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Investigation of the Structure of Quinoa Saponins by HPLC and MASS-SPEC

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Quinoa is a grain grown mainly by Natives in diverse areas throughout Latin America that is considered by many nutritional scientists as possibly the most nourishing grain available. Its contents include high levels of protein, an exceptional composition of amino acids, and many carbohydrates. Unfortunately, quinoa is very climate sensitive, and currently can only be grown in certain areas of the world, due to a low tolerance for intense heat. For this reason, quinoa is not grown everywhere and its annual yields are nowhere near the enormous quantities of rice and grain harvested annually. There are many different known varieties of quinoa grown throughout the world, each relatively specific to the region in which grown.

All species of quinoa are known to have soapy residues, called saponin, on the exterior of the grain as a natural defensive mechanism to discourage animals and insects from eating it. For general human consumption of quinoa, one must simply wash the grain in water to remove the soapy residue, which results in the formation of foam and bubbles. The amount of saponin per grain varies per each variety of saponin. Little is known about these saponins, their chemical compositions, and their differences from variety to variety.

The very positive health benefits from quinoa warrant the need for a thorough study of the grain in order to better understand its composition, nutritional benefits, and investigate potential ways to increase its worldwide distribution, growth, and harvest. A complete study of quinoa must investigate each variety of the grain to understand their differences and similarities. This study must include a careful examination of the saponin residue on each variety of quinoa, to discover its chemical composition and relative amounts of saponin per grain. This information is critical to someday create a species of quinoa practical for extensive, widespread agricultural production and distribution.

The particular emphasis of research on which we focused was developing an accurate, consistent, and reliable extraction procedure to analyze the composition of the saponins found on different samples of seeds. It was requisite to develop a procedure that could be easily repeatable for very small samples of seeds. This extraction procedure will be used in the future to study and compare the saponin compositions of the various species of quinoa.

Our successful extraction method that yielded the most pure sample of saponins involved three parts: (1) A reflux system to release the saponins from the ground seed, (2) a butanol wash to separate the organic saponin compounds from other hydrophilic debris, and (3) a wash with hexanes to remove the remaining oily residue from the sample. After extracting the saponin from the quinoa flour, a sample is injected into the HPLC. The HPLC is required so that the components of the saponins in the samples can be compared. A non-polar solvent is added so that the sample

will separate and those compositions that are not attracted to the column will bind to this solvent. The column is made up of silicon and four carbon chains. The HPLC displays a “fingerprint” of the components of the saponins.

Data from the HPLC results are compiled in such a way to allow for easy comparison between samples. Each peak is individually evaluated to determine its corresponding peak or lack thereof on the other samples. Using MATLAB software, all of the peak areas were entered into data matrices allowing for easy manipulation of the data and to apply principal component analysis. Principal Component Analysis (PCA) is a way of simplifying multidimensional data sets, such as the peak area data, into smaller dimensions, or principal components. The completed principal component analysis leaves little question that the different saponin samples were very unique in their properties. Thus, there must be a qualitative difference between the saponins of each bulk.

Using the PCA models, we have identified which peaks from the HPLC are most relevant to the qualitative differences. We must now develop a procedure on a larger scale that will enable us to remove significant aliquots for each peak of the HPLC. Mass spectrometry will then produce data from which we can determine the composition of each peak. We will look at published literature as a starting point to help us determine what these compounds might be. This project is ongoing and more research will be needed to continually improve extraction methods and improve ability to identify the specific compounds found in the various saponins.