Raman microspectroscopy enables one to acquire spectra of Raman scattering with a spatial resolution in the order of a few μm^3 and thus to study the natural composition of biological objects such as tissues, single cells and cellular organelles in a non-invasive way. In this work, we used Raman microspectroscopy to investigate vacuoles of the opportunistic human yeast pathogen *Candida albicans*. Large sets of Raman spectra of vacuoles were collected based on different cultivation protocols. The sets of the spectra were evaluated using the multivariate statistical method of singular value decomposition. Based on the spectral analysis, we characterized the chemical composition of the vacuoles. We found out that the vacuoles of cells cultured differently or in different media vary particularly in the concentration of polyphosphate, represented in the spectra by the peak near 1155 cm^{-1}. Interestingly, the wavenumber position of the polyphosphate peak may also be shifted by several cm^{-1}. We studied these shifts *in vitro* with sodium hexametaphosphate as a model of vacuolar polyphosphate. Based on these experiments, we suggest that the peak position is significantly influenced by the concentration of divalent cations.