Supplemental Figure S1. Photos of samples at various stages during extraction in Protocol B. Two samples from Protocol B following alkali hydrolysis for two hours are shown in (A). As the reaction takes place, the sample should turn orange or an orange-brown. As indicated by the arrows, portions of the sample could not be mixed with the NaOH, thereby preventing the reaction from taking place. The difficulty with which HCl, NaCl, and ethyl acetate were mixed following the alkali hydrolysis is exemplified in (B). When neutralized by HCl, the sample should turn from dark orange (or orange-brown) to white. In Protocol A, this took place with ease with little mixing required. However, in Protocol B, the swollen starch was so thick in some samples that only the top portion of the gelatinized mass would be exposed to HCl, even after vortexing. Occasionally, the separation of the ethyl acetate layer after centrifugation was acceptable (C). However, the gelatinous mass in the bottom of the vial was unable to be mixed well (as indicated by the yellow-orange color which should be white). Furthermore, there should only be a small white maize pellet at the bottom of the tube, but the starch was swollen to varying degrees in the two vials shown.
Supplemental Figure S2. Cost efficiencies for different scenarios. The cost efficiency will differ on a case by case basis, but Protocol A is cost efficient in all scenarios plotted above. Two different values of \( n_T \) were evaluated, those being 1,000 and 5,000 samples. The salaries shown are potential annual employee salaries. To calculate the values above, \( n_B \) was assumed to be 72 and the additional cost per sample due to the use of \( \alpha \)-amylase was assumed to be $0.16. In the most extreme scenario, the use of Protocol A saved approximately $50,000.
Supplemental Figure S3. Overview of similarities and differences between Protocol A and Protocol B. Shared steps are shown in blue, steps unique to Protocol A are shown in green, and steps unique to Protocol B are shown in orange. Most steps in Protocol A and Protocol B are shared. However, there are a few key differences. The main difference in the two protocols is the inclusion of a starch digestion step in Protocol A prior to the addition of NaOH. The omission of the starch digestion step in Protocol B made the extraction of ferulic acid much more difficult. To better separate the layers, the centrifugation time in Protocol B was increased in comparison to the centrifugation time used in Protocol A.
Supplemental Figure S4. Chromatogram of compounds extracted in the ethyl acetate fraction. Peaks indicate the phytochemicals present in the samples analyzed. Each peak is labeled with its corresponding phytochemical and retention time (in minutes).