CHAPTER THREE. The Instrumental Measurement of Gelatine Colour.

INTRODUCTION.

The colour of gelatine was of commercial importance for two reasons:

1. It was possibly a cultural phenomenon but white or colourlessness had a connotation of purity and it was therefore desirable. Hence, all other things being equal a paler gelatine colour had the advantage in the market place.

2. There was a technical demand for pale or non-coloured gelatines for the production of non-coloured confectionery jellies of high clarity, furthermore, the paler the gelatine the less it modified the colours of dyes used in the manufacture of gelatine-based gels and films.

Currently, there was no generally accepted instrumental or absolute method for the measurement of the colour of gelatine and it was apparent that such a system would be of value to the industry as a whole.

Without detailed knowledge of gelatine there would appear to be little difficulty in the instrumental measurement of gelatine colour (Saunders and Ward, 1953). However, there was considerable difficulty in obtaining spectrophotometer values that were well correlated with the subjective or visual colour of dry gelatine or gelatine in solution.

Because of the commercial importance of gelatine colour, manufacturers had developed "in house" methods for comparing gelatines to colour standards by eye using methods which conformed to Beer's Law or which were completely arbitrary. Hence, the commercial "specification" of gelatine colour by the manufacturer or the consumer was extremely difficult.

In the dry state gelatine was produced in many forms from sheets or pieces of dried film to powders with a particle size of 100 microns or finer. As the physical form of the gelatine played a significant role in the visual

assessment of dry colour and because gelatine was mostly used as a solution in water for food applications, it was most common for gelatine colour to be assessed in aqueous solution. As the gel state could introduce additional problems to colour assessment due to a variable loss of clarity close to the isoelectric pH (pH 5 for Type B gelatine) (Veis, 1964) it was therefore common practice to assess gelatine colour in solution at a temperature above the gel melting temperature i.e. in the vicinity of 40°C.

The visual assessment of gelatine colour could be achieved but the repeatability was affected by day-to-day personal errors and bias. Also it was not practical, on a day-to-day basis, to have several experienced assessors determine the colours of all the production gelatines being tested and as a consequence it was accepted that there would be differences between two assessments of colour. In this study a 10% error was accepted or ± 0.8 colour units between colours of 0 and 12. Above 12 an error of approximately 1.6 units was regarded as acceptable, hence, the agreement between the "instrumental" colour and the visual colour should have been within 1.5 units up to colour 12 and within 3 units above colour 12.

The eye had a unique ability to compensate for small differences in solution turbidity which was caused by the scattering of light due to molecular size and by insoluble colloidal matter in suspension. To the spectrophotometer scattered light was "absorbed" and it was common knowledge that the amount of scatter was a variable depending on the wavelength of the light (Sears and Zemansky, 1964).

In this study an attempt was made to solve the problems associated with the instrumental determination of gelatine colour.

LITERATURE REVIEW.

A literature search showed that there was only one reference made to the instrumental measurement of gelatine colour. Saunders and Ward (1953) determined the absorption spectrum of gelatine between 350 and 900 nm. They also recognized the problem of light scatter due to suspended matter and recommended that gelatine solutions be filtered through a "Seitz" (sterilizing) filter before absorbance determination. They claimed that colour could be expressed as optical densities (OD) at approximately 425, 520 and 700 nm using a colorimeter and filters. No statistical correlations of absorbance

at the different wavelengths and visual colour were performed. The failure of the industry to adopt the above method was probably due to a lack of suitable data to correlate the instrumental determination of gelatine colour with conventional visual methods.

EXPERIMENTAL MATERIALS and METHODS.

Unless otherwise stated Reagent Grade chemicals were used in all the experiments. The analytical data on the gelatines used in this study are detailed in ADDENDUM 6. In order to ascertain that the methods were generally applicable, gelatines from a number of other manufacturers, as shown in ADDENDUM 6, were included in the study.

Solution of gelatine.

Due to the low absorbance of the paler gelatines it was an advantage to use a high concentration of gelatine in order to maximize the sensitivity of the method. Hence, it was decided to use a concentration of 6.67 %. This was the internationally accepted concentration used for the determination of Bloom gel strength (British Standards Institution, 1975).

Gelatine solutions were made by weighing 0.714 ± 0.0005 g aliquots of gelatine into 50 ml glass containers fitted with screw caps or into 30 ml test tubes. Water (10 ml) or an aqueous solvent (see below) was pipetted into the containers. These were swirled using a mechanical vortex mixer to wet the particles of gelatine. The gelatine was allowed to swell for 20 to 30 min. It was then placed in a 40°C to 50°C waterbath. The mixtures were swirled from time to time until completely dissolved (usually 10 min.). The presence of striations at the bottom of a sample indicated that solution was incomplete. When a proteolytic enzyme (Alcalase, Trypsin, Papain) solution was used an additional reaction time of 1 hour at 40°C was allowed for the enzymic hydrolysis of the gelatine samples (see below).

Visual measurement of gelatine colour.

The traditional method of estimating colour was conducted by comparing 100 ml of a 4% solution of an unknown sample to 100 ml of a 4% solution of a standard sample in Nessler tubes at a temperature of approximately 40°C. The standard sample had been arbitrarily assigned the colour value or colour concentration

of 8. The tubes were examined down their length against a white background and solution was poured out of the darker tube until a match was obtained. According to Beer's law:

$$V_1C_1 = V_2C_2$$

Where V = volume and C = concentration.

If 50 ml of unknown sample was equal to 100 ml of the standard sample then the colour of the unknown was 100 x 8 ÷ 50 = 16, or if 70 ml standard sample = 100 ml unknown sample, then the colour of the unknown was 70 x 8 ÷ 100 = 5.6 etc.

Instrumental measurement of gelatine colour.

Preliminary transmittance results were obtained using a Perkin-Elmar 500 UV-VIS double beam spectrophotometer with a 1 cm path length and 1mm beam width. Quartz cells were used. The 6.67% gelatine solutions were scanned manually at 10 nm intervals between 150 and 700 nm.

Appraisal of the problem of gelatine colour measurement.

The basis of the problem of the measurement of gelatine colour appeared to be linked to the following:

1. Gelatine did not have a convenient absorption maximum in the visible region of the spectrum, hence, its visible colour was the cumulative result of the absorption of light over the visible spectrum. To imitate this optical effect it would be necessary to scan the light absorbance over the visible spectrum and integrate the area under the absorption curve. A Beckman DU70 spectrophotometer (Beckman Instruments, Tonetti St, Halfway House, Johannesburg, R.S.A.) with these capabilities was selected.

2. Variable light scattering by solutions of gelatine would be mainly due to variances in gelatine clarity which was largely the result of suspended impurities. In addition scatter due to molecular size was particularly noticeable at the isoelectric point and was known as the "isoelectric effect". This effect was enhanced by high gelatine quality (high Bloom strength and viscosity), low concentration and low temperature (Veis, 1964).

3. High viscosity gelatine solutions were difficult to filter, hence filtration through sterilizing 0.45μm filters to obtain a uniform clarity was often quite impractical. A possible solution could be to hydrolyse the gelatine to a uniform low molecular weight prior to filtration. Filtration to uniform clarity could then be practical. Furthermore, such a procedure could reduce the scatter due to molecular size to a constant. Hence, if the procedure did not affect the colour of the gelatine it could well lead to a practical pretreatment which would permit the instrumental measurement of colour.

It was decided to use Novo Alcalase 0.6L (Novo Enzymes ex Enzymes S.A. (Pty) Ltd, PO Box 651216, Benmore 2010. R.S.A.) as a practical pretreatment to hydrolyse gelatine prior to colour measurement using the Beckman DU70 spectrophotometer. Alcalase was widely used commercially hence it was readily available. It was also a proteolytic enzyme of good stability when kept refrigerated. The cost of approximately R 15/kg was low when compared to proteolytic enzymes like Trypsin at R 1/g. The only objection to Alcalase was its intrinsic colour but this could be compensated for by using the enzyme solution as the blank. The quantity chosen for use was based on personal experience, as were the conditions required for gelatine hydrolysis. Also, it was known that from the point of view of viscosity reduction, the hydrolysis of gelatine was practically complete in less than 10 minutes, however, an hydrolysis time of 1 hour was allowed throughout this study to ensure complete hydrolysis.

Beckman DU70 Spectrophotometer.

The Beckman DU70 was a single beam spectrophotometer with 2 nm slit width, using a 1 cm path length cell. A glass cell was used for readings in the visible region of the spectrum (400 to 700 nm) and a quartz cell was used for scans with readings below 400 nm. The operation of the instrument was computer controlled and permitted spectral scans to be down-loaded to other computers for manipulation or analysis of data. The Beckman Data Leader software (Ver 2.3) provided facilities for base line modification which was used to move the base line to the 700 nm absorbance value. The software also allowed the calculation of the area under the curve between 400 and 700 nm. Also, use was made of the Zoom facility which enabled detailed curve analysis, and of the Trace function for the determination of the absorbance at any required wavelength. In this study, the instrument was set to scan between 400 and 700

nm at a scan speed of 600 nm/min giving two data points per nm. There were no other variable parameters.

Procedures.

The cuvette was washed three times with approximately 1 ml aliquots of the gelatine solution to be tested. It was then fillet with test solution and scanned by the spectrophotometer. The machine automatically recorded the scan. At the end of a series of scans or when the spectrophotometer's memory was full the scans were transferred from the spectrophotometer's memory to a computer, running the Data Leader software, for permanent storage and manipulation. During each scan the 700 nm absorbance value was noted from the instrument. As a precaution the 400 nm absorbance was also noted. If either of these values was missed then it could be obtained using the software Trace Home or Trace End facilities. It was occasionally noted during the course of this study that the Manual Baseline facility had not always contained the correct 700 nm absorbance value for area calculations. It was necessary, therefore, to check that the value displayed was the same as that recorded during the scanning of the solution. If it was not correct the Manual Baseline value for the 700 nm absorbance could be edited appropriately.

To avoid the formation of a gel in the cuvette the absorbance or transmittance of 40°C gelatine solutions in water were measured in duplicate as quickly as possible. Hydrolysed gelatine solutions were allowed to cool to room temperature (20-25°C) prior to taking readings. The optical densities of determinations on duplicate samples were recorded and averaged.

On each day that absorbance data was determined there was one sample which was used as a control to establish that the instrument and the procedure were giving substantially constant results.

The usefulness of the instrumental data for the measurement of gelatine colour was evaluated by entering the instrumental and visual colour data into a Quattro spreadsheet (Borland International Inc, 1800 Green Hills Rd, Scotts Valley CA 95067 USA) and calculating the linear correlation between the instrumental and visual data. Data on gelatines with colours of less than 3.2 and gelatines of very poor clarity were excluded from the correlation calculations. By definition a zero colour gelatine must have zero absorbance at all wavelengths, hence the linear regression constant must be zero. The

Quattro regression calculation allowed this to be specified.

Aqueous Solvents.

Enzyme solution No. 1. (ES1).

Enzyme Solution No.1 (ES1) was prepared by diluting 1.0 ml of Novo Alcalase 0.6L (*loc sit*). with distilled water to 1000 cm^3 in a volumetric flask. Alcalase 0.6L had a specified activity of 0.6 AU/ml. (AU = Anson Unit).

Trypsin solution.

The Trypsin enzyme preparation was made by dissolving 0.1g Crystalline Porcine Trypsin Novo (Novo Enzymes *loc sit*) in distilled water containing 0.2 ml of 0.3N hydrochloric acid according to the manufacturers instructions and diluting to 500 ml in a volumetric flask. Trypsin solutions were always slightly hazy. The PTN 3.0 S, Trypsin had a specified activity of 3.0 AU/g.

In the trials using Trypsin, duplicate samples of gelatine were treated with the two solvents, ES1 and Trypsin solution, and the areas under the absorbance curves were recorded as well as the 400 nm and 700 nm absorbances.

Enzyme solution No. 2 (ES2).

The development of ES2 was necessary because on treatment of pigskin gelatines with ES1 it was found that they could not be filtered easily and on refrigeration the samples gelled indicating inadequate hydrolysis. The reason was attributed to the low pH (4.5) of these gelatines which was on the edge of the activity range of Alcalase. ES2 was prepared with 0.05% ammonia to adjust gelatine solutions with a pH of about 4.5 to a pH of about 6. Ammonia was chosen as the preferred alkali because it was a weak base. Thus it would alter the pH of gelatine gently. Also Alcalase could be exposed to ammonia solutions (pH 11) without damage whereas dilute sodium hydroxide (pH 13) could inactivate Alcalase.

The solution was prepared as follows:

Alcalase 0.6L 0.5 ml,
1:4 Ammonia soln. (5%) 5.0 ml.
Distilled water to 500 ml in a volumetric flask.

Enzyme solution No. 3 (ES3).

Due to the precipitation caused with some gelatines by the use of solvent ES2 it was decided that this was not a viable solution to the problem of inadequate hydrolysis of gelatines with a low pH. ES3 was made up by dissolving 0.62 g Papain 6100P (supplied by Enzymes S.A. Pty Ltd. *loc cit*) in distilled water and diluting to 200 ml in a volumetric flask. Papain was a vegetable protease derived from *circa papaya* with a specified activity of 6100 National Formulary units (NFu/mg). This enzyme solution was also slightly hazy.

Acid hydrolysis.

Hydrochloric acid (20%) for 16 hours at 100°C, was normally used for the hydrolysis of gelatine for amino acid analysis (Heidemann, 1981). As only partial hydrolysis was required for the colour determination, duplicate 0.714 g samples of gelatine were treated with 10 ml x 15% hydrochloric acid for 16 hours at 100°C. Another pair of samples of the same gelatine were treated with ES1 in the usual way. As membrane filters cannot be used with strong acid, the acid hydrolysed gelatine samples were filtered using Whatman GF/A filter papers prior to absorbance determination.

Acid hydrolysis solution (1:1 HCl) was prepared by diluting concentrated hydrochloric acid (30%) with an equal volume of distilled water.

Gelatine Filtration.

In preliminary trials an attempt was made to simplify sample preparation by filtration of gelatine to standard clarity using 10 cm circles of Whatman GF/A filter paper (1.6 μm pore size). Subsequently it was found to be necessary to use 0.45μm membrane filters. The procedure used was to fit Millipore Swinex filter holders (Microsep. PO Box 391647 Bramley 2018.) with Millipore 25 mm

HVLP 02500 low protein binding membrane filters. The 10 ml samples of enzyme treated gelatine solution were poured into the barrel of a 10 ml plastic syringe while closing the outlet with a finger. The syringe plunger was fitted and the air expelled from the syringe. A filter holder was then pressed onto the syringe outlet and the contents of the syringe were filtered into the spectrophotometer cuvette using 3 x 1ml portions to wash out the cuvette and then approximately 4 ml to fill the cuvette for the absorbance scan. The remaining solution in the syringe was filtered into the original glass container which was then placed into a refrigerator (2°C). Failure to gel ensured that the gelatine solution had been hydrolysed sufficiently.

The BYK-Gardner Color-View Reflectance Spectrophotometer.

In a study on gelatine colour measurement it was considered that one of the "tristimulus" (L, a, b system) instruments should be used, especially when the conventional spectrophotometric approach was giving controversial results.

A BYK-Gardner Colour-View was obtained on loan (from Premier Technologies (Pty) Ltd. PO Box 173, Northriding, Randburg). This instrument is described in the attachments in Addendum 7. The main features were:

a. 45° circumferential illumination, with viewing normal to the sample surface. Illumination was achieved by the use of multiple fibre optics.

b. Analysis of the light reflected / transmitted by the sample was on the L, a, b, colour scale. The computer program was used to convert this data to the ASTM and DIN indices of "Yellowness".

c. The L, a, b, colour of a sample could be obtained as an absolute value or as a difference between the sample value and a standard value. For this investigation it was decided to maintain the normal spectrophotometric procedure and use the difference between the solvent (blank) and the sample.

d. The instrument had to be connected to a host computer running under Microsoft Windows (Ver 3.1) (Microsoft SA (Pty) Ltd. Katherine St, Sandown, Rivonia, R.S.A.). The software provided was Gardner-soft QC Manager (Ver 1.3) which both controlled the instrument and recorded the results. Each colour value was the average of three readings taken on each sample.

e. The sample cup was a quartz glass cylindrical vessel with an external diameter of 60 mm and a capacity of 90 ml. The base through which the light entered the sample was a high quality optically plane surface. In order that most of the light entering a clear sample was not lost from the upper surface, the top of the sample cup was covered with the white standard tile provided with the instrument.

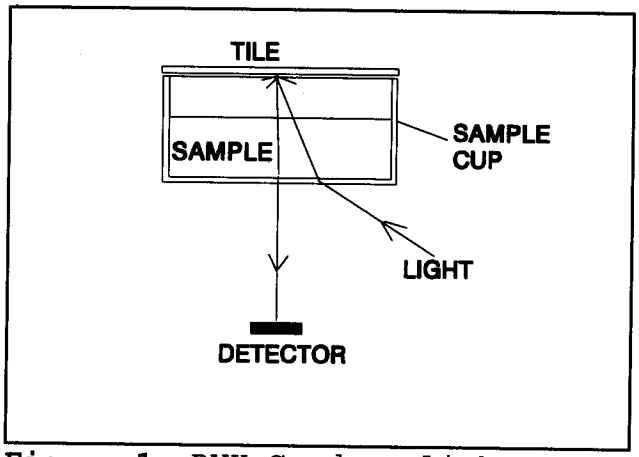

Figure 1. BYK-Gardner light path.

To establish the effect of changing the cell, trials were made using a "Grainer" cylindrical vessel of similar dimensions filled to the same depth as the quartz cell.

The effect of sample depth was established by comparing a 75 ml sample and a 50 ml sample in the Gardner sample cup.

As could be seen from Figure 1, the light entering the sample was scattered by the tile and a large portion was reflected back to the detector. This had two consequences:

1. Moisture condensed on the tile hence the amount of light scattered back to the detector was not constant resulting in reduced repeatability.

2. The amount of light scattered by the tile was considerably more than was scattered by a solution of normal clarity (<80 NTU) hence the Color-View readings appeared to be relatively insensitive to variances in gelatine molecular scatter and clarity.

Experimental procedure.

Each day the instrument was calibrated against the black and white tiles provided. The program was set to record the results of each reading. Using a 50 ml grade B measuring cylinder, 50 ml water was measured into the sample cup. After use the measuring cylinder was inverted so that it could drain between samples. The white tile was placed on top of the cup and then the water was read as the standard. The instrument was then set to read samples.

Each sample consisted of a 6.67% solution of molten gelatine at about 40°C. (This allowed the direct measurement of molten Bloom samples). Amounts of 50 ml were measured into the sample cup which was then placed on the instrument. The white tile was positioned on top of the cup. The sample was then read. The sample was returned to the original container and the cup was inverted so that it could drain on absorbent tissue until required for the next sample. The L, a, b, and "Yellowness" (differences from standard) were noted from the computer screen (CRT). The values were entered into a Quattro spread sheet for statistical analysis. Each day a control sample was included in order to be able to assess the instrument's performance and the repeatability of the method.

RESULTS and DISCUSSION.

Gelatine spectra.

Figure 1, depicts the transmission spectra of three gelatines. It was noted that:

1. A higher %Transmission was recorded for the pale gelatine N33/3 than the dark gelatine N33/1 over the spectral range 150 to 750 nm.
2. Chrome gelatine C542/2 showed a spectral anomaly in the region 300 to 330 nm which had been observed with most of the SPA process (Drew, 1930) gelatines derived from chrome tanned leather. No explanation for the anomaly could be provided.

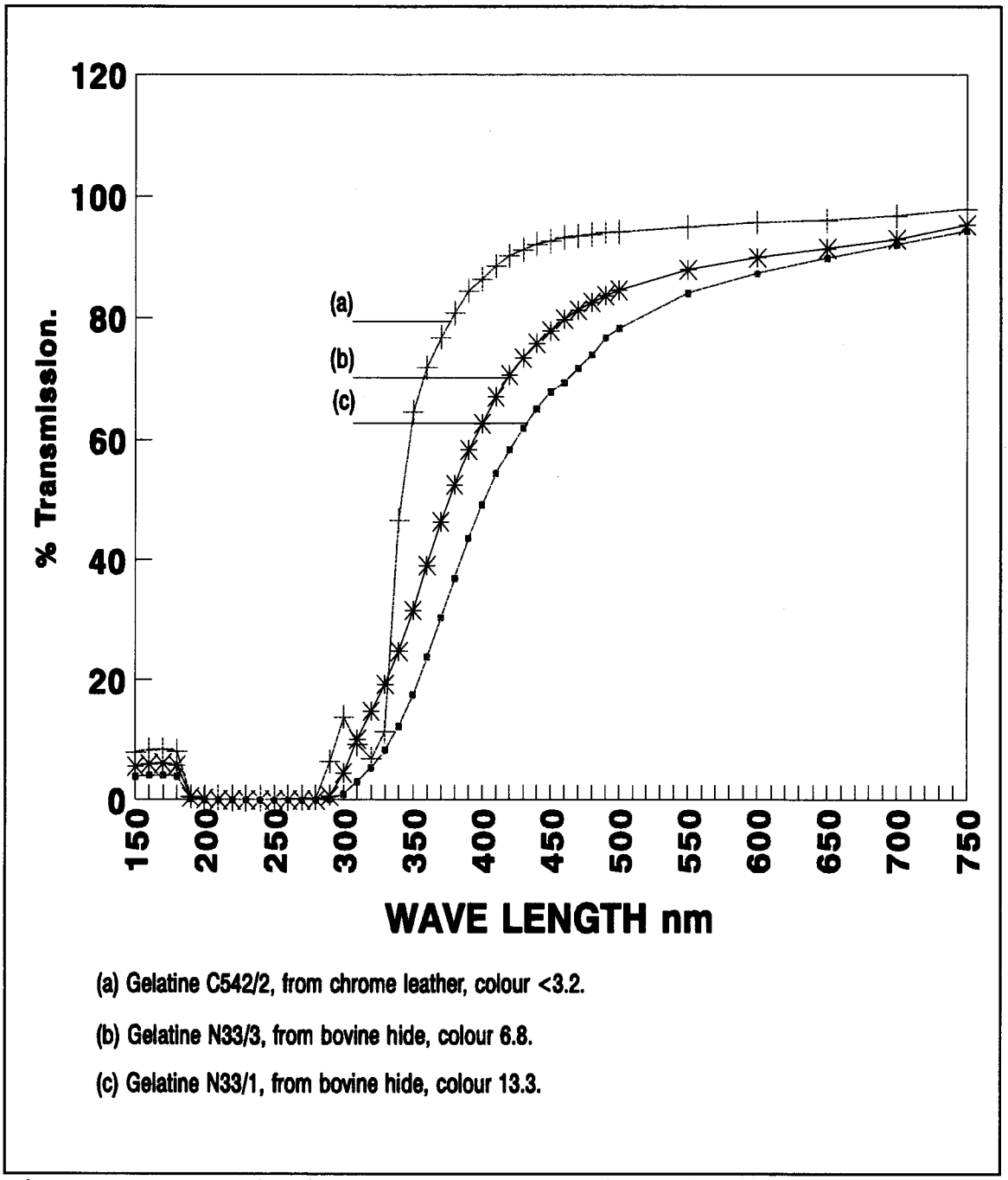

Figure 2 Transmission spectra of bovine hide gelatins.

Introduction to the problem of gelatine colour measurement.

From Figure 2 it was observed that gelatine transmits light relatively well in the visible region of the spectrum (400 to 750nm). Hence, the integrated transmission data should be related to visual colour intensity better than the transmission at a single wavelength. For this reason it was decided to evaluate the integration of the %T values over the visible spectrum. To this end the %T values for a number of gelatines at 400, 450, 500, 550, 600, 650, 700 and 750nm were summed. It was assumed that if gelatine had 100% transmission at all wavelengths (Σ%T = 800) this would equate to colour 0.0. The second order polynomial regression equation coefficients for the correlation of the integrated %T and visual colour were:

B(0) = 211.3185

B(1) = -0.4839819, B(2) = 2.741259E-04

R Square = 0.8256, R = 0.909

Using these coefficients and the values of Σ%T, the colour values for the various gelatines were calculated as shown in Table 1.

Table 1. *The correlation of visual colour and colour value calculated from Σ%T.*

Sample	Visual Colour	Σ%T	Calculated Colour Value
N33/4	14.5	627.6	15.5
N33/1	13.3	643.3	13.4
WT3/3	13.3	667.9	10.4
WT3/1	10.7	684.2	8.5*
N33/3	6.8	685.2	8.4
M8884	6.8	687.8	8.1
N33/2	9.4	702.0	6.7*
ES1	3.2	703.9	6.5*
ES1	3.2	729.0	4.1
	0.0	800.0	-0.4

The data in Table 1 showed that there were too many instances (*) where the disparity between the visual colour and the "calculated" colour was unacceptably large from the commercial point of view for the procedure to be acceptable.

Verification of the problems of colour determination.

The data in Table 2 shows that the correlation of area under the absorbance curve and visual colour was slightly better than the correlation of 400 nm absorbance and colour. Furthermore, the value of the "standard error of the estimate" of colour derived from the instrumental data, was very significant. This was the standard deviation of the difference between the estimated colour from the regression equation and the visually measured value of the colour.

Table 2. *The correlation of visual colour and absorbance data for gelatines dissolved in water.*

Absorbance Parameter X.	700 nm	400 nm	Area under the Curve
Regression Constant.	0	0	0
Std. Error of Estimated Colour.	8.54	4.48	4.37
Correlation Coefficient.	0.0	.555	0.586
Number of Observations.	83	83	83
Degrees of Freedom.	82	82	82
X Coefficient.	113.4	30.58	0.4922
Std. Error of Coefficient.	18.04	1.61	0.0251

For additional details see Addendum 1

The use of enzymic hydrolysis and filtration.

Tables 3 & 4 show the results of a series of preliminary comparisons of the area under absorbance curve for gelatines dissolved in water and in solvent ES1 (after filtration through a GF/A paper).

Table 3. *The area under the absorbance curves between 400 and 700 nm for three gelatines.*

Gelatine.	178(a)		183(b)		542(c)	
Solvent	Absorbance area	Mean	Absorbance area	Mean	Absorbance Area.	Mean.
Water	30.416	31.4	22.120		5.307	
Water	32.167	[0.89]	23.007	22.7	6.430	5.8
Water	31.541		23.095	[0.51]	5.711	[0.57]
ES1	28.026	29.0	22.972	21.9	3.543	4.2
ES1	29.951	[1.36]	20.758	[1.57]	4.774	[0.87]

(a) colour = 17.8; (b) colour = 9.0; (c) colour = 3
[ ] = Standard deviation.

Comments:

(i) The standard deviations of sample 542 were far too high for the area under the curve to be meaningful as an estimate of colour.

(ii) Based on water solvent, the area/colour regression equation was: Colour = -1.75 + 0.62 x Area. (r = 0.939 - not significant; probability = 0.1).

(iii) Based on ES1 solvent, the regression equation was: Colour = - 0.75 + 0.62 x Area. (r = 0.91 - not significant).

Table 3. shows that the regression equation correlation coefficient had not improved with the use of enzyme in the solvent. This was problematic, however, the absorbance curves shown in Figure 3. suggested that the area between the absorbance at 700 nm and the baseline could contribute to unwanted variation. The invisible absorbance at 700 nm and higher wavelengths could not contribute to the visible colour hence it became apparent that the baseline should be at the 700 nm absorbance value and not at 0.00 absorbance.

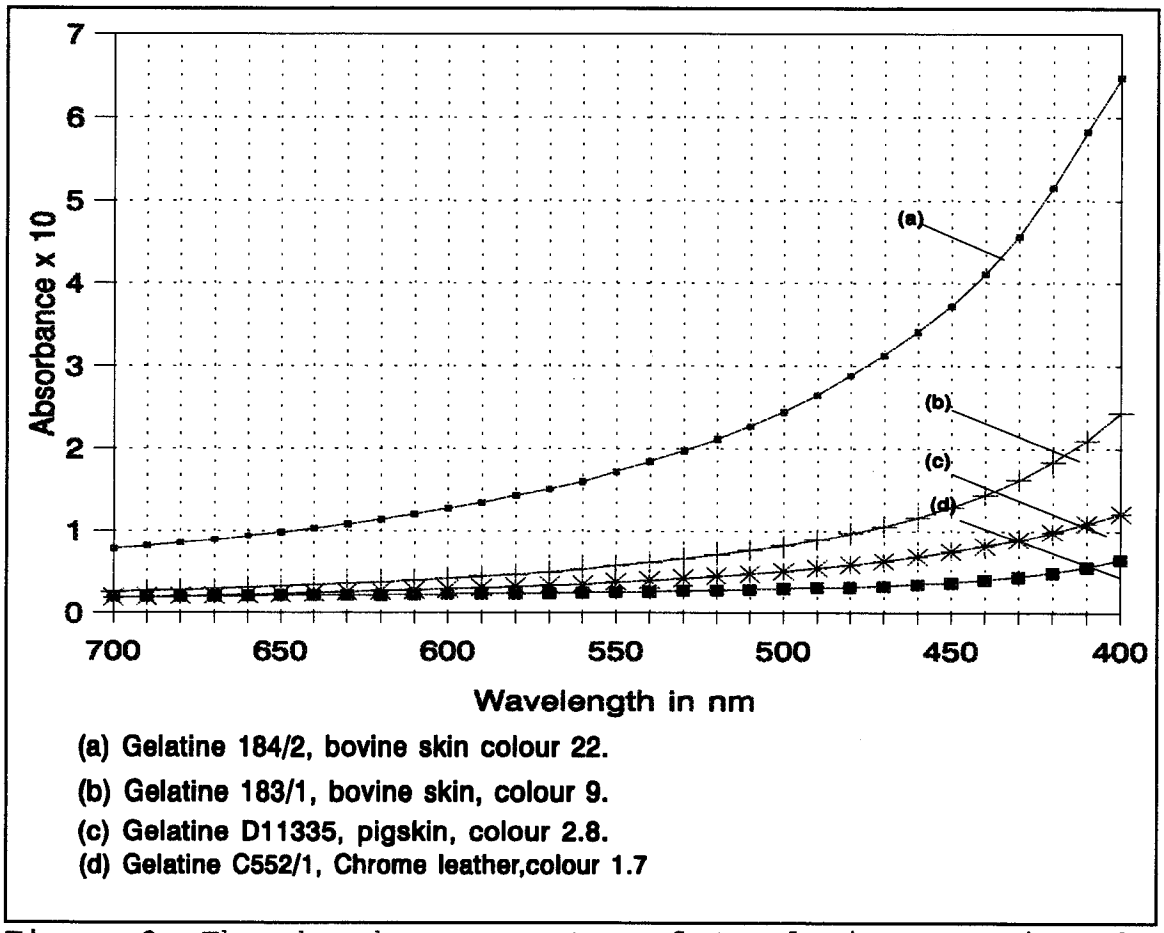

Figure 3. The absorbance spectra of 4 gelatins spanning the normal colour range.

Table 4. *The area under the absorbance curves between 400 and 700 nm for three gelatines, calculated with the baseline at the 700 nm absorbance value.*

Gelatine.	178(a)		183(b)		542(c)	
Solvent	Absorbance area	Mean	Absorbance area	Mean	Absorbance area.	Mean
Water	24.371	24.585	15.820		3.522	
Water	24.982	[0.34]	15.766	15.770	4.279	3.808
Water	24.401		15.626	[0.05]	3.624	[0.41]
ES1	22.347	22.379	(15.43)	13.54	2.942	2.951
ES1	22.411	[0.05]	13.543		2.959	[0.01]

(a) colour = 17.8; (b) colour = 9.0; (c) colour = 3
[ ] = Standard deviation.

The results shown in Table 4 were a distinct improvement on those shown in Table 3 and were attributable to the moving of the baseline to the 700 nm absorbance value:

(i) They show an improvement in repeatabilities. The standard deviations using solvent ES1 became acceptable because a weighing variance of ±0.002 g on 0.714 g would be expected to result in variances 0.3% in absorbance i.e. 0.01 on an area of 3.00 or 0.1 on an area of 30 which was the order of magnitude of the standard deviations of the ES1 samples.

(ii) It was noted that even with the pale colour and good clarity of sample 542, the enzyme-filtration combination treatment had the effect of markedly reducing the sample absorbance indicating reduced light scattering.

(iii) The colour / area regression line for water as solvent was:

Col. = -1.176 + 0.803 x Area. r = 0.968

The colour / area regression line for ES solvent was:

Col. = - 0.541 + 0.865 x Area. r = 0.9756.

These values of r were significant at the 0.1 level of probability (1 degree of freedom).

Based on these preliminary findings all subsequent determinations of the area under the absorbance curve were performed with the base line at the 700 nm absorbance.

Analysis of the use of ES1 solvent & filtration (GF/A)

Having shown that enzyme treatment and filtration of the gelatine solutions together with changing the base line for the absorbance area determination would probably give a useful correlation to visual colour, the next step was to apply the technique to a large number of gelatine samples. Using the data in Addendum 2 but excluding data from gelatines of unknown visual colour value, the linear regression results shown in Table 5. were obtained.

In comparison to the unfiltered data (Table 2) there was a small improvement in the standard error of the estimate of the colour but otherwise the improvement in correlation coefficients was disappointing due largely to the poor clarity achieved by filtration of the G35 and G36 samples using GF/A paper. The decision was then made to try 0.45μm membrane filters.

Table 5. *The correlation of visual colour and absorbance data for gelatines dissolved in solvent ES1 and then filtered using GF/A paper.*

Absorbance Parameter X.	700 nm	400 nm	Area under the Curve
Regression Constant.	0	0	0
Std. Error of Estimated Colour.	6.21	3.49	4.01
Correlation Coefficient.	0.0	0.637	0.466
Number of Observations.	31	31	31
Degrees of Freedom.	30	30	30
X Coefficient.	259	35.68	0.54
Std. Error of Coefficient.	39	2.51	0.04

Solvent = ES1.
Filtration = Whatman GF/A papers. (For detail see Addendum 2.)

A comparison of the results obtained using GF/A filter papers and 0.45μm membrane filters is shown in Table 6. from which it was observed that:
1. Fresh enzyme solution daily gave more reproducible results.
2. The repeatability of absorbance area determinations were better than 1 unit of absorbance using 0.45μm filters.
3. That the area under the absorbance curve increased from 13.6 using GF/A filters to 14.1 using membrane filters. This indicated that if the removal of suspended matter by the membrane was the same or better than with the GF/A filters then it was likely that the GF/A had removed more colour from solution than had the membrane.

Furthermore, by comparing Tables 5 and 7 it was concluded that the use of 0.45μ filters greatly improved the accuracy of the estimate of colour from the absorbance data and statistically the 400 nm absorbance could be expected to give better results than the area under the absorbance curve.

It should be mentioned that initially the results using the 0.45μ filters were hardly better than those using the GF/A papers.

Table 6. *A comparison of the daily repeatability of absorbance data on gelatine control sample 183/1.*

	Solvent = Water			Solvent = ES1			
Day Number	Absorbance			Filter	Absorbance.		
	400 nm	700 nm	Area		400 nm	700 nm	Area
0	-	-	15.77		-	-	14.49
1	- -	0.024 0.029	15.47 17.38	GF/A	0.230 0.232	0.024 0.024	15.03 14.92
2	0.236 0.239	0.021 0.022	15.42 15.55	GF/A *	0.200 0.193	0.021 0.020	12.02 11.56
3	0.247 -	0.024 0.025	15.79 16.19	GF/A	0.225 0.223	0.024 0.023	14.36 14.33
4	0.242	0.025	15.70	GF/A *	0.204 0.200	0.019 0.021	13.03 12.55
	Mean, [Standard Deviation]		15.91 [0.64]		Mean, [Standard Deviation]		13.59 [1.31]
5	0.241	0.023	15.91	0.45μm*	0.212	0.021	14.06
6	0.251 0.251	0.026 0.027	16.43 16.16	0.45μm*	0.222 0.221	0.020 0.021	14.35 14.13
7	0.254	0.025	16.23	0.45μm*	0.224 0.213	0.019 0.019	14.65 13.55
	Mean, [Standard Deviation]		16.18 [0.21]		Mean, [Standard Deviation]		14.15 [0.41]

* Fresh Enzyme Solution.
\- Data not recorded.

It was noted that the poor correlation was mainly due to 6 (dark) gelatines, hence it was decided to ask the Davis Gelatine Laboratory to reassess the visual colours of these gelatines with the proviso that two experienced lab assistants should agree on the results. Additionally it was noted that the G35 and G36 gelatines could not be adequately filtered (700 nm absorbance > 0.04) so they were excluded from the correlation as well.

Table 7. *The correlation of visual colour and absorbance data for gelatine dissolved in ES1 and filtered using 0.45 µ membrane filters.*

Absorbance Parameter X.	700 nm	400 nm	Area under the Curve
Regression Constant.	0	0	0
Std. Error of Estimated Colour.	4.61	1.308	1.685
Correlation Coefficient.	0.535	0.971	0.951
Number of Observations.	31	31	31
Degrees of Freedom.	30	30	30
X Coefficient.	647	44.2	0.6946
Std. Error of Coefficient.	49.28	0.880	0.0178

Solvent = ES1.
Filtration = Millipore 0.45µm membrane filters. (For detail see Addendum 3.)

The results of the reassessment of the visual colours mentioned above are shown in Table 8. These result were in line with the anticipated error of 5 to 10 ml when using 100 ml Nessler Tubes, and the correlation coefficients were improved to 0.95 and 0.97 as a result.

Table 8. *The result of the reassessments of the visual colours of some gelatines.*

Sample Number	Original Colour	Reassessed Colour	Change in Volume to equal Standard. - ml.
LF752/1	6.0	5.2	10
850/6	16.0	18.8	5
183/4	14.5	13.3	5
186/6	16.0	16.0	0
178/12	20.0	17.8	5
184/10	22.8	22.8	0

The inclusion of Type A (pigskin) gelatines.

Due to the apparent success of the enzyme filtration combination treatment for the instrumental determination of gelatine colour, it was decided to assess whether Type A gelatines would present any problems to this colour assessment

technique. To this end a manufacturer was asked to provide pigskin gelatines with a range of colours. The nine samples they provided were assessed using solvent ES1. The result of the inclusion of this data with the data used to generate the correlation shown in Table 7 was to reduce the 400 nm correlation coefficient to 0.95 and the Absorbance area correlation coefficient to 0.93.

Based on the observation that the solutions were very difficult to filter it was decided to modify solvent ES1 by including some ammonia to give solvent ES2. This change, however, required the reassessment of the Type B gelatines under the same circumstances. The results are summarised in Table 9. The complete data is presented in Addendum 4.

Table 9. *The correlation of visual colour and absorbance data for gelatines dissolved in solvent ES2.*

Absorbance Parameter X.	700 nm	400 nm	Area under the Curve
Regression Constant.	0	0	0
Std. Error of Estimated Colour.	5.718	1.808	2.173
Correlation Coefficient.	0.387	0.571	0.9366
Number of Observations.	34	34	34
Degrees of Freedom.	33	33	33

Solvent = ES2.
Filtration = Millipore 0.45μm membrane filters. (For detail see Addendum 4.)

A comparison of the data in Tables 9 and 7 indicated that the use of ES2 solvent system had not improved the correlation coefficients for the instrumental method. The main reason was attributed to the observation that the change in pH sometimes caused precipitation and consequent loss of colour with the Type B gelatines.

Summary of data using solvent system ES1.

Some gelatine absorbances were measured repeatedly in order to assess the reproducibility of the method and to act as a check on the instrument. It was considered that the inclusion of the data on a single gelatine several times could distort the calculated correlation coefficients. It was decided therefore, to average the results of multiple absorbance measurements made using solvent ES1 and to use this to calculate the correlations between

absorbance data and visual colour as shown in Table 10. The X coefficient was then used to calculate Table 11.

Table 10. *The correlation of visual colour and absorbance data for both Type A and Type B gelatines dissolved in solvent ES1 and filtered through 0.45µ filters.*

Absorbance Parameter X.	400 nm	Area under the Curve
Regression Constant.	0	0
Std. Error of Estimated Colour.	1.463	1.769
Correlation Coefficient.	0.959	0.939
Number of Observations.	35	35
Degrees of Freedom.	34	34
X Coefficient.	43.66	0.6851
Std. Error of Coefficient.	1.771	0.0223

Solvent = ES1. (Alcalase solution)
Filtration = Millipore 0.45µm membrane filters. (For detail see Addendum 3.)
Each gelatine was included only once.

Table 11. *The correlation of the visual and estimated colours of gelatines based on their absorbance data when dissolved in solvent ES1 and filtered through 0.45µ membrane filters.*

Gelatine Sample	Visual Colour	400 nm Absorbance Colour Estimate		Absorbance Area Colour Estimate	
178/12	17.8	16.2		15.7	
180/6	16.0	17.3		17.1	
183/1	9.0	9.5		9.7	
184/10	22.8	23.7		25.5	
184/5	10.0	9.8		9.7	
185/2	6.8	7.6		7.1	
CT6/1	5.2	5.4		4.8	
CT6/2	5.6	7.0		6.6	
CT6/3	6.0	6.9		6.2	
D11335	2.8	3.0		3.4	
D1287	2.0	3.4		3.7	
D147	2.0	3.6	*H	4.1	*H
D1512	2.4	3.5		3.9	
D2116	2.4	2.9		3.1	
D2227	2.0	3.1		3.4	
D2249	2.0	3.0	*H	3.3	
D31108	2.8	5.4	*H	6.0	*H
D32117	4.0	6.8	*H	7.6	*H
D3264	3.6	5.9		6.5	*H
N - TY/A	4.8	5.4		4.9	
FISH 824/1	11.4	6.9	*L	6.7	*L
HORSE/1	14.0	12.5		12.5	
L752/1	5.2	5.4		5.2	
LCS	7.6	9.9	*H	8.9	
LF752/1	6.0	5.2		4.2	*L
LF752/1	6.0	5.0		4.1	*L

Table 11 Contd.

Gelatine Sample	Visual Colour	400 nm Absorbance Colour Estimate		Absorbance Area Colour Estimate	
826/1	8.0	7.5		6.9	
OCS	8.0	8.7		8.3	
PIS PP1	11.4	9.0	*L	7.5	*L
SBG	4.8	4.1		3.9	
ST26/1	11.4	10.9		10.6	
ST26/2	11.4	11.7		11.4	
ST26/3	16.0	15.4		15.0	

*H unacceptable high estimate.
*L unacceptable low estimate.

Table 11 showed that:

1. In 21% of cases the colours estimated from 400 nm absorbance were not acceptable (*) and that there were both high and low estimates.

2. In 24% of cases the colours estimated from absorbance area were not acceptable (*) and that there were both high and low estimates.

These differences were in line with the differences in correlation coefficients and the conclusion reached was that in spite of the highly significant correlation coefficients of 0.95 and 0.93 the instrumental method of colour measurement was not commercially viable.

The next approach to the problem was to find a proteolytic enzyme that could hydrolyse acidic gelatines.

The use of solvent ES3 on Gelatines with a pH of 4 to 5.

The enzyme Papain which had good proteolytic activity in the pH range of 4 to 5 was evaluated using gelatine. Although the enzyme was said to have had adequate activity in the range pH 3 to 10 it was found that above pH 5 the hydrolysis of Type B gelatines was inadequate, that is, the gelation of 6.67%

solutions at room temperature was prevented but gelation at 2°C still occurred and the solution viscosity at room temperature was such as to make filtration very difficult. A mixed solution of Alcalase and Papain was made. It appeared to work well on both Type A and Type B gelatines from the point of view of hydrolysis. The mixture, however, was markedly cloudy and particularly with gelatine 178/12 with which it caused a filterable precipitate. The absorbance of the filtrate was low, indicating loss of colour with the precipitate. Hence, Enzyme Solution (ES3) which only contained Papain, was used on the acid process pigskin gelatines. The results are shown in Table 12.

Table 12. *A comparison of the areas under the absorbance curves between 400 and 700 nm for low pH gelatines using solvents ES1 and ES3.*

Solvent	DGI	ES1	ES3	Estimated Colour
Sample Number	Colour	Absorbance Area	Absorbance Area	= ES3 Abs. x 0.7
D2227	2.0	5.01	3.92	2.7
D3264	3.6	9.53	6.90	4.8
D1512	2.4	5.62	3.81	2.7
D32117	4.0	11.13	8.15	5.7*
D2116	2.4	4.57	3.54	2.5
D147	2.0	5.99	4.55	3.2
D31108	2.8	8.82	6.64	4.6*
D1287	2.0	5.39	4.29	3.0
D2249	2.0	4.75	3.78	2.6

* denotes an unacceptable instrumental estimate of colour.

It should be noted that there were differences in clarity between solvents ES1 and ES3 but as all absorbances were measured with the solvent as the blank the absorbance values at 400 nm could also be correlated with visual colour.

On average, the absorbance areas of low pH gelatines determined in solvent ES1 were 1.7 absorbance units higher than in solvent ES3 presumably due to inadequate hydrolysis in solvent ES1. This equates to an average of 1.2 colour units darker than the visual colour which was very significant when the visual colour was about 3.

The absorbance area data obtained by the use of ES3 with the low pH gelatines,

was substituted for the data obtained with ES1 on these gelatines the statistical results shown in Table 13 were obtained.

Table 13. *The correlation of visual colour and absorbance data for gelatines with pH <5 dissolved in solvent ES3 and gelatines with pH >5 dissolved in solvent ES1.*

Absorbance Parameter X.	400 nm	Area under the Curve
Regression Constant.	0	0
Std. Error of Estimated Colour.	1.290	1.509
Correlation Coefficient.	0.968	0.953
Number of Observations.	35	35
Degrees of Freedom.	34	34
X Coefficient.	44.299	0.6999
Std. Error of Coefficient.	1.044	0.01936

Each gelatine was included only once.
Solvent = ES3.(Papain solution).
Filtration = Millipore 0.45μm membrane filters. (For detail see Addendum 5.)

The small improvements in correlation coefficients due to the use of ES3 absorbance values in place of ES1 absorbance values resulted in the "unacceptable" estimates of colour being reduced from 24% to 18%.

The effect of ES1 and ES2 on solution pH.

The pHs of a number of pairs of gelatine samples in water and in solvent ES1 and ES2 are recorded in Table 14. As can be seen the change in pH due to enzymic hydrolysis (ES1) were negligible due to the strong buffering properties of gelatine and the limited ability of enzymes to hydrolyse gelatine.

Table 14. *A comparison of gelatine pHs in water and solvents ES1 and ES2.*

Sample	pH in Water	pH in ES1	pH in ES2
185/2	5.15	5.14	-
178/12	5.30	5.29	-
183/1	4.92	4.91	-
185/2	5.12	-	7.30
824/1	5.65	-	7.42
D1512	4.52	-	6.64
N - TyA	5.78	-	6.18
N - TyA	5.81	-	6.2
D2227	4.50	-	6.8

- Not applicable.

Repeatability of the area under the absorbance curve.

The daily control sample data presented in Table 15 shows the repeatability of colour values. From the data it could be concluded with a probability of 0.95, that determinations of absorbance area should not differ by more than 2 units of absorbance (which equates to 1.4 colour units on the Davis scale). In confirmation it was found that in ADDENDUM 2 there were a number of gelatines with two or more determinations of absorbance area all of which agreed to better than 1 unit of absorbance.

Table 15. *The repeatability of absorbance data from day-to-day.*

Sample	Date	Absorbance Area	Date	Absorbance Area
183/1	17 JAN 19 JAN 28 JAN 02 FEB	14.8, 15.0 14.3, 14.3 14.1 14.6, 13.6	18 JAN 20 JAN 01 FEB	12.0, 11.8 13.0, 12.8 14.4, 14.2
183/1		Mean = 13.75	Standard Deviation = 1.04	Number of Determinations = 13
178/12	25 MAR 12 APR 20 APR 27 APR	22.6 24.3, 24.4 24.6, 24.1 23.0, 23.0	11 APR 14 APR 22 APR	22.8, 22.6 24.2, 24.1 24.4, 24.4
178/12		Mean = 23.73	Standard Deviation = 0.79	Number of Determinations = 13

The use of an alternative enzyme.

It was desired to show whether the method was dependent on the use of Alcalase. Trials were undertaken using Pancreatic Trypsin Novo in place of Alcalase in solvent ES1. Using the computer program Epistat (T.L. Gustafson, 2011 Cap Rock Circle, Richardson, Texas, 75080, USA), the Student's t-test was applied to the Absorbance Areas in Table 16. The value of t(8) for the difference between the two means was 1.061445, with a probability exceeding 0.3. This indicated that there was no significant difference between the means.

In this experiment, due to the observed haze in the Trypsin solution both enzyme solution blanks were filtered (0.45μ) prior to use. However, as a result of residual haze in the trypsin solution there was a statistically significant difference between both the 400 nm and the 700 nm absorbances obtained with ES1 or Trypsin. This demonstrated the value of absorbance area determination.

Table 16. *A comparison of the absorbance data obtained with Alcalase and Trypsin hydrolysis of Type B gelatines.*

	A L C A L A S E Absorbances.			T R Y P S I N Absorbances.		
Sample.	400 nm.	700 nm.	Area 400 - 700 nm.	400 nm.	700 nm.	Area 400 - 700 nm.
C200/1	0.0392	0.001	2.09	0.041	0.001	2.35
WT3/3	0.3262	0.017	20.58	0.3292	0.018	20.65
YSA/1	0.0843	0.007	5.29	0.0805	0.007	5.04
INOE/2	0.2815	0.013	18.70	0.2918	0.025	18.26
877/1	0.1432	0.003	8.14	0.1562	0.014	8.33
155/1	0.1657	0.006	9.90	0.1771	0.018	9.80
5Y4/3	0.2022	0.012	12.61	0.2127	0.023	12.57
178/12	0.3690	0.011	22.65	0.3774	0.028	22.47
CT4/2	0.1524	0.011	9.16	0.1592	0.020	8.98

The Effect of acid hydrolysis of gelatine.

Again it was desirable to know whether a hydrolysis system other than an enzymic system could be used in preparation for the instrumental determination of colour. The results are shown in Table 17.

Table 17. *A comparison of the absorbance data for gelatines hydrolysed with 1:1 hydrochloric acid and Alcalase solution ES1.*

SOLVENT - 15% Hydrochloric Acid.				
	Colour	A B S O R B A N C E.		
Sample		400 nm.	700 nm.	Area 400 - 700 nm.
178/12	17.8	1.57, 1.53	0.10, 0.09	124.5, 120.7
185/2	6.0	1.12, 1.12	0.07, 0.06	89.7, 89.6
SOLVENT - ES1.				
178/12	17.8	0.37, 0.37	0.02, 0.02	23.01, 23.00
185/5	6.0	0.18, 0.18	0.01, 0.01	10.44, 10.46

From Table 17 it was clear that partial acid hydrolysis was accompanied by considerable darkening and for this reason alone it was decided that the

procedure was not worth pursuing. It was noted, however, that the paler gelatine hydrolysate had a markedly lower absorbance than the darker, hence there did seem to be a relationship between gelatine colour and hydrolysate colour.

The BYK-Gardner Color-View Reflectance Spectrophotometer.

The results of the measurements made on a range of gelatines are recorded in Addendum 8.

Preliminary trials were made comparing the results using 50 and 75 ml of sample. The L values of the samples was some 3 units or 6% paler using 75 ml sample size indicating a need to control the sample size used for the determination and the need for the smaller sample size to maximise the effect of colour. Furthermore, the differences caused by a change from the Gardner sample cell to a Grainer glass cell filled to the same depth were such as to indicate the need to use the same cell for all determinations. This aspect would preclude the possibility of the routine determination of colour on gelled solutions.

The sample quantity of 50 ml was chosen because it half filled the Gardner cell which was convenient from the point of view of handling and measurement and it appeared better than 75 ml from the point of view of the optics of the system. The maximum sample size would have been 90 ml which was the capacity of the cell but this would have been impractical from the handling point of view.

Initially trials were made using the white tile as the standard and the following linear correlation coefficients were obtained after excluding the G35, G36 and G37 gelatines with unmeasurable clarities:

L and Colour.	r = 0.98;	a and Colour.	r = 0.56
b and Colour.	r = 0.91;	Y1 and Colour.	r = 0.95
Y2 and Colour.	r = 0.92		

It was realised, however, that it would be best to stay with the conventional procedure of comparing the colour of the solution to a solvent blank thus eliminating any differences which might arise due to differences in standard tiles.

Using water as the standard, the following linear correlation coefficients were obtained after eliminating gelatines with clarities of ≥ 100 NTU:

L and Colour.	r = 0.96;	a and Colour.	r = 0.84
b and Colour.	r = 0.89;	Y1 and Colour.	r = 0.91
Y2 and Colour.	r = 0.88		

From the above it was concluded that for the 86 gelatines tested, the L or darkness component gave the best correlation to visual colour and the correlation coefficient was very slightly better than that obtained in Table 10. The Davis colours of all the gelatines were calculated from the linear coefficients (Colour = -1.345 -1.582 x L) and there were 15% where the instrumental colour deviated unacceptably (marked with a *) from the visual colour. From inspection it was clear that where the instrumental colour was low, in most cases the clarity was also unusually low. Furthermore, the gelatines with clarities > 100 NTU caused the instrumental colour to be high.

The gelatines used in this experiment were as diverse as those used previously and included the pigskin and fish skin gelatines as well as Type A cow hide gelatines, and gelatines from other manufacturers, hence, it appeared that the BYK-Gardner reflectance spectrophotometer gave better results than the Beckman DU70 spectrophotometer without the need to prepare samples specially for colour measurement. In addition, it was concluded that given the inherent error of the subjective measurement of colour, the BYK-Gardner instrument would give colour results as reliable or with a better reliability than visual assessment, as long as the results obtained with poor clarity gelatines (>80 NTU) were accepted as being unreliable, as were visual colours on poor clarity gelatines. Finally, examination of the data in ADDENDUM 8 showed that the maximum error between determinations of colour should not exceed 0.7 Davis colour units. This error could largely be attributed to differences in reflection from the white tile (with water in the cell, the difference in colour between a clean tile and one with condensed moisture on it, was 0.4 Davis colour units).

CONCLUSIONS.

1. Table 8 shows the uncertainty associated with the subjective visual determination of the colour of gelatine in solution. To this must be added the fact that such a system of colour determination was comparative and hence it was very difficult or impractical to use it for "specification" purposes. Hence, it would be an advantage to be able to measure gelatine solution colour instrumentally.

2. Table 2 showed that without the elimination of interferences there was a poor correlation between optical density (OD) and visual colour. Table 3 showed that even with filtration there was little improvement in the correlation coefficient. Table 4 showed that if in addition the base line was moved, then a significant correlation between:

a) the area under the absorbance curve between 400 and 700 nm and visual colour, and

b) the 400 nm absorbance and visual colour.

could be obtained.

3. The use of enzymic hydrolysis and filtration of the gelatine solution through a 0.45μ membrane filter refined the correlation between 400 nm absorbance or 'area under the curve and colour to the level where a commercially useful method was available for Type B gelatine with a pH of greater than 5.

4. The inclusion of acid pigskin gelatines upset the correlation between absorbance measurements and visual colour. It was realised that the low pH of these gelatines made the hydrolysis by Alcalase ineffective. It was found that the enzyme papain could be used on gelatines with a pH between 4 and 5 and this modification returned the correlation coefficients between absorbance area and visual colour to 0.95. The correlation coefficient between 400 nm OD and colour was increased to almost 0.97 (Table 13). In other words, absorbance data and visual colour agreed reasonably in 88% of the determinations even though every effort had been made to upset the correlation by including a variety of gelatines from all available sources.

The method was based on the principle of reducing the light scatter of

gelatine solutions to a constant. This was not possible with the bad clarity G35, G36 and G37 gelatines and these had to be eliminated from the correlation. Hence, it must be stated that the method could only be applied to gelatines with a natural clarity of better than 80 NTU measured on 6.67% solutions at about 40°C. This translates to a filtered OD at 700 nm not exceeding 0.030.

5. The BYK-Gardner Color-View reflectance spectrophotometer gave a better correlation of the L component of colour to the visual colour than was obtained with the Beckman DU70 spectrophotometer. Given the inherent error of the visual measurement of colour and that solution colour could be reliably measured on remelted 6.67% Bloom sample without any further manipulation. This instrument could be recommended for the measurement of gelatine colour with the probability that the difference between two determinations would not exceed 0.7 Davis colour units which was half the error currently accepted using the visual determination of colour.

6. A number of minor observations were made:

a) Whatman GF/A filter papers absorbed some of the colour from darker gelatine solutions.

b) The enzymes Alcalase and Trypsin could be used interchangeably (Table 6). If absorbance data from different solvent systems was to be used to measure gelatine colours then the best correlation was given by the absorbance area between 400 and 700 nm. The reason for this was that the effective subtraction of the 700 nm absorbance had not fully compensate for differences in solvent clarity.

c) If, during enzymic hydrolysis there was precipitation then this was likely to be accompanied by absorption of some of the colour by the precipitate, resulting in a low OD or colour value (Addendum 5 & Table 9).

d) The pH change in 6.67% gelatine solutions due to enzymic hydrolysis was small (Table 14) both because of the buffering power of gelatine and because the enzymes could only partially hydrolyse the protein.

e) The possibility of acid hydrolysis was investigated (Table 17) but it was found that this led to a marked darkening of the solutions so the process was

not pursued.

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ADDENDA.

ADDENDUM 1. DU70 Absorbance data on gelatines.
Solvent = Water.
Filtration. Nil.

DATE	SAMPLE	COLOUR	CLARITY	ABS 400 nm	ABS 700 nm	AREA 400-700
12/1	178/12	17.8	7.5	0.393		31.4
12/1	183/1	9	6.5	0.24		22.7
12/1	C542/2L	<3.2	13	0.064		5.8
	BASE LINE = ABS 700nm					
13/1	C542/2L	<3.2	13			3.808
19/1	G35	4.8	NM	1.1101	0.37	71.8
19/1	G36	8.9	NM	0.6687	0.153	45.71
18/1	YSB/1	<3.2	10	0.0953	0.016	5.92
19/1	C552/1	<3.2	12.5	0.0502	0.011	2.57
20/1	C542/1L	<3.2	13.3	0.0738	0.016	3.99
19/1	C542/3L			0.114	0.015	6.47
28/1	C542/3L			0.1244	0.012	5.42
28/1	YSB/1	<3.2	10	0.0845	0.012	5.22
02/2	C542/1L	<3.2	13	0.074	0.016	3.8
02/2	C542/2L	<3.2	13.3	0.0671	0.008	3.99
13/1	178/12	17.8	7.5			24.58
13/1	183/1	9	6.5			15.77
17/1	183/1	9	6.5		0.026	16.42
17/1	184/10	22.8	4	0.6412	0.077	44.25
17/1	LF752/1	6	11	0.178	0.03	8.63
17/1	185/2	6.8	10.5	0.1838	0.01	10.95
17/1	L752/1	4.8	10	0.0293	0.066	10.29
17/1	180/6	16	6.5	0.4239	0.034	26.69
17/1	183/4	14.5	10.5	0.3023	0.021	18.69
18/1	183/1	9	6.5	0.2374	0.022	15.48
18/1	CT6/1	5.2	11.5	0.1471	0.02	8.52
18/1	CT6/2	5.6	11.5	0.1865	0.032	9.81
18/1	CT6/3	6	12.5	0.1757	0.016	9.93
18/1	YSB/3	4.8	9	0.1394	0.027	8.37
18/1	ST26/1	11.4	11.8	0.2826	0.033	16.79
18/1	ST26/2	11.4	11.8	0.285	0.022	17.01
18/1	ST26/3	16	10	0.371	0.027	22.69
19/1	183/1	9	6.5	0.247	0.025	16.09
19/1	826/2	8	26	0.211	0.027	11.96
19/1	OCS	8	20	0.2204	0.016	13.33
19/1	LCS	7.6	7.5	0.2635	0.015	15.73
19/1	YSB/2	5.2	7.5	0.1621	0.047	9.73
19/1	184/5	10	11.1	0.242	0.021	15.33
20/1	183/1	9	6.5	0.2421	0.025	15.7

ADDENDUM 1. Continued...

DATE	SAMPLE	COLOUR	CLARITY	ABS 400 nm	ABS 700 nm	AREA 400-700
20/1	D11335	2.8	10	0.1204	0.017	8.452
20/1	D94025	2.4	9	0.1903	0.042	13.61
20/1	HORSE/90	14	4.5	0.4034	0.017	25.47
20/1	CALF-A/3	5.2	9	0.2474	0.052	16.77
20/1	PIS-PP1	11.4	10.5	0.273	0.036	14.14
20/1	PIZ/1	3.6	9	0.1652	0.049	9.1
20/1	SBG	6.4	8	0.1544	0.019	9.72
20/1	N CALF-TyB	5.6	12	0.1581	0.015	9.27
28/1	183/1	9	6.5	0.243	0.022	16.08
28/1	L752/1	4.8	10	0.1868	0.054	9.13
28/1	N CALF TyB	5.6	12	0.1552	0.011	9.44
28/1	LF752/1	6	11	0.1725	0.023	9.41
28/1	178/12	17.8	7.5	0.3974	0.022	24.67
28/1	183/4	14.5	10.5	0.3001	0.018	19.03
28/1	185/2	6.8	10.5	0.1804	0.008	10.78
28/1	184/10	22.8	4	0.6442	0.076	44.52
28/1	YSB/3	4.8	9	0.1431	0.025	9.26
28/1	180/6	16	6.5	0.408	0.023	26.17
28/1	YSB/2	5.2	7.5	0.1436	0.033	9.03
28/1	826/2	8	9	0.1947	0.015	11.21
28/1	CT6/3	6	12.5	0.1654	0.012	8.93
28/1	CT6/1	5.2	11.5	0.1344	0.012	8
28/1	184/5	10	11.1	0.2198	0.008	13.93
28/1	OCS	8	10	0.2008	0.007	12.39
28/1	CT6/2	5.6	11.5	0.1625	0.015	9.39
01/2	183/1	9	6.5	0.2511	0.027	16.3
01/2	SBG	6.4	8	0.6136	0.019	10.57
01/2	HORSE/1	14	4.5	0.4139	0.075	25.66
01/2	G35	4.8	NM	1.1143	0.369	72.65
01/2	LF752/1	6	11	0.1802	0.03	8.91
01/2	184/10	22.8	4	0.6483	0.079	44.76
01/2	LCS	7.6	7.5	0.2682	0.016	15.87
01/2	180/6	16	6.5	0.4274	0.034	26.9
01/2	178/12	20	7.5	0.4098	0.025	25.37
01/2	183/4	14.5	10.5	0.3093	0.021	19.2
02/2	183/1	9	6.5	0.2537	0.025	16.23
02/2	D11335	2.8	10	0.114	0.014	8.14
02/2	D94025	2.4	9	0.1884	0.038	13.7
02/2	PIS PP1	11.4	10.5	0.2667	0.036	14.91
02/2	ST26/2	11.4	11.8	0.2864	0.022	17.73
02/2	ST26/3	16	10	0.3761	0.028	23.28
02/2	ST26/1	11.4	11.8	0.2987	0.04	17.43
02/2	850/6 + C	17.8	5	0.4876	0.063	28.63
02/2	850/6	17.8	5	0.4999	0.063	30.45
02/2	852/1 + C	6	11	0.1783	0.027	9.39
02/2	852/1	6	11	0.1719	0.031	9.56
11/4	178/12	17.8	7.5	0.3964	0.025	24.33
11/4	D31108	2.8	36	0.1914	0.035	13.65
11/4	D2249	2	11	0.0731	0.006	5.05
11/4	D1512	2.4	30	0.1284	0.022	9.55
11/4	D2116	2.4	17	0.0897	0.011	6.52
11/4	D2227	2	21	0.096	0.015	6.81

ADDENDUM 1. Continued...

DATE	SAMPLE	COLOUR	CLARITY	ABS 400 nm	ABS 700 nm	AREA 400-700
11/4	N - TyA	4.8	17	0.1545	0.016	9.44
28/4	D32117	4	50	0.2334	0.046	16.21
28/4	D1287	2	31	0.1171	0.019	8.31
28/4	D3264	3.6	42	0.1946	0.035	13.66
28/4	D147	2	44	0.1571	0.031	11.36
28/4	FISH 824/1	11.4	8.5	0.2157	0.038	12.3
28/4	FISH COD2/1	6.0	11.0	0.1532	0.012	9.07
28/4	178/12	17.8	7.5	0.3957	0.022	24.73
28/4	185/2	6	11	0.1838	0.01	10.66

ADDENDUM 2. Solvent = ES1 (1 ml Alcalase 0.6L/l)
Filtration = Whatman papers (GF/A).
BASE LINE = ABS. 700nm for area calculation.

DU70 Gelatine Absorbance Data.

DATE	SAMPLE	COLOUR	CLARITY	ABS 400 nm	ABS 700 nm	AREA 400-700
12/1	C542/2L	<3.2	13	0.051		4.2
13/1	C542/2L	<3.2	13			2.95
18/1	YSB/1	<3.2	10	0.085	0.018	4.73
19/1	C542/3L			0.0964	0.006	5.24
19/1	C552/1	<3.2	12.5	0.0435	0.006	2.34
20/1	C542/1L	<3.2	13.3	0.052	0.008	2.52
12/1	178/12	17.8	7.5	0.371		29
12/1	183/1	9	6.5	0.238		21.9
13/1	178/12	17.8	7.5			22.38
13/1	183/1	9	6.5			13.54
17/1	183/1	9	6.5	0.2309	0.024	14.92
17/1	184/10	22.8	4	0.4843	0.037	32.2
17/1	LF752/1	6	11	0.1272	0.01	6.34
17/1	185/2	6.8	10.5	0.1886	0.014	10.98
17/1	L752/1	4.8	10	0.1313	0.012	7.44
17/1	180/6	16	6.5	0.3504	0.024	21.03
17/1	183/4	14.5	10.5	0.2798	0.018	17
18/1	183/1	9	6.5	0.1962	0.021	11.79
18/1	CT6/1	5.2	11.5	0.1413	0.017	8.29
18/1	CT6/2	5.6	11.5	0.1671	0.017	9.74
18/1	CT6/3	6	12.5	0.174	0.018	9.82
18/1	YSB/3	4.8	9	0.1094	0.014	6.61
18/1	ST26/1	11.4	11.8	0.2591	0.018	16.12
18/1	ST26/2	11.4	11.8	0.2843	0.021	17.34
18/1	ST26/3	16	10	0.3703	0.028	22.65
19/1	183/1	9	6.5	0.2237	0.024	14.35
19/1	826/2	8	26	0.186	0.013	10.74
19/1	OCS	8	20	0.2155	0.018	13.01
19/1	LCS	7.6	7.5	0.2329	0.014	13.39
19/1	YSB/2	5.2	7.5	0.1151	0.021	7.2
19/1	184/5	10	11.1	0.2341	0.018	14.38
19/1	G35	4.8	NM	0.4721	0.077	32.73
19/1	G36	8.9	NM	0.5282	0.088	37.26
20/1	183/1	9	6.5	0.202	0.02	12.79
20/1	D11335	2.8	10	0.0662	0.007	4.37
20/1	D94025	2.4	9	0.0535	0.011	3.47
20/1	HORSE/90	14	4.5	0.322	0.039	20.64
20/1	CALF-A/3	5.2	9	0.1675	0.023	10.36
20/1	PIS-PP1	11.4	10.5	0.2072	0.02	10.52
20/1	PIZ/1	3.6	9	0.0801	0.007	4.09
20/1	SBG	6.4	8	0.1256	0.016	7.63
20/1	N CALF-TyB	5.6	12	0.1396	0.011	7.86

ADDENDUM 3. Solvent = ES1.
Filtration = membrane (0.45 μm).

DU70 Gelatine absorbance data.

DATE	SAMPLE	COLOUR	CLARITY	ABS 400 nm	ABS 700 nm	AREA 400-700
28/1	C542/3L			0.0794	-0.004	4.41
28/1	YSB/1	<3.2	10	0.0624	0.005	3.76
02/2	C542/1L	<3.2	13	0.0454	0	2.25
02/2	C542/2L	<3.2	13.3	0.0457	0	2.71
01/2	G35	4.8	NM	0.3956	0.052	27.09
28/1	183/1	9	6.5	0.2115	0.012	14.07
28/1	L752/1	5.2	10	0.1237	0.001	7.59
28/1	LF752/1	6	11	0.1138	-0.004	6.05
28/1	178/12	17.8	7.5	0.3662	0.01	22.67
28/1	183/4	13.3	10.5	0.2808	0.009	17.78
28/1	185/2	6.8	10.5	0.1681	0	10.31
28/1	184/10	22.8	4	0.538	0.037	37.22
28/1	YSB/3	4.8	9	0.0995	0.006	6.41
28/1	180/6	16	6.5	0.3924	0.025	24.63
28/1	YSB/2	5.2	7.5	0.093	0.009	5.94
28/1	826/2	8	9	0.1724	0.006	10.02
28/1	CT6/3	6	12.5	0.1581	0.008	9.07
28/1	CT6/1	5.2	11.5	0.1228	0.009	7.06
28/1	184/5	10	11.1	0.2254	0.014	14.09
28/1	OLD C/S	8	10	0.2001	0.012	12.11
28/1	CT6/2	5.6	11.5	0.1601	0.012	9.57
01/2	183/1	9	6.5	0.2215	0.021	14.24
01/2	SBG	6.4	8	0.0946	0.003	5.74
01/2	HORSE/1	14	4.5	0.2871	0.027	18.22
01/2	LF752/1	6	11	0.1222	0.006	6.08
01/2	184/10	22.8	4	0.5453	0.045	37.2
01/2	LCS	7.6	7.5	0.2271	0.009	12.97
01/2	180/6	16	6.5	0.4012	0.027	25.16
01/2	178/12	20	7.5	0.3774	0.017	22.99
01/2	183/4	14.5	10.5	0.2925	0.017	18.12
02/2	183/1	9	6.5	0.2183	0.019	14.1
02/2	D11335	2.8	10	0.0687	0.005	5
02/2	PIK SHP	11.4	10.5	0.205	0.007	10.9
02/2	ST26/2	11.4	11.8	0.2679	0.013	16.6
02/2	ST26/3	16	10	0.3517	0.019	21.83
02/2	ST26/1	11.4	11.8	0.2495	0.013	15.49

SOLVENT ES1, FILTRATION 0.45μm.
ABSORBANCE AREA v/s Colour. Regression Output:

Constant		0
Std Err of Y Est		1.684898
R Squared		0.904779
No. of Observations		31
Degrees of Freedom		30
X Coefficient(s)	0.691605	
Std Err of Coef.	0.017813	

ADDENDUM 3. Continued...

ABSORBANCE-400nm v/s Colour. Regression Output:

Constant	0
Std Err of Y Est	1.308228
R Squared	0.942595
No. of Observations	31
Degrees of Freedom	30
X Coefficient(s)	44.20141
Std Err of Coef.	0.880448

ABSORBANCE-700nm v/s Colour. Regression Output:

Constant	0
Std Err of Y Est	4.613375
R Squared	0.286124
No. of Observations	31
Degrees of Freedom	30
X Coefficient(s)	647.7928
Std Err of Coef.	48.95671

Additional data including pigskin gelatines.
SOLVENT =.ES1. FILTER = 0.45μm.

DATE	SAMPLE	COLOUR	CLARITY	ABS 400 nm	ABS 700 nm	AREA 400-700
11/4	178/12	17.8	7.5	0.3679	0.016	22.74
11/4	D31108	3.8	36	0.124	0.011	8.82
11/4	D2249	2.4	11	0.0676	0.005	4.75
11/4	D1512	2.2	30	0.0791	0.009	5.62
11/4	D2116	2	17	0.0662	0.005	4.57
11/4	D2227	2.2	21	0.0711	0.006	5.01
11/4	N - TyA	4.8	17	0.1247	0.006	7.17
28/4	D32117	4.7	50	0.1566	0.017	11.13
28/4	D1287	2.2	31	0.077	0.007	5.39
28/4	D3264	4	42	0.1351	0.014	9.53
28/4	D147	2.4	44	0.0826	0.01	5.99
28/4	FISH 824/1	11.4	8.5	0.1579	0.004	9.85
28/4	FISH COD2/1	6	11	0.1718	0.016	10.7
28/4	178/12	17.8	7.5	0.3748	0.018	23.01
28/4	185/2	6.8	11	0.1795	0.011	10.45

ABSORBANCE AREA V/S COLOUR. Regression Output:

Constant	0
Std Err of Y Est	1.820664
R Squared	0.901143
No. of Observations	46
Degrees of Freedom	45
X Coefficient(s)	0.69094
Std Err of Coef.	0.0175

ADDENDUM 3. Continued...

ABSORBANCE - 400 nm V/S COLOUR.	Regression Output:
Constant	0
Std Err of Y Est	1.472233
R Squared	0.93536
No. of Observations	46
Degrees of Freedom	45
X Coefficient(s)	44.15308
Std Err of Coef.	0.899812

ABSORBANCE - 700nm v/s COLOUR.	**Regression Output:**
Constant	0
Std Err of Y Est	4.106796
R Squared	0.579047
No. of Observations	57
Degrees of Freedom	56
X Coefficient(s)	635.9171
Std Err of Coef.	39.49756

ADDENDUM 4. Solvent = ES2. (Contained ammonia).
Filtration. = membrane (0.45 μm).

DU70 Gelatine Absorbance data.

DATE	SAMPLE	COLOUR	CLARITY	ABS 400 nm	ABS 700 nm	AREA 400-700
14/4	C200/1	<3.2	11	0.048	0	2.74
14/4	YSB/1	<3.2		0.072	0.005	4.72
12/4	D2116	2.4		0.055	0.004	3.84
12/4	D2249	2		0.055	0.003	3.87
14/4	D1287	2	10	0.058	0.005	4.05
12/4	D2227	2		0.061	0.006	4.27
12/4	D1512	2.4		0.062	0.006	4.5
14/4	D147	2	8	0.065	0.007	4.7
12/4	D31108	2.8	36	0.103	0.01	7.19
14/4	5Y4/3	8.4	5.6	0.126	0.005	7.24
14/4	D3264	3.6	8	0.102	0.009	7.31
14/4	D32117	4	7	0.125	0.013	9.09
14/4	N -TyA	4.8	11.5	0.167	0.003	10.02
14/4	WT3/3	16	4.8	0.308	0.008	19.17
14/4	IOE/2	12.3	11	0.319	0.015	21.29
12/4	178/12	17.8	7.5	0.386	0.014	24.02
14/4	178/12	17.8		0.384	0.014	24.4
20/4	ST24/1	8.9		0.1163	-0.001	6.6
20/4	5Y4/2	6.8		0.1287	0	7.49
20/4	5Y4/3	8.4		0.1694	0.003	10.4
20/4	ST24/3	13.3		0.3015	0.007	18.99
20/4	ST24/2	12.3		0.2122	0.003	12.82
20/4	5Y4/1	5.6		0.0942	0	5.71
20/4	YSB/3	4.8		0.1157	0.01	7.91
20/4	YSB/2	5.2		0.1053	0.012	7.283
20/4	178/12	17.8	7.5	0.3853	0.014	24.32
22/4	SBG	6.4	8	0.1026	0.002	6.7
22/4	178/12	17.8	7.5	0.3837	0.014	24.38
22/4	850/6+C	17.8	5	0.409	0.019	25.87
22/4	850/6	17.8	5	0.4282	0.025	27.66
22/4	FISH824/1	11.4	8.5	0.1423	0.004	8.22
22/4	183/4	13.3	10.5	0.3056	0.013	19.94
22/4	180/6	16	6.5	0.3899	0.021	24.95
22/4	184/10	22.8	4	0.5721	0.045	40
22/4	184/5	10	11	0.2451	0.015	16.21
22/4	185/2	6	11	0.1957	0.012	12

ADDENDUM 5. Pigskin gelatines.
Solvent = ES3. (Contained papain).
Filtration = membrane (0.45μm).

DU70 absorbance data on Pigskin gelatines with pH < 5.

DATE	SAMPLE	COLOUR	CLARITY	ABS 400 nm	ABS 700 nm	AREA 400-700
6/6	D2227	2.2		0.0559	0.001	3.92
6/6	D3264	4		0.1023	0.006	6.9
6/6	D1512	2.2		0.0563	0.003	3.81
6/6	D32117	4.7		0.1191	0.008	8.15
6/6	D2116	2		0.0524	0.001	3.54
9/6	D147	2.4		0.0656	0.006	4.55
9/6	D31108	3.8		0.1	0.005	6.64
9/6	D1287	2.2		0.0623	0.005	4.29
9/6	D2249	2.4		0.0537	0.001	3.78
9/6	OCS	8		0.1751	0.007	10.11
9/6	UNFILTERED ES3			0.025	0.0131	1.34

MIXED ENZYME Solution.
0.62g PAPAIN + 0.2 ml ALCALASE TO 200 ml.
10 ml per sample.

DATE	SAMPLE	COLOUR	CLARITY	ABS 400 nm	ABS 700 nm	AREA 400-700
6/6	178/12	17.8		0.2979	0.012	17.57
6/6	877/1	5.6		0.139	0.003	7.77
6/6	155/1	6.8		0.1612	0.006	9.24
6/6	D2227	2.2		0.0527	0.002	3.39
6/6	D3264	4		0.0938	0.005	6.11
6/6	D1512	2.2		0.049	0.001	3.27
6/6	UNFILTERED MIXED ENZYME.			0.0984	0.046	6.57

ADDENDUM 6. Gelatine analytical data.

NOTES - ORIGINS	SAMPLE	COLOUR	CLAR	BLOOM	pH	% Moist	% Ash	Visc.ms	Type
Bovine hide Prodn. Gelatine	155/1	6.8	12.5	269	5.7	10.36	0.4	36.4	B
Bovine hide Prodn. Gelatine	178/12	17.8	7.5	125	5.7		1.3		B
Bovine hide Prodn. Gelatine	180/6	16	6.5	204	5.2		0.98		B
Bovine hide Prodn. Gelatine	183/1	9	6.5	256	5.3	8.68	0.78	36.5	B
Bovine hide Prodn. Gelatine	183/4	14.5	10.5	160	5	7.9	0.89		B
Bovine hide Prodn. Gelatine	184/10	22.8	4	98	5.9	9.3	1.56		B
Bovine hide Prodn. Gelatine	184/5	10	11.1	219	5.2		1.25		B
Bovine hide Prodn. Gelatine	185/2	6.8	10.5	266	5.2		0.4	39.5	B
5 year old bovine gelatine	5Y4/3	8.4	11	226	5.6	11.5	2.41	33.2	B
Bovine hide Prodn. Gelatine	826/2	8	26	263	5.1	9.55	0.82	39.8	B
Bovine hide Prodn. Gelatine	850/6	17.8	5	182	5.3	10.4	0.76	29.8	B
Bovine hide Prodn. Gelatine	850/6 + C	17.8	5	178	5.3	9.25	0.78	29.9	B
Bovine hide Prodn. Gelatine	852/1	6	11	240	5.3	13.23	0.35	42.4	B
Bovine hide Prodn. Gelatine	852/1 + C	6	11	231	5.1	15.46	0.34	40.1	B
Bovine hide Prodn. Gelatine	877/1	5.6	11.5	267	5.1	11.59	0.42	42.1	B
Calf Skin - Type A	CALF-A/3	5.2	9	284	6.5	13.8	0.1	32.6	A
18 month old Brahman	CT4/2	5.6	11	324	5.4	8.93	0.58	48	B
18 month old Brahman skin	CT6/1	5.2	11.5	327	5.3	10.04	0.85	48	B
18 month old Brahman skin	CT6/2	5.6	11.5	308	5.6	9.34	0.64	55.6	B
18 month old Brahman skin	CT6/3	6	12.5	277	5.6	9.62	0.59	49.7	B
SPA process gelatine	ES1	3.2	12.5	0	Not Analysed				
SPA process gelatines	C200/1	<3.2	11.1	15	6.3	11.4	1.75	15.6	B
SPA process gelatines	C542/1L	<3.2	13.3	60	5.7	12.02	1.28	12.5	B
SPA process gelatines	C542/2L	<3.2	13.3	6	4.3	10.75	1.36	12.5	B
SPA process gelatines	C542/3L			0	NOT	ANALYS-	ED		B
SPA process gelatines	C552/1	<3.2	12.5	4	5.6	14.2	0.92		B
Pigskin Gelatine.	D11335	2.8	10	233	4.6	9.5	0.21	35	A
Pigskin Gelatine.	D1287	2	31	268	4.6	8.68	0.27	44	A
Pigskin Gelatine.	D147	2	44	289	4.5	8.44	0.2	58.4	A
Pigskin Gelatine.	D1512	2.4	30	281	4.4	7.88	0.16	47.4	A
Pigskin Gelatine.	D2116	2.4	17	246	4.5	8.48	0.14	36.1	A
Pigskin Gelatine.	D2227	2	21	206	4.5	8.86	0.22	30.6	A
Pigskin Gelatine.	D2249	2	11	227	4.5	8.26	0.17	32.1	A
Pigskin Gelatine.	D31108	2.8	36	109	4.2	8.45	0.33	25.3	A
Pigskin Gelatine.	D32117	4	50	86	4.3	9.78	0.3	21.5	A
Pigskin Gelatine.	D3264	3.6	42	121	4.2	8.38	0.29	25	A
Pigskin Gelatine.	D94025	2.4	9	282	4.6	8.6	0.14	69	A
Type A bovine hide gelatine	N - TyA	4.8	17	228	4.4	10.3	0.77	34.2	A
Fish Skin Gelatine	FISH 824/1	11.4	8.5	210	5.1	7.71	1.46	61.5	A
Fish Skin Gelatine	FISH COD2/1	6	11	243	5	10.95	0.49	23.5	A
Bovine hide Prodn. Gelatine	G35	4.8	NM	205	5.3	9.1	1.52	36.3	B
Bovine hide Prodn. Gelatine	G36	8.9	NM	245	5.2	8.8	1.02	34.3	B
Horse Skin Gelatine	HORSE/90	14	4.5	296	5.4	10.4	3	28.8	B
Bovine hide Prodn. Gelatine	LCS	7.6	7.5	86	5.3	12.5	0.74	22.4	B
Bovine hide Prodn. Gelatine	M8884	6.8	6.5	236	5.2	10.7	0.56	41.0	B
Lab gelatine.	LF752/1	6	11	267	5.5	12.35	4.12	22.2	B
Production Calf - Type B	N CALF TyB	5.6	12	232	4.7	9.97	1.66	32	B
Bovine hide Prodn. Gelatine	N33/3	6.8	11	245	4.5	11.5	0.89	34.8	B
Bovine hide Prodn. Gelatine	N33/1	13.3	11	109	4.3	10.8	0.86	20.7	B
Bovine hide Prodn. Gelatine	N33/4	14.5	11	135	4.6	10.8	0.92	27.3	B
Bovine hide Prodn. Gelatine	OCS	8	10		4.8				B

ADDENDUM 6. Continued....

NOTES - ORIGINS	SAMPLE	COLOUR	CLAR	BLOOM	pH	% Moist	% Ash	Visc.ms	Type
Pickled Sheep skin	PIS-PP1	11.4	10.5	307	5.3	8.6	4.3	39.4	B
Ex Pizzles.	PIZ/1	3.6	9	328	5	10.8	2.2	36	B
Bone Glue	SBG	4.8	8	77	6.1	12.5	0.34	25.7	
12 year old bovine hide	ST26/1	11.4	11.8	307	5.2	12.5	3.44	30.5	B
12 year old bovine hide	ST26/2	11.4	11.8	280	5.1	12.4	1.82	28.9	B
12 year old bovine hide	ST26/3	16	10	246	5.2	9.6	2.14	31.5	B
12 year old bovine hide	WT3/3	16	4.8	241	5.5	10.6	1.74	32.1	B
10 month old bovine hide	YSB/1	<3.2	10	314	5.5	9.08	0.47	53.7	B
10 month old bovine hide	YSB/2	5.2	7.5	312	5.5	9.76	0.43	52.6	B
10 month old bovine hide	YSB/3	4.8	9	287	5.6	10.79	0.42	38.7	B
12 year old Inguni	IOE/2	12.3	11	233	5.3	10.89	0.62	26.9	B
Lab extracted	L752/1	4.8	10	289	5.4	11.64	3.54	127.1	B

Included in the above samples were production or experimental gelatines from the following manufacturers:

DynaGel Incorporated, Calumet City, USA.
Gelatinas Ecuatorianas S.A. (Gelec S.A.). Quito, Ecuador.
Leiner Davis Gelatin (Brasil). Sao Paulo, Brasil.
Leiner Davis Gelatin (South Africa). Krugersdorp, South Africa.
Leiner Davis Gelatin (New Zealand). Christchurch, New Zealand.
Productoria de Gelatina SA (Progel). Manizales, Colombia.
Systemes Bio-Industries (SANOFI). Boulogne Cedex, France.
Smits Vuren B.V. Gorinchem, Nederland.

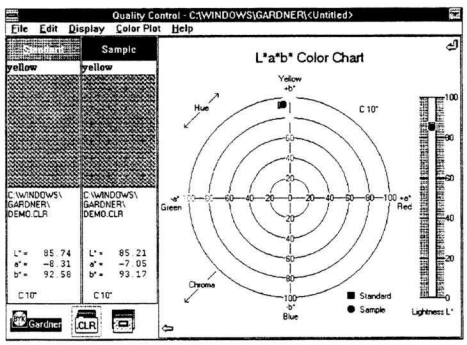
ADDENDUM 7. BYK-Gardner Color-View Reflectance Spectrophotometer.

BYK Gardner

15
15.1.1

Color
color-view™
Spectrophotometer

Catalog 92

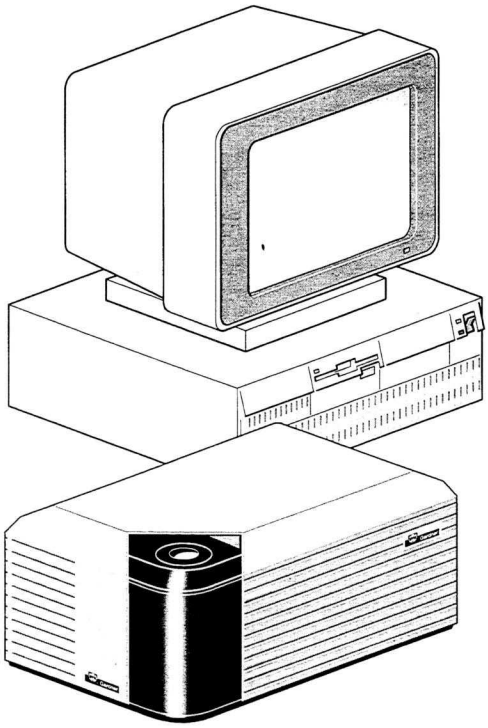


Color Measurement
in accordance with DIN 5033, DIN 5036, ISO 7724, NF X 08-012, ASTM D 2244, E 97, E 308, E 313,

color-view is a compact and versatile array spectrophotometer which integrates gardner-soft color-insights, a new Microsoft® Windows™* based quality control software to provide the user a cost-effective solution for quality control needs in virtually any application.

color-view is designed using 45/0 geometry (45° circumferential illumination / 0° viewing), which provides the best agreement with visual assessment.

Experiences gained from the reliable **TCM** spectrophotometer led to the consistent development of the **color-view**. Circumferential illumination minimizes the need for multiple measurements of directional or textured products.



color-view allows the rapid color measurement of a wide variety of materials. Such materials include:

- Paint and Coatings
- Automotive
- Plastics
- Raw Materials
- Adhesives
- Inks & Graphic Arts
- Agricultural
- Ceramics
- Paper
- Food
- Detergents
- Textiles

An internal paddle mechanism blocks the light path except for the rapid measurement cycle and minimizes the risk of material falling into the sensing head.
In addition, glass fiber optics prevent transmission of IR radiation, thus minimizing the effect of photochromism and thermochromism respectively, which can adversely effect sample measurement.

color-view provides colorimetric measurements under commonly used standardized illuminants, color scales, observers, and indices.

Software
- gardner-soft color-insights software
- Communications library to facilitate programming for custom link to third party color quality control packages (Option)

*Windows™ is a registered trademark of Microsoft® Corporation

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ADDENDUM 7 Continued....

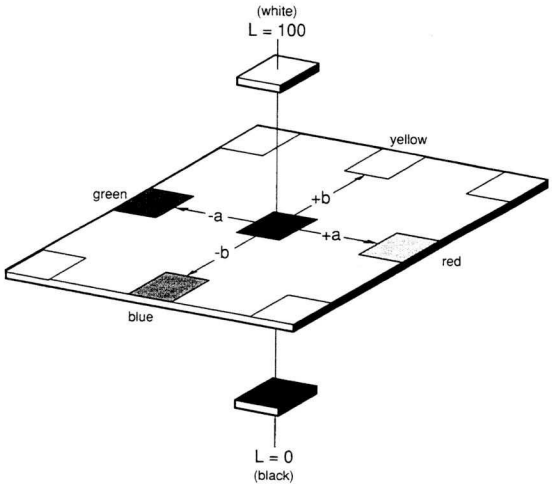
BYK Gardner

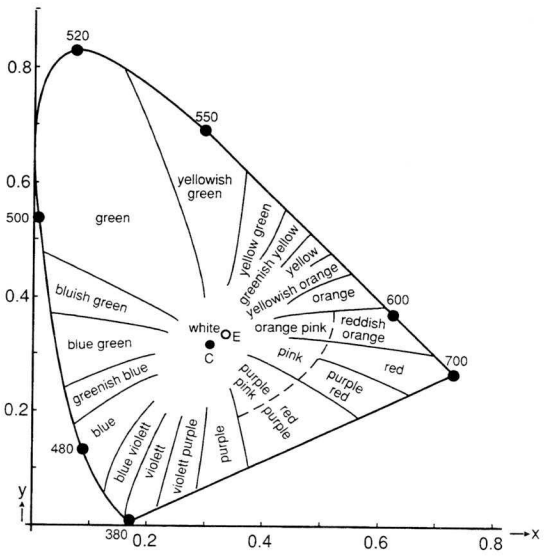
15
15.1.2

Color
color-view™ Spectrophotometer

Catalog 92

L, a, b - System





Chromaticity Diagram in accordance with CIE publication 15.2, ASTM E 308 and DIN 5033

Features

• Compact Size
color-view is one of the smallest 45/0 bench-top spectrophotometers produced today. It takes up less bench space while allowing convenient positioning of the sensor.

• Patented Auto-standardization
An internal paddle mechanism performs a reference standardization prior to every measurement, reducing operator error while increasing certainty of correct calibration.

• Rugged Construction
With its enclosed optics and its temperature and humidity operating range, **color-view** can go in more environments than other bench top instruments of the same class.

• Sample Viewer (Standard Accessory)
The sample viewer enables the operator to positively place and view small specimen areas prior to measurement.

• Quality Control Software
color-view incorporates gardner-soft color-insights software for applications ranging from pass/fail to in depth color analysis. color-insights, based on the Microsoft® Windows™ operating environment, is both extensive in performance and easy to use.

• Patented 60° Gloss Measurement Option
With this option, the user simultaneously measures 60° gloss values and color values according to international standards. Use of this option will save the operator's time.

• Foot Switch for Remote Control (Option)
The Unsolicited Data Option gives the operator the ability to measure samples directly at the sensor by use of a foot switch or switch closure. This option improves at-line measurement and allows the sensor to be placed into more rugged environments than a computer can withstand.

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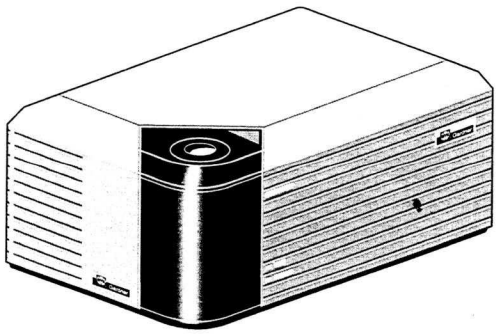
ADDENDUM 7 Continued....

BYK Gardner

15
15.1.3

Color
color-view™ Spectrophotometer

Catalog 92



BYK-Gardner color-view
Color Measuring System
- Spectrophotometer, 45° circumferential/0° geometry
- in accordance with DIN, ISO, ASTM
- **PC or Printer not Included**

Principle: Reflectance measurement of visible spectrum from 380 nm to 720 nm in 10 nm intervals. 60° gloss measurement.

Catalog No.	Voltage	Gloss Measurement 60°
6501	230 V/50 Hz	no
6502	230 V/50 Hz	yes

Standard System Configuration
color-view 45/0 grating/diode array spectrophotometer,
color-insights QC Manager software,
Integrated sample viewer,
Standard 1" (32 mm) area of view,
Holder with scratch-resistant calibration standards,
Inspection standard, Spare lamp and fuses,
Mains cable, Serial cable,
Instrument User's guide, Software operator's manual

Options:
60° gloss measurement per ASTM D 523,
Port down configuration with stand and sample presentation elevator (on request),
Small area of view (11 mm),
Microsoft® Windows™,
Foot switch for remote control,
Foreign language options: English, German, French, Italian, Spanish

The complete system requires:
IBM-PC, PS/2 or true compatible
- 386 processor, 2 MB RAM,
10 MB available on hard drive,
Enhanced Color Graphics (VGA) or any Microsoft Windows supported monitor,
DOS version 3.3 is required, 5.0 is recommended,
Microsoft Windows 3.X,
Microsoft Windows supported printer,
Microsoft Mouse,
Math coprocessor recommended

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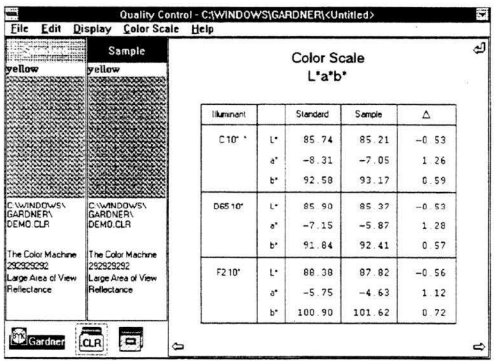
ADDENDUM 7 Continued....

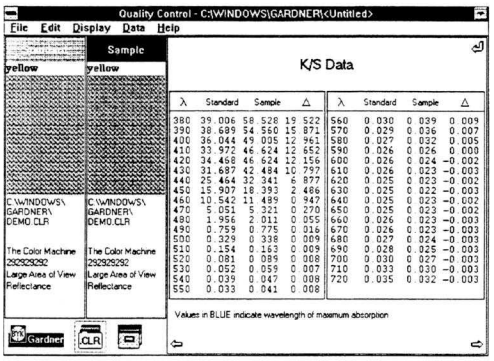
BYK Gardner

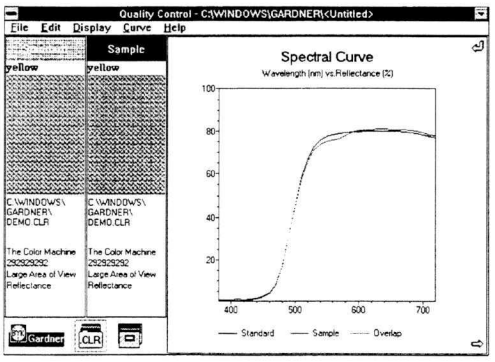
15
15.1.4

Color
color-view™ Spectrophotometer

Catalog 92







Technical Data

Power Supply:	230 volts 50/60 Hz or 115 volts 50/60 Hz
Repeatability:	Less than 0.01 ΔE* 1 σ * Standard Deviation Based on 300 consecutive readings of white tile
Reproducibility:	0.1 ΔE*, 1σ * Standard Deviation Based on average of 12 BCRA II tiles (complete set)
Standardization:	Uses internal white and black standards as continuous reference to minimize the use of external standards. External calibration also achievable
Measurement Geometry:	45° circumferential illumination 0° receiver optics
Sample Port Aperture: Options:	32 mm ø (1.26 in.) 11 mm ø (0.43 in.)
Spectral Response:	10 nm data collection with 10 nm bandpass measures visible spectrum from 380 to 720 nm
Illumination:	Tungsten-Halogen Lamp
Illuminants*:	A, C, D65 & F
Color Scales*:	X, Y, Z L*, a*, b* (CIELAB) L, a, b (Hunter) L*, C*, h Y, x, y L*, u*, v*
Indices*:	Metamerism Yellowness per DIN & ASTM Whiteness per CIE & ASTM
Color Differences*:	Component Color Scale Differences FMC II, CMC
CIE Observers:	2° & 10°
Dimensions: H x W x D Instrument Weight:	162 x 381 x 273 mm 6.4 x 15.0 x 10.8 in. 11.1 kgs / 24.6 lbs

Operating Environment

Operating Temp.:	+15 °C to +35 °C (65 °F to 95 °F)
Relative Humidity:	5 % to 85 % (non-condensing)

* A wide variety of illuminants, scales, indices and differences for special applications are standard. Please contact BYK-Gardner for information.

ADDENDUM 8. Gardner Tri-stimulus data.

GARDNER SPECTROPHOTOMETER. STANDARD = WHITE TILE
6.67% GELATINE SOLUTIONS COVERED WITH THE WHITE TILE.
(CONDENSATION WAS NOTED)

VOLUME ml	SAMPLE	COLOUR	CALC. COLOUR	CLARITY	L	a	b	Y1	Y2
75 ml	155/1	6.8		10	-51.8	-2.19	16.23	42.42	38.01
75 ml	970/3	8.7		15	-52.94	-0.2	26.07	66.89	56.52
50 ml	WATER	0.0		0	-50.47	-1.44	-2.43	-10.71	-7.28
50 ml	C79/1-2	4.8		14	-52.95	-2.46	7.65	19.84	19.45
50 ml	C79/1A	4.8		14	-52.71	-2.45	7.20	18.51	18.28
50 ml	C6/1-3	5.2		25	-53.58	-2.45	8.67	23.06	22.16
50 ml	C6/1-2	5.2		25	-54.42	-2.35	8.57	23.20	22.18
50 ml	C6/1	5.2		25	-54.09	-2.33	8.72	23.57	22.45
50 ml	764/1	5.2		13	-53.19	-2.56	8.03	20.88	20.43
50 ml	764/1-2	5.2		13	-53.13	-2.54	8.11	21.14	20.62
50 ml	877/1-2	5.6		17	-54.05	-2.53	10.5	28.22	26.6
50 ml	877/1	5.6		17	-53.83	-2.66	10.22	27.16	25.84
50 ml	155/1	6.8		10	-55.18	-2.35	10.90	30.13	28.01
50 ml	764/6	12.3		25	-58.10	-0.86	18.65	54.32	46.72
50 ml	764/6-2	12.3		25	-58.12	-0.87	18.66	54.36	46.76
50 ml	183/4-2	14.5		25	-58.07	-1.19	17.49	50.99	44.30
50 ml	183/4	14.5		25	-58.04	-1.16	17.65	51.39	44.60
50 ml	180/6	16.0		54	-59.68	-0.22	18.54	56.46	47.69
50 ml	111/3A-2	20.0		21	-62.51	1.84	23.68	74.17	59.92
50 ml	111/3A	20.0		21	-62.29	1.79	23.64	73.76	59.66
50 ml	184/10	22.8		73	-64.77	-2.48	19.7	68.76	54.24
50 ml	184/10	22.8		73	-64.47	-2.23	18.96	66.26	52.49
50 ml	D2249	2.4		11	-52.08	-2.31	3.85	8.51	9.81
50 ml	OCS	8		29	-56.01	-2.04	12.71	35.97	32.61
50 ml	155/1	6.8		10	-55.02	-2.43	11.71	32.1	29.79
50 ml	D1287	2.2		31	-53.04	-1.96	3.91	9.38	10.19
50 ml	L752/1	4.8		10	-55.19	-2.17	8.61	23.82	22.54
50 ml	D147	2.4		44	-53.9	-1.71	4.23	10.95	11.26
50 ml	970/3	8.7		15	-56.36	-1.8	16.53	46.38	41.14
50 ml	970/3-2	8.7		15	-56.36	-1.77	16.67	46.77	41.44
50 ml	G36	8.9		NM	-63.25	0.64	13.58	47.76	39.23
50 ml	G37	10		NM	-60.73	-0.43	10.69	35.51	30.43
50 ml	G35	4.8		NM	-66.8	0.8	11.09	43.31	35.19

Linear Regression - visual Colour v/s Gardner values. Blank = White Tile.

COL/L Regression Output:	
Constant	-85.98
Std Err of Y Est	1.2444
R Squared	0.9643
No. of Observations	28
Degrees of Freedom	26

X Coefficient(s) -1.693
Std Err of Coef. 0.0639

COL/a Regression Output:	
Constant	14.36923
Std Err of Y Est	5.41556
R Squared	0.323752
No. of Observations	28
Degrees of Freedom	26

X Coefficient 3.135633
Std Err of Coef. 0.8887

ADDENDUM 8. Continued.

SAMPLE SIZE = 50 ml. COLOUR STANDARD = 50 ml WATER
SAMPLES COVERED WITH THE WHITE TILE.

SAMPLE	COLOUR	CALC: COLOUR	CLARITY	L	a	b	Y1	Y2
111/3A	20.0	18.4	21	-12.5	3.33	26.16	85.2	67.41
111/3A	20.0	18.6	21	-12.6	3.4	26.18	85.46	67.52
143/1	7	6.5	60	-4.97	-0.65	15.1	46.09	39.44
143/1	7	6.3	60	-4.86	-0.59	15.36	46.93	40.08
155/1	6.8	6.5	10	-4.96	-0.99	14.37	43.51	37.66
155/1	6.8	6.5	10	-4.99	-0.97	14.46	43.79	37.87
155/1	6.8	6.7	25	-5.1	-0.93	14.33	43.56	37.63
155/1	6.8	6.1	10	-4.68	-1.01	14.19	43.07	37.3
155/1	6.8	6.8	10	-5.18	-0.98	14.08	42.95	37.18
157/1	7.2	6.7	21	-5.09	-0.65	17.87	53.45	45.65
160/7	11.7	11.9	10	-8.36	0.84	24.28	73.19	60.61
161/1	5.2	5.3	9	-4.22	-0.97	13.22	40.16	34.79
161/2	6.1	6.1	10	-4.68	-0.93	14.74	44.6	38.48
161/3	6.1	6.4	13	-4.88	-0.91	14.77	44.8	38.63
161/4	7.9	9.0	10	-6.52	0.38	18.98	57.49	48.78
161/5	9.7	10.8	9	-7.68	0.17	21.84	66.06	55.39
161/6	10.9	12.0	8	-8.44	0.62	23.25	70.61	58.68
161/7	10.9	11.8	7	-8.28	0.55	23.9	71.82	59.79
162/1	5.2	5.0	7	-3.99	-1.07	13.3	40.14	34.89
162/2	5.7	5.8	9	-4.53	-0.98	14.19	42.95	37.17
162/3	6.6	7.2	12	-5.4	-0.77	15.98	48.52	41.64
162/4	9.2	10.3	12	-7.34	-0.03	20.38	62.02	52.22
162/5	11.7	12.3	12	-8.63	0.77	23.83	72.32	59.96
162/6	12.5	12.7	12	-8.9	0.98	24.64	74.66	61.67
163/7	10.9	10.1	10	-7.21	0.32	22.86	68.39	57.16
164/1	6.1	5.3	10	-4.22	-1.25	15.21	45.16	39.3
164/1	6.1	5.3	10	-4.23	-1.24	15.19	45.14	39.27
164/2	7.4	6.9	16	-5.21	-0.98	16.89	50.54	43.56
164/3	10.3	10.9	16	-7.71	0.36	22.09	67.1	56.02
164/4	12.5	13.0	21	-9.09	1.21	24.68	75.44	62.04
164/5	14.6	15.4	24	-10.59	2.1	27.03	83.25	67.47
177/2	10	8.4 *L	40	-6.18	-0.29	16.19	50.25	42.52
179/6	12.3	10.7 *L	36	-7.62	0.03	19.2	59.43	49.94
180/6	16.0	14.2	54	-9.84	1.37	21.87	69.74	57.02
183/1	8.9	8.5	54	-6.24	-0.27	14.48	45.72	38.74
183/1	8.9	8.7	54	-6.35	-0.24	14.43	45.7	38.69
183/4	14.5	12.6 *L	25	-8.82	0.39	20.72	64.51	53.82
184/10	22.8	21.9	73	-14.7	3.96	22.42	80.3	62.19
752/1	4.8	6.8 *H	10	-5.16	-0.74	11.09	34.68	29.96
764/1	5.2	3.4 *L	13	-3.02	-1.13	11.45	34.37	30.05
764/6	12.3	11.4	25	-8.05	0.61	21.63	66.45	55.18
824/1-FISH	11.8	9.7 *L	39	-6.98	-0.65	20.54	42.32	35.41
877/1	5.6	5.0	17	-3.98	-1.08	12.93	38.95	33.9
970/3	8.7	8.6	15	-6.29	-0.35	19.31	58.05	49.23
970/3	8.7	8.8	15	-6.41	-0.31	19.32	58.22	49.32
BL 539	7	6.8	28	-5.12	-0.58	15.9	48.59	41.48
BL 539	7	6.9 1	28	-5.2	-0.62	15.93	48.63	41.57
BL 540	6.1	5.2	19	-4.11	-1.03	14.81	44.42	38.45
BL-534	7.9	8.6	24	-6.27	-0.32	18.29	55.7	47.21

ADDENDUM 8 Continued..

SAMPLE	COLOUR	CALC: COLOUR		CLARITY	L	a	b	Y1	Y2
BL-536	6.1	6.1		33	-4.72	-0.89	13.75	41.98	36.25
BL-537	6.1	6.4		16	-4.92	-0.83	15.62	47.21	40.6
C6/1	5.2	4.4		25	-3.65	-0.99	11.22	34.14	29.73
C6/1	5.2	4.4		25	-3.64	-1.01	11.19	34.03	29.66
C79/1	4.8	3.2	*L	12	-2.88	-1.01	10.24	31.05	27.13
C82/1L	2.8	2.8		21	-2.59	-0.48	6.37	20.19	17.5
C82/1L	2.8	2.8		21	-2.59	-0.46	6.45	20.47	17.71
C92/2	5.7	4.5		12	-3.68	-1.15	14.04	41.97	36.52
C93/3	2.6	2.3		6	-2.31	-1.12	8.7	26.24	23.21
C94/2	2.8	2.3		8	-2.28	-1.15	8.94	26.93	23.82
C95/3	5.2	4.5		16	-3.69	-1.25	13.54	40.45	35.33
D1287	2.2	3.4		31	-3.02	-0.51	6.44	20.42	17.73
D1287	2.2	3.3		31	-2.92	-0.51	6.51	20.62	17.9
D147	2.4	4.7	*H	44	-3.83	-0.26	6.69	21.73	18.59
D1512	2.2	3.4		30	-3.02	-0.49	6.29	20.03	17.37
D2116	2	2.5		17	-2.4	-0.71	6.1	18.97	16.72
D2227	2.2	2.7		21	-2.57	-0.72	6.56	20.45	17.98
D2227	2.2	2.8		21	-2.65	-0.7	6.55	20.47	17.97
D2249	2.4	1.9		11	-2.03	-0.83	6.3	19.32	17.14
D2249	2.4	2.2		11	-2.27	-0.83	6.22	19.1	16.96
D31108	3.8	5.4	*H	36	-4.26	-0.77	10.26	32.05	27.82
D32117	4.7	7.3	*H	50	-5.47	0.75	11.64	36.63	31.7
D3264	4	5.9	*H	42	-4.56	-0.74	10.45	32.77	28.4
LCS	7.6	7.5		48	-5.62	-0.88	18.31	54.48	46.81
LDP	7.2	6.5		44	-4.98	-1.04	14.18	43	37.26
LPC	7.2	7.1		48	-5.32	-0.86	13.84	42.53	36.7
LPG	7.4	9.1	*H	50	-6.63	-0.6	15.91	49.27	42.1
LPJ	6.1	6.4		36	-4.88	-0.87	12.62	38.91	33.64
OCS	8	8.1		29	-5.99	-0.62	15.04	46.45	39.73
OCS	8	8.0		29	-5.91	-0.61	15.22	46.9	40
PG94	5.2	12.1	*H	89	-8.47	-0.18	12.62	61.27	52.41
SFI	3.5	2.8		26	-2.6	-0.7	8	24.98	21.75
SFI	3.5	3.5		26	-3.06	-0.62	8.44	26.54	22.98
SBG	4.8	4.3		44	-3.58	-0.81	10.91	33.75	29.24
SBG	4.8	3.9		44	-3.33	-0.85	10.9	33.57	29.13
X084	6.1	5.5		11	-4.3	-0.95	14.67	44.27	38.23
X091	7	6.4		13	-4.92	-0.82	15.75	47.67	40.98
GELATINES WITH UNMEASURABLE CLARITY									
STD-185	15.9	18.8		NM	-12.75	1.7	11.22	58.34	46.89
STD-185	15.9	19.0		NM	-12.88	1.71	16.19	58.18	46.76
G37	10	15.9	*H	NM	-10.88	0.99	13.19	46.62	38.01
G36	8.9	19.9	*H	NM	-13.46	2.07	16.02	58.61	46.65
G35	4.8	25.5	*H	NM	-16.97	2.24	13.48	54.04	42.5

ADDENDUM 8 Continued..

WATER STANDARD MEASURED AS A SAMPLE

	Calc: Colour	L	a	b	Y1	Y2
WATER	-1.9	0.34	-0.05	-0.10	-0.35	-0.24
WATER	-1.9	0.35	-0.05	-0.09	-0.33	-0.23
CLEAN TILE + WATER	-1.5	0.10	-0.03	-0.09	-0.33	-0.25
TILE ONLY		50.39	1.42	2.52	11.03	7.57

STANDARD = 50 ml WATER FOR THE GARDNER CELL, 57 ml WATER FOR THE GRAINER CELL.

	SAMPLE	COLOUR	CALC. COLOUR	CLARITY	L	a	b	Y1	Y2
GRAINER	752/1	4.8		10	-3.7	-0.68	7.21	29.84	26.09
GARDNER		4.8		10	-5.16	-0.74	11.09	34.68	29.96
GRAINER	970/3	8.7		15	-4.15	-0.64	12.81	51.33	44.15
GARDNER		8.7		15	-6.41	-0.31	19.32	58.22	49.32

Linear Regression - Gardner L value and visual Colour. Blank = 50 ml water.

COL/L Regression Output:		COL/a Regression Output:	
Constant	-1.345	Constant	8.483555
Std Err of Y Est	1.2225	Std Err of Y Est	2.2578
R Squared	0.9124	R Squared	0.701165
No. of Observations	86	No. of Observations	86
Degrees of Freedom	84	Degrees of Freedom	84
X Coefficient(s)	-1.582	X Coefficient	3.4322
Std Err of Coef.	0.0535	Std Err of Coef	0.2445

Included in the above samples were production or experimental gelatines from the following manufacturers:
DynaGel Incorporated, Calumet City, USA.
Gelatinas Ecuatorianas S.A. (Gelec S.A.). Quito, Ecuador.
Leiner Davis Gelatin (Brasil). Sao Paulo, Brasil.
Leiner Davis Gelatin (South Africa). Krugersdorp, South Africa.
Leiner Davis Gelatin (New Zealand). Christchurch, New Zealand.
Productoria de Gelatina SA (Progel). Manizales, Colombia.
Systemes Bio-Industries (SANOFI). Boulogne Cedex, France.
Smits Vuren B.V. Gorinchem, Nederland.