SAMPLE SIZE DETERMINATION IN POST HARVEST EXPERIMENTS

by

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ABSTRACT

Nine sets of data representing nine characters or variables were analyzed with the objective of determining the optimum sample size for post harvest experiments on mango. The estimated values of the variance components $\hat{\sigma}_E^2$ and $\hat{\sigma}_S^2$ were used to relate the precision of a treatment mean with the number of replications r and number of subsamples s. For any desired degree of precision, one can refer to the graph obtained to determine the sample size n which is the product of r and s. On the other hand, if the cost of an experiment is known or can be estimated, then the optimum numbers r, s and n can be determined from the tabulated values.

INTRODUCTION

One very important consideration that a research worker has to consider in the planning of his experiment in sample size. In this case of comparative experiments, sample size may refer to the number of experimental units used per treatment which is also called replications as in a completely randomized design, or it may refer to the combination of the number of replications and number of subsamples per replication as in a completely randomized design with subsampling. Researchers can be guided in this particular problem by examining and using the results of the statistical analysis of past experiments in which the variance components due to identified sources can be estimated. By using the estimated values of those variance components, the researcher can determine the precision of a treatment mean from which the sample size of future experiments can be determined. Thus, in this study, the focus of the analyses were some data from the past experiments of the Post Harvest and Training Research Center (PHTRC) at the University of the Philippines at Los Banos with the aim of determining the appropriate sample size for future post harvest experiments on mango.

In many of the experiments that have been conducted at PHTRC, the statistical design used was the completely randomized design with subsampling. In such design, the experimental error variation of the data comes from two sources, namely, from the variation due to the differences between experimental units treated alike and from the variations due to the differences between the sampling units within experimental units. Thus, if the experimental error variance of a character x is denoted by σ_X^2, then

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$$\sigma X^2 = \sigma E^2 + \sigma S^2 \quad (1)$$

where σE^2 is the variance components due to the experimental units or replications and σS^2 is the variance components due to the sampling units. Without any knowledge of the magnitudes of these variance components, the researcher would not know the appropriate sample size to be used in order to obtain reliable and precise experimental results, hence, he may just utilize whatever materials are available. If the sample size used happened to be too small, then the experimental results may not be able to detect real treatment differences, while if the sample size used happened to be too large, the results of the experiment might be more precise than what would be required statistically.

In the light of the problems stated above, this study was conducted with the main objective of determining the appropriate and optimum sample size for mango post harvest experiments. The specific objectives of the study were: (i) to obtain estimates for the variance components due to experimental units and due to sampling units for various mango post harvest characters, (ii) to obtain the appropriate sample size for mango post harvest experiment that will yield results with given degree of precision, and (iii) to obtain the optimum sample size for mango post harvest experiments that will give optimal results for a given fixed cost per treatment.

REVIEW LITERATURE

Anderson (1947) used the analysis of variance to test the significance of variance components that affects the price of hog meat in two markets. Marcuse (1949) obtained an estimate of the reciprocals of n1, n1n2, and n1n2n3. Anderson and Bancroft (1952) utilized a general estimation procedure for the variance components, such as the method of maximum likelihood.

Kempthorne (1952) derived the optimum number of secondary sampling units and optimum of primary sampling unit for sampling in field experiments. Goldsmith and Gaylor (1970) used the three stage nested design for the estimation of variance components, however, unbalanced the arrangements may be. Sahai (1976) studied various estimators of the variance components for the balanced three stage nested design.

In sampling for laboratory brix, Solivas (1978) found that in raw and adjusted sugar rendement, the variance components among the rows and the experimental error variance components were significantly greater than zero. In the study of the avocado fruit characters, Ledesma (1983) found various variance components that gave higher contribution to the total variation. In sampling for coconut characters, Alforja (1983) found that the sample size n considered optimum varies depending with the uniformity of the cultivars. He also found out that 24 palms is sufficiently enough to obtain reliable information for nuts per tree estimation.

MATERIALS AND METHODS

A. The Data

The data used in this study were obtained from the past post harvest experiments on mango that were conducted at PHTRC. Table 1 shows the description of the experiments. There were four experiments and the number of treatments range from four to eight; the number of replications from three to four; and the number of subsamples per replication from three to nine. Nine post harvest characters or variables were measured, two from experiment 1, two from experiment II, four from experiment III and one from experiment IV. These characters were color index at day 0 and 5, total soluble solids,

titratable acidity, percent cumulative weight loss, firmness, disease incidence, PH and visual quality rating.

B.The Statistical Design and Model

All the four experiments were conducted in a completely randomized design with subsampling. A typical example of such design is where the treatments are heating temperatures, the experimental units or replications are boxes of fruits, and the sampling units are the individual fruits in the boxes. An experiment may then involve, say, t treatments, r boxes of fruits per treatment, and s fruits per box. Thus, if a character x is measured on sampling unit, the statistical model is of the form

$$x_{ijk} = \mu + \tau_i + \varepsilon_{ij} + d_{ijk} \qquad (2)$$

$$i = 1, 2, \ldots, t$$

$$j = 1, 2, \ldots, r$$

$$k = 1, 2, \ldots, s$$

where x_{ijk} is the observed value in the kth sampling unit of the jth replication and ith treatment, μ is the effect of the ith treatment, e_{ij} is the random error effects due to the jth experimental unit of the ith treatment, and d_{ijk} is the sampling error effect due to the kth sampling unit of the jth replicate. For each of the nine characters used in the model it was assumed that the treatment effects τ_i are fixed and $\Sigma\tau_i = 0$. Also, the experimental error e_{ij} were assumed to be normally and independently distributed with mean 0 and variance σ_E^2 or $e_{ij} \sim NID(0, \sigma_E^2)$, and the sampling error d_{ijk} were assumed to be normally and independently distributed with mean 0 and variance σ_S^2 or $d_{ijk} \sim NID(0, \sigma_S^2)$.

C. Estimation of Variance Components

The variance components to be estimated for each character are those due to the differences between experimental units or replications which was denoted by σ_E^2 and due to the differences between the sampling units within the experimental units denoted by σ_S^2.

The method used in estimating these variance components was by analysis of variance. Essentially, the steps involved in the estimation of σ_S^2 and σ_E^2 were:

(1) construction of the analysis of variance table (Table 2), and

(2) equating the actual mean square and the expected mean square for the sampling error and experimental error. Hence, if

(i) $MS(SE) = \sigma_S^2$

(ii) $MSE = \sigma_S^2 + s\,\sigma_E^2$

then

$$\sigma_S^2 = MS(SE) \qquad (3)$$

$$\sigma_E^2 = (MSE - MS(SE))/s \qquad (4)$$

where MSE is the mean square due to experimental error defined as

$$MSE = s \sum_i \sum_j (x_{ij.} - x_{i..})^2/t(r-1), \qquad (5)$$

and MS(SE) is the mean square due to the sampling units defined as

$$MS(SE) = \sum_i \sum_j \sum_k (x_{ijk} - x_{ij.})^2/tr(s-1) \qquad (6)$$

In these formulas, the quantities

$x_{i..} = \sum_j \sum_k x_{ijk}/rs$, the treatment mean, and

$x_{ij.} = \sum_k x_{ijk}/s$, the replication mean.

D. Determining the Sample Size for a Given Degree of Precision

The basis of determining the sample size, which in this study is the combination of the number of replications r and the number of subsample s or n = rs, was by the use of a formula for the precision of a treatment mean. In a completely randomized design with subsampling, the variance of a treatment mean $x_{i..}$ is given by the formula

$$\text{var}(\bar{x}_{i..}) = \text{MSE}/rs \qquad (7)$$

In terms of the estimated variance components $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$, the variance of a treatment mean is

$$\text{var}(\bar{x}_{i..}) = \hat{\sigma}_E^2/r + \hat{\sigma}_S^2/rs \qquad (8)$$

Therefore, the standard error of a treatment mean is

$$\text{s.e.}(\bar{x}_{i..}) = \sqrt{\hat{\sigma}_E^2/r + \hat{\sigma}_S^2/rs} \qquad (9)$$

In terms of the coefficient of variation, the precision is

$$CV(\bar{x}_{i..}) = \text{s.e.}(\bar{x}_{i..})/\bar{x}_{...}$$

where $x_{...} = \sum_i \sum_j \sum_k x_{ijk}/trs$, the grand mean or mean of the experiment

Thus, the final form of the precision formula used in this study is

$$CV(\bar{x}_{i..}) = \left(\sqrt{\hat{\sigma}_E^2/r + \hat{\sigma}_S^2/rs}\right)/\bar{x}_{...} \qquad (10)$$

The values of the $CV(\bar{x}_{i..})$ were then computed and their graphs were drawn against r and s for values of r = 2,3,4,5 and s = 1,2,...,10.

E. Determining the Optimum Sample Size at a Given Experiment Cost per Treatment

Kempthorne (1952) defined the information on each treatment mean as

$$I = \frac{rs}{\hat{\sigma}_E^2 + s\hat{\sigma}_E^2} \qquad (11)$$

By assuming a cost function of the form

$$C_O = r(C_E + s C_S) \qquad (12)$$

where C_O is the cost of the experiment per treatment, C_E is the cost per experimental unit, and C_S is the cost per sampling unit. Solving for r, in (12) and substituting the result in (11) gave the formula for the information as

$$I = \frac{(s)(C_O)}{(C_E + s C_S)(\hat{\sigma}_S^2 + s\hat{\sigma}_E^2)} \qquad (13)$$

Maximizing (13) with respect to s gave

$$s = \sqrt{(C_E/C_S)(\sigma_S^2/\sigma_E^2)} \qquad (14)$$

The optimum value of r was then found to be

$$r = \frac{C_O}{C_E + \sqrt{C_E C_S \hat{\sigma}_S^2/\hat{\sigma}_E^2}} \qquad (15)$$

Since estimates of C_E and C_S were not available, various ratios of C_E to C_S were assumed and then the value of s and r were computed by formulas (14) and (15) using the known values of $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$ for each character.

RESULTS AND DISCUSSION

A. The Analysis of Variance

The results of the analysis of variance for the nine post harvest characters of mango showing only the degrees of freedom (DF) and mean square (MS) for treatment, experimental error and sampling error are given in Table 3. In these results, the estimates for the mean square error, MSE, and the sampling error mean square, MS(SE), may be considered as stable since these were based on relatively large degrees of freedom.

The mean squares for the treatment, MSTr are marked to indicate that they are either significant (x or xx) or not significant (NS) as compared with mean square error . Out of the nine characters, seven were identified for which the treatment effects were significant. Only two characters, color index at day 0 and 5 did not show the significant effects of treatments.

With respect to the magnitude of the mean square error and mean square sampling error, it was noted that all but one character, the values of the former were larger than the latter. These results indicate that the experimental error variance components in such characters are all positive. The only character which showed a negative estimate for the error variance components was pH. However, in testing the significance of the experimental error variance components, $\hat{\sigma}_E^2$, resulted only with two significant mean square error, those for color index at day 5 and total soluble solids.

B. Estimates of Variance Components

The two variance components, $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$ were estimated by formulas (3) and (4) using the values of MSE and MS(SE) given in Table 3.. These estimates of variance components, $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$ are given in Table 4 which are expressed in absolute form or as percentage of their total. For instance, the values of $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$ for color index at 0 are 0.20 and .0055, or they are 97% and 3% respectively, of the total variance component 0.2055. These results indicate that most of the variability in post harvest characters of mango comes from the differences between the sampling units and very little comes from the differences between the experimental units. In such cases, the implication is that the experiments were conducted with very little control given to keep the sampling units more uniform, such as using more uniform fruits with respect to weights or size, etc. The use of other experimental design, such as randomized complete block design with subsampling may even bring about more efficient results.

C. The Precision of a Treatment Mean as a Function of Sample Size

The precision of a treatment mean may be expressed as a function of the number of replications r and number of sampling units s after having obtained the estimates of $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$. By using equation (11) and the values of $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$ given in Table 4, the values of coefficient of variation of a treatment mean, $CV(\bar{x}_{i...})$ were computed for r ranging from 2 to 5 and s ranging from 1 to 10. The graphs of the $CV(\bar{x}_{i...})$ values versus r and s were drawn and they are shown in Figures 1 (a) to (i). The graph for each character is the function

$$CV(\bar{x}_{i...}) = \frac{\sqrt{\hat{\sigma}_E^2/r + \hat{\sigma}_S^2/rs}}{\bar{x}_{...}} \cdot$$

For total soluble solids, for instance, the graph shown in Figure 1 (c) is the function

$$CV(\bar{x}_{i...}) = (\sqrt{.038/r + 0.18/rs})/(6.74)$$

where r = 2,3,4,5 and s = 1,2,...,10.

The graph of $CV(\bar{x}_{i..})$ against the subsample size s for a given number of replications r behave like a negative exponential with maximum value at ∞ when s = 0 and a minimum value at $\hat{\sigma}_E^2/r$ at s = ∞. By proper choice of r and s, one can bring down the value of $CV(\bar{x}_{i...})$ to any prescribed percentage, such as 10%, 5% or 1%. To use the graph for any character, the desired precision level can be set and one simply locates the combination of r and s. For example, using Figure 1 (a), one can make the precision of the treatment mean equal to 10% for choices of (r) (s) as (2) (7), 3(5), (4) (4) and (5) (3). These choices on the average led to a sample size of about n = 15.

D. The Optimum Sample Size

The formulas for determining the optimum number of subsamples s and optimum number or replication r are given as

$$s = \sqrt{\left(\frac{C_E}{C_S}\right)\left(\frac{\hat{\sigma}_S^2}{\hat{\sigma}_E^2}\right)}$$

$$r = \frac{C_O}{C_E + \sqrt{C_E C_S \, \hat{\sigma}_S^2 / \hat{\sigma}_E^2}}$$

If one knows the cost of the experiment per treatment (C_O), the cost per experimental unit (C_E) and the cost per sampling unit (C_S), then the optimum value for s and r can be computed since $\hat{\sigma}_S^2$ and $\hat{\sigma}_E^2$ are already known. To see the behavior of the estimates of s and r, certain ration of the cost estimate as $C_E : C_S$ were given and then the values of s and r computed for each character. For example, if the ratio is 4:1, say, then

$$C_O = r' (4 + s' (1))$$

where r' and s' were the numbers of replications and subsamples in the actual experiment. Therefore, the value of

$$S = \sqrt{\frac{4}{1} \cdot \frac{\hat{\sigma}_S^2}{\hat{\sigma}_E^2}}$$

and $$r = \frac{C_O}{4 + \sqrt{(4)(\hat{\sigma}_S^2)/\hat{\sigma}_E^2}}$$

The computed values of s and r for the cost ratios 1:4, 1:2, 1:1, 2:1 and 4:1 are given in Table 5. Thus, for a cost ratio of 4:1, say, the optimum numbers r, s and n were computed as 3, 11, 33 for color index at day 0; 5, 6, 30 for color index at day 5;5,4,20 for total soluble solids; 4,7,28 for titratable acidity; 3,5,15 for percent cumulative weight loss; 3,824 for firmness; 2,10,20 for disease incidence; and 2,11,22 for visual quality rating. As Kempthorne had pointed out, these numbers maximize the information on each treatment mean for a given cost per treatment.

SUMMARY AND CONCLUSION

Nine sets of data obtained from the experiments conducted at the Post Harvest Training and Research Center of U.P. Los Banos were analyzed using a completely randomized design with subsampling model for main purpose of obtaining estimates for two variance components that will be used for determining optimum sample size for post harvest experiments on mango. One set of data represent one post harvest character or variable and those characters were color index at day 0, color index at day 5, total soluble solids, titratable acidity, percent cumulative weight loss, firmness, disease incidence, pH and visual quality rating.

The analysis of variance of the characters showed that the mean square error (MSE) were larger than the mean square sampling error (MS(SE)) except for the character pH. Those results meant that the estimates for the variance component due to the experimental unit or replication, σ_E^2 were all positive. Further tests of significance, however, revealed that only two characters indicated significant estimates of σ_E^2.

From the results of the analysis of variance, the values of MSE and MS(SE) were used to estimate the two variance components, σ_E^2 and σ_S^2, the variance component due to sampling units. The comparison of the two estimated variance components indicated that $\hat{\sigma}_S^2$ represents from about 85 to 97 percent of the experimental error variance among the nine post harvest

characters. In terms of ratio, values of $\hat{\sigma}_S^2$ were larger than the values of σ_E^2 by as much as 5 to 36 times.

By expressing the precision of a treatment mean, s.e.(x_i) in terms of the coefficient of variation of treatment mean, CV($\bar{x}_i$), a function relating the CV($\bar{x}_i$), with the number of replications r and number of subsamples s were obtained for each character using the estimated values of $\hat{\sigma}_E^2$ and $\hat{\sigma}_S^2$. The graph of each function was then drawn for each character by varying the values of r from 2 to 4 and s from 1 to 10. The graphs showed that for a particular value of r, the values of the CV($\bar{x}_i$) decreases exponentially with increasing s and the points of inflection where somewhere between 4 and 6. Thus, if one wishes to obtain the right combination of r and s that will give the desired precision, he would simply refer to the graph of a particular character.

With respect to the determination of optimum sample size, the formulas derived by Kempthorne were used. Various ratios of the cost per experimental unit, C_E to the cost per sampling unit, C_S were used in the formula to get the optimum number of subsamples s and optimum number of replications r for each character. Thus, for any given cost ratio that is within the cost ratios used in this study, one simply refer to the tabulated values of r and s to get the optimum sample size n = rs.

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Table 1. Description of the Four Post Harvest Experiments on Mango from which the Data of the Study Were Obtained.

Experiment no.	Number of Treatments (t)	Number of replications (r)	Number of subsamples (s)	Characters measured
I	4	4	8	Color index at day 0 and 5.
II	6	3	9	Total soluble solids and titratable acidity.
III	8	4	3	Percent cumulative weight loss, firmness, disease incidence and pH.
IV	6	4	4	Visual quality rating.

Source: Post Harvest Training and Research Center, U.P. at Los Banos.

Table 2. Format of the Analysis of Variance for the Nine Post Harvest Characters of Mango.

Source of variation	Degrees of freedom	Sum of squares	Mean square	Expected mean square
Treatment	t-1	$rs \sum_i (\bar{x}_{i..} - \bar{x}_{...})^2$	MS(tr)	$\sigma S^2 + s + s\sigma E^2 + \sum_i T_i^2/(t-1)$
Experimental error	t(r-1)	$s \sum_i \sum_j (\bar{x}_{ij..} - \bar{x}_{i..})^2$	MSE	$\sigma S^2 + s\sigma E^2$
Sampling	tr(s-1)	$\sum_i \sum_j \sum_k (\bar{x}_{ijk} - \bar{x}_{ij.})^2$	MS(SE)	σS^2
Total	trs-1	$\sum_i \sum_j \sum_k (\bar{x}_{ijk} - \bar{x}_{...})^2$		

Table 3. Results of the Analysis of Variance for the Nine Post Harvest Characters of Mango.

Source of	CI (0)		CI (5)		TSS		TA		CWL	
variation	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
Treatment	3	.19^{ns}	3	2.59^{ns}	5	4.5**	5	10.03**	7	.7**
Experimental error	12	.25^{ns}	12	1.15*	12	.56**	12	.18^{ns}	24	.24**
Sampling error	123	.20	128	.57	162	.18	162	.11	96	.07

Source of	F		DI		PH		VOR	
Variation	DF	MS	DF	MS	DF	MS	DF	MS
Treatment	7	.08*	7	2.39**	7	.43**	5	6.23*
Experimental error	24	.03^{ns}	24	.52^{ns}	24	.04^{ns}	18	1.49^{ns}
Sampling error	96	.02	96	.44	96	.06	96	1.29

CI (0) - Color index at day 0 TA - titratable acidity DI - Disease incidence

CI (5) - Color index at day 5 CWL - Cumulative Weight Loss VOR - Visual quality rating

TSS - Total soluble solids F - Firmness DF - Degree of freedom

MS - Mean sqaure error

Table 4. Estimate of Experiment Mean (x), Coefficient of Variation (CV), and Variance Components for the Nine Post Harvest Characters of Mango.

CHARACTER	MEAN	CV($\bar{x}$)	VARIANCE COMPONENTS					
	($\bar{x}$)	(%)	$\hat{\sigma}^2_S$	%	$\hat{\sigma}^2_E$	%	$\hat{\sigma}^2_x = \hat{\sigma}^2_S + \hat{\sigma}^2_E$	%
Color index at day 0	1.29	38.7	0.20	97	0.0055	3	0.2055	100
Color index at day 5	4.44	24.2	0.57	90	0.064	10	0.634	100
Total soluble solids	6.74	11.1	0.18	83	0.038	17	0.218	100
Titratable acidity	3.33	12.7	0.11	94	0.007	6	0.117	100
% Weight cumulative loss	0.99	35.0	0.07	85	0.0125	15	0.825	100
Firmness	0.99	17.5	0.02	89	0.0025	11	0.0225	100
Disease incidence	0.42	171.1	0.44	96	0.02	4	0.46	100
PH	4.54	4.44	0.66	100	0	0	0.66	100
Visual quality rating	6.25	19.50	1.29	97	0.04	3	1.33	100

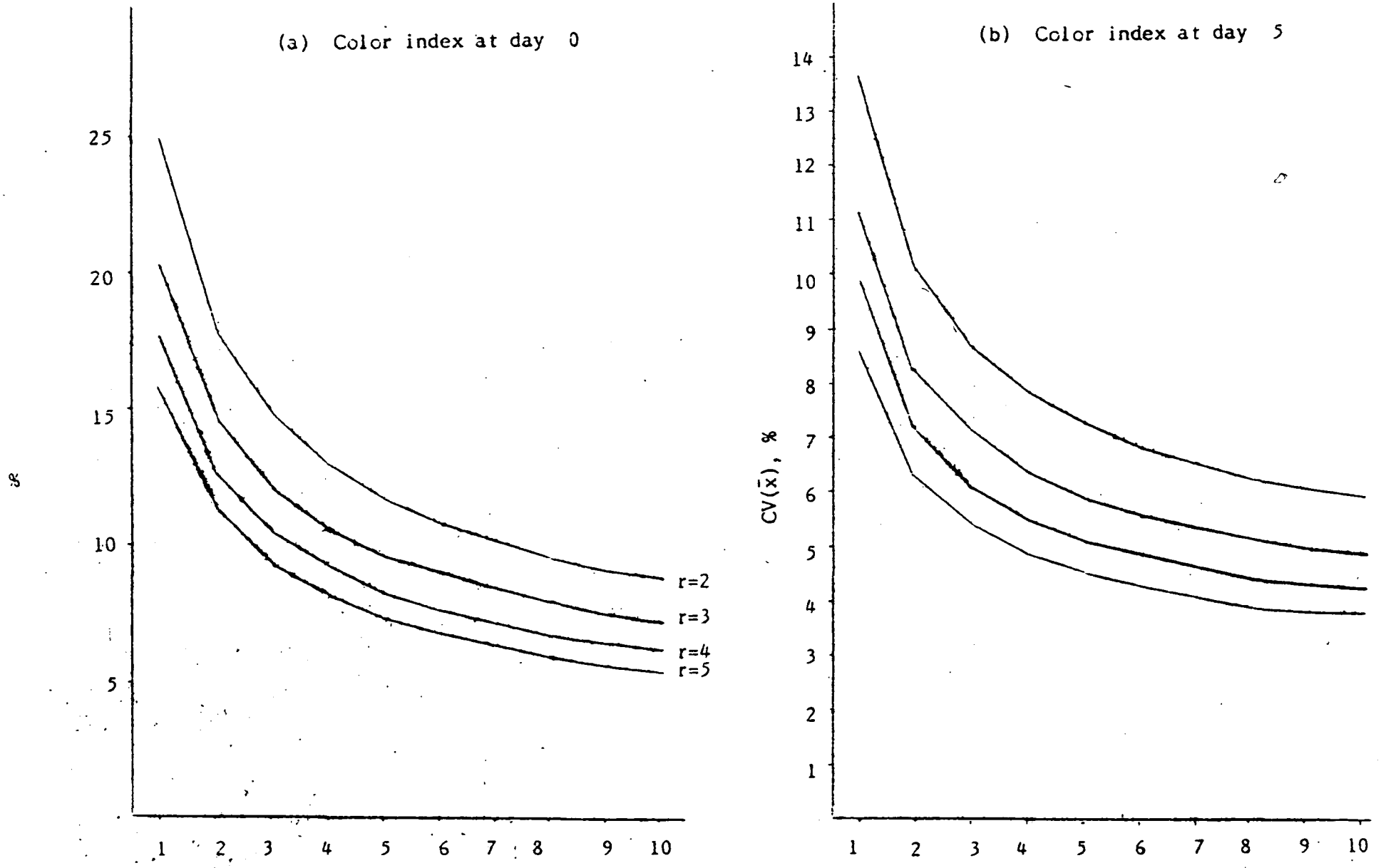

(a) Color index at day 0
(b) Color index at day 5
CV(x̄), %
25
20
15
10
5
14
13
12
11
10
9
8
7
6
5
4
3
2
1
r=2
r=3
r=4
r=5
1 2 3 4 5 6 7 8 9 10
NUMBER OF SUBSAMPLE(S)

Figure 1

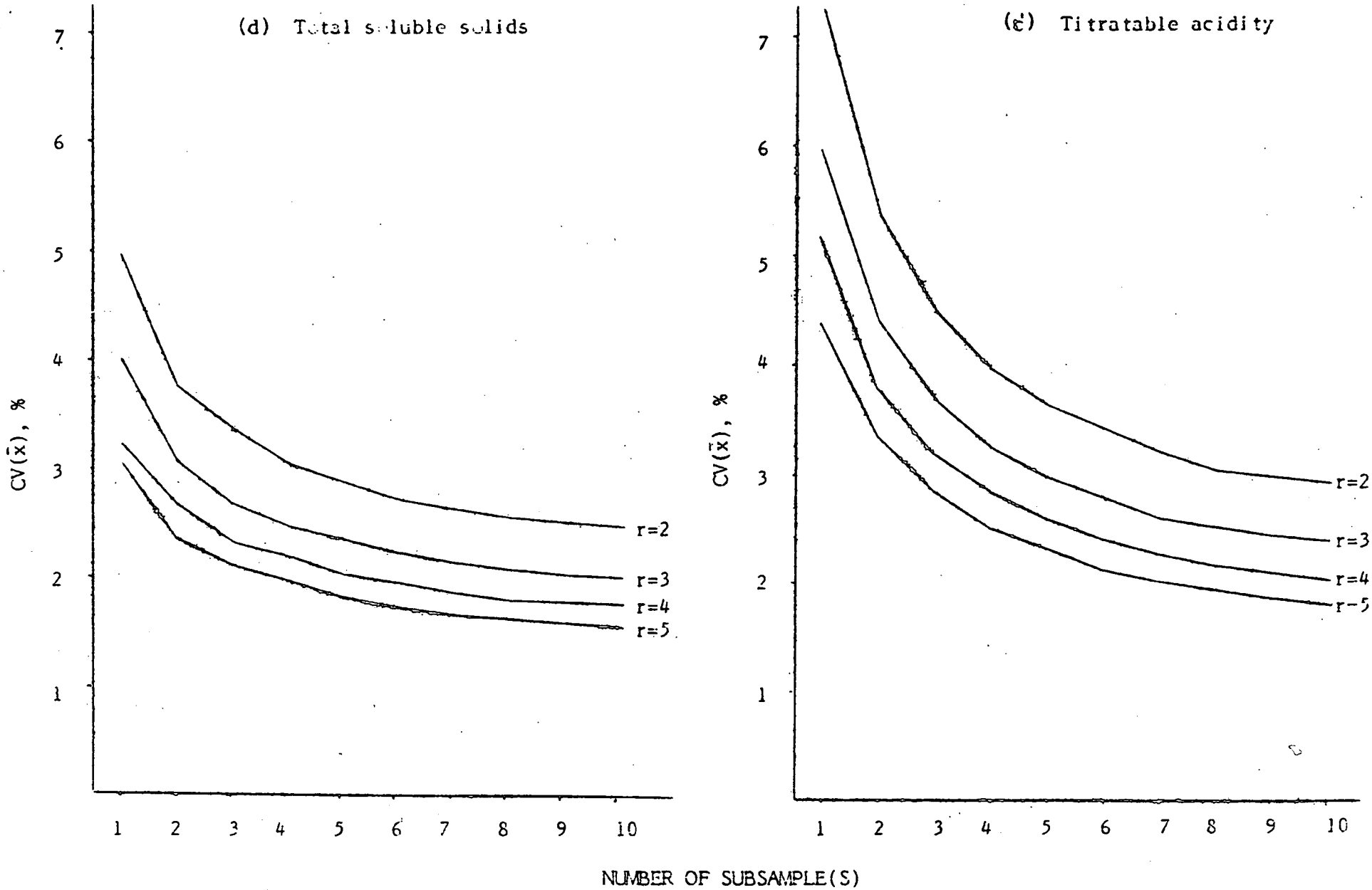
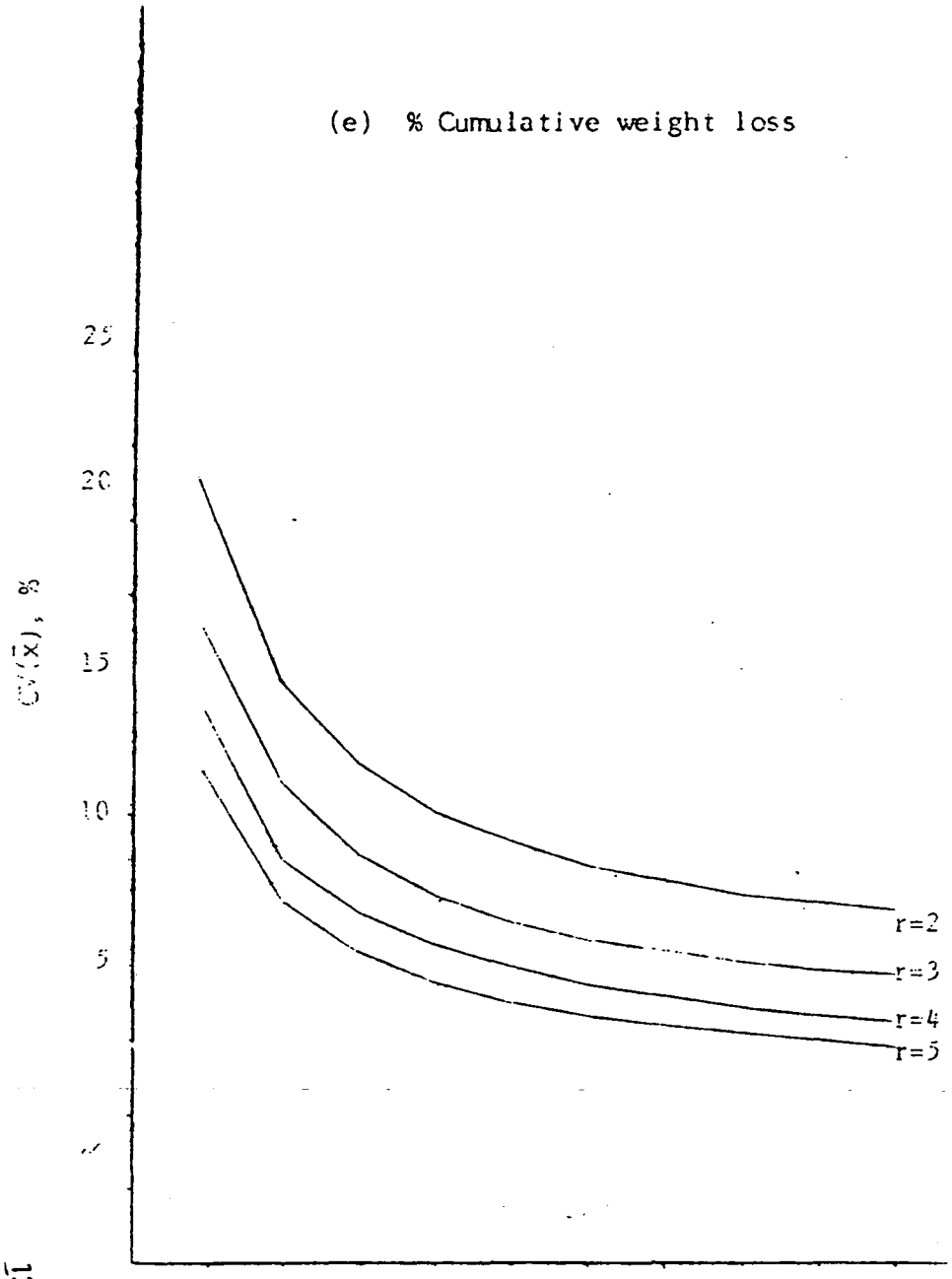
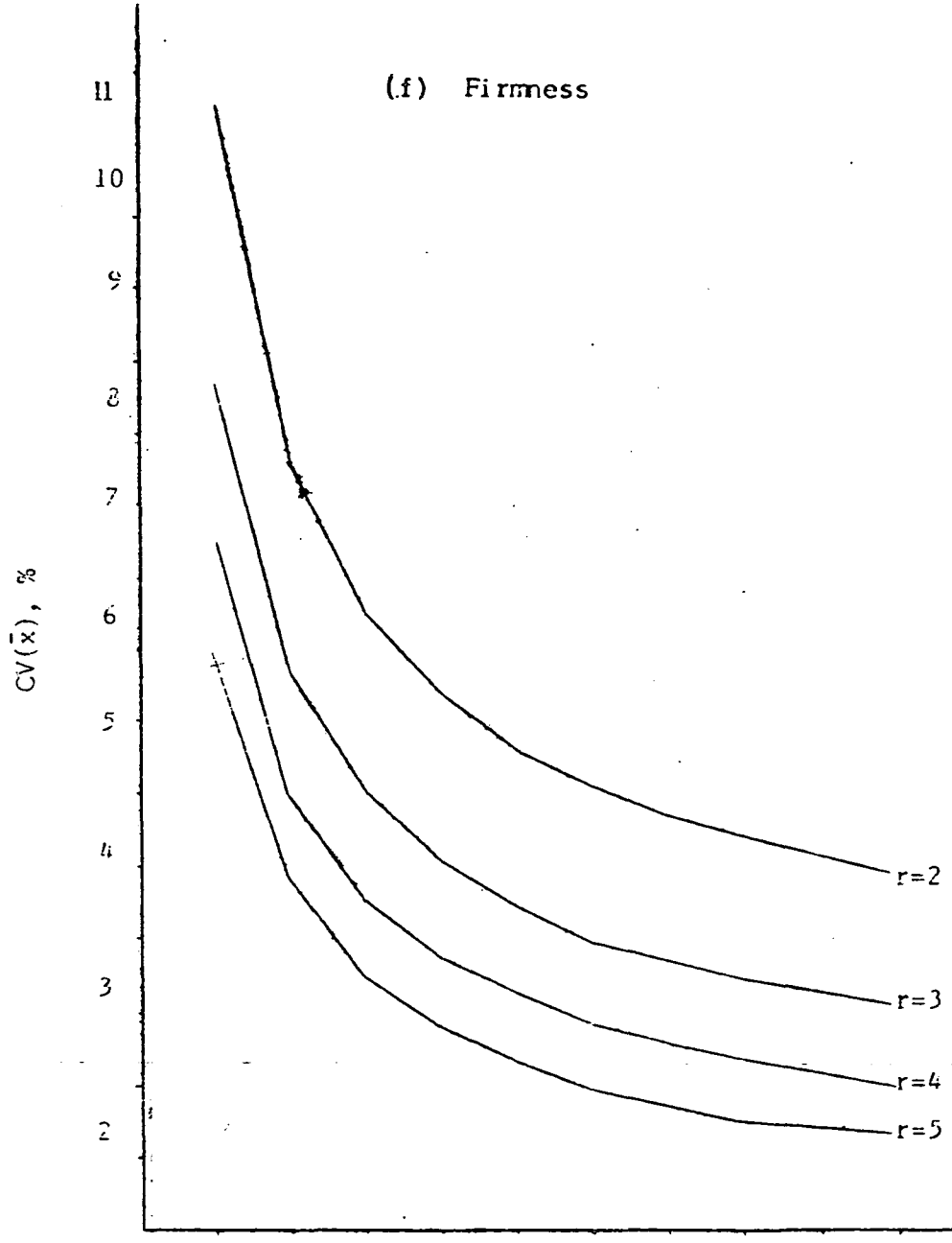

(d) Total soluble solids
(e) Titratable acidity
CV(x̄), %
7
6
5
4
3
2
1
r=2
r=3
r=4
r=5
1 2 3 4 5 6 7 8 9 10
NUMBER OF SUBSAMPLE(S)

Figure 1





NUMBER OF SAMPLE(S)

Figure 1

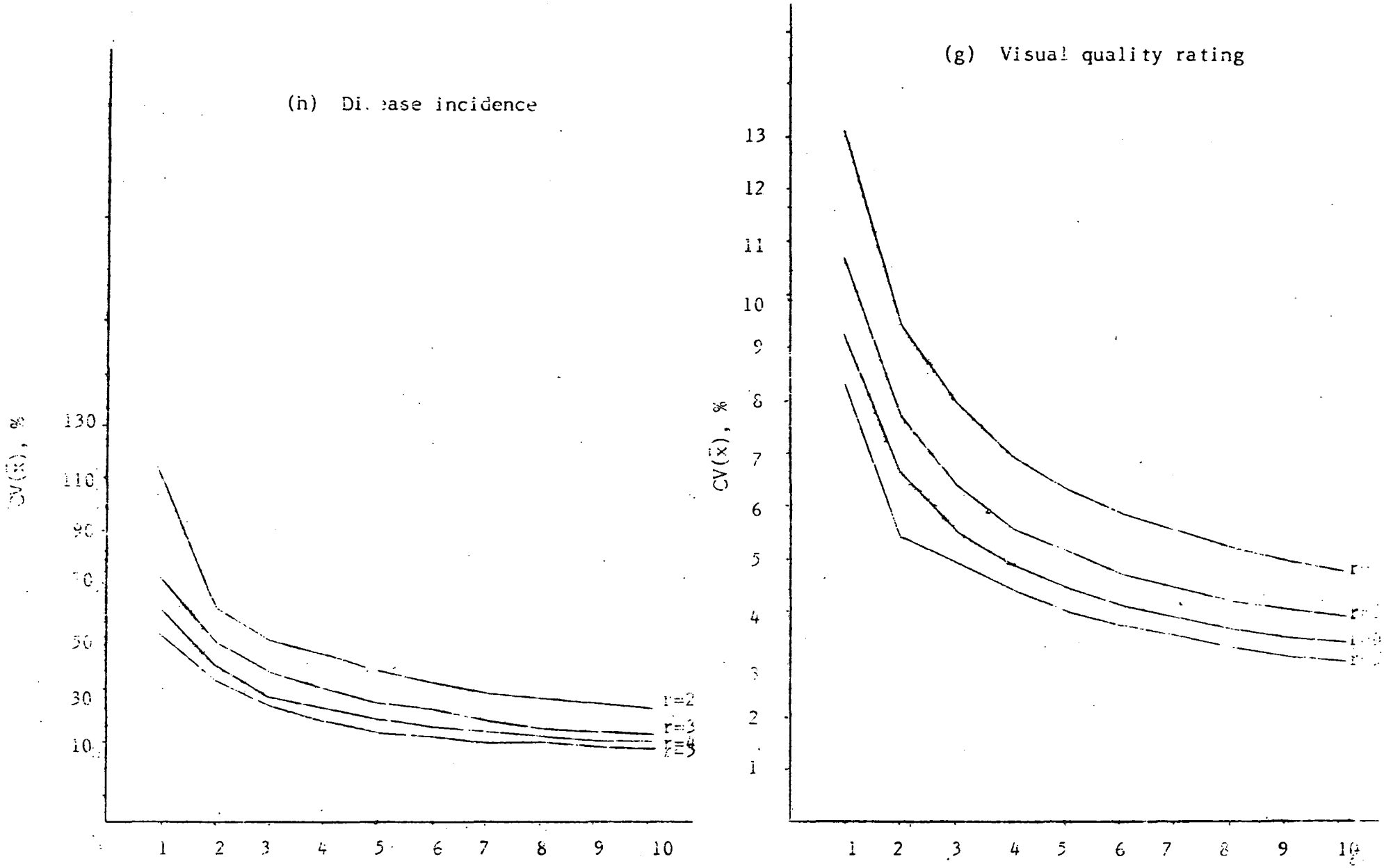

(h) Disease incidence
CV(x̄), %
130
110
90
70
50
30
10
r=2
r=3
r=4
r=5
1 2 3 4 5 6 7 8 9 10
(g) Visual quality rating
CV(x̄), %
13
12
11
10
9
8
7
6
5
4
3
2
1
1 2 3 4 5 6 7 8 9 10
NUMBER OF SUBSAMPLE(S)

Figure 1

Table 5. Optimum Numbers of Replications (r), Subsamples (s) and Sample Size (n = rs) at a Given Cost Ratio (C_E : C_S) for the Eight Post Harvest Characters of Mango.

CHARACTER	SIZE	COST RATIO, C_E : C_S				
		1:4	1:2	1:1	2:1	4·1
Color index	r	12	8	6	4	3
at day 0	s	3	4	6	8	11
	n	36	32	36	32	33
Color index	r	21	15	10	7	5
at day 5	s	2	2	3	4	6
	n	42	30	30	28	30
Total soluble	r	23	16	10	7	5
solids	s	1	2	2	3	4
	n	23	32	20	21	20
Titratable	r	14	10	7	5	4
acidity	s	2	3	4	7	7
	n	28	30	28	35	28
% Cumulative	r	9	6	5	4	3
weight loss	s	1	2	2	3	5
	n	9	12	14	12	15
Firmness	r	8	6	4	3	3
	s	1	2	3	4	8
	n	8	12	12	12	24
Disease	r	5	3	3	2	2
incidence	s	2	3	5	7	10
	n	10	9	15	14	20
Visual quality	r	6	4	3	2	2
rating	s	3	5	6	8	11
	n	18	20	18	16	22