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Meat and Muscle Biology™

Statistical Analysis of GC/MS Data from Beef Steaks Cooked with Different Grill Temperatures and Steak Thicknesses Containing Missing Values, Lacking Normal Distributions, or Unequal Variance

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Objectives

Our objectives were to determine the degree of normality in selected volatile compound samples, the improvement to normality transformations may make, and the changes in interpretation transformations could induce.

Materials and Methods

Beef strip loins (n = 32) were sliced 3.81 or 1.27 cm thick. Steaks were cooked on an electric flat grill set at either 176°C or 232°C to an internal cook temperature of 71°C. Cubes (1.27 square) were transferred to a 470 mL glass jar and the static headspace was collected with SPME for 2 h. The SPME was injected into a multi-dimensional GC/MS identification of volatile aroma compounds. Volatiles were selected with a MS quality score above 75 and occurred in a minimum of 10% of the total steak samples. Absence of an observation for a volatile compound may be due to treatment effect, instrumentation limits, or simply missing from the sample. To analyze the normality of volatiles, representative volatiles selected were benzaldehyde, methyl-pyrazine, nonenal, and 2-octanone, as they were present in 95, 63, 42, or 20% of the total steak samples, respectively. These selected volatiles were then subjected to square root, $\log_{10}$, or Box-Cox transformations. If a volatile was absent in a steak sample, that cell was designated as either a missing value (MV) or as a zero (0). Shapiro-Wilk, box-plot, and normality distribution plots were used to measure normality for each of the volatiles across all conditions. Brown-Forsythe and Bartlett's tests were used to determine homogeneity of variance.

Results

Volatiles designated as a zero when no GC total ion counts (TIC) were present had residuals within treatment cells that were not normally distributed ($P < 0.05$). Volatile compounds nonenal and 2-octanone were normally distributed ($P > 0.05$) when empty cells were designated as MV, but benzaldehyde and methyl-pyrazine were not ($P < 0.05$). Square-root transformation of the data resulted in all the data designated with MV to be normally distributed ($P > 0.05$) while data with empty cells designated with 0 were unchanged and not normal ($P < 0.05$). Furthermore, Box-Cox transformations of MV data had lambda values of 0.23, 0.11, 0.18, and 0.10 for benzaldehyde, methyl pyrazine, nonenal, and 2-octanone, respectively. Brown-Forsythe and Bartlett's test indicated that as the percentage of volatiles present decreased, the treatment mean responses became less homogeneous in their variance ($P < 0.05$) as indicated by the fact that only benzaldehyde upheld the assumption of homoscedastic behavior ($P > 0.05$).

Conclusion

Analysis of volatile aroma compounds from cooked beef results in numerous missing values in the data, and by nature the data are not normally distributed, nor do they have homogenous variance as a result. Analyzing data with missing values rather than zeroes improves normality and additionally, transformation of the data using square root or Box-Cox significantly improved normality but had only minor impact on homogeneity of variance. ANOVA F-ratios were consistently highest on data that were entered with missing values rather than zeroes and were not transformed. Care should be taken analyzing volatile GC data to take into account the basic assumptions regarding the data and steps can be taken to conform to those assumptions.