“Which locally grown fruits work best for DNA extraction?

1. Introduction:

With this experiment, we aimed to investigate which locally grown fruits yield optimal results for DNA extraction, therefore, to gain insight into which fruits are better suited for genetic analysis in our ecosystem.

1.1 Background Research (

1.1.1. Research on the Romanian Local Fruits.

Firstly, we did extensive research on the Romanian local fruits. We chose 7 top fruits, taking into consideration the following criteria: diversity, size, water content, DNA characteristics and growing regions in Romania. In aligning with these criteria and aiming for diversity among the chosen fruits, we ultimately settled on plums, apples, pears, peaches, strawberries, grapes, and quince**.**

1.1.2 **Locally Grown Fruits**:

Selected fruits for this experiment include plums, apples, pears, peaches, strawberries, grapes, and quince. Each fruit has unique characteristics that may impact DNA extraction.

1.PLUMS:

The dominance of plum cultivation remains pronounced in Romanian orchards, comprising roughly 50% of the total. Romania holds the top position in plum cultivation area across the EU, with plums occupying 34,899 hectares out of the country's total orchard area of 77,883 hectares**.**

- **Growing Regions in Romania:** Argeș and Mehedinți.
- **Soil Characteristics:** Well-drained soils with a pH ranging from slightly acidic to neutral.
- **Water Content:** Approximately 87%.
- **DNA Characteristics:** Diploid with a characteristic chromosome number.

*** We purchased the plums from a local farmer in Argeș County.**

2. APPLES:

The largest apple orchards are found on the hills and valleys of Bistrița-Năsăud County and Mehedinti.

- **Growing Regions in Romania:** Maramureș, Sibiu, Cluj.
- **Soil Characteristics:** Loamy, well-drained soils. Good drainage is essential.
- **Water Content:** Around 86%.
- **DNA Characteristics:** Diploid with a characteristic chromosome number (e.g., 34).

*** We purchased the plums from a local farmer in Bistrita County.**

3.PEARS:

Pear trees, being adaptable, can thrive in various regions across the country. The expansive 180-hectare wild pear plantation in Chegea, Satu Mare County, claimed to be the largest in Europe by the Babeș-Bolyai Institute in Cluj. Despite the modest soil quality, the consistent emergence of young pear trees annually underscores their resilience and preference for the environment.

- **Growing Regions in Romania:** Satu-Mare , Timiș, Sibiu, Bacău.
- **Soil Characteristics:** Well-drained, loamy soils with a slightly acidic to neutral pH.
- **Water Content:** About 84%.
- **DNA Characteristics:** Diploid with a characteristic chromosome number.

*** We purchased the pears from a local farmer in Satu Mare County.**

4. PEACHES:

The top peach-producing county in our region is Constanta, followed by Bihor, Dolj, Timis and the area around Bucharest. Peach cultivation is low in the eastern and northeastern parts of Transylvania and in northern Moldova, where the climate is too cold for this species.

- **Growing Regions in Romania:** Constanta, Bihor ,Dolj, Olt.
- **Soil Characteristics:** Well-drained soils in southern regions with slightly acidic to neutral ph.
- **Water Content:** Approximately 88%.
- **DNA Characteristics:** Diploid with a characteristic chromosome number.

*** We purchased the pears from a local farmer in Constanta County.**

5. STRAWBERRIES:

The most extensive area dedicated to strawberry cultivation is in Satu Mare county, covering 1,084 hectares and projecting a yield of over 11,000 tons. Counties like Giurgiu (293 hectares), Valcea (176 hectares), and Gorj (99 hectares) also cultivate strawberries on significant scales, with several others ranging between 25 and 45 hectares. Strawberries thrive in both southern and northern regions of the country, but optimal results are seen in areas receiving annual rainfall between 600-900 millimeters.

- **Growing Regions in Romania:** Satu Mare, Cluj, Brașov, Prahova.
- **Soil Characteristics:** Well-drained, sandy-loam soils.
- **Water Content:** High, around 90%.
- **DNA Characteristics:** Octoploid with a characteristic chromosome number (e.g., 56).

*** We purchased the pears from a local farmer in Constanta County.**

6. GRAPES:

Our nation stands as a significant player in the global wine industry, boasting a vine cultivation heritage spanning at least 2,000 years. Despite a decrease from nearly 300,000 hectares three decades ago, our country still maintains around 190,000 hectares of vineyards. Vrancea county takes the lead nationally, both in vineyard size and employment in this sector. With over 22,000 hectares dedicated to noble vines, comprising more than 10% of the national vineyard area, nearly 80,000 residents of Vrancea are engaged in vineyard-related activities.

- **Growing Regions in Romania:** Vrancea ,Timiș, Mehedinți.
- **Soil Characteristics:** Well-drained, slightly acidic to neutral soils.
- **Water Content:** About 81%.
- **DNA Characteristics:** Diploid with a characteristic chromosome number.

***We purchased the pears from a local farmer in Vrancea County**

7.QUINCE:

Quinces thrive in the central and southern regions, benefiting from warm summers conducive to ripening. Giurgiu hosts Romania's largest organic quince orchard, established in 2008, sprawling over 5.5 hectares and hosting 4,000 trees.

- **Growing Regions in Romania:** Giurgiu, Arad, Bihor, Alba.
- **Soil Characteristics:** Well-drained soils with a slightly acidic to neutral pH.
- **Water Content:** Approximately 81%.
- **DNA Characteristics:** Diploid with a characteristic chromosome number.

***We purchased the pears from a local farmer in Giurgiu County**

1.1.3 Factors Influencing DNA Extraction:

- **Water Content:** Ranges from approximately 81% to 90% in selected fruits.
- **Chromosome Number:** Generally diploid for most fruits, except strawberries (octoploid).

1.1.4. Fruit-Specific Factors:

Plums:

- **Cell Wall Toughness:** May impact the efficiency of cell wall breakdown.
- **Pectin Content:** Can influence the viscosity of the extraction buffer.

Apples:

- **Cellular Integrity:** The structure of apple cells influences DNA release.

- **Polyphenol Oxidase:** Enzymes affect DNA extraction by promoting browning reactions.

Pears:

- **Cellulose Content:** Influences the efficiency of cell disruption.
- **Enzymatic Activity:** Enzymes may affect the stability of extracted DNA.

Peaches:

- **Enzymatic Activity:** Presence of enzymes may affect DNA yield.
- **Flesh Texture:** Soft and juicy texture may impact DNA release.

Strawberries:

- **Achenes:** Small seeds on the surface may affect the extraction process.
- **Enzymatic Inhibition:** Inhibiting enzymes is crucial.

Grapes:

- **Skin Thickness:** Influences the efficiency of cell disruption.
- **Tannin Content:** Affects extraction process and DNA stability.

Quince:

- **Cell Wall Rigidity:** Firmness of cell walls may impact DNA release.
- **Pectinase Activity:** Enzymatic activity may influence the viscosity of the extraction buffer.

1.1.5. Fruits Commonly Used for DNA Extraction:

- **Strawberries:** Strawberries are frequently used for DNA extraction due to their high water content, octoploid nature, and relatively simple cellular structure.
- **Bananas:** Bananas are also commonly used for DNA extraction as they have a high DNA yield and a less complex cellular structure.
- **Spinach:** Spinach leaves are used in DNA extraction experiments due to their high chloroplast content and relatively simple cell structure.

1.1.6. Reasons for Common Use:

- **High Water Content:** Fruits with high water content often yield more DNA during extraction.
- **Simple Cellular Structure:** Fruits with a less complex cellular structure may facilitate easier DNA extraction.

1.1.7. Influence of Soil Characteristics on DNA Extraction:

- **Soil Composition:** Indirectly affects nutrient availability to plants, impacting overall health and DNA content.
- **Soil pH and Composition:** Influences the availability of minerals essential for plant growth and DNA synthesis.

2. DNA EXTRACT PROCEDURE

Materials used:

• Fruits (mentioned above)
• Zip-closure sandwich bag
• DNA extracting solution (mix about 1 tablespoon of dish detergent and 1 teaspoon of salt into 1 cup of water):
• Plastic cup
• Gauze, cheesecloth, or coffee filter
•Rubber band
• beakers
• Dropper (or spoon)
• Denatured alcohol: ethanol- put in the freezer for best results.
• Paper towels

•Balance

Procedure:

Step 1: Preparation:

- We washed the fruits thoroughly to remove any external contaminants.
- We weighted and used the same quantity of each fruit for extraction to maintain consistency across samples.

Step 2: Cell Lysis:

- We chopped the fruits into small pieces and transfer them to a zip-closure sandwich bag. We removed most of the air before sealing the bag.
- We also mashed the fruits through the bag in our hand. We did not hit against the table as this might damage the DNA.
- We added 2 tablespoons of the DNA extracting solution to break down cell membranes and release cellular contents. The dishwashing liquid bursts open the cells of the fruits, releasing the DNA and the salt it ensures that the proteins in the cell are kept separate from the DNA.

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Step 3: DNA Precipitation:

- We placed a piece of gauze over the opening of the cup, securing it with a rubber band.
- We carefully poured the mashed fruits mixture into the beaker making sure to catch the solids with the gauze.
- We took a spoonful of the liquid in the cup and placed in the beaker.

- We added a dropper for spoonful of the alcohol to the beaker. We made sure not to tilt or tip the beaker; didn't mix the two liquids.

Step 4: DNA suspension and observation:

- We observed the line between the fruits (DNA) mixture and the alcohol.
- You noticed a white thread-like cloud appearing at this line, which we identified as being the DNA. The DNA clumped together and float to the top of the alcohol layer.

Variables

1. Independent Variable:

- **Type of Fruit:** The type of fruit used is the primary independent variable in this experiment. This includes strawberries, grapes, apples, pears, peaches, plums, and quince. Each fruit was chosen based on its unique properties that may affect the efficiency of DNA extraction.

2. Dependent Variable:

- **Yield of DNA:** The quantity of DNA extracted from each fruit type is the dependent variable. This is measured by the visibility of DNA precipitate formed during the extraction process.

3. Controlled Variables:

- **Quantity of Fruit Used:** The same weight of each type of fruit was used to ensure that the volume of fruit material did not affect the amount of DNA extracted**. We used 100 grams of fruit**
- **Extraction Solution Composition and Volume:** The same DNA extraction solution (a mix of detergent and salt) and the same volume were used for each fruit to maintain consistency in cell lysis conditions.
- **Extraction Technique:** The method of physically breaking down the fruit (chopping and mashing) and the subsequent steps were standardized.
- **Temperature:** All extractions were carried out at room temperature to avoid temperature fluctuations affecting the results.
- **Duration of Extraction:** Each fruit sample was subjected to the same duration of extraction steps to ensure that the timing did not variably affect the DNA yield**. The entire extraction process took us about 45 per each extraction**
- **Alcohol Type and Temperature:** Ethanol was used for all samples, kept at a consistent low temperature to ensure effective DNA precipitation.

4. Extraneous Variables:

- **Ripeness of Fruit:** The stage of fruit ripeness can affect the water content and cellular structure, impacting DNA yield. This was controlled by selecting fruits at a similar ripeness stage.
- **Handling and Storage:** The way fruits were handled and stored prior to the experiment could affect their condition. Standard protocols for handling and immediate processing after purchase were used to minimize this impact.

Hazards and Precautions:

- **Hazard**: Chemical exposure to detergents, alcohol.
- **Precautions:** We wore gloves and worked in a well-ventilated area. We followed proper handling and disposal procedures for hazardous chemicals, avoid skin and eye contact with chemicals.

3. Hypothesis:

Based on the background research, it is hypothesized that strawberries will yield the highest quantity of DNA during the extraction process, followed by grapes, apples, pears, peaches, plums, and quince. This hypothesis is formulated considering the relatively high-water content, soft texture, and presence of small seeds , which may facilitate efficient cell lysis and DNA release. Strawberries and grapes are expected to yield a substantial amount of DNA due to their juiciness and soft pulp. Apples and pears may yield moderate amounts of DNA, while peaches, plums, and quince are anticipated to yield lower quantities due to their firmer texture and potentially lower water content.

4. Results

1. **Strawberries:** As hypothesized, strawberries yielded the highest quantity of DNA. The factors contributing to this result include their high-water content, soft texture, and numerous small seeds, which enhance the breakdown of cell walls and release of cellular contents during the extraction process. This confirms that the structural characteristics of the fruit can significantly influence DNA yield.
2. **Grapes**: Following closely were grapes, which also produced a substantial amount of DNA. Their juiciness and soft pulp likely facilitated the release of DNA, supporting the hypothesis that fruits with higher moisture content and softer textures are more amenable to DNA extraction.
3. **Apples**: Apples yielded a moderate amount of DNA. While they have a firmer texture compared to strawberries and grapes, their relatively high water content still allowed for a decent extraction yield.
4. **Pears**: Similar to apples, pears provided a moderate yield of DNA. Their composition and texture, akin to apples, support the hypothesis that such characteristics result in moderate DNA extraction efficiency.
5. **Peaches:** Peaches, with their slightly firmer texture and possibly lower water content compared to the fruits listed above, yielded less DNA. This aligns with the hypothesis predicting a lower yield for fruits with these characteristics.
6. **Plums**: Plums produced a slightly lower yield than peaches. Their firm texture and skin might have posed challenges in breaking down the cell walls effectively, leading to less DNA being extracted.
7. **Quince**: Yielding the least amount of DNA, quince confirmed the hypothesis that fruits with the firmest texture and lowest water content would be the most challenging from which to extract DNA. The tough texture and lower juiciness likely hindered the lysis process and DNA release**.**

   ****** In terms of results and measuring the dependent variables in our experiment, we encountered challenges in quantifying the exact amount of DNA extracted due to limitations in our equipment and methodology. As a result, we opted to measure the DNA based on its visible quantity. This approach involved assessing the amount of DNA by visually estimating the size and thickness of the DNA precipitate formed during the extraction process. While this method does not provide precise quantitative data, it allows us to compare the relative yields of DNA across different fruit samples and gives a practical indication of which fruits are more effective for DNA extraction in a more accessible and less technically demanding way.**

5. Conclusions

The experiment's results strongly support the initial hypothesis that fruit characteristics such as water content, texture, and the presence of small seeds significantly affect DNA yield during the extraction process. Strawberries, with their optimal structure for lysis, topped the list, while quince, with its challenging texture, produced the least DNA. These findings highlight the importance of considering physical and cellular properties of biological materials when performing DNA extraction.

This study not only underscores the variability in DNA yield based on fruit characteristics but also could serve as a foundational reference for selecting materials in genetic studies or educational demonstrations. Moreover, the outcomes emphasize the potential need for varying the extraction methodology based on the specific type of biological material to maximize DNA yield.

5. Optimization of Extraction Protocol:

Use of Enzymes: Incorporating enzymes like pectinase or cellulase can help break down cell walls more effectively, especially in fruits with high pectin and cellulose content. This can increase the yield of DNA by ensuring more cells are lysed during the extraction process.

Optimizing Lysis Buffer: Experiment with different concentrations of the lysis buffer components, such as varying the detergent strength or salt concentration, to find the optimal conditions for each type of fruit.

Quantitative Methods for DNA Yield:

Spectrophotometry: Use a spectrophotometer to measure the absorbance at 260 nm, which is a common method for quantifying nucleic acids. The ratio of absorbance at 260 nm and 280 nm can also indicate the purity of the DNA.

Fluorometry: More sensitive than spectrophotometry, fluorometry involves using fluorescent dyes that bind to DNA, such as PicoGreen, which is highly specific to double-stranded DNA and can provide a more accurate measurement of the DNA concentration.

Agarose Gel Electrophoresis: Although primarily qualitative, running the extracted DNA on an agarose gel can provide a rough estimation of yield and size distribution. Densitometry software can analyse the intensity of the DNA bands to approximate the quantity.

Improved Precision and Control:

Standard Curves: Establish a standard curve using known concentrations of DNA to calibrate measurements and improve the accuracy of DNA quantification via spectrophotometry or fluorometry.

Replication and Controls: Increase the number of replicates for each fruit type to ensure consistency and reliability in the results. Include negative controls (no fruit sample) and positive controls (known quantities of DNA) to validate the extraction efficiency and measurement accuracy.

Technological Enhancements:

Automated DNA Extraction Systems: For higher investment, automated systems can standardize the extraction process, reduce manual errors, and increase throughput. These systems are particularly useful in high-resource settings or when processing large numbers of samples.

Microvolume Spectrophotometers: Devices like the NanoDrop allow for DNA concentration measurements with very small sample volumes, reducing the amount of DNA required for accurate quantification.

Documentation and Analysis:

Detailed Protocol Documentation: Keep detailed records of all experimental conditions and adjustments to track what works best for different types of fruits.

Statistical Analysis: Employ statistical software to analyze the data obtained from multiple experiments or replicates. This can help in understanding the variability and establishing the significance of the results.

7. Importance of Determining DNA Quantity in Plants

7.1 **Educational Value:**

- Students gain practical experience in molecular biology techniques, such as DNA extraction, which is fundamental in many areas of biological research
- It introduces students to the concepts of genetics and genomics, providing a good way to understand where and how genetic information is stored in living organisms.

7.2 **Scientific and Research Implications:**

- **Genetic Research:** Knowledge of DNA quantity in plants can help researchers determine the genetic variability and stability among species, which is essential for conservation genetics and studying evolutionary biology.
- **Agriculture:** In agriculture, knowing the DNA content helps in the genetic modification of plants, aiming to produce varieties that are more resistant to pests, diseases, or adverse climatic conditions.
- **Medicine and Pharmacology:** Plants are a source of numerous medicinal compounds. Understanding their genetic makeup can lead to the discovery of new pharmaceuticals or enhancements in the efficacy of existing ones.
- **Biotechnology:** Genetic information from plants can be used to engineer biofuels, bioplastics, and other environmentally friendly technologies.

7.3. Practical Applications:

- **Breeding Programs:** Plant breeders can use DNA quantity along with genetic information to select traits that improve crop yield, quality, and disease resistance.

- **Conservation Efforts:** For endangered plant species, DNA studies are essential for conservation planning and execution, ensuring genetic diversity is maintained.

- **Climate Change Research:** Studying plant genetics helps understand how plants adapt to changing climates, which can inform strategies to manage ecosystems and agriculture under global warming conditions.

8. Detailed Project Timeline

Weeks 1-2: Initial Research and Planning

- Conducted comprehensive research on the types of fruits grown locally in Romania.
- Selected seven fruits: strawberries, grapes, apples, pears, peaches, plums, and quince based on diversity, size, water content, DNA characteristics, and growing regions.

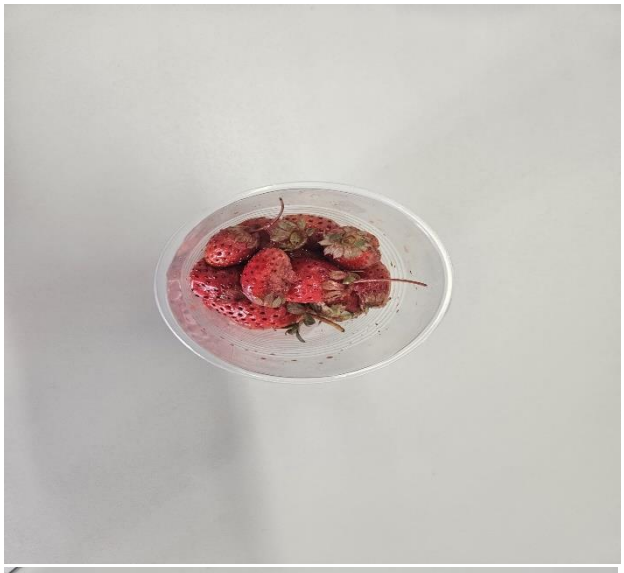
Weeks 3-4: Collection of Fruits

- Collaborated with local farmers across different counties to source fresh fruits.
- Purchased the fruits

Weeks 5-6: DNA Extraction Process

- **Week 5 focused on preparing and extracting DNA from grapes and strawberries and apples**
- **Week 6 extended the extraction process to pears, peaches, plums, and quince, applying the same methods for consistency.**

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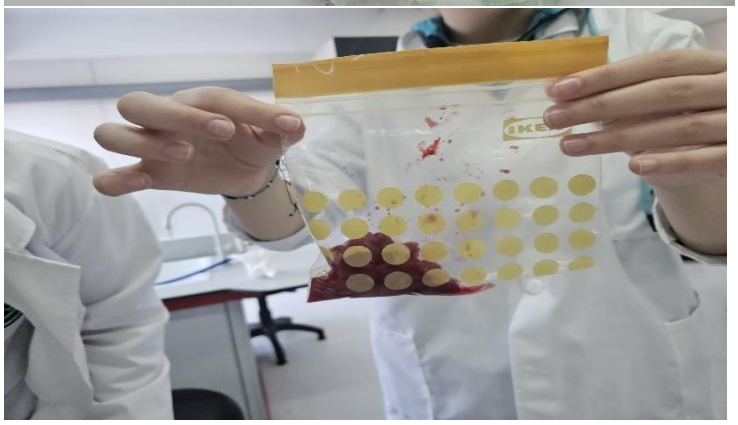
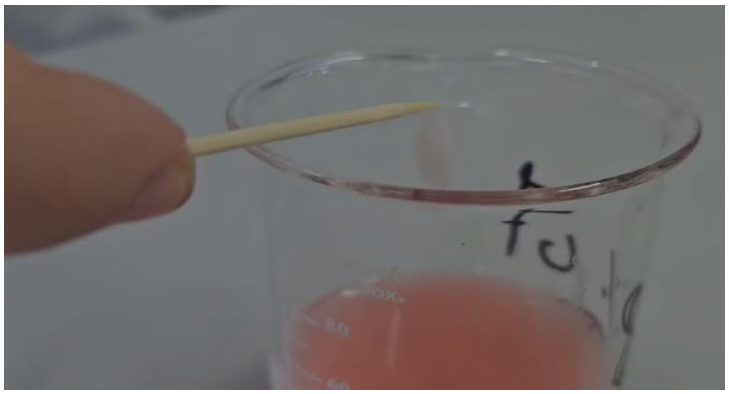
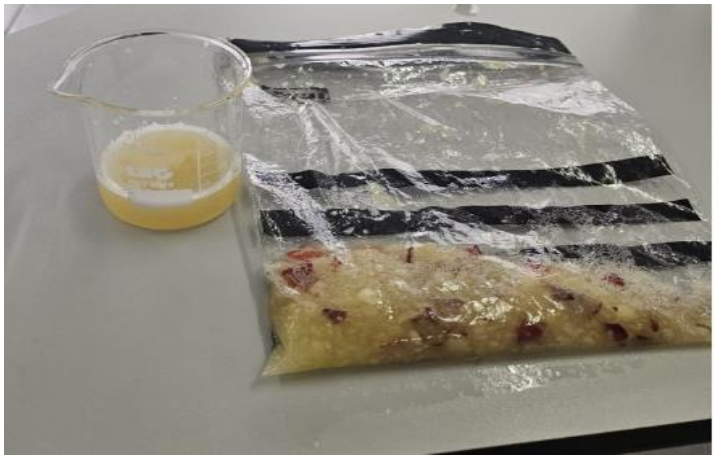
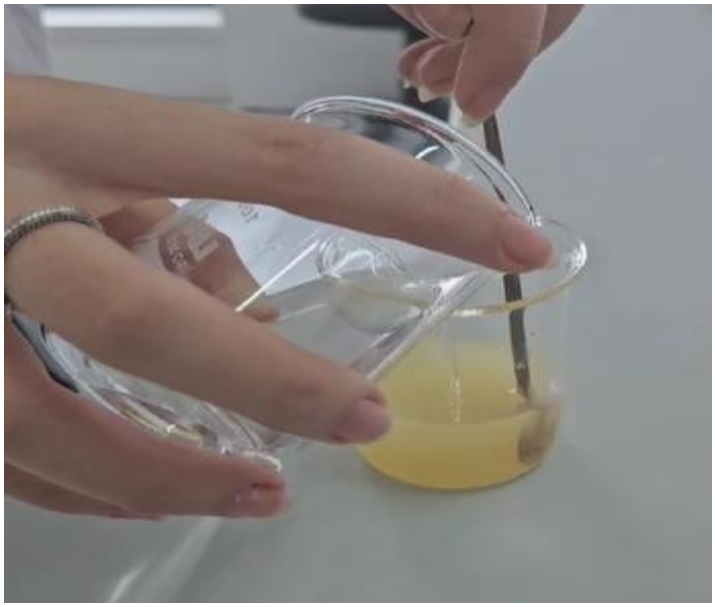
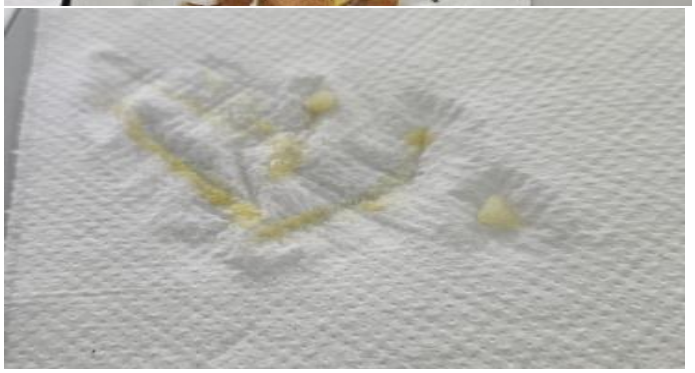











Strawberry

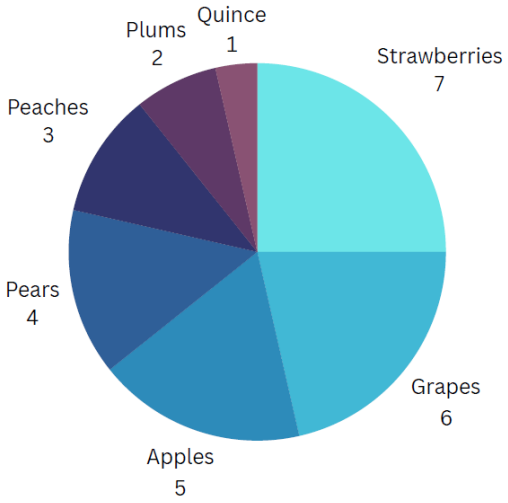

PEACH


PEACH


PLUMS

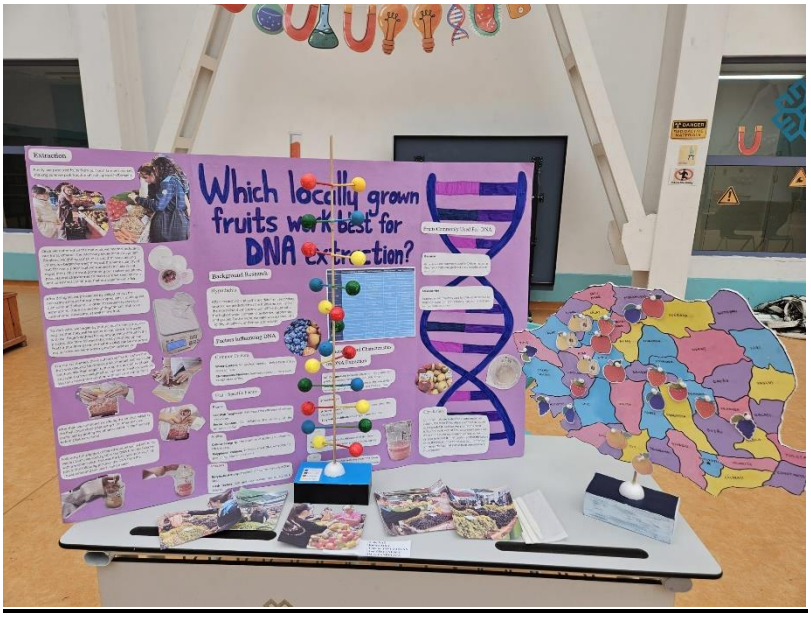
Week 7: Data Analysis and Conclusions

- Analysed the DNA yield from each fruit, noting significant differences in the amount of DNA extracted.
- Compiled results and drew conclusions about which fruits were most effective for DNA extraction and why.



Weeks 8 : Presentation and Recognition

- Prepared and delivered a detailed presentation of the project at the internal Science Fair competition within our school.
- **Our project was awarded first prize, recognizing the meticulous research, effective execution, and insightful conclusions.**



Summary of Activities by Weeks

- Weeks 1-2: Background research and fruit selection.
- Weeks 3-4: Collecting fruits from local farms.
- Weeks 5-6: Conducting DNA extraction experiments.
- Week 7: Comparing results and formulating conclusions.
- Weeks 12-14 of April: Final presentation at the science fair, culminating in first prize recognition.

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