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Applications of biostatistics in food science – Review

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Abstract:

Statistical methods are important aids to detect trends, explore relationships and draw conclusions from experimental data. The aim

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of this paper is to present some of statistical techniques and to highlight their uses based on practical examples in Food Science and Technology like Standard Deviation, Normality, ANOVA, Correlation analysis and other statistical methods.

Key words: Biostatistics, Statistical methods, Standard Deviation, Normality, ANOVA, Correlation analysis.

INTRODUCTION

Statistical analysis, which is defined as the process of making scientific inferences from data that contain variability, has historically played an integral role in advancing nutritional sciences. This tool has gained an increasingly important role in the systems biology era to analyze large, complex data sets generated from genomics, proteomics and metabolomics studies[1-2].Particularly, analyses of data from the reverse transcriptase-polymerase chain reaction (RT-PCR) as well as microarray, proteomics and other bioinformatics studies requires statistical models to account for various sources of variations[3-4].Appropriate statistical methods can minimize systemic errors, optimize data analysis, and identify which genes are differentially expressed in the face of substantial biological and technical variations[5-4].Statistics is essentially a branch of mathematics applied to analysis of data. In Food Science, statistical procedures are required in the planning, analysis and interpretation of experimental work. Such work may include surveys of the chemical, physical, sensory and microbiological composition of food and beverages during development and manufacture, including changes to these properties as a consequence of process optimization. Currently, many software packages are available that facilitate statistical analysis of data; when used properly they provide a valuable tool to enable different types of statistical and mathematical analyses to be done rapidly. Such software packages take

seconds to generate linear/non-linear models, draw graphs or resolve complex numerical algorithms that used to take a considerable amount of time using manual procedures.

The importance of proper application of statistics in Food Research cannot be ignored; it is essential if one is to understand data and make decisions that take account of the statistical variability of measurement and process control systems, summarize experimental.

CONCEPTS OF STATISTICS APPLIED IN FOOD SCIENCE

Use of the correct statistical tools is essential since the researcher needs to extract as much information as possible from experimental results. When work is published in a journal sufficient detail must be provided to permit the reader to understand fully the aims and outcome of the research and, should it be appropriate.

The total variance of a specific sampling plan (TV, also indicated as "Total error"), may be expressed by using statistic variance as a measure of variability and may be described as the sum of sampling variance (SV), sample preparation variance (SPV), and analytical variance (AV) as follows:

$$TV=SV+SPV+AV.$$

The reported analysis of results is often restricted to descriptive statistics (mean, median, minimum, maximum values, standard deviation and/or coefficient of variation). These, and other statistical tests such as correlation, regression, and comparison of mean values, are often based on the slavish use of 'statistical packages' that may, or may not, be appropriate for the purpose.

All of these considerations should be addressed prior to setting up an experimental plan and all are generally within the control of the researcher. Sometimes experimental results

may fall outside the limits of an analytical method; for instance, the level of an analyte in a sample may be below the lowest limit or, more rarely, above the highest limit of detection or quantification of a method. Such results are referred to as left- or right-censored values, respectively. How should such results be handled? This is a subject much under discussion in many fields, including (food) chemistry, microbiology and toxicology and several questions still need to be addressed with respect to the suitability of the procedure used to handle censored [6-7].

A procedure, known as the Tobit regression, for evaluation of censored data in food microbiology has been described by [8].— the concepts are equally applicable in other areas of Food Science.

Two characteristics of data sets must be considered prior to the application of any inferential tests:

1. Do the data conform to the principles of 'normality', i.e. to a 'normal' distribution (ND)
2. Do the data satisfy an assumption of homoscedasticity, i.e. uniformity of variance.

What do we mean by a 'normal' distribution (ND). A population ND can be described as a bell-shaped curve (Fig. 1) under which approximately 95% of values lie within the range mean (μ) ± 2 standard deviations (σ) and approximately 99% lie within the range μ ± 3σ. The standard deviation is a measure of the dispersion of values around the mean value and is determined as the square root of the variance, i.e.σ ¼ $=\sqrt{\sigma^2}$. The mean value (x) and standard deviation (s) of a set of data obtained by analysis of random samples provide estimates of the population statistics.

If a number of random samples from a 'lot' or 'batch' of food, or indeed of other test matrix, is analyzed for some particular attribute (e.g. sugar content, acidity, pH level) it would be unrealistic to assume that the analytical results will be absolutely identical between the different samples, or even

between subsamples of the same product. The reasons relate to the measurement uncertainty of the analytical method used for the test and the intrinsic variation in composition that occurs both within and between samples.

We would therefore expect to obtain a range of values from the analyses. If only a few samples are analyzed, the results may appear to be randomly distributed between the lowest and highest levels (Fig. 2A); but if we were able to examine at least 20 samples, we would expect to obtain a distribution of results that conform reasonably well to a ND (Fig. 2B) with an even spread of results on either side of the mean value. However, in some cases, the distribution will not be 'normal' and may show considerable skewness (Fig. 2C) — such results would be expected, for instance, in the case of microbiological colony counts.

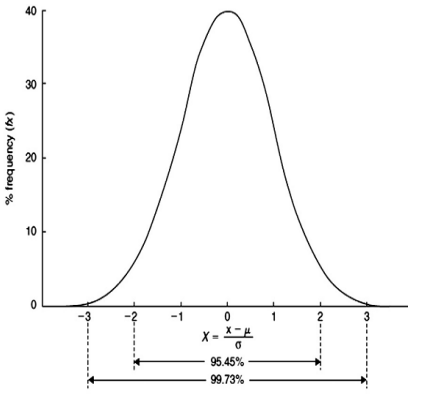


Fig.1. A population normal distribution (ND) curve showing that approximately 95.45% of all results lie within ±2 standard deviations (s) of the mean and 99.73% lie within ±3s. Modified from [9]

STATISTICAL MODELING AND DATA ANALYSIS

Depending on study design and the type of response variables, data will be analyzed with use of different statistical models to reflect the data structure and potential correlation between observations. Categorical response variables are usually

analyzed using contingency tables, logistic regressions, or generalized estimation equations (GEE) models. The contingency tables can also be used to test the homogeneity of distributions for categorical response or explanatory variables. In contrast, continuous response variables are analyzed using the t test, analysis of variance (ANOVA), correlation, and regression [10].Statistical modeling is the data processing step to sort out information from a study. This can be achieved by building a quantitative relationship between the outcome or response variables and the explanatory or independent variables through a mathematical model or equation that characterizes the dependence of the former on the latter. In modeling response variables, their correlations should receive special attention, because the responses (e.g., body weights of the same subject at different time points) are highly correlated and thus the correlation structure should be incorporated into data analysis. Therefore, longitudinal studies should be carefully analyzed for the following reasons: First, subjects are monitored with multiple observations at different time points. Second, the correlation structure between observations affects estimation accuracy and subsequent inference [11].

Sample size

A large sample size yields a powerful test to detect a difference between populations. Therefore, sample size calculation is needed to ensure desirable power in hypothesis testing. For this purpose, a difference in the parameters of distributions between study populations needs to be specified [10]. Sample size determination is a major issue in planning quantitative research. Accurate estimation of sample size will not only ensure the planned study to achieve a sufficient power to detect significant differences, but also save time and money by recruiting no more subjects than needed. Many factors affect sample size calculation, including Type I error rate, the power of test and the expected significance of detection. Sample size

calculation for studies not involving microarray or other high-throughput technologies can be found in many biostatistics books [12-13].

The aims of microarray studies include identification of differentially expressed genes between cases and controls, as well as profiling of subjects based on gene expression levels, the main objective of microarray studies is to discriminate cases fromcontrols. Two major classes of statistical models have been studied so far. One class of models focuses on gene expression, including the ANOVA method [14].and the t test-like method, such as significance analysis of microarrays [15].

The ANOVA model-based approach

This approach rigorously depends on a statistical model for data (i.e., the ANOVA model) where individual gene expression or its transformation (usually a log transformation to ensure the normality of intensity data) is assumed to be normally distributed and analyzed using the ANOVA model. Popular models are one-way or two-way ANOVA, incorporating experimental design factors[16].described detailed modeling and calculation based on the classical approach to sample size determination for linear models with adjustments for multiple comparisons through controlling type I error rate, FWER and FDR. They then considered detailed sample size for several standard microarray study designs, including matched-pair data, completely randomized design, and isolated effect design. A sample size table was also provided for each design. This method can be assisted with a software package sizepower in R [17].

Calculating the Standard Deviation

The standard deviation (s) is a measure of the deviation from the mean. Note the dependence of S of the number of data points. If the terms are all about the same, then the precision should increase (S decreases) as N increases. So, it is

statistically advantageous to make more measurements, although this must be balanced with practical considerations.

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \overline{x})^2}.$$

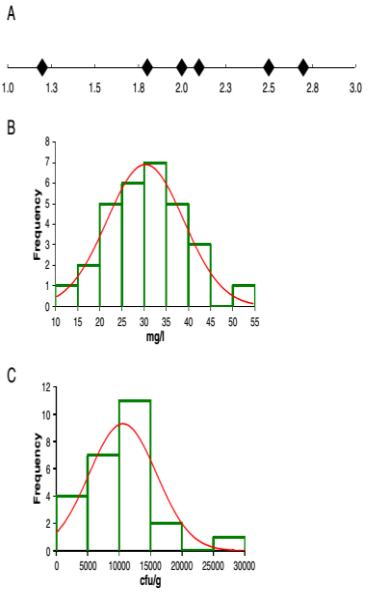


Fig. 2. (A) Plot of six analytical values, mean 2.05, SD = 0.532; range 1.20 to 2.70; (B) plot of analytical values from 30 replicate samples, overlaid with a ND curve forx = 30.3, s = 8.6. The data values are very slightly skewed but otherwise conform well to a ND; (C) plot of microbiological colony counts on 25 samples (as colony forming units/g) overlaid with a ND plot for x = 10,600 and s = 5360. Note that the data distribution shows a marked left-hand skew and kurtosis. The data do not conform to a ND.

Normality of data

The normality of experimental results is an important premise for the use of parametric statistical tests, such as analysis of variance (ANOVA), correlation analysis, simple and multiple regression and t-tests. If the assumption of normality is not confirmed by relevant tests, interpretation and inference from any statistical test may not be reliable or valid [18].

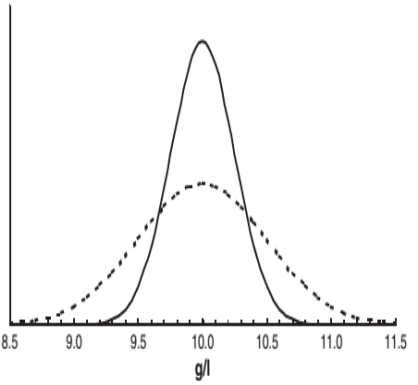


Fig. 3. Comparison of two ND curves both having x = 10 g/l; curve A ( ) has s = 0.25 and B has s = 0.5 (…). Note that more than 25% of the data values for curve B fall outside the 95% CLs (9.5, 10.5) of the data in curve A.

Normality tests assess the likelihood that the given data set {x1, …, xn}conforms to a ND. Typically, the null hypothesis H0 is that the observations are distributed normally, with population mean μ and population variance σ2; the alternative hypothesis Ha is that the distribution is not normal. It is essential that the analyst identify the statistical distribution of the data. Most chemical constituents and contaminants conform well, or reasonably well, to a ND, but it is generally recognized that microbiological data do not. Whilst microbial colony counts generally conform to a lognormal distribution, the numbers of cells in dilute suspensions generally approximate to a Poisson distribution. The prevalence of very low levels of specific organisms, especially pathogenic organisms such as Cronobacter spp. and Salmonella spp., in infant feeds and other dried foods show evidence of over-dispersion that is best described by a negative-binomial or a beta-Poisson distribution [19].

In practice, there are two ways to check experimental results for conformance to a ND: graphically or by using numerical methods. The graphical method, usually displayed by normal quantile–quantile plots, histograms or box plots, is the simplest and easiest way to assess the normality of data

[20].Numerical approaches are the best way to test for the normality of data, including determination of kurtosis and skewness; for example, tests such as those attributed to Anderson–Darling (AD), Kolmogorov–Smirnov (KS), Shapiro–Wilk (SW), Lilliefors (LF), and Cramér von Mises (CM). Frequently, people use histograms or probability plot graphs to test for normality (when they do!), but it can be risky since it does not provide quantitative proof that data follow ND. The shape of the graph depends on the number of samples examined and the number of bins used. Due to the small number of values the data shown in Fig. 4 do not appear to follow a normal distribution but the hypothesis of normality is not rejected by tests.

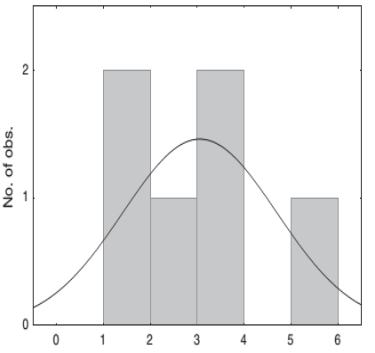


Fig. 4. Histogram of data values overlaid with a ND plot. Although the data do not appear to conform to a ND, tests for normality do not reject the null hypothesis due to the small number of data: Kolmogorov–Smirnov: pKS N 0.20; Lillifors: pLillifors N 0.10, and Shapiro– Wilk: pSW = 0.68666.

[20]studied the power and efficiency of four tests (AD, KS, SW, and LF) using Monte Carlo simulation and concluded that SW is the most powerful test for all types of distribution and sample sizes, whereas KS is the least accurate test. They also confirmed that AD is almost comparable with SW and that LF always outperforms KS. Using the example of **Fig. 5**, the SW test gives p = 0.02355 b 0.05, so the null hypothesis is rejected and the alternative hypothesis, that the data do not follow a normal distribution, is accepted but if the KS test had been

used p = 0.217 N 0.05 so the hypothesis of normality is not rejected.

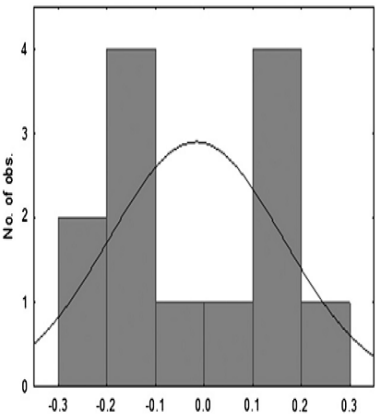


Fig. 5. Histogram of data values overlaid with a ND curve; the Shapiro–Wilk (SW) rejects the hypothesis for normality (p = 0.0236) but the Kolmogorov–Smirnov (KS) test (p N 0.20) does not reject the null hypothesis. The result from the Lilliefors tests (p b 0.10) is indeterminate.

The moral is to choose your test with care and to understand its limitations. In sensory and microbiological studies, for example, it is very common to obtain results that do not follow a ND [21].

Parametric statistics in Food Science

Depending on the statistical distribution of data, sample size, and homoscedasticity, samples and treatments can be compared using parametric or non-parametric tests. Parametric tests should be used when data are normally distributed and there is homogeneity of variances, as shown by the Shapiro–Wilk and Levene (or F) tests, respectively. Then, a Student's t-test is used to check for differences between two mean values or an ANOVA is used when three or more mean values need to be compared (Fig. 6).

The ANOVA model-based approach

This approach rigorously depends on a statistical model for data analysis (i.e., the ANOVA model) where individual gene

expression or its transformation (usually a log transformation to ensure the normality of intensity data) is assumed to be normally distributed and analyzed using the ANOVA model. Popular models are one-way or two-way ANOVA, incorporating experimental design factors [10].

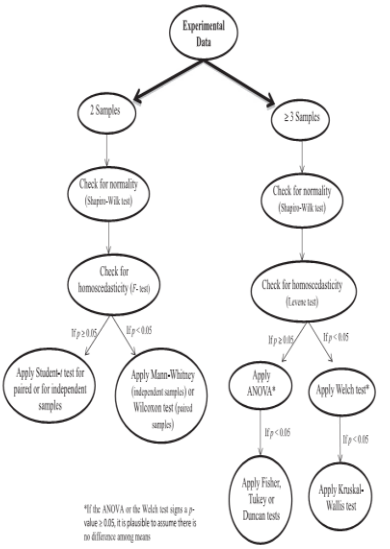


Fig. 6. Statistical steps and tests to compare two or more samples in relation to a quantitative response variable.

Analysis of variances for three or more data sets

Analysis of variances (ANOVA) is a parametric statistical tool that partitions the observed variance into components that arise from different sources of variation. In its simplest form, ANOVA provides a statistical test of whether or not the means of several groups are all equal. In this sense, the null hypothesis, H0, says there are no differences among results from different treatments or sample sets; the alternative hypothesis (Ha) is that the results to differ. If the null hypothesis is rejected then the alternative hypothesis, Ha, is accepted, i.e. at least one set of results differs from the others. The ANOVA procedure should be used to compare the mean values of three or more data sets. One practical example of

application of analysis of variance is provided by [22] authors investigated the rheological behavior of honeys from Spain under different temperatures (25 °C, 30 °C, 35 °C, 40 °C, 45 °C, and 50 °C) and concentrations and compared the samples using one-way ANOVA followed by a test of multiple comparison of means. Three alternative models can be used in an ANOVA: fixed effects, random effects or mixed effects models. The fixed effects model is appropriate when the levels of the independent variables (factors) are set by the experimental design. The random effects model, which is often of greatest interest to a researcher, assumes that the levels of the effects are randomly selected from an infinite population of possible levels [23].

Depending on the number of factors to be analyzed, we can have:

-A one-way ANOVA in which only one factor is assessed. This is the case for relatively simple comparisons of physicochemical, colorimetric, chemical and microbiological analyses [24-25-26-27]. For example, if five samples of apple are analyzed for catechin content, the "apples" are the independent variable and the "catechin content" is the dependent response variable. Another important application of one-way ANOVA is when different groups of test animals that are treated with an extract/drug and compared to a control group [28].

-A 2-way ANOVA is used for two factors in which only the main effects are analyzed. The 2-way ANOVA determines the differences and possible interactions when response variables are from two or more categories. The use of 2-way ANOVA enables comparison and contrast of variables resulting from independent or joint actions [29].This type of ANOVA can be employed in sensory evaluation when both panelists and samples are sources of variation [30]. Or when the consistency of the panelists needs to be assessed.

-A factorial ANOVA for n factors, that analyzes the main and the interaction effects is the most usual approach for many experiments, such as in a descriptive sensory or microbiological

evaluation of foods and beverages[31].A repeated-measures (RM) ANOVA is used to analyze designs in which responses on two or more dependent variables correspond to measurements at different levels of one or more varying conditions.[32].used a RM-ANOVA to examine results from assessments of different instrumental color attributes for a mixture of juices from yacón (Peruvian ground apple) tubers and yellow passion fruit as a function of storage time.

The choice of post-hoc tests to be used should be decided in advance so that bias is not attributed to any one set of data. In recent times, so-called 'robust' ANOVA methods have been developed that are not affected by outlier data and can be used when data do not conform strictly to ND. They were developed following a need to analyze inter-laboratory studies during validation of analytical methods for use in chemistry and microbiology and are important also in determination of measurement uncertainty estimates that are nowadays required as part of laboratory accreditation [33].

Non-parametric statistics in Food Science

Non-parametric procedures use ranked data rather than actual data values. The data are ranked from the lowest to the highest and each value is assigned, in order, the integer values from 1 to n (where n = total sample size.. actual difference between populations. Ranking for non parametric procedures preserves information about the order of the data but discards the actual values. Because information is discarded, non-parametric procedures can never be as powerful (i.e. less able to detect differences) as their parametric counterparts [34].

Table 1: Use of the Wilcoxon Signed Ranks test to determine the level of patulin in lots of apple compote (legal limit 25 µg/kg) using two analytical methods (A & B)

Production lot	Patulin (µg/kg)		Difference	Sign	Rank (R)	Signed R
	A	B	A – B			
1	12.5	10.5	2.0	+	8.5	+8.5
2	11.5	10.8	0.7	+	6	+6
3	12.5	13.0	−0.5	−	4	−4
4	12.0	12.0	0		−	−
5	14.0	12.0	2.0	+	8.5	+8.5
6	12.5	12.4	1.0	+	1	+1
7	14.0	12.3	1.7	+	7	+7
8	12.5	12.7	−0.2	−	−2	−2
9	14.0	13.5	0.5	+	4	+4
10	13.0	12.5	0.5	+	4	+4
Mean	12.85	12.17	Sum R+			+39
			Sum R−			−6

Tabulate the results for methods A & B, then determine the difference (A − B). Ignoring the sign and any zero value allocate a rank to each difference, using an average rank if results are identical. Then allocate the relevant sign to each rank. Add the rank scores for R+ and R− and, for the number of pairs (in this case n = 9), compare the smaller rank total with the tabulated value in tables of Wilcoxon's signed ranks. If, as in this case, the smaller rank total is ≤ published value then the difference is statistically significant at p = 0.05. Hence the null hypothesis, that results from both methods are equal, should be rejected as method A gives higher results. Whether the differences are of practical importance is another matter!

Table 2: Comparison of bacterial numbers on cotton and plastic sponge swabs taken from chicken neck skins immediately after evisceration.

Number of bacteria (CFU × 10^{-4}/25 cm^2)			
Cotton swab (A)		Sponge swab (B)	
Count	Rank	Count	Rank
110	14.5	20	7
16	6	200	20
24	8	5	2
105	13	89	11
155	18	125	16
2	1	140	17
104	12	180	19
10	4	49	10
7	3	15	5
28	9	110	14.5

Allocate ranks (1–20) across both sets of data; average the rank for identical counts (in this case, counts of 110). Calculate the rank totals: RA = 88.5; RB = 121.5. Calculate the UA statistic for data set A: UA = [nA(nA + 1) / 2 + nAnB − RA] = 66.5. Similarly, calculate the UB statistic for data set B: UB = 33.5. Take the smaller value of U as the test statistic and compare it with the Mann–Whitney tabulated value for p = 0.05 with nA = nB = 10. The calculated value of UB (33.5) N Ucritical (23) so the null hypothesis that both methods give equal results is not rejected — the 2-tailed probability is p = 0.25.

Table 3. Non-parametric analysis of the scores from a wine tasting

Wine	Score for taster no.				
	1	2	3	4	5
A	1	1	2	1	3
B	5	5	4	5	5
C	2	3	3	1	3
D	1	3	1	3	2
E	1	2	1	2	2
F	2	2	2	4	2

Number of samples = n = 6. We determine a value M using the equation: M ¼ nk k ð Þ 12 þ1 ∑R2 k−3n k ð Þ þ 1. For these data, M = 4.233 with υ = k − 1 = 4 degrees of freedom.

From the Tables, we find that the critical value for χ2(p = 0.05, υ = 4) is 9.49 which is greater than M and therefore we do not reject the null hypothesis that the tasters have scored the samples uniformly. Full details of these, and other nonparametric and parametric tests, are given in standard works including [35].

Bivariate correlation analysis

Correlation is a method of analysis used to study the possible association between two continuous variables. The correlation coefficient (r) is a measure that shows the degree of association between both variables [36].This parametric test requires both

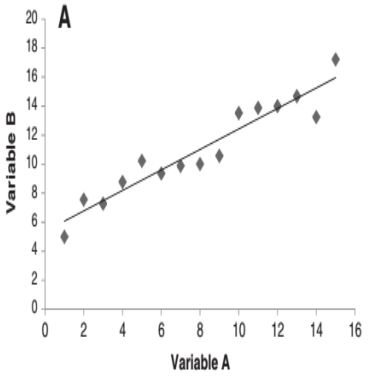
data sets to consist of independent random variables that conform to ND. The correlation coefficient measures the degree of linear association between the two sets of data (A and B), and its value lies between −1 and +1. The closer the absolute value, $|r|$, is to 1, the stronger the correlation between the data values [37].The correlation between two variables is positive (Fig. 7A) if high values for one variable are associated with high values for the other variable and negative (Fig. 7B) if one variable is low when the other is high. A correlation close to $r = 0$ (Fig. 7C) indicates that there is no linear relation, or at best a very weak correlation, between the two variables. However, a low r-value does not necessarily imply that there is no relationship between the responses; a low value can be due to the existence of a non-linear correlation between these variables, but the presence of outliers in one or both data sets may also affect the r-value [38].

Many workers calculate the Pearson linear correlation coefficient in order to seek to determine the strength of association between data sets. However, when more than five variables are analyzed, the analysis is compromised because correlation coefficients do not assess simultaneous association among results for all variables, which makes it difficult to understand and interpret the structure and patterns of the data. For example, if one considers five sets of response variables (A, B, C, D and E), it is necessary to calculate the correlation coefficients, and their significance, for each data set pair, i.e. AB, AC, AD, AE, BC, BD, BE, CD, CE, and DE. It is easy to understand and interpret up to three correlations coefficients but, in order to better understand multiple responses, a more sophisticated multivariate statistical approach, such as principal component analysis, clustering techniques, linear discriminant analysis should be used [39].

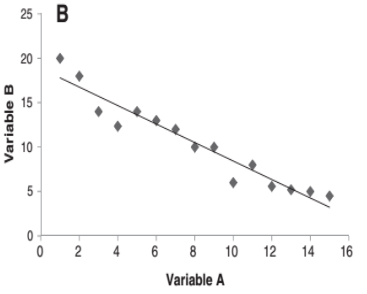
When large sets of results (≥30) are analyzed, data should be formally checked for normality. If the data do not follow a normal distribution a non-parametric approach, such

as the Spearman's rank correlation coefficient, should be used to analyze for any correlation between the responses. Fig. 9 shows the steps to follow when two data sets (each with $n \geq 8$) are to be analyzed with respect to correlation. Spearman's correlation coefficient (ρ) should be used when either or both data sets do not conform to ND, when the sample size is small, or when the variables are measured as ordinals i.e. first, fifth, eighth, etc. in a sequence of values. The Spearman correlation coefficient does not require the assumption that the relationship between variables is linear. One good example to compare both Pearson and Spearman correlation coefficients can be obtained by analyzing the data sets below:

A: 12.56; 14.46; 16.65; 25.68; 16.80; 28.95; 32.25; 30.33; 32.81; 28.29; 29.98; 30.32; 33.57



B: 53.25; 65.33; 53.68; 62.74; 61.53; 64.89; 66.40; 60.99; 68.50; 56.30; 66.36; 68.25; 73.89



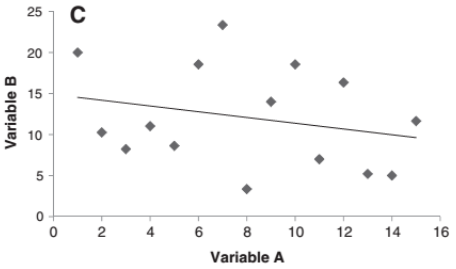


Fig. 7. (A) Positive correlation, (B) negative correlation, and (C) almost null correlation.

There are two major concerns regarding correlation tests: the significance of the correlation and the interpretation of results. Firstly, to assume a statistically significant association between variables the p-value of the correlation coefficient should be b<.05[40].showed that with large data sets, the correlation coefficient is often statistically significant even at a moderate or low r value. On the other hand, when the data set is small [41].

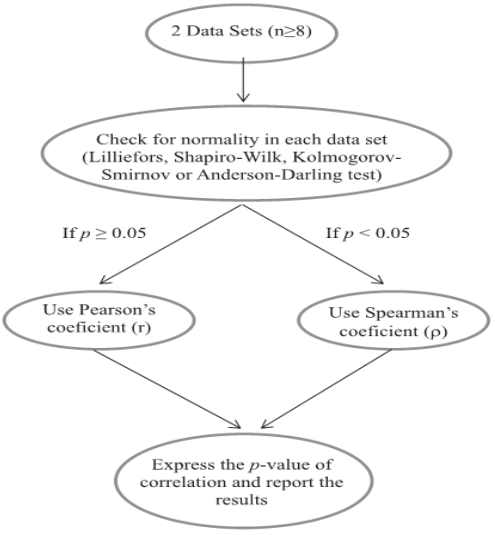


Fig.8. Steps to follow when two data sets (usually wi)
the n ≥ 8 are to be analyzed for correlation.

Regression analysis

Assumptions for regression require that:

– The samples are representative of the population for the inference prediction.

– The concentrations of the independent variables are measured with error, i.e. they are 'absolute' values; if this is not the case then the more complex orthogonal linear regression must be used in order to correct for errors in the predictor variable[42].

– The error term of the estimates is a random variable with zero mean;

– The predictors are linearly independent, i.e. each value cannot be expressed as a linear combination of the other values.

1. The variance of the errors is homoscedastic. In order to perform a regression analysis it is essential to:
2. Test data for the presence of outliers (at 95 or 99% of confidence) using the Grubb's test for each concentration level.
3. Ensure the homogeneity of variances in the concentration levels of the calibration curve by using one the tests.
4. Test the significance of the regression and its lack of fit through the F-test and a one-factor ANOVA. Provided that the response is directly and linearly correlated to the concentrations then the regression coefficient should be significant. Evidence for lack of fit ($p < 0.05$), may be due to a non-linear response, to excessive variation in the replicates at one or more of the test values or the use of an over-extended independent variable range. In this case, removing the highest values and repeating the statistical analysis should reduce the range of concentrations. Evidence for lack of linearity may indicate that a nonlinear model (quadratic, for example) might be more appropriate for the method, and therefore, alternative models should be evaluated.

5. Determine the following statistical parameters by means of the regression analysis:

• The regression equation ($y = ax + b$), where y is the dependent estimate at independent concentration level (x), (a) is the slope of the line and b is the linear intercept when $x = 0$.

• The standard deviation of the estimated parameters and model;

• The statistical significance of the estimated parameters;

• The coefficient of determination (R^2; regression coefficient) and the adjusted R^2.

The regression model is considered suitable to the experimental data when:

1. The standard deviation of the parameter is at least 10% lower than the corresponding parameter value.

2. The standard deviation of the proposed mathematical model is small;

3. The parameters of a model are statistically significant otherwise they will not contribute to the model.

It is a myth to consider that if $R^2 > 90\%$ the model is excellent this is only one criterion to evaluate the goodness of fit of the model. If R^2 is low (<70%), the mathematical model is not good; on the other hand, if R^2 is high (>90%), it means that you should continue the analysis and check the other criteria. It is noteworthy that in some applications, depending on the type of analysis, e.g. evaluation of sensory data, the coefficient of determination may be considered well if $R^2 > 60\%$.

4. The statistical significance, obtained from the F-test of an ANOVA analysis of the proposed mathematical model is at least $p < 0.05$.

5. Analysis of the residuals (experimental value for a response variable minus value predicted by the mathematical model) must conform to ND and have a constant variance, as described above. This is a necessary condition for the application of some post-hoc tests such as t and F. It is important to recognize that the regression and correlation coefficients describe different

parameters. Regression describes the goodness of fit of a model; correlation estimates the linear relationship of two variables. A common mistake is to use R^2 to compare models. R^2 is always higher if we increase the order of a model (linear in comparison to quadratic, for example). For example, a third order polynomial has a higher R^2 than a second order polynomial because there are more terms, but it does not necessarily mean that the first is the better model. An analysis of the degrees of freedom (number of experimental points minus number of parameters from the model) needs to be carried out. A model with more terms requires estimation of more coefficients so fewer degrees of freedom remain. Thus, another criterion needs to be used: the adjusted regression coefficient — R^2_{adj} . This coefficient adjusts for the number of explanatory terms in a model relative to the number of data points and its value is usually less than or equal to that of R^2. When comparing models, the one with the highest adjusted coefficient is the best model.

We have noted above that in addition to simple 'Generalized Linear Models' of regression other, more complex, forms of regression are available for use in specific circumstances. The reader is directed to other works, such as [43].for information and guidance on such procedures.

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