Final Report DNA extraction and concentration analysis project

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This project was organized by the Marianopolis Research Team and conducted in the laboratories of Marianopolis College.

Introduction

DNA extraction and analysis is a procedure that has many real-world applications even outside of the world of research. It can be useful for food safety and authentication, medical diagnostics, forensic science, genetic engineering and more. As DNA extraction is often used to detect food fraud or contamination, this research project will focus more on the DNA extraction of fruits.

Objective

Extract and analyze DNA of fruits grown in different conditions to determine how growing conditions affect DNA concentration. More specifically, compare the DNA concentration of organic and non-organic labeled fruits.

Current Research Done on the Subject

A Comparative Study of Some Procedures for Isolation of Fruit DNA of Sufficient Quality for PCR-Based Assays

This study focuses on the most effective methods to extract DNA from fruits through polymerase chain reaction-based tests. In order for the PCR to be accurate, this study compares different DNA extraction methods and identifies the best one. The main challenge in this process is the polysaccharides and the phenolics (aromatic chemical compounds) found in the fruits that prevent complete DNA extraction and interfere with the results of PCR tests. PCR tests help with identifying food fraud given that there is often mislabeling of the ingredients and adulteration by replacing a fruit with a cheaper fruit, for example, which misleads the consumer. Isolating and extracting DNA from fruits ensures that what is being advertised in an ingredient list of a product is in fact true. The DNA extraction methods conducted are the following: commercial DNA extraction kits, CTAB (detergent that breaks down the cell wall of plants) method, SDS method (detergent that breaks down the cell membrane), mechanical grinding, in-house methods (suited to the fruit and the extraction challenges they have), and magnetic bead-based method (magnetic beads that bind to DNA). The conclusion of this study is that there is not necessarily a “better” extraction method, it all depends on the type of fruit. [1] (Maria Lemontzis, Eliana Gasparrini)

DNA extraction optimization and authentication of Vaccinium berries and their products by high-resolution DNA melting analysis - ScienceDirect

This research was done to find an easy kit-based method to differentiate fruits that look similar or are similar colours. This is to be used not only for fresh fruits, but also for processed products such as jams, freeze-dried berries, and powders. DNA is extracted from certain berries to examine whether any deliberate or unintentional adulteration has occurred. High-resolution

melting for DNA barcoding (Bar-HRM) is commonly used for fruit identification, to make sure certain berries haven't been mistaken for others, or switched out for more common ones to increase profit. However, extracting the DNA from these fruits presents a challenge because the DNA denatures when the fruits are processed (drying, heating, grinding, or long-term storage). Researchers have found some merits to using DNA extraction kits over traditional methods. This may help recover genetic diversity, because it would for example call out companies passing blueberries as bilberries because they are cheaper to produce. [2] (Maria Lemontzis, Eliana Gasparrini)

Biochemical and Molecular Aspects of DNA in Raw and Ripen Fruit and Vegetables – Biosciences Biotechnology Research Asia

This study examined the concentration of DNA in fruits and vegetables. The food was processed in a blender instead of crushed by hand, and then the DNA is separated with soap and ethanol. There was an additional step where the precipitate was placed in a freezer. The researchers found that DNA concentration was higher in ripe fruits vs. unripe (raw) fruits, and the fruits used were cucumbers, tomatoes, bananas and papayas. This may indicate that we should choose fruits as ripe as possible when conducting our experiment. Also, if the organic and non-organic version of a fruit are at different ripe-ness, this may affect how accurate the difference in DNA concentration is. [3] (Maria Lemontzis, Eliana Gasparrini)

GMO Fruits and effect on DNA concentration

GMO stands for "genetically modified organism", and the term is often used in agricultural technology domains and the food sector. With genetic engineering, gene editing and special breeding techniques, humans have created crops that suit their specific nutritional needs. In some cases, genetic modifications are necessary for some plants to resist parasites or fatal diseases, allowing us to cultivate them without worrying about an entire crop being wiped out. It is also possible to modify these organisms to increase their nutritional value or improve their taste and appearance. In 2016, the first animal GMO was approved for sale in Canada.

For the purpose of this lab, we can assume that the GMO fruits we are studying were modified using a gene editing technique, which requires the manipulation of the crop's genome (the density of the DNA in fruits modifies using selective breeding is not likely to be changed), like Horizontal (or vertical) gene transfer (HGT). To give a plant a new trait, such as resistance to disease or pesticides, the modification process would require the removal of a certain strand of DNA from an organism having evolved that trait (such as a species of bacteria) to then add the DNA into the fruit's genome. This method results in the addition of new DNA strands into the organism's genome, however, some small deletions of base pairs have been observed. Therefore we can expect the GMO fruits to have a higher DNA density than their non-GMO counterparts, that is, if the modified organisms went through HGT. [4, 5, 16] (Nicholas Huni)

Unfortunately, in Canada, companies are not obligated to label GMO fruits, making it harder to determine whether the fruits tested are GMO.

Manipulations

The protocol is comprised of two main parts : DNA extraction and concentration analysis. The DNA is extracted through precipitation with ethanol and isopropanol. The amount of DNA extracted is then determined using a diphenylamine assay. The fruits that were tested includes: strawberries, bananas, kiwis and cucumbers. Organic and non-organic brands were only compared for strawberries. The organic brand is from *Nature Fresh Farms: Little Obsessions*, while the non-organic brand is from *Driscoll's*.

Full Protocol (Nicholas Huni)

https://docs.google.com/document/d/1b953l1SLCjQf7sBPCz2c-eSN2XfroXqDhgnVo4O3Dko/edit?tab=t.0#heading=h.5x0d5h95i329

Why does ethanol precipitate DNA?

DNA precipitation is achieved by adding salt and alcohol to a DNA solution to concentrate, desalt, and recover nucleic acids. In an aqueous solution mixed with salt, the cations created from the salt have difficulties binding with the nucleic acid to neutralize its charge and precipitate. This predicament is caused by water's high dielectric constant, which surrounds the DNA polymers with water molecules creating hydration shells. Consequently, alcohols such as ethanol are added to lower the dielectric constant of the solution, which increases the attraction between the cations and the negative backbone of the DNA, allowing monovalent cations to bind with DNA. Isopropanol, which has a lower dielectric constant than ethanol, can be used at half the volume of ethanol for precipitation. However, its lower polarity can lead to salt co-precipitation, and its reduced volatility results in longer drying times for the precipitated DNA to evaporate the isopropanol. Adding a buffer, such as TE buffer, to the solution will prevent the DNA from decomposing by contaminated DNA enzymes, and keep the solution in neutral pH for low pH impacts DNA precipitation. [6,7,8,9] (Nam Nguyen-Huynh)

Choice of Fruits

For this experiment, soft fruits should be used because they are easier to mash into smaller pieces to extract DNA from them. In fact, it is recommended to use strawberries, kiwis, and even bananas. All three of these fruits have a softer texture, making it easier to pulverize and break them into smaller fragments. Strawberries are used because they also have 8 of each type of chromosome in each cell (octoploid), which leads to a larger amount of genetic material that can be extracted. Kiwis are useful because they contain an enzyme called proteinase that breaks down proteins. This facilitates DNA extraction from the fruit because the genetic material is separated from the proteins. Bananas have three copies of each chromosome in each cell (triploid), which allows for a larger amount of genetic material to be extracted. The growing conditions that should be tested in this experiment are GMO (genetically modified organisms) and non-GMO fruits. This is due to the fact that GMO fruits have been genetically modified using genetic engineering, so their DNA will differ from that of a fruit grown naturally. This will allow for a comparison of the differences in the DNA extracted from each type of growing condition. [10,11,12,13] (Laura De Angelis)

Effect of pH

DNA is affected by pH because the presence of high concentration H+ ions in acidic conditions and OH ions in basic conditions has an impact on the hydrogen bonds in the backbone of the molecule. If the pH drops below 5 and becomes acidic, the DNA molecule will destabilize because the high concentration of hydrogen bonds will break the phosphodiester bonds between base-pairs. This will lead to depurination where the molecule will lose its purines. If the pH rises above 9 and becomes basic, the DNA molecule will denature because of the high concentration of hydroxide ions. These ions will remove hydrogen from the backbone of the molecule and break the bonds. When the pH is in the neutral range, which is considered 5 to 9 for DNA molecules, it will be stable. Buffers play an important role because they reduce the effects of pH changes on a solution or molecule by attempting to equalize and balance the concentration of H+ and OH- ions, leading to a neutral solution. Therefore, the buffers affect DNA because it helps to ensure that the DNA molecule stays intact and does not denature due to modifications in pH. [14,15] (Laura De Angelis)

Results

Due to time constraints and difficulties with the manipulations, results were not collected for all fruits and for the standard curve. Furthermore results were not distinguished between the organic and non-organic strawberries.

Average mass of strawberries used for each extraction: 59.3g

Absorbances Measured For Strawberries
0.879
0.77
0.376
1.243
1.163
0.62
0.197
1.07
0.583
0.14
1.262

Average Absorbance	0.755
Standard deviation	0.4071267169
Confidence interval (95)	[0.481, 1.029]

Analysis

Without a standard curve or differentiated results between organic and non-organic fruits, it is difficult to state many observations. There is a large variation within the results with a confidence interval of 95% of [0.481, 1.029]. This makes our results less reliable and is most likely due to the sources of uncertainty.

Sources of Uncertainty

DNA extraction

Only strawberries, had successful DNA extractions, with the other fruits not showing a visible phase separation. This is most likely due to the octoploid nature of the strawberries, making the fruits have a higher DNA concentration. Isopropanol was more efficient than ethanol at precipitating DNA. Nonetheless, the precipitated DNA didn't clump together as expected, making it harder to separated the precipitated DNA from the initial solution and causing a lower reading for the diphenylamine assay. Thus, some of the initial solutions could have been transferred into the diphenylamine assay, affecting the absorbance readings.

DNA concentration analysis

For most of the readings, the test tubes did not turn blue as expected for a diphenylamine assay. This is most likely due the test tubes not being exposed long enough in the hot water bath. Thus, the final data collected, might be a result of the cloudiness in the solution rather than the chemical reaction between DNA and the diphenylamine reagent. This makes our results much less likely to be the accurate concentration of DNA in strawberries.

Conclusion

No definitive conclusions can be made regarding the DNA concentration in organic vs non-organic fruits. The protocol for both the extraction and analysis would need to be adjusted in order to get more accurate results. To obtain more accurate and reliable results, improvements to both the extraction and analysis protocols are necessary. Nevertheless, this remains a valuable area of scientific research. Refining these methods could eventually enable researchers to better assess the impact of growing conditions on fruit DNA and potentially aid in detecting mislabeling, helping verify whether produce is truly organic.

Special thanks to all the members of the Marianopolis Research Team that contributed to the project!

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