

New Statistical Approach to Evaluation of Lemon Juice

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Data, collected between 1959 and 1969 from 289 fresh and commercial samples of lemon juice from California, Arizona, Florida, and Italy, were reexamined with respect to detecting citric acid adulteration. Ratios of amino acids and *l*-malic acid to total phenolics were calculated and used in a multiple regression equation to predict the citric acid-total phenolics (CA:TP) ratio. The coefficient of determination (R^2) was 0.918. A ratio of the measured-to-predicted CA:TP

values produced a new function which had a mean value of 1.00 and a standard deviation of 0.112. The new function did not differ significantly from the normal distribution curve. Critical values at various probabilities and sample sizes are given. With five samples a 12, 14, 18, and 22% adulteration could be detected 90% of the time at significance levels of 0.1, 0.05, 0.01, and 0.001, respectively.

Lemon juice is sold commercially on the basis of its total acidity, which is primarily citric acid. Since synthetic citric acid costs about one-fifth the price of the acid in lemon juice, the temptation exists to adulterate the juice with citric acid. Rolle and Vandercook (1963) presented a multiple regression approach for determining added citric acid in lemon juice. This was based on predicting citric acid concentration from an expression involving the concentrations of the total amino acids, malic, and total phenolics. The predicted citric acid was compared with the measured value, and if the difference exceeded 19.1 mequiv/100 ml, the juice was considered to be outside the 99% confidence limits. The approach was originally designed for single-strength juice. Thus, due to the nature of the equation, the detection sensitivity was dependent upon the final concentration. Recently Lifshitz *et al.* (1971) devised a procedure for detecting adulterations in a single-strength Israeli lemon juice based on the χ -square distribution. Their procedure is fairly sensitive to added citric acid, but also is limited to single-strength juice, and their best determination depends upon inexpensive substances (citric acid, Ca, Mg, K) which could be added to compensate for dilution.

Since our original study was published, we have analyzed a considerable number of additional samples and now have a broad statistical sampling. The purpose of the present investigation was to reevaluate all of the data with respect to detecting adulterations with citric acid by procedures which could be independent of dilution.

EXPERIMENTAL SECTION

There were 289 samples of fresh and authentic commercial lemon juice used in this study, which covered the period of 1959 through 1969. The samples were distributed as follows: fresh coastal California lemons, 36; fresh Arizona desert lemons, 61; commercial California concentrate (unknown mixtures of coastal, valley, and desert lemons), 119; commercial Arizona concentrate, 30; commercial Florida concentrate, 25; and Italian lemon juice (commercial and fresh), 18. The commercial samples were from major processors, four in California and two in Florida. The commercial concentrates (6 to 12 oz samples) were reconstituted to single strength prior to analysis. The fresh samples represented from 10 to 30 lemons hand-reamed in our laboratory. All samples were analyzed according to published methods for total acidity, total amino acids,

and *l*-malic acid in mequiv/100 ml (Vandercook *et al.*, 1963) and total phenolics in absorbance units (Vandercook and Rolle, 1963). The resulting data were converted to ratios with total phenolics in the denominator: citric acid-total phenolics (CA:TP); amino acids-total phenolics (AA:TP); and malic acid-total phenolics (MA:TP). The ratios AA:TP and MA:TP were treated as the independent variables, while CA:TP was used as the dependent variable. The statistical calculations were done on an IBM 1800 computer using standard programs.

RESULTS AND DISCUSSION

The data were expressed in terms of ratios rather than concentrations, since ratios represent the amount of one parameter relative to another in the same juice. The reasoning behind this was that in the manufacture of lemon juice concentrate, water normally is removed until the acidity reaches the desired level. Thus, a larger volume of low-acid juice or a lesser volume of a high-acid juice is required to make a particular concentrated product. The volume-concentration factor normally is not known, therefore, reconstitution is to an approximate or average value. Any approach to detecting adulteration which is based on concentrations of the juice constituents is limited to single-strength products or is made less sensitive by an unknown dilution factor.

The ratios AA:TP and MA:TP would be independent of dilution or added citric acid; CA:TP is also independent of dilution but would reflect added citric acid. Table I lists the averages, standard deviations, coefficients of variation, and ranges for all the ratios and shows the large variability in the parameters. The range and variance of the CA:TP ratio make it valueless by itself in detecting adulterations with citric acid.

The two-dimensional relationship between CA:TP and AA:TP is shown in Figure 1 and between CA:TP and MA:TP in Figure 2. These plots show the correlations between the variables. The standard deviation of CA:TP independent of MA:TP is 35.2 and that independent of AA:TP is 30.3. This represents a highly significant reduction in variability of CA:TP, as compared with a standard deviation of 72.3 for the overall data.

When the data are combined by means of a multiple regression expression, the variance in CA:TP is reduced even further. The multiple regression equation is

$$\text{CA:TP} = 10.22 + 31.06 (\text{AA:TP}) + 6.36 (\text{MA:TP})$$

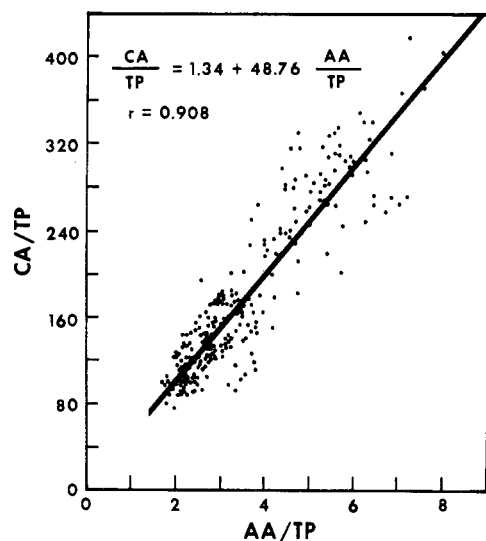
The standard deviation of CA:TP, independent of AA:TP and MA:TP, is reduced to 20.8. The coefficient of determination (R^2) is 0.918, which means that 91.8% of the variability of CA:TP is explained by linear relationships with AA:TP and MA:TP. Extension of the regression model to include quadratic and interaction components only increased the value of R^2 to 0.925.

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Table I. Statistics for Ratios of Citric Acid, Amino Acids, and Malic Acid to Total Phenolics in Lemon Juice

	Avg ^a	Min	Max	Std dev	Coeff var
CA:TP	177	76	403	72.3	40.7%
AA:TP	3.61	1.51	8.05	1.35	37.3
MA:TP	8.66	1.28	28.1	5.11	59.0

^a 289 samples.**Figure 1.** Relationship between the citric acid-total phenolics ratio (CA:TP) and the amino acid-total phenolics ratio (AA:TP) in lemon juice samples.

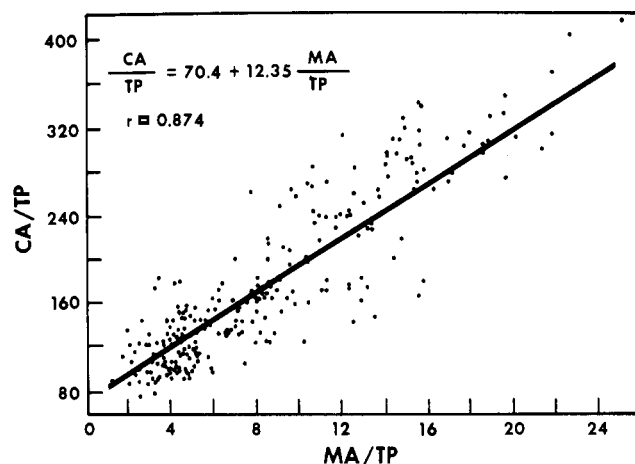
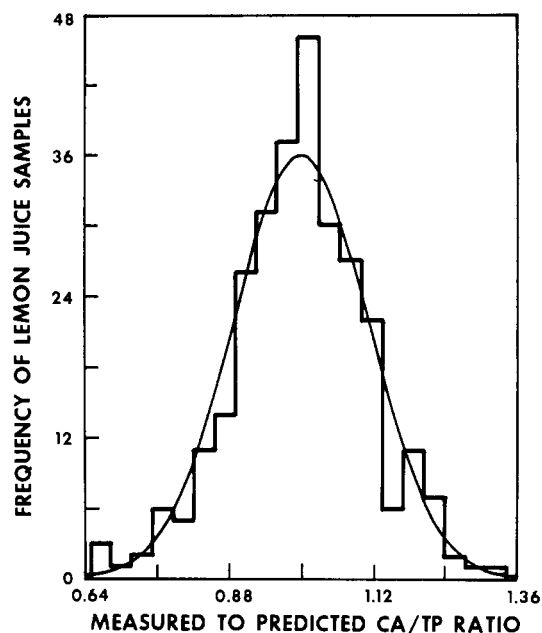
Adulteration of lemon juice can take either the form of citric acid and water added to single-strength juice or citric acid alone added to a concentrate. In either case, the net effect is to increase citric acid relative to total phenolics, thus making the measured value of CA:TP higher than the predicted value. The absolute difference between the measured and predicted CA:TP increased as the measured ratio increased. On the other hand, measured CA:TP divided by predicted CA:TP was symmetrically distributed with a mean value of 1.00 and a standard deviation of 0.112 (90% confidence interval = 0.105, 0.120). This distribution was evaluated by the Kolmogorov-Smirnov test (Ostle, 1963) and was found to be not significantly different from the normal distribution. According to the test, the maximum absolute difference was 0.0223, which is well below the critical values of 0.0629 and 0.0800 for α 0.20 and α 0.05, respectively. The appropriate normal density curve is shown in Figure 3, superimposed on the frequency distribution of the measured-to-predicted CA:TP ratio. As would be expected from the results of the goodness of fit test, there is good agreement between normal density and observed frequencies.

If we can assume normality and that our estimate of the standard deviation is sufficiently well known and stable to substitute for the population parameter, a statistical test can be derived to detect significant increases in the measured-to-predicted ratio above its mean of 1.00 (Ostle, 1963). A critical value, Θ , is calculated as follows.

$$\Theta = 1.00 + Z_{\alpha} (0.112) / \sqrt{n}$$

where Z = standard deviate for a one-tail test and α = probability of exceeding Θ when the mean is 1.00. If the observed mean of n observations exceeds the critical value, then it is significantly higher than 1.00 at the α probability level. Table II gives some critical values for various numbers of samples and probability levels.

Significantly higher ratios can be interpreted as evi-

**Figure 2.** Relationship between the citric acid-total phenolics ratio (CA:TP) and the malic acid-total phenolics ratio (MA:TP) in lemon juice samples.**Figure 3.** Normal density curve and frequency distribution of measured-to-predicted (CA:TP) ratios.

dence of adulteration with citric acid with two precautions to be kept in mind. First, the statistics are based on the randomness of the future sampling. Care must be taken that any future sample validly represents the lot, population, or source of juice to which the results are extrapolated. Secondly, small differences would be expected between natural juices of different types and locations resulting in many subpopulations. For example, one subgroup of fresh desert lemons (42 ten-lemon samples picked from six trees over a 6-week period) had an average measured-to-predicted ratio of 1.09. However, a more comprehensive sampling of lemons from the same area (18 samplings of about 30 fruit each from a packinghouse over three seasons) resulted in an average ratio of 0.99. A subgroup of Florida lemon concentrate (seven samples) had an average ratio of 1.12, whereas the overall Florida average was 1.03 (22 samples). There would also be some uncertainty in extending the test to lemon juices from areas not included in this study until they were shown to follow the same distribution. The majority of the United States' lemon crop comes from California and Arizona, both of which were adequately sampled. The Florida and Italian sampling was limited, but the fruit appears to be

Table II. Critical Values of the Measured-to-Predicted CA:TP Ratio at Various Probabilities

Number of samples	Probability level			
	0.10	0.05	0.01	0.001
1	1.14 ^a	1.18	1.26	1.35
2	1.10	1.13	1.18	1.24
3	1.08	1.11	1.15	1.20
4	1.07	1.09	1.13	1.17
5	1.06	1.08	1.12	1.15
10	1.05	1.06	1.08	1.11

^a Calculated from the equation $\theta = 1.00 + Z_{\alpha} (0.112)/\sqrt{n}$, where $Z_{.10} = 1.282$, $Z_{.05} = 1.645$, $Z_{.01} = 2.330$, and $Z_{.001} = 3.09$ for a one-tail test.

part of the general population. Because of the small differences which still exist between subgroups after the statistical model has accounted for 91.8% of the variability, a significant difference can be shown if the number of samples is large enough. That is, the probability of rejection increases as the sample size increases when a sample mean is greater than 1.00. Thus the number of samples must be determined by considering realistic levels of adulteration that are to be detected. These generally would be in excess of from 10 to 20% adulteration, depending upon the application.

To further clarify the latter point, a series of power function curves are graphed in Figure 4. For example, to detect a 20% adulteration 90% of the time, at α probability levels of 0.10, 0.05, 0.01, and 0.001, respectively, two, three, four, or six samples would have to be analyzed (the normality assumption is used again to obtain these power curves). The expression

$$\delta = \theta + Z_{\beta} (0.112)/\sqrt{n}$$

where θ is the critical value as calculated above, gives the population value for the measured-to-predicted CA:TP ratio that gives $1 - \beta$ probability of concluding a sample mean is significantly higher than 1.00. A percent difference in this ratio above 1.00 could be interpreted as the percent addition of citric acid. Again this assumes that natural juice has a value of 1.0 and that any other population value results from addition of citric acid.

When applying the statistical techniques presented in this paper, the user should be aware of the problems of sampling and statistics in general. For example, the $p = 0.05$ level means a 5% chance of being wrong, as well as a 95% chance of being right. Practical consideration also must be kept in mind when the decision is made to accept or reject the sample. The level of probability selected should be determined by the end use of the data. The economic and legal ramifications should determine the balance between the chance of accepting an adulterated sample and of rejecting an authentic one. Furthermore, there

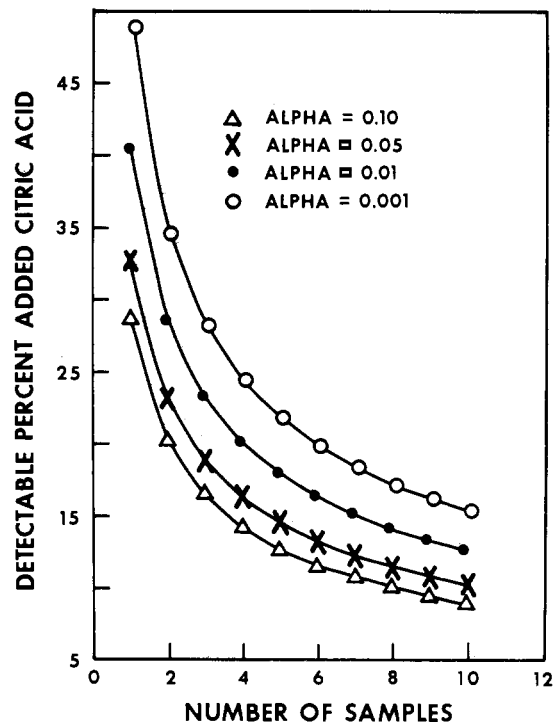


Figure 4. Number of samples required to give 90% probability of detecting a given percent added citric acid with a probability α of wrongly rejecting an unadulterated juice.

are dangers in attempting to push the system to detecting very low differences since, as was pointed out above, there will be subpopulations. Thus, in the final analysis, a judgment still must be made but this system will provide a statistical basis for that decision.

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