

**R&D REPORT**

**NO. 16**

**Physical Techniques for Establishing  
the Authenticity of Rice**

**June 1995**



**Campden & Chorleywood**  
Food Research Association





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R&D Report No. 16

Project No. 21068

## Physical Techniques for Establishing the Authenticity of Rice

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June 1995

Final Report on MAFF ANO 634

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## SUMMARY

The incentive for adulteration of rice by dishonest traders has been recognised by MAFF. Particular authenticity issues concern the adulteration of Basmati rice with other types, and of US long grain rice with rice originating from other countries. Work was therefore commissioned in a previous study to develop methods for detecting the authenticity of Basmati rice. The purpose of the current project was to develop further and validate these methods, and to establish their suitability to US long grain rice authenticity in addition to Basmati.

The methods developed were Image Analysis, Rapid Visco-Analysis (RVA), and Near Infrared (NIR) spectroscopy. These have been used to test a database of about 100 samples of rice of various types to evaluate their ability to discriminate them.

Image Analysis involves the cooking and automatic size and shape measurement by computer, of individual rice grains. The project has confirmed previous findings that this is a highly successful test for Basmati authenticity, subject to introduction of additional calibration procedures.

The RVA test involves the heating and stirring of a mixture of rice flour and water. Measurement of the viscosity (resistance to stirring) after various time intervals established in the first study provides the ability to distinguish many of the rice samples. The current project demonstrated that the method is not as good as image analysis for identification of Basmati, but achieves some (although imperfect) discrimination of US long grain rice, of which image analysis is incapable. Although pure samples can be distinguished, the method would be unsuitable for identification of mixed samples, due to the use of a flour rather than individual grains.

NIR spectroscopy is a highly convenient and rapid method widely used for several common analytical tasks, such as moisture determination in various foods. It has also been applied to the authenticity of orange juice. It was tested for suitability to rice partly because of the convenience a successful test would offer, and the previous study had suggested that it could provide some ability to distinguish both rice types of interest. NIR requires careful calibration

against known sample sets, and the calibration previously established was unsuccessful with the new sample set. A new calibration showed an ability to discriminate US long grain rice, particularly if used in combination with image analysis to first reject Basmati rices. However, there is some evidence that the test may simply be detecting systematic moisture differences between the particular samples used in the test.

The project suggests that image analysis can be used to distinguish authentic Basmati rice samples from almost all the potential adulterants tested. RVA can be used to discriminate US long grain samples from those of other origins, although with less certainty. Further testing will be necessary to establish whether the apparent success of NIR is in fact only an accident of moisture differences between the samples tested. If its success is genuine, it could provide an alternative to RVA for US rice authenticity determination, with even better results if used in combination with image analysis.

## **CONTENTS**

### **Page No..**

#### **SUMMARY**

<b>1. INTRODUCTION</b>	<b>1</b>
<b>2. MATERIALS AND METHODS</b>	<b>4</b>
2.1 Sample collection	4
2.2 NIR transmittance	4
2.3 Rapid Visco-Analyser (RVA)	5
2.4 Image Analysis	9
<b>3. RESULTS</b>	<b>15</b>
3.1 Introduction	15
3.2 Image analysis	15
3.2.1 Basmati	15
3.2.2 US	16
3.3 NIR	16
3.3.1 Basmati	16
3.3.2 US	17
3.4 RVA	17
3.4.1 Basmati	17
3.4.2 US	17
3.5 Combinations of methods	24
3.5.1 Basmati	24
3.5.2 US	24
<b>4. DISCUSSION AND CONCLUSIONS</b>	<b>25</b>
4.1 Image analysis	25
4.2 NIR	25
4.3 RVA	25
4.4 Combination of methods	25
4.5 Basmati	26
4.6 US	26
4.7 Further data analysis	26
<b>REFERENCES</b>	<b>28</b>
<b>ACKNOWLEDGEMENTS</b>	<b>28</b>
<b>Appendix 1. Database of rice samples</b>	<b>29</b>





## 1. INTRODUCTION

Long grain accounts for around 160,000 tonnes out of a total UK rice market of 250,000 tonnes with a retail value of £200 million. Basmati, a class of rice grown in the Punjab region of India and Pakistan, is recognised as a market sector in its own right with about 20% of total rice sales. Basmati can only be grown once a year with a yield half that of other rices; its eating quality cannot be duplicated by growing the same seed in other regions. As a consequence of its scarcity and its popularity in the UK, Basmati commands a high price compared to other rices. The incentive for adulteration was recognised by the Ministry of Agriculture, Fisheries and Food (MAFF) who in 1990 commissioned work at FMBRA (now CCFRA) to develop methods for determining the authenticity of Basmati rice, and by the Grain and Feed Trade Association (GAFTA) whose Rice Standards Section introduced a Code of Practice in January 1992.

The work commissioned by MAFF on the separation of rice proteins by polyacrylamide gel electrophoresis (Scanlon *et al.*, 1991) was only partially successful. For other crops, notably wheat and barley, it is well established practice to identify varieties by biochemical analysis of gene products specific to the seed, particularly the nitrogen storage proteins from the endosperm, known as gliadins or hordeins. These have the merit of being abundant and having many allelic forms that are readily separated into fingerprint patterns by gel electrophoresis or high performance liquid chromatography (HPLC). Unfortunately, rice has adopted a different nitrogen storage mechanism, as have oats, in which prolamine proteins (gliadin type) play a minor role. The abundant glutelin proteins apparently cannot tolerate the many slight mutations evident in the prolamines of most cereals; hence the major rice proteins do not give fingerprint patterns in gel electrophoresis or HPLC. The previous work attempted to derive patterns from the minor prolamine fraction of rice proteins. However, this requires very high resolution and sensitivity which is difficult to reproduce in routine operation. Furthermore, Basmati is defined by origin as well as genotype. Gene-specific components would be expected to be consistent across samples of the same genotype from different origins. As a result of these considerations, further research focused on objective physical methods.

CCFRA has developed three tests (Image Analysis, Rapid Visco-Analyzer (RVA) and near infrared spectroscopy (NIR) ) for evaluating the authenticity of Basmati rice (Osborne *et al.* 1993b). Each of the tests is based on a physical measurement which is characteristic of both the genetic make-up of Basmati and of the environment under which it was grown. The tests have been applied to approximately 95 (see 2.1 for details) samples of milled rice of known provenance to provide a database against which samples of unknown provenance may be compared.

Near infrared spectroscopy is widely used for the compositional analysis of food and has previously been applied to testing the authenticity of orange juice. In this method, NIR spectra are measured by transmittance through individual rice grains using the Tecator Infratec 1225 Grain Analyzer fitted with a single seed adaptor. The spectra are reduced to the first four principal components and discriminant analysis is used to derive a rule for classification for rice types (Osborne *et al.* 1993a). With two groups, Basmati and other, this procedure results in a set of weights that are applied to the spectral data to calculate a single number, the linear discriminant function (LDF) for each sample. The approach was able to classify correctly all the 17 Basmati samples in the database, but incorrectly classified 20 of the remaining 100 as Basmati also. This it would appear that the possible verdicts based on this test alone are 'definitely non-Basmati' or 'possibly Basmati'.

Image Analysis of cooked rice exploits the characteristic shape change which Basmati rice undergoes during cooking. Unbroken grains from the sample are cooked then placed on a light box. A video camera is used to capture their silhouette images for computer processing. First, the computer makes a correction for background lighting, then it thresholds the image to identify dark regions and stores those measurements consistent with rice grains. For each grain, several size and shape measurements are combined into a single value (Rice Parameter 3, or RP3), which is greater for non-Basmati than for Basmati grains, and can be used for discriminant purposes. In a previous study (Osborne *et al.*, 1993b), grains were cooked loose in a beaker. For the purposes of the present study, grains were held in individual holes in a special tray during cooking in the beaker, allowing image analysis data to be combined with a previously determined NIR spectrum for each grain, with the aim of improving the discrimination.

The Rapid Visco-Analyzer (RVA, manufactured by Newport Scientific, Sydney, Australia) measures the electrical energy required to maintain a constant stirring speed of a starch-water dispersion as it is taken through a profile of temperature changes with time. The RVA is controlled by a computer which presents the measurements in the form of a plot of viscosity against time referred to as a hot pasting curve. The shape of this curve is dominated by the sequential gelatinisation, swelling, shearing and gelling of the starch component of the sample. Two parameters from the curves have been identified as being useful for discriminating between different types of ground rice. These are known as D70 (the rate of increase of viscosity at a particular time during the initial swelling of the starch granules) and V120 (the viscosity at a time point early in the cooling-induced gelling of the hot paste resulting from disruption of the swollen starch granules by mechanical shear and/or enzymic hydrolysis). A two-dimensional plot of D70 versus V120 for the 98 samples in the database was interpreted in terms of the type of origin of the samples. The RVA method cannot be applied to single rice grains and is not quantitative, but it can be used to compare the intact and broken sub-fractions of a grain sample. It should be regarded as complementary to other techniques.

The three techniques NIR, Image Analysis and RVA are complementary to one another in that potential adulterants indistinguishable from authentic Basmati by one technique may be distinguishable by a different technique. No one of the three techniques alone is 100% reliable for establishing the authenticity of Basmati rice, although the image analysis comes close. However, it may be possible to achieve even better results by combining the data from the three techniques into a single discriminate score.

A second problem in the area of rice authenticity concerns authenticity of origin, in particular, origin of rice labelled as 'US' or 'product of USA'. This issue is also covered by the GAFTA code of practice but, as yet, there is no objective method available for establishing the authenticity of US long grain rice. The existing database contained 32 samples of authentic US long grain rice and the new data collected for this project included 43 such samples. Thus it is possible to use these data to investigate the feasibility of establishing the authenticity of US long grain rice using the three techniques already established for Basmati

rice. If it proves possible to achieve this then the data from the three techniques can be combined in a similar way to that proposed for Basmati.

This project therefore aimed to develop and validate methods using three physical techniques (Near Infrared Spectroscopy, Image Analysis and Rapid Visco-Analyser) for establishing the authenticity of Basmati and of US long grain from other long grain rice types.

## **2. MATERIALS AND METHODS**

### **2.1 Sample collection**

A database of 132 rice samples was collected for the previous project (Osborne *et al.*, 1993b). Of these samples 98 (17 Basmati, 32 US, 49 other) were studied by Image Analysis and RVA, and 117 (17 Basmati, 32 US, 68 other) were studied by NIR analysis of single grains. Only 89 samples were studied by all three techniques. This will be referred to as database 1.

A further set of 95 samples (Appendix 1) was collected for this project. This database (database 2) comprised 19 Basmati, 9 Indian non-Basmati, 43 US long grain, and 24 other rice samples. Samples MR94/421-430 were obtained from retail sources in Madras, samples MR94/431 - 436 were provided by the Directorate of Rice Research, Hyderabad and samples MR94/1905 - 1947 were provided by the USA Rice Council. Samples MR95/140 - 155 from the Natural Resources Institute were freshly milled samples of paddy rices that had been used for database 1. All 95 samples were studied by all three techniques.

Each sample of rice had been milled (whitened) prior to receipt at CCFRA.

### **2.2 NIR transmittance**

A Tecator Infratec 1225 Grain Analyzer was used to measure the NIR transmission spectra of the 95 new rice samples. This instrument uses a monochromator based on a 50W tungsten lamp and a diffraction grating to scan the spectrum from 850 to 1050nm in increments of 2nm, giving 100 datapoints. The illumination is collimated, shines through the rice grain and is detected on the far side. The NIR instrument used in this study enables spectra to be measured on single grains, with the aid of an accessory. For each sample, 23 grains were

placed in the cassette (Figure 1), each grain being identified by its position, and individual scans were stored. The data were stored on floppy discs in a dedicated format and were transferred to PC discs for analysis. This process could be done by the manufacturers, Tecator, but problems with creating the datafiles necessitated verification at the time of creation. The necessary proprietary software was unavailable. Therefore, data were transferred to a PC computer over a serial link, using the 'Kermit' terminal emulation program. A program was written at CCFRA to convert the original binary files to ASCII for analysis.

### **2.3 Rapid Visco-Analyser (RVA)**

The procedures used to determine the pasting behaviour of the rice samples were essentially the same as used in the previous MAFF project (Osborne et al., 1993b), and these in turn were broadly the same as those that have subsequently become AACC Approved Method 61-02 (American Association of Cereal Chemists, 1995). Unfortunately, in the course of participation in the international ring tests conducted by the manufacturers of the RVA (Newport Scientific Pty. Ltd.), in order to establish the Approved Method, it transpired that the particular instrument at CCFRA was significantly out of calibration electromechanically. The manufacturers had not previously been aware that the instruments were liable to drift to such an extent. As described below, a calibration method has now been instituted to maintain comparability of the RVA data, but the unfortunate consequence has been that the RVA data of the previous MAFF project cannot be combined with that from the present work.

The differences in procedure were minor, but possibly significant. Firstly, the grinding of the rice grains was, as before, done with the KT hammer mill (fitted with a 0.5mm screen, as for wheat samples being ground for the Hagberg Falling Number Test), rather than with the specified Udy or Cyclotec sample mills fitted with 0.5mm screens. The method states that use of different grinders can significantly affect the results, and therefore the KT mill was kept for the present work in the hope of maintaining comparability with the results of the previous MAFF project. Secondly, the final constant temperature period of the time-temperature profile used to paste the flours was extended by 0.4 min, a change that could have no effect on the results but which was introduced for the sake of conformity with the AACC Method.

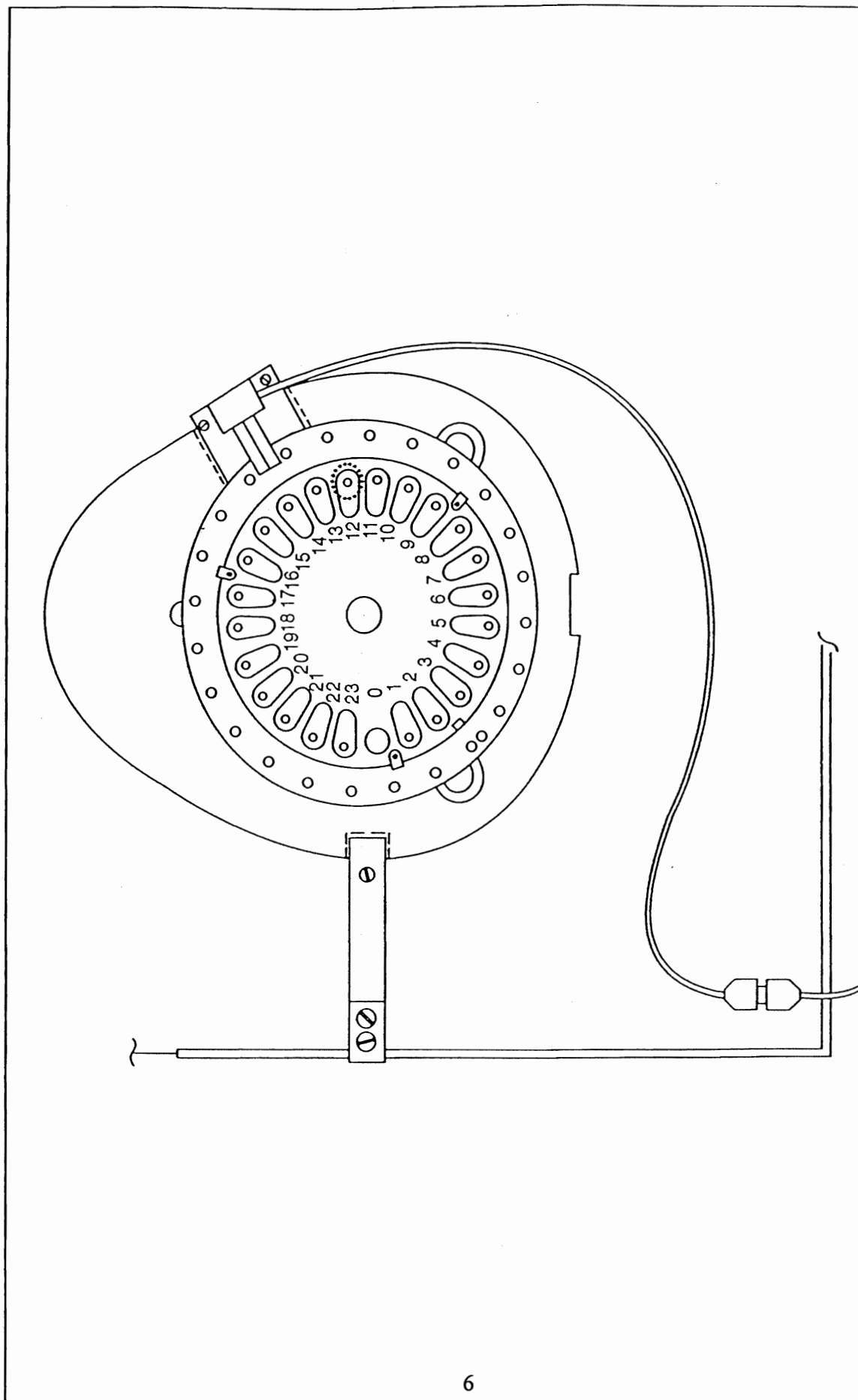


Fig.1 Infratec 1225 single seed adaptor

### *Grinding of milled rice samples*

50g of each rice sample was milled to a flour using a KT hammer mill (Koneteollisuus Oy, Helsinki) fitted with a 0.5 mm screen. Care was taken to open up and vacuum-clean the mill between samples, particularly at the start of work when a 50g portion of long grain rice (retail sample) was milled and discarded, to flush through any traces of wheat flour possibly present in the mill. Ideally, all samples would have been tempered to a standard moisture content, but this was impracticable for a rapid test on such small samples, which were therefore milled as received (typical moisture contents for milled long grain rice are  $12 \pm 1\%$  - personal communication from D.E. Kohlwey, Riviana Foods, Texas).

### *Hot pasting of rice flours*

Hot pasting curves were measured on the Rapid Visco-Analyzer Model RVA-3D under the control of the customised Thermocline and Thermoview computer software run on an IBM PC-compatible computer. At the beginning of each substantial series of measurements, the electromechanical calibration of the instrument was checked by pasting a standard starch sample supplied by the manufacturers, and checking that certain parameters from the pasting curve fell within certain specified limits. If this is not the case then it is necessary to have internal adjustments made to the instrument by the manufacturers or their approved agents (Calibre Control International Ltd., in the U.K.). This has been called for only once between the normal annual overhauls of the instrument.

Portions of water (25.0ml) were pipetted into the disposable aluminium sample canisters supplied with the RVA. Immediately before each duplicate portion (3.00g) of each flour was processed, it was slurried into the water by ten manual plunges of the disposable plastic stirrer. The samples were subjected to the following time-temperature profile, which was as specified in AACC Approved Method 61-02:

Temperature/°C	Time/min
50	0 - start
50	1.0
95	4.8
95	7.3
50	11.1
50	12.5 - end

All the resultant pasting curves were gathered as a file of 199 viscosity values measured at 4 second intervals, and exported into a specially written programme for calculation of the following parameters:

$$D70 = [(\text{mean visc. at points 71-75}) - (\text{mean visc. at points 65-69})] \times 2.5$$

- this is the rate of increase of viscosity in Stirring Number Units (SNU)/min at 4.65 min, calculated as the slope of the chord between one minute average viscosity values at approx. 4.45 and 4.85 min.

$$V120 = \text{average visc. in SNU over points 115 to 129.}$$

- this is the viscosity at approximately 8.1 min, averaged over a one minute period.

Since repeatability of the whole curves was good, the duplicate values of D70 and V120 were averaged for assessment of each rice sample. Earlier work on the sample set of the previous MAFF project (Osborne *et al.* 1993b) had suggested that these two parameters could be used to distinguish Basmati rices from other long grain rices, via the criteria that for Basmati rice  $D70 < 130$ ,  $V120 > 80$  and  $V120 + 20 > D70$ . The other rices failed either or both of these conditions, as did some mixtures of the two types. The two parameters were therefore plotted against one another.

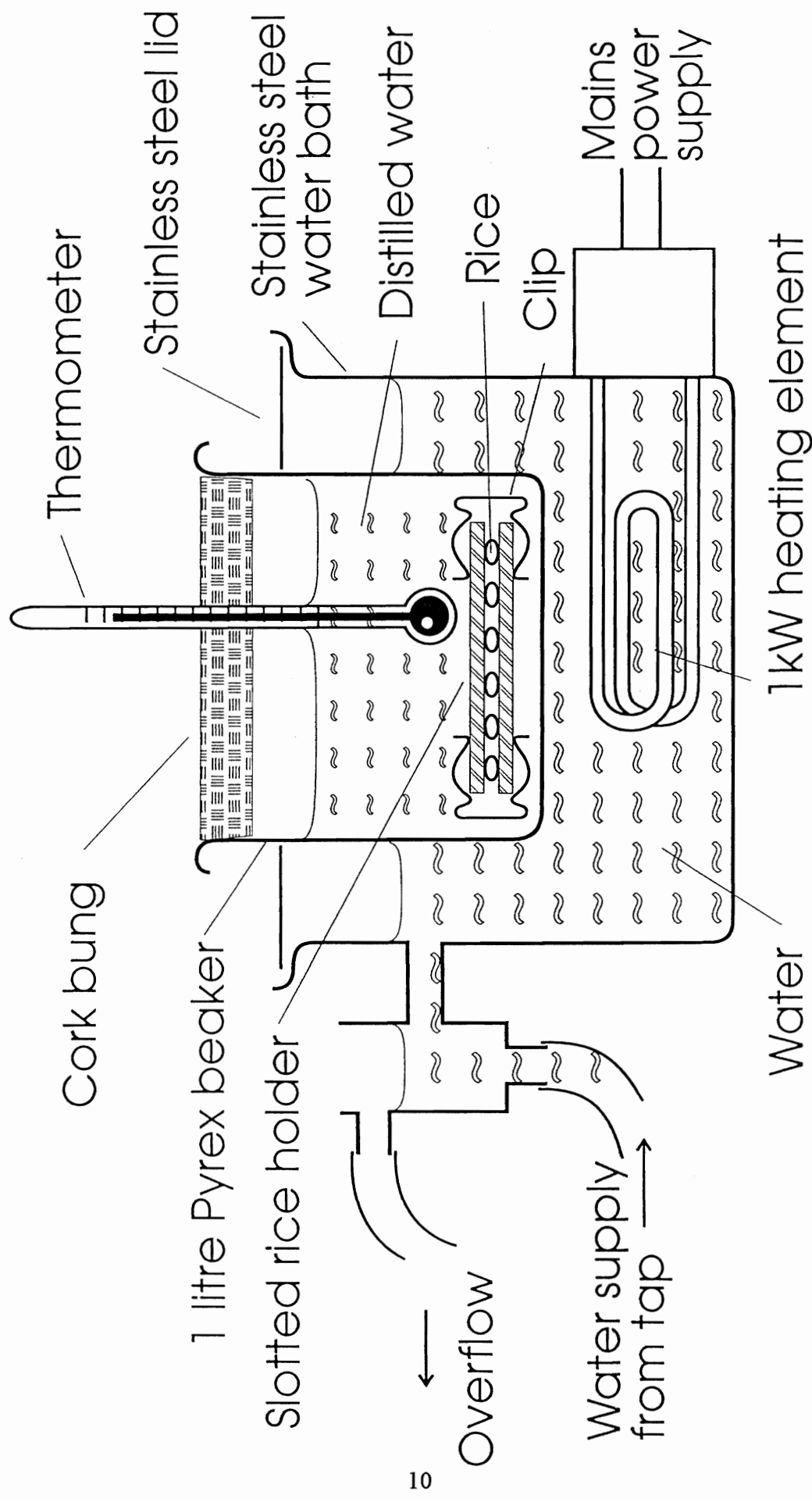


## 2.4 Image Analysis

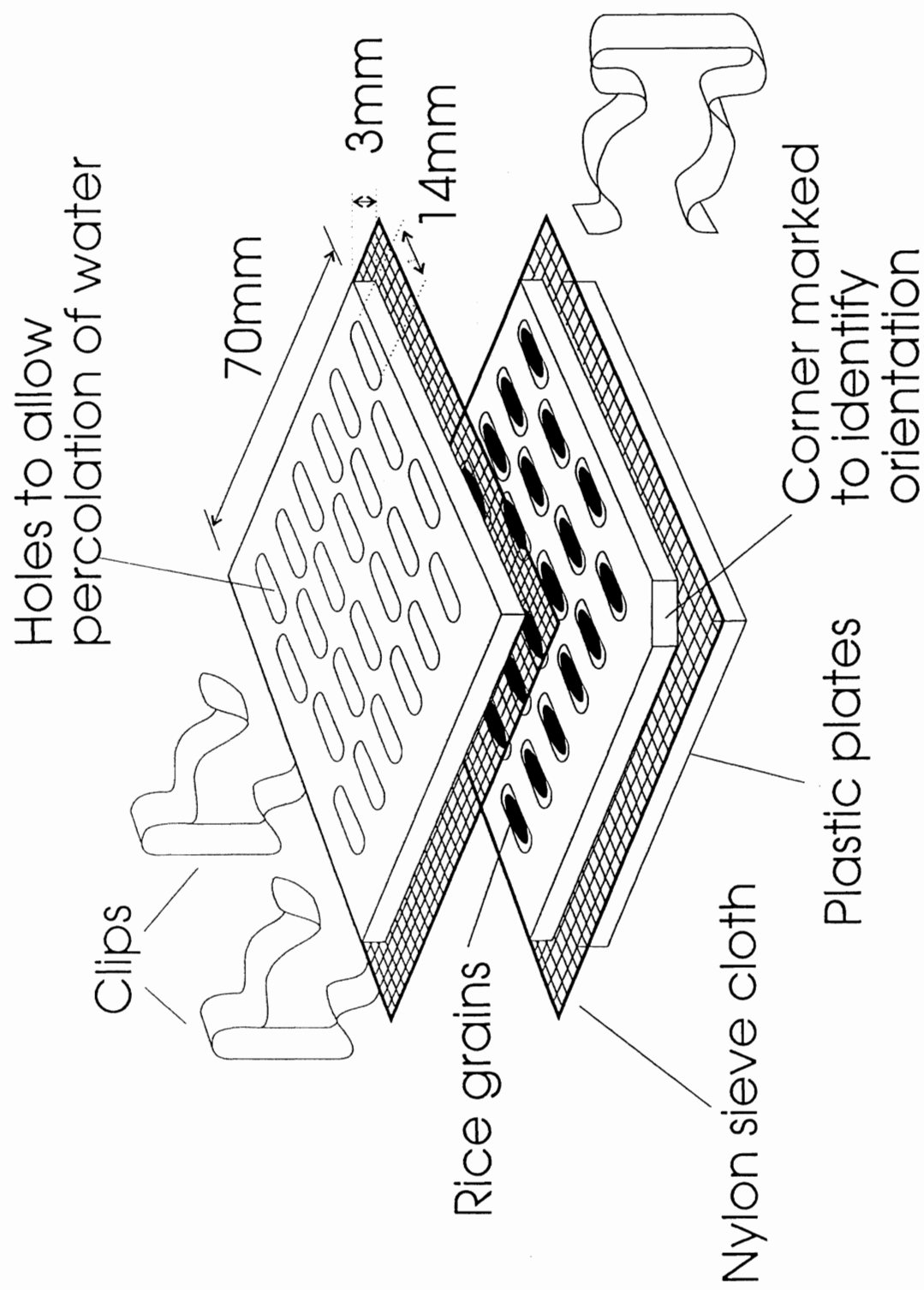
Rice grains were boiled for analysis on the premise that the difference in size and shape of Basmati and other rices is greater when they are cooked than when uncooked. Grains first analysed by single grain NIR were placed in individual holes in a perforated tray, cooked and measured by image analysis.

### *Cooking Procedure*

Figure 2 shows the apparatus used to cook rice grains. A 1 kW boiling water bath (210mm diameter x 125mm height) was filled with tap water and brought to the boil. A 1 litre Pyrex beaker was washed. It was gripped at its rim with a clamp mounted on a retort stand and was suspended in the boiling water, hanging through a circular hole in the lid of the water bath. The beaker was filled with about 600 ml of boiling distilled water. This had been boiled in a plastic kettle which had been used for distilled water only. A cork bung was fitted to the beaker. A thermometer fitted through a hole in the bung was used to measure the temperature of the water in the beaker. Time was allowed for the water in the beaker to reach at least 96°C. It is not possible for it to reach 100°C because heat is transferred from the water bath by conduction through the beaker. This requires a temperature gradient, and so the interior of the beaker is always at a lower temperature than the exterior. While the water was heating, rice grains were prepared for cooking. In a previous study (Osborne *et al.*, 1993b), grains were placed loose in the beaker. However, for comparison with individual NIR spectra in this study, it was necessary to be able to identify grains after cooking. Therefore, they were placed in individual holes in a purpose built plastic tray (Figure 3). The tray had 24 holes sufficient to accommodate the 23 grains held in each carousel of the NIR instrument. The holes had dimensions of 7 mm x 14 mm x 3 mm, designed on the basis of grain measurements in the previous study to be large enough to accommodate any cooked grains without restricting their expansion. The bottom surface of the tray upon which the grains lay was made of nylon sifter cloth with 1 mm square apertures, sufficient to contain the grains but to allow water to flow through easily. This was attached to the plastic by cotton stitching. In initial trials, the cloth was found to fray, and the stitches were therefore covered with araldite epoxy resin to prevent this. The tray was covered with a further piece of sifter cloth and perforated plastic sheet of similar construction, such that each grain was held between two layers of sifter cloth. The grains analysed by NIR were placed in the tray in order such



**Fig 2: Rice cooking apparatus**



**Fig 3: Holder for rice grains during cooking**

that they were identified by their position. The orientation of the tray was uniquely identified by cutting off one corner. When the grains had been loaded, the tray and its cover were held together with three sprung clips. The tray was tipped and tapped gently to ensure that all the grains lay along the length of their holes, allowing space for them to swell during cooking. The tray was immersed in the hot water in the beaker, a timer was started and the bung was replaced. Care is needed to ensure that the operator is not scalded by steam, and protective gloves were employed. The rice was left in the beaker until 10 minutes had elapsed, as in the previous study. The bung was then removed, and the tray of rice removed from the water with tongs. This was washed thoroughly with cold water, and the clips removed. The cover was slid from the main tray to prevent rice sticking to it, as would be the case if it were simply lifted. A paper towel was folded in four, soaked thoroughly in distilled water and placed over the tray. The towel and tray were inverted together and the tray was then lifted carefully while tapping it to ensure that all the grains fell out onto the paper towel in their correct positions. The purpose of the wet paper towel was to prevent the rice grains drying out during the time required to analyse them, thus minimising shrinkage. The grains were immediately subjected to image analysis. About 3 minutes had typically elapsed between removing the beaker from the water and commencing analysis. Grains which had broken during cooking or which had become wedged perpendicular to the axis of the holes, restricting their elongation, were rejected; their identities were noted for later identification in the data files.

### **Measurement**

A JVC CCD camera was mounted above a light box on a photographic enlarger stand. The camera generates an RGB and a PAL output, of which the PAL signal was used. The field of view was approximately 30mm x 40mm. Frames were captured in monochrome (the brightness component of the PAL signal) to a resolution of 512 x 512 pixels x 7 bits using a Solitaire computer manufactured by Seescan Imaging. This computer also performed the analysis of the images.

Immediately before analysis of each sample, an image of the light box was captured, and stored. Grains were then laid out on the light box with their symmetry planes horizontal, such that they did not abut, and such that they did not overlap the edges of the video frame. The

frame was then grabbed and was divided by the previously stored image of the light box to correct for the small variation of lighting intensity across the width of the light box. The fact that a fresh image of the light box was acquired for each sample ensured that the results were insensitive to any long term variation in the brightness of the light box. A binary threshold was applied to the corrected image at a grey level of 118 (full scale is 0 (black) to 127 (white)). Objects darker than the threshold were identified as candidate rice grains, and were measured. Grains are measured and their results stored by the system in order of the position of the left most pixel of their top row in the image. The ordering is in rows from top to bottom, and from left to right within rows. Grains were therefore placed in staggered rows on the light box to ensure that they were analysed in order and that the measurements could be identified against the correct NIR spectra. The ordering of processing was monitored by watching the computer screen to ensure that the intended ordering had been achieved. Any deviations from the intended order were noted and later corrected in the datafiles.

Each grain was measured to determine the following standard measurements:

Circularity	=	$4\pi \text{ Area/Perimeter}^2$
Aspect Ratio	=	Length/Breadth (where length is the longest caliper measurement, and breadth is perpendicular to it).
Area	=	The projected area of the grain ( $\text{mm}^2$ )
Perimeter	=	The perimeter of the grain (mm)
Shortest	=	The shortest caliper width of the grain (mm)
ShortPerp	=	The caliper measurement made in a direction perpendicular to Shortest. This is thus a measure of length.

Objects were considered to be single rice grains if they satisfied the criteria

$$\begin{aligned} \text{ShortPerp} &> 3 \text{ mm} \\ 5 \text{ mm}^2 &< \text{Area} < 40 \text{ mm}^2 \\ \text{Aspect Ratio} &< 6 \end{aligned}$$

Abutting grains, and specks of dust did not satisfy the criteria and their measurements were rejected. The remaining measurements were stored and later transferred to a PC computer for analysis.

Further variables were derived from combinations of those described above and used to discriminate rice types.

Comparison of the data with those determined in the previous study (Osborne *et al.*, 1993b) shows systematic changes in some of the measured variables, and in particular, an increase in the perimeter of grain images without an equivalent increase in area. While this may indicate a reduction in the smoothness of grain surfaces resulting from the modified cooking procedure (ie incorporation of a tray for grains) or from any changes in rice milling procedures between the studies, it is thought that it probably results from an instrumental effect with a decrease in the stability of the images during digitisation. A severe example of this has been noticed occasionally when the horizontal sync signal is lost and the image becomes highly unstable. This represents a distinct and recognisable fault in the image processing computer and measurements affected by this were rejected. The systematic change in measurements between the two studies reflects a less perceptible effect. While it is still possible to discriminate rice samples within either dataset, it will be necessary to implement a calibration and validation procedure to ensure that future measurements remain consistent. A more modern image processing system now available at CCFRA provides more stable images reflecting advances in image digitisation technology. To ensure that the image stability remains consistent, reference objects with well defined perimeters could be measured periodically to ensure that no changes occur over time. A calibration on objects of similar dimensions to rice would also be desirable. If rice grains themselves are used, they should be uncooked to ensure that they have a stable size. Additionally, they should be presented in a consistent manner (eg glued to a transparent rigid base) to remove random errors resulting from changes in orientation.

### **3. RESULTS**

#### **3.1 Introduction**

With two problems (authenticity of Basmati and of US long grain) to study, using 3 techniques either separately or in combination, and two databases, the number of options is large. The analysis to date has considered the measurements only at the level of samples, ie both the NIR and Image Analysis results for single grains have been averaged over all grains in the sample. The general strategy was to examine the three techniques separately first, and then to consider them in combination. In each case the first step was to attempt to validate the existing discriminant rule on database 2. Then, typically, the second step was to seek improved rules using database 2. Approaches here used linear and quadratic discriminant analysis (LDA and QDA), applied to principal components in the case of NIR and RVA, and also studied first and second derivative curves for the NIR and RVA. For the US long grain problem there was no previous discriminant rule, so the analysis began with step 2.

#### **3.2 Image Analysis**

As noted earlier, occasional problems with unstable imaging caused loss of some data. Generally this resulted in measurements for fewer than the planned number of grains; however, for two samples in database 2 (MR95/126 and MR95/142, both Indian non-Basmati) all data were lost, reducing the total number of samples to 93.

##### **3.2.1 Basmati**

First the performance of the derived measurement RP3 was assessed. For simplicity, and because there was no intention to investigate mixtures here, only the mean value of RP3, averaged over all grains measured for the sample, was considered. This is in contrast to previous work where the percentage of grains in the sample exceeding a given value was used as the criterion.

For database 1 all the 17 Basmati samples had RP3 values less than 39, whilst only 6 of the other 81 samples (3 Indian non-Basmati samples and 3 Iranian varieties grown in Italy) had RP3 values below this cutoff.

For database 2 the maximum RP3 value for any of the 19 Basmati samples was 37.1, and only 3 of the other 74 samples (2 Indian non-Basmati, 1 Italian) had RP3 values below 37.1. Two others, both Italian, had values between 38.5 and 39, the rest exceeded 39.

Comparison of RP3 values between the two sets, both for the small number of samples included in both sets and for the totality of samples, indicated a general shift in the RP3 measurements of approximately 2 to 3 units between the two sets, the second set having lower RP3 values. This appeared to be mainly due to higher perimeter measurements in the later set, possible causes and validation procedures for which were suggested earlier. The change in the appropriate cutoff for Basmati from 39 to approximately 37 was consistent with this general shift.

An attempt was made, using discriminant analysis on database 2, to find combinations of the basic image analysis measurements that performed better than RP3. This met with no success, although alternative combinations were found that performed about as well.

### **3.2.2 US**

The ability of the image analysis measurements, either as calculated or in new combinations, to discriminate between US and non-US samples was also studied, using both databases. There was little evidence of any success beyond the already known ability of the method to distinguish between US and Basmati samples.

## **3.3 NIR**

### **3.3.1 Basmati**

A linear discriminant function (LDF) based on 4 principal components was derived from database 1 in the earlier study. It successfully classified all 17 Basmati samples, but classified 20 of the other 100 samples in the database as Basmati. This LDF produced a much lower success rate when applied to database 2. Of the 19 Basmati samples, 14 were correctly classified and 5 were classified as non-Basmati, whilst 28 of the 76 non-Basmati samples were classified as Basmati. Shifting the cut-off far enough to include all the Basmati samples would have included well over half of the non-Basmati samples as well.



A new LDF, based on 5 principal components, was derived from database 2. This incorrectly classified 4 of the 19 Basmati samples and 15 of the 76 non-Basmati. When applied to database 1 it performed very badly. The use of first and second derivative spectra was also investigated, using database 2, but no better results were obtained.

### **3.3.2 US**

A discriminant rule was sought on database 2. Raw spectra, and first and second derivative spectra were all considered. The most promising rule used a second derivative at the 59th spectral point (966nm). The separation of the second derivative spectra in the region of this point can be seen in figure 4, which shows mean second derivative spectra for 6 groups of samples (defined in the key to figs. 4 to 8). Even this measurement was not very successful when used in isolation, but it was particularly successful in combination with the RP3 parameter from the image analysis. These results are described in 3.5.2 below.

## **3.4 RVA**

The mean RVA viscosity curves for 6 groups of samples are shown in figure 5.

### **3.4.1 Basmati**

Calculating the parameters D70 and V120 for database 2 gave the results in figure 6. Separation between US and Indian samples was good, but there was little separation between Basmati and Indian non-Basmati samples, and the other, mainly European, samples overlapped both groups.

Database 2 was then investigated using linear and quadratic discriminant analysis on principal components, and searching for alternative parameters to D70 and V120. None of the results showed success levels approaching those of the image analysis.

### **3.4.2 US**

A discriminant rule was sought on database 2. Raw curves, and first and second derivative curves were all considered. It was clear that the RVA did have some discriminatory power for US samples. A quadratic discriminant rule based on 5 principal components correctly

### **Key to line types in figures 4 and 5**

Long dashes	US long grain samples
Dashes and dots	US long grain samples, aromatic varieties
Solid line	Authentic Basmati samples
Short dashes	Non-Basmati samples from the Indian sub-continent
Dots	South American long grain samples
Close dots	European samples, mainly from Italy

### **Key to symbols used in figures 6, 7 and 8**

Solid circles	US long grain samples
Open circles	US long grain samples, aromatic varieties
Solid triangles	Authentic Basmati samples
Open triangles	Non-Basmati samples from the Indian sub-continent
Plus signs, +	South American long grain samples
Crosses, x	European samples, mainly from Italy

Fig 4. NIR on database 2, mean spectra for 6 groups of samples

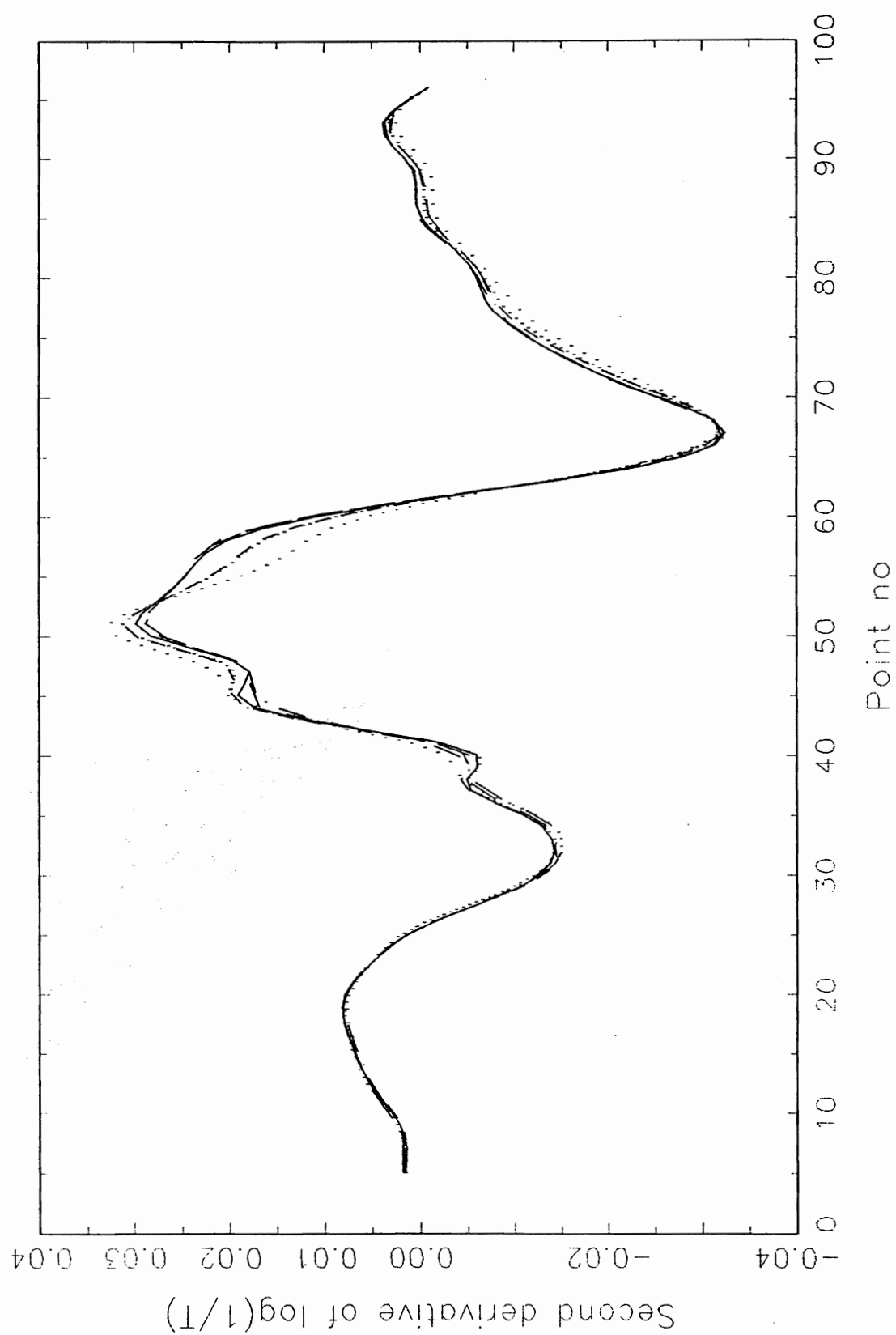


Fig 5. RVA on database 2, mean curves for 6 groups of samples

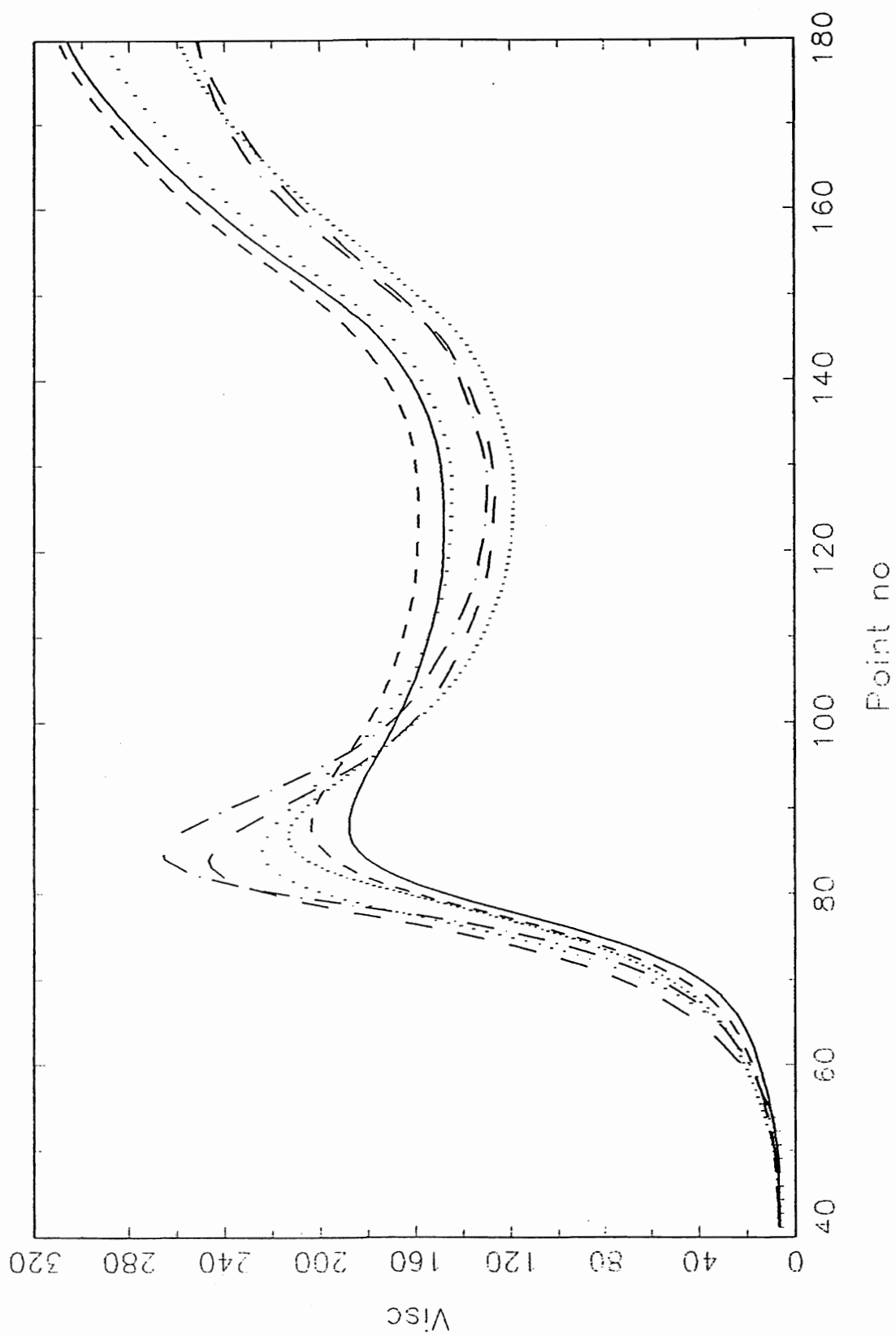


Fig 6. RVA on database 2, D70 and V120 for 6 groups of samples

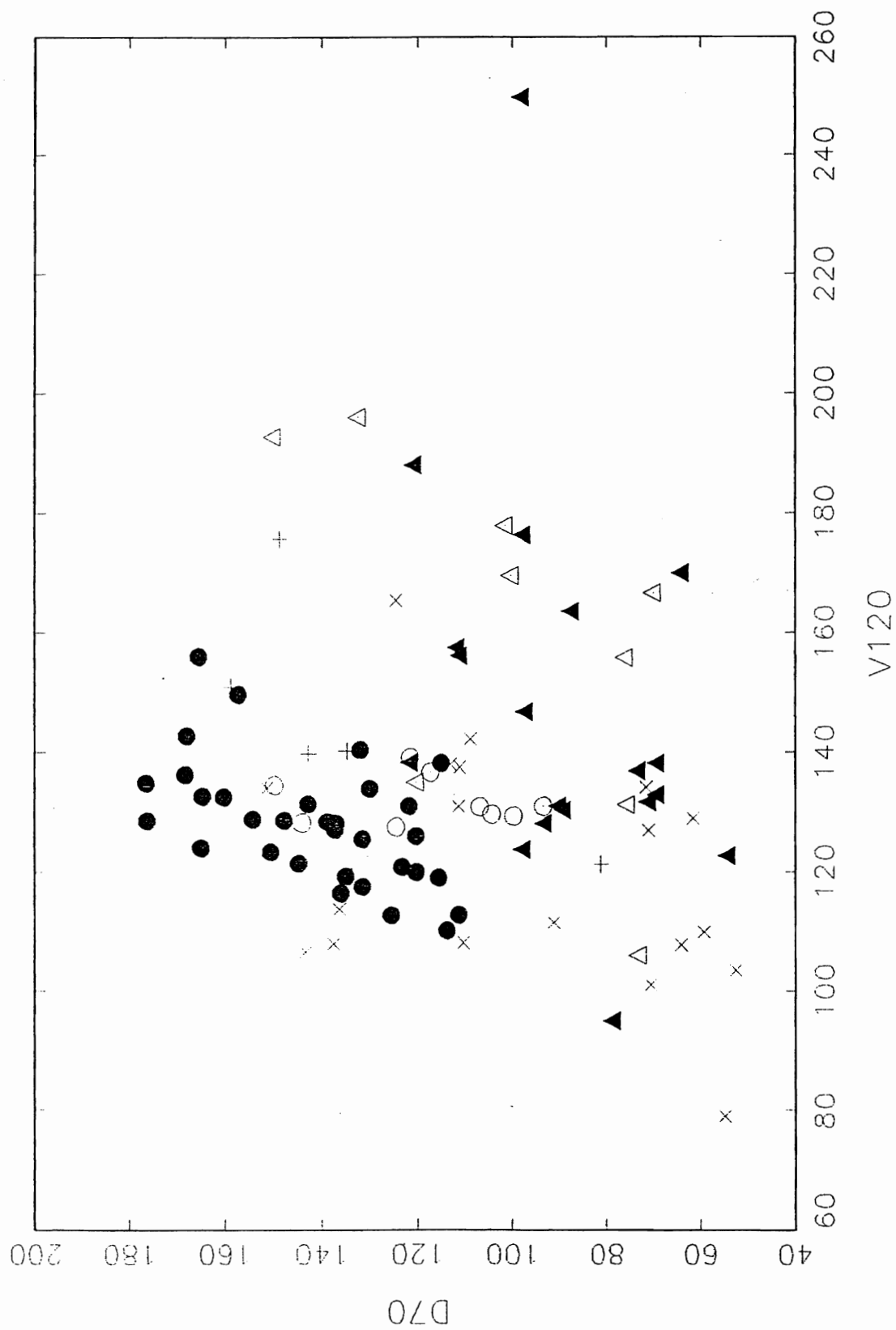


Fig 7. IA and NIR on database 2, RP3 and NIR for 6 groups of samples

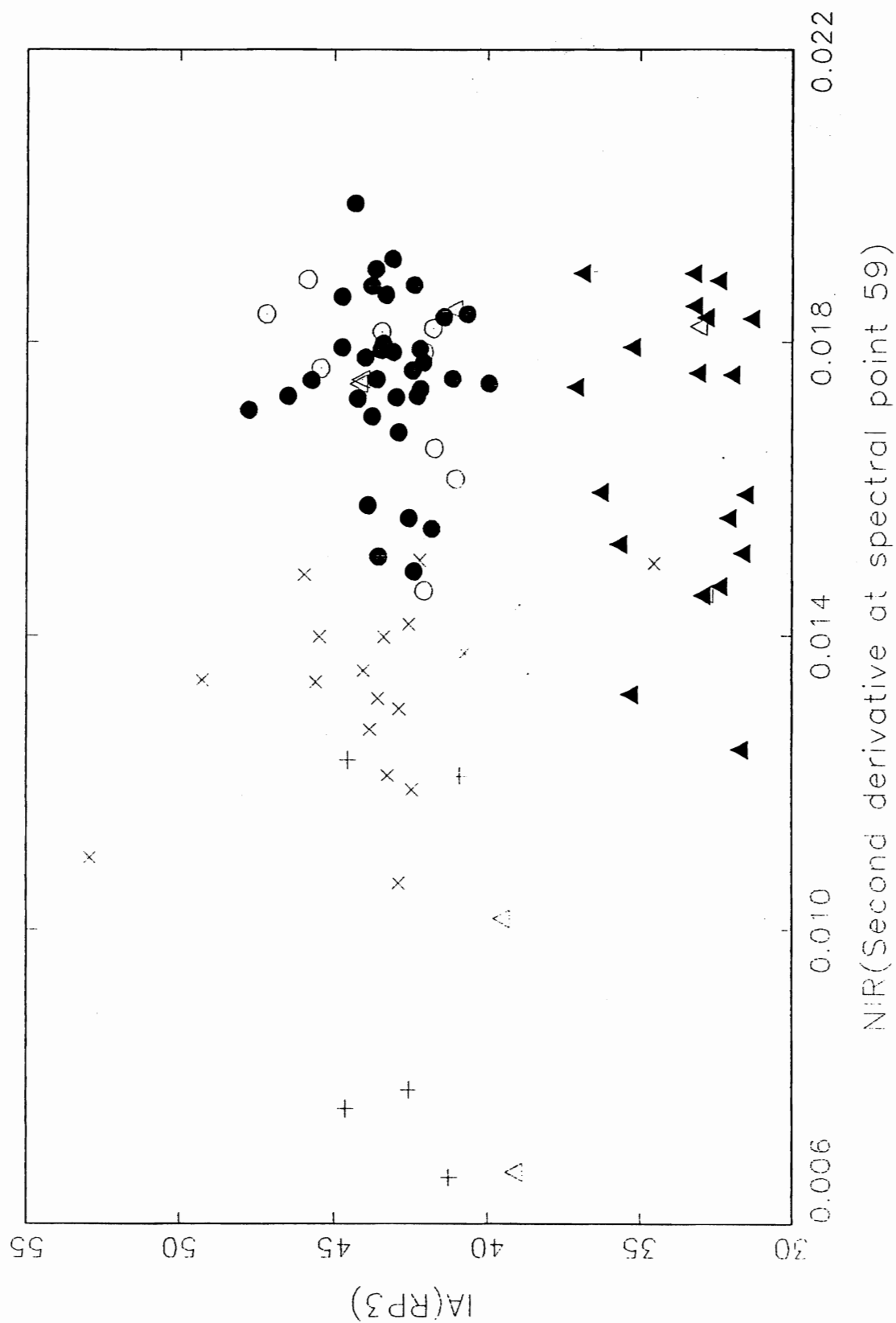
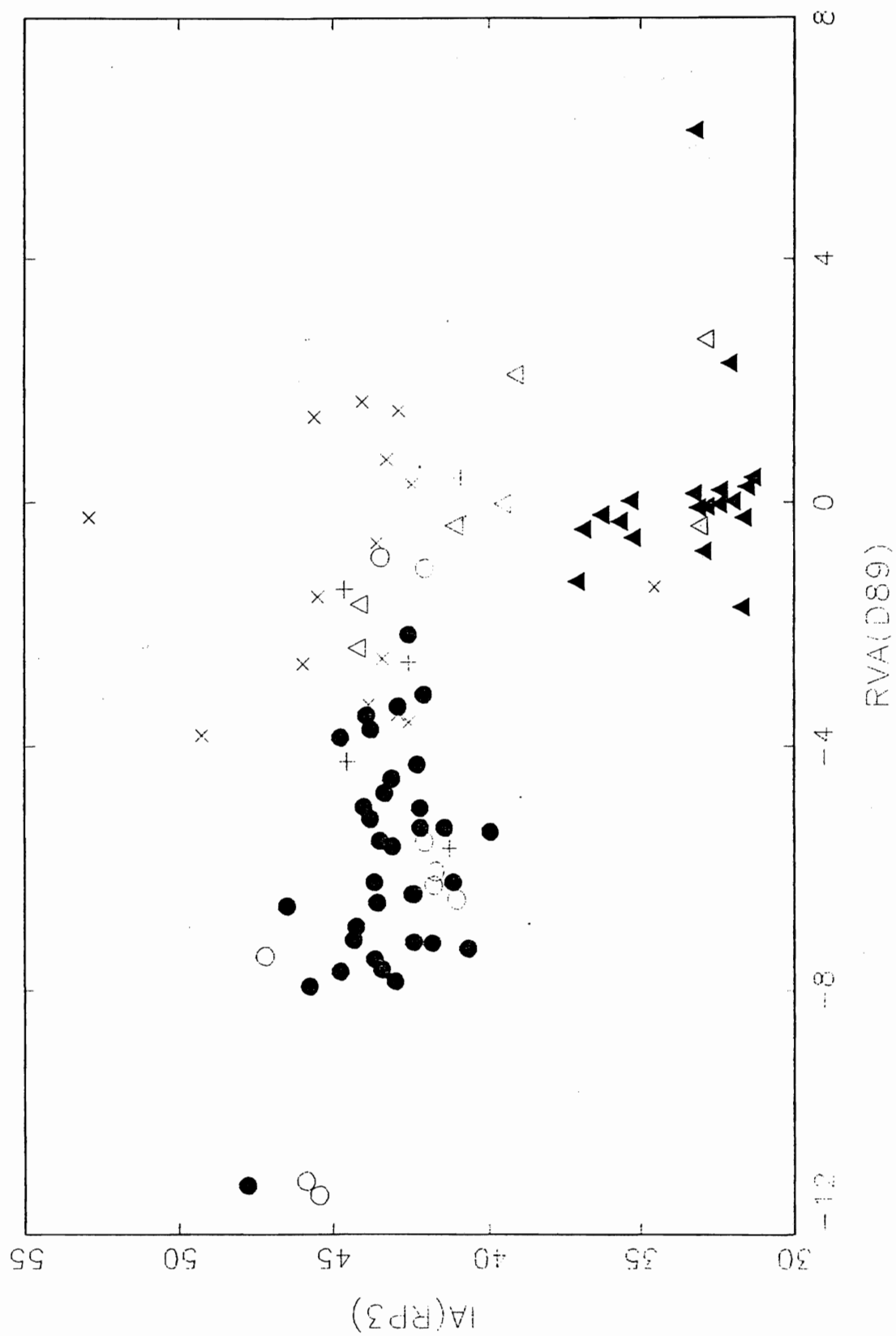


Fig 8. IA and RVA on database 2, RP3 and D89 for 6 groups of samples



classified 90 of the 95 samples. One US sample was incorrectly classified as non-US and 4 European samples (3 Italian, 1 French) were classified as US. A much simpler slope measurement at the 89th point (henceforth referred to as D89 by analogy with the D70 used earlier) also showed promise, classifying 41 of the 43 US samples correctly and including 12 of the 52 non-US samples with the US group. The combination of this slope measurement with RP3 was also investigated and is described in 3.5.2 below.

### **3.5 Combinations of methods**

#### **3.5.1 Basmati**

With such a large discrepancy between the success rates of the image analysis and the other two methods the scope for combining the methods in a single score is limited. This was studied, using database 2, but with no real success. Essentially the three samples misclassified by image analysis are not easy to separate using either (or both) RVA or NIR.

#### **3.5.2 US**

The potential for combining methods is much greater in the US problem, because all three methods show some power. What was initially a very encouraging result was obtained when the RP3 parameter was combined with the second derivative NIR measurement described in 3.3.2. Figure 7 shows the result. RP3 separates the Basmati samples from the US ones, and the NIR measurement separates most of the others. There is a serious caveat however. The NIR wavelength used (966nm) is in a region known to be associated with the measurement of water, and it is very probable that the discriminatory power results from systematic differences in moisture content between the samples from different sources. When the same approach was applied to database 1, the NIR measurement showed almost no discriminatory power.

The relationship between RP3 and the RVA measurement D89 was also investigated for possible synergy. In fact figure 8 shows that there is very little. The RVA alone is able to separate Basmati and US, and RP3 is useful only in adding confidence near the boundary.



With doubts about the validity of the basis for the NIR discrimination it did not seem worth expending much effort studying combinations of the three. A limited investigation suggested that such combinations performed about as well as NIR plus RP3.

## **4. DISCUSSION AND CONCLUSIONS**

### **4.1 Image analysis**

The RP3 measurement derived in previous work continued to perform well in discriminating Basmati from non-Basmati samples. With the cut-off set so that all Basmati samples pass the test, a small number of non-Basmati samples do get through. It was not possible to improve on this measurement, either by using alternative image analysis parameters or by combining RP3 with measurements from the other techniques. A shift in the image analysis measurements between the two studies was detected, and development of a calibration procedure is recommended to ensure stability in the future. The image analysis measurements alone were of little use in discriminating between US and non-US samples.

### **4.2 NIR**

Although NIR still showed some discriminatory power for Basmati, it was less successful on database 2 than database 1 even when a fresh discriminant rule was derived. This may reflect the greater variability, particularly in the Basmati samples, in database 2. Applied to the US problem, the NIR seemed of value only when combined with image analysis (see 4.4 below).

### **4.3 RVA**

The RVA also continued to show some discriminatory power for Basmati, but like NIR was less successful on database 2. The method did show promise for application to the US discrimination problem, although the most successful approach, a quadratic discriminant rule based on 5 principal components, is a complex one and will need further validation.

### **4.4 Combinations of methods**

Combining methods was not advantageous in the case of Basmati, but the Image Analysis was so successful on its own that this was not surprising. The apparently good result from combining NIR and Image Analysis for the US problem is of doubtful validity and should not

be relied on without further testing with samples of known moisture content. There is some limited advantage in combining RVA and Image Analysis measurements for the US problem.

#### **4.5 Basmati**

The only one of the three methods to hold up satisfactorily on validation on database 2 was the Image Analysis. The other two techniques seem to offer little more than a very crude screening, and future effort should probably concentrate on Image Analysis. Because of the bias between measurements made in this and the previous study, it is recommended that further work should be carried out to establish a validation procedure for routine use, based on measurement of calibration objects and making use of improved digitisation hardware. Re-testing of only a small number of database samples would allow the bias in previous measurements to be identified and a stable cutoff value of RP3 to be chosen, against which mean test values could be compared for routine testing purposes. On the evidence of this study, such a method would be suitable for discriminating Basmati samples from a large number of other rice types.

#### **4.6 US**

The most promising technique for this problem was RVA, possibly used in combination with Image Analysis. This needs further investigation, because the discriminant rule is complex and calibration problems mean that validation on database 1 is not a serious option. Further work is therefore recommended for validation of this procedure and if successful, for selection of a test criterion for routine use and to establish confidence limits for such a test.

The NIR result is probably an artifact of moisture contents, but would be so useful if it were true that a small amount of further study, using samples equilibrated to the same moisture content, is recommended to assess whether a more extensive study is justified.

#### **4.7 Further analysis**

The analysis of the data collected in this project is not complete. With so many possibilities to explore it is necessary to draw a line at some point and report the findings.

Although the scope of this project concerned only the problem of discriminating authentic samples of Basmati and US long grain samples from potential substitutes, the techniques identified also have potential for application to the alternative problem of adulteration of authentic samples by admixture of other rice types, and could potentially be developed for this purpose in future work. In particular, the Image Analysis and NIR techniques involve measurement of individual grains, and the current database could therefore be used as the basis for such a study. Further tests should also be conducted on a series of mixtures of known samples to establish the sensitivity to different potential adulterants achievable in practice, and should concentrate primarily on the Basmati problem for which the Image Analysis test in particular is most effective. For the purpose of testing for admixture to US long grain rice, the sensitivity of the RVA technique should be evaluated. Since this does not use individual grains, statistical evaluation of the existing pure sample databases is inappropriate, and evaluation should depend primarily on testing of mixtures.

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## ACKNOWLEDGEMENTS

The authors would like to thank MAFF for funding this work, and the Natural Resources Institute, USA rice council, CIRAD, Ente Nazionale Risi, Indian Directorate of Rice Research, Stevens and Brotherton Ltd., Whitworths Ltd., and GAFTA for assistance in collecting samples and for advice on their relevance.

## Appendix 1 Database of rice samples collected for this study

\* Basmati samples † U.S. long grain samples

<u>Sample number</u>	<u>Place of origin</u>	<u>Description</u>
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Retail samples purchased in Madras, India

MR94/...			
421	India	Non-Basmati	- (no brand name)
422	India	Non-Basmati	- (no brand name)
* 423	India	Basmati	- (no brand name)
* 424	India	Basmati	Super Silly Kohinoor
* 425	India	Basmati	Double Bull
* 426	India	Basmati	Neel Kamal
* 427	India	Basmati	Double Deer
* 428	India	Basmati	Lal Qilla Brand
* 429	India	Basmati	Da Awat
* 430	India	Basmati	Flying Horse

From Directorate of Rice Research, Hyderabad, India

MR94/...			
* 431	India	Basmati-370	Kh-93
* 432	India	Pusa Basmati-1	
* 433	India	Basmati-385	Kh-93
* 434	India	Dehradun Basmati	
435	India	Sonasali	Kh-93
436	India	Pr-109	Kh-93

From USA rice council, Houston, Texas

	MR94/...		
†	1905	Texas	Alan
†	1906	Arkansas	Alan
†	1907	Mississippi	Alan
†	1908	Texas	Maybelle
†	1909	Arkansas	Maybelle
†	1910	Mississippi	Maybelle
†	1911	Texas	Jackson
†	1912	Arkansas	Jackson
†	1913	Mississippi	Jackson
†	1914	Texas	Kaybonnet
†	1915	Arkansas	Kaybonnet
†	1916	Mississippi	Kaybonnet
†	1917	Louisiana	Lemont
†	1918	Texas	Gulfmont
†	1919	Arkansas	Gulfmont
†	1920	Mississippi	Gulfmont
†	1921	Texas	Katy
†	1922	Arkansas	Katy
†	1923	Mississippi	Katy
†	1924	Texas	Cypress
†	1925	Louisiana	Cypress
†	1926	Arkansas	Cypress
†	1927	Mississippi	Cypress
†	1928	Texas	Newbonnet
†	1929	Arkansas	Newbonnet
†	1930	Mississippi	Newbonnet
†	1931	Texas	Toro 2
†	1932	Arkansas	Toro 2
†	1933	Mississippi	Toro 2

†	1934	Texas	L202	
†	1935	Louisiana	L202	
†	1936	Arkansas	L202	
†	1937	Mississippi	L202	
†	1938	Mississippi	Rexmont	
†	1939	Texas	Della	Aromatic
†	1940	Arkansas	Della	Aromatic
†	1941	Mississippi	Della	Aromatic
†	1942	Texas	Jasmine 85	Aromatic
†	1943	Arkansas	Jasmine 85	Aromatic
†	1944	Mississippi	Jasmine 85	Aromatic
†	1945	Texas	A301	Aromatic
†	1946	Arkansas	A301	Aromatic
†	1947	Mississippi	A301	Aromatic

From Stevens and Brotherton Ltd, Orpington, UK

MR95/...

79	Guyana	White long grain
80	Surinam	White long grain

From CIRAD, Montpellier, France

MR95/...

81	French Guyana	L7	Long grain
82	French Guyana	L20	Long grain
83	French Guyana	Mana	Long grain
84	Camargue, France	Thaibonnet	Long grain
85	Camargue, France	Ariete	Long grain
86	US variety grown in Camargue	A301	Long grain
87	US variety grown in Camargue	Dwarf japonica	LG perfumed

From Whitworths Ltd., Wellingborough, UK

(These varieties are probably common adulterants of Basmati)

MR95/...

124	India	Terrycot
125	India	Guarav
126	India	Duplicate

From Ente Nazionale Risi, Milan, Italy

MR95/...

128	Italy	Porto
129	Italy	Graldo
130	Italy	Thaibonnet
131	Italy	Dedalo
132	Italy	Pegaso
133	Italy	Panda
134	Italy	Zena
135	Italy	Artiglio

From Natural Resources Institute (NRI), Chatham, UK

MR95/..., (number from previous study: MW/...)

*	140 (351)	India	Basmati 370
	141 (353)	India	Kasturi
	142 (348)	India	Karnal local (2)
*	143 (352)	India	Pusa Basmati-1
*	144 (339)	Pakistan	Basmati 198
*	145 (337)	Pakistan	Basmati 370
*	146 (336)	Pakistan	Basmati 385
*	147 (335)	Pakistan	Basmati PAK
*	148 (338)	Pakistan	Line 4048



From Natural Resources Institute (NRI), Chatham, UK (continued)

MR95/..., (number from previous study: MW/...)

149 (305)	Italy	Artiglio
150 (311)	Italy	Icaro
151 (322)	Italy	Vela
152 (321)	Italy	Thaibonnet (L202)
153 (346)	Italy	Koral
154 (347)	Italy	Panda
155 (345)	Italy	Strella