electrode from the components of the oxidation reaction.

Workpackage 3 (Biosensor arrays)

Task 3.2.3 (multi-enzyme calibration) has been completed and inhibition profiles have been demonstrated for all the target pesticides against all the enzymes. This showed that it should be possible to differentiate between the different target pesticides using the six biosensors. Plans for large production runs of biosensors have been made to supply the instrument during the evaluation phase of the project.

Task 3.3.2 has been extended to the end of the project as this was deemed the most appropriate phase of the project for programming the neural network. This reflected the discussions held in previous Project Management boards. Pattern recognition software has been programmed using data from the laboratory inhibition studies. There was insufficient data to show any conclusive pattern recognition by the software. More data is being collected

and final programming of the neural network will take place during the evaluation phase in an intake laboratory.

Task 3.3.3 has now been deleted as it is no longer required.

Task 3.5 (biosensor optimisation and modification) is ongoing.

Workpackage 4 (Instrumentation)
This workpackage is complete apart from one task which runs to the end of the project (Task 4.5.2 – field evaluation). This task is now been incorporated into workpackage by, see below.

Workpackage 5 (Evaluation)
This workpackage has been rewritten to take compensate for problems with the oxidation step, supply of biosensors and fluidics on the instrument. The evaluation will now take place in an analytical laboratory at Weetabix until November 2003. Following this, the instrument will be sent to an intake laboratory at RHM Technology for final evaluation. The instrument will be sent to CCFRA for assessment of fruit and vegetable applications.

The instrument has been in place in the analytical laboratory (Task 5.1.1) but required some reconfiguration of the fluidics and manifold, this had to be performed at Jenway. It is due back at Weetabix in the middle of September. Therefore, there has been a delay in the analytical evaluation.

5. Project changes

Workpackage 5 is now being lead by Derek Finnegan of Weetabix.

6. Publications

Not refereed

I. R Luxton. Pesticide Biosensors. FoodLink News, No 35, June 2001.

II. R Luxton. Development of a new rapid analytical system for raw produce. Food Safety Express, vol. 2, issue 3, September 2001.

III. A presentation was given to the HGCA reviewing committee, November 2001.

IV. CCFRA publish a yearly summary of current research projects and will include a one-page summary of this Link project. The summary will be circulated to approximately 1600 companies.

V. A presentation was given to the HGCA reviewing committee, November 2002.

VI. R Luxton. Rapid pesticide sensor. FoodLink News, No 41, December 2002.

Refereed

I. A poster was presented at two different conferences, it had the same tile and same authors but slightly different content. It was presented at: Biosensors 2002, Kyoto, Japan and Electrochem 2002, Preston, UK

II. Studies towards an amperometric biosensor array to measure organophosphate residue concentrations in raw produce. R. Wedge, J.P. Hart, J.L. Marty, D. Fournier & Paul Millner

III. A poster will be presented at Electrochem 2003: Studies towards an amperometric biosensor array to measure organophosphate residue concentrations in raw produce.

IV. A poster will be presented at an international biosensors meeting, being held at Marrakech, in October 2003.

V. A poster presentation: Immobilization and stabilization of AchE on carbon electrode in biosensors for pesticide detection.

7. PhD students employed

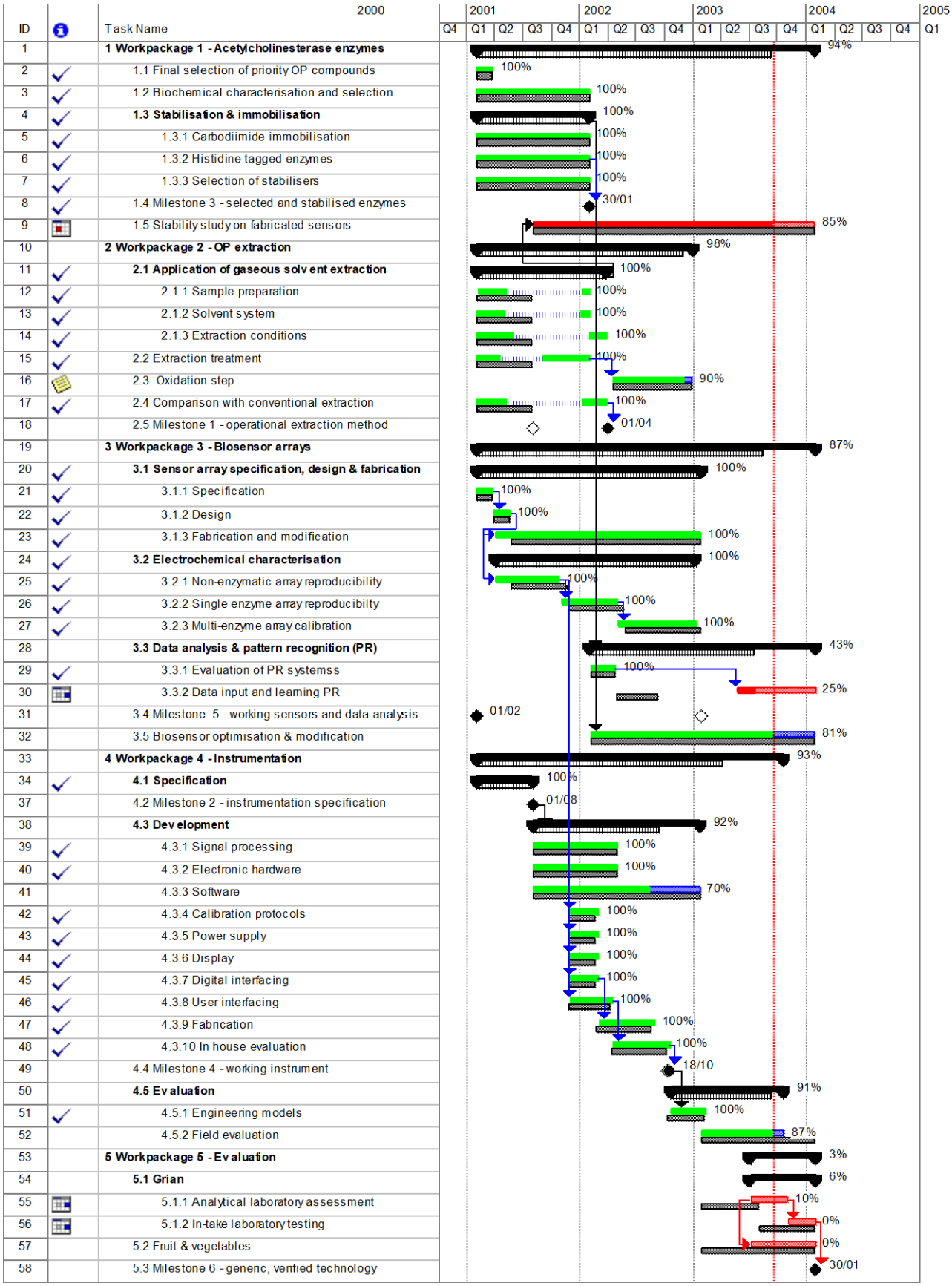
No PhD students are employed on this project.

8. Exploitation report

The Exploitation Committee has been unable to meet in the last six months but the position regarding the supply of Phytosol solvent has been clarified in so much that Advanced Phytonics are happy to supply the solvent but at present the cost of this is still unknown.
A number of enquiries have been made into the use of the technology for measuring pesticides in other applications such as tea and spices. IPR ownership is still to be resolved and will be addressed at the next Exploitation Committee meeting. It is hoped that the instrument will be demonstrated at Grain 2003 in November.
A new project proposal will be submitted to DEFRA building on the technology developed in this project to measure mycotoxins using antibodies in the biosensors.

9. Project Monitoring Officer's comments

Tracking Gantt Chart as of: 15-09-2003



ID	Task Name
1	**1 Workpackage 1 - Acetylcholinesterase enzymes**
2	1.1 Final selection of priority OP compounds
3	1.2 Biochemical characterisation and selection
4	**1.3 Stabilisation & immobilisation**
5	1.3.1 Carbodiimide immobilisation
6	1.3.2 Histidine tagged enzymes
7	1.3.3 Selection of stabilisers
8	1.4 Milestone 3 - selected and stabilised enzymes
9	1.5 Stability study on fabricated sensors
10	**2 Workpackage 2 - OP extraction**
11	**2.1 Application of gaseous solvent extraction**
12	2.1.1 Sample preparation
13	2.1.2 Solvent system
14	2.1.3 Extraction conditions
15	2.2 Extraction treatment
16	2.3 Oxidation step
17	2.4 Comparison with conventional extraction
18	2.5 Milestone 1 - operational extraction method
19	**3 Workpackage 3 - Biosensor arrays**
20	**3.1 Sensor array specification, design & fabrication**
21	3.1.1 Specification
22	3.1.2 Design
23	3.1.3 Fabrication and modification
24	**3.2 Electrochemical characterisation**
25	3.2.1 Non-enzymatic array reproducibility
26	3.2.2 Single enzyme array reproducibilty
27	3.2.3 Multi-enzyme array calibration
28	**3.3 Data analysis & pattern recognition (PR)**
29	3.3.1 Evaluation of PR systemss
30	3.3.2 Data input and learning PR
31	3.4 Milestone 5 - working sensors and data analysis
32	3.5 Biosensor optimisation & modification
33	**4 Workpackage 4 - Instrumentation**
34	**4.1 Specification**
37	4.2 Milestone 2 - instrumentation specification
38	**4.3 Development**
39	4.3.1 Signal processing
40	4.3.2 Electronic hardware
41	4.3.3 Software
42	4.3.4 Calibration protocols
43	4.3.5 Power supply
44	4.3.6 Display
45	4.3.7 Digital interfacing
46	4.3.8 User interfacing
47	4.3.9 Fabrication
48	4.3.10 In house evaluation
49	4.4 Milestone 4 - working instrument
50	**4.5 Evaluation**
51	4.5.1 Engineering models
52	4.5.2 Field evaluation
53	**5 Workpackage 5 - Evaluation**
54	**5.1 Grian**
55	5.1.1 Analytical laboratory assessment
56	5.1.2 In-take laboratory testing
57	5.2 Fruit & vegetables
58	5.3 Milestone 6 - generic, verified technology