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Evaluation of DNA extraction methods for PCR analysis of maize and sunflower oils

Tamara Kutateladze[1], Kakha Bitskinashvili[1], Boris Vishnepolsky[1], Kakha Karchkhadze[2], Tata Ninidze[1, 2], Nelly Datukishvili[1, 2]

[1] Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia

[2] School of Natural Sciences and Medicine, Ilia State University, Tbilisi, Georgia

Challenges of the Modern Food Industry

Oil plants differ in the following indicators:

- Chemical and molecular composition
- Toxic and allergenic properties
- Nutritional value and safety
- industrial value

The modern food industry has a big challenge dealing with the adulteration of different types of plant oils

CODEX
International Food Standards
ALIMENTARIUS

- **International legislation requires food authentication and traceability at all stages of production**
- It is necessary to provide accurate information about the presence of food ingredients and allergens through labeling

A food monitoring system needs effective methods of detecting food ingredients!

Study Aim:

To develop efficient PCR technology for plant DNA traceability in oils

Objects:

Sunflower and Maize

Seeds
Flours
cold-pressed oils
refined oils

Sunflower samples

Sunflower and maize seeds and flours were purchased from a supermarket

Cold-pressed sunflower oil was provided by the company „AgroPro Ltd”

Maize samples

Refined and cold-pressed corn oils were purchased from a supermarket

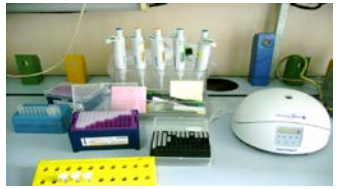
Preparation of DNA samples of appropriate quality and sufficient quantity from oils remains a major drawback for successful PCR detection!

Our study examines several approaches to DNA enrichment and extraction

DNA enrichment

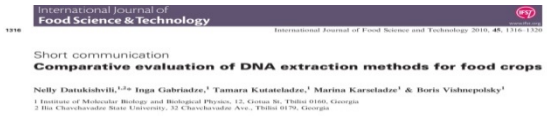
- Centrifugation of oils at 18,000 g, 4 ºC

- Adding of TE Buffer and centrifugation in microcentrifuge at 25^{0}C



DNA extraction

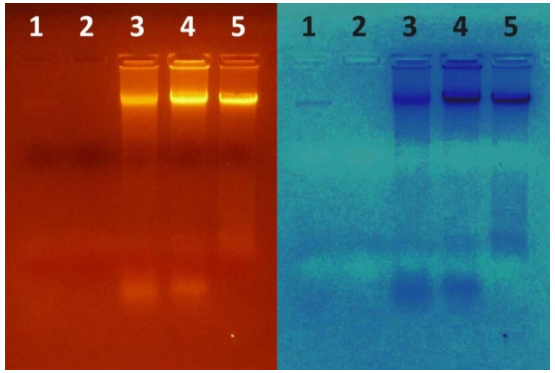
- Standard CTAB method



- Modified CTAB method - Developed in this study
- NucleoSpin Food Mini Kit

- Olive Oil DNA Isolation Kit

Genomic DNA extraction



Agarose gel electrophoresis of genomic DNAs

Oil DNA enrichment – Centrifugation of 30 ml cold-pressed sunflower oil at 18,000 g, 4 ºC

DNA samples:

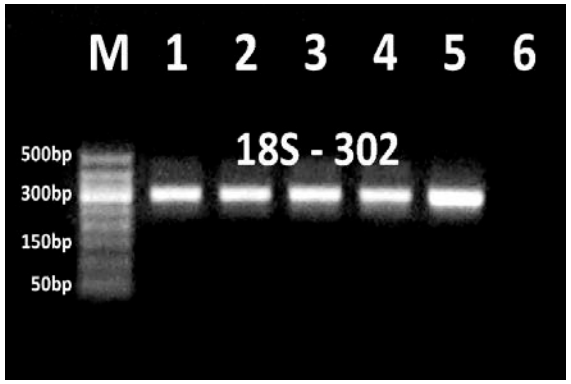
1. cold-pressed sunflower oil - extraction by modified CTAB method
2. cold-pressed sunflower oil - extraction by standard CTAB method

3 - 4. sunflower seeds - extraction by standard CTAB method

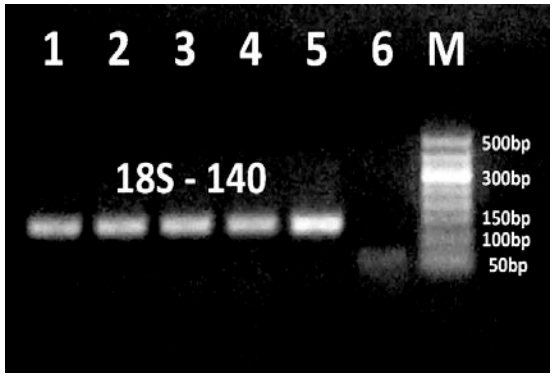
5. Maize flour - extraction by standard CTAB method

The genomic DNA band visible on the agarose gel was obtained from cold-pressed sunflower oil!

PCRs specific to 18S ribosomal RNA gene



Amplicon 18S-302



Amplicon 18S-140

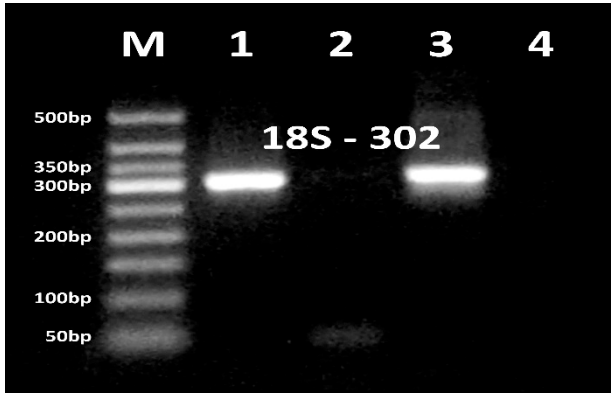
Samples

1-2. cold-pressed sunflower oil, **Olive Oil kit**
3-4. cold-pressed sunflower oil, **NucleoSpin Food kit**
5. Sunflower seeds, plant mini kit
6. Water negative control

Results

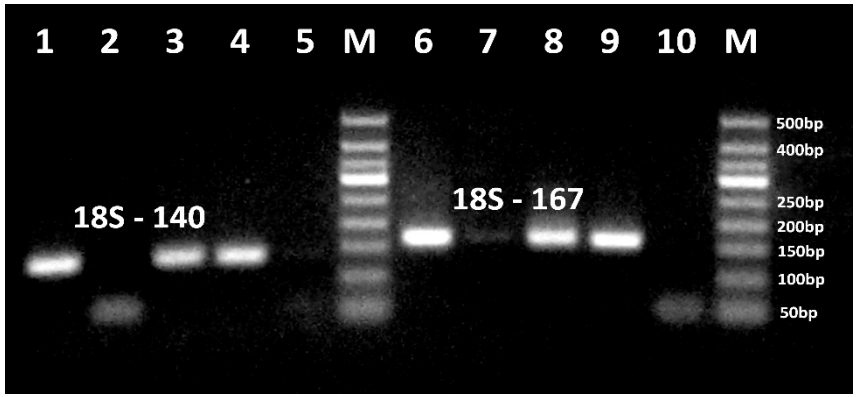
- DNA enrichment by oil centrifugation at 18,000 g, 4 ºC gave best results
- Olive oil kit and Nucleospin food kit gave amplifiable DNA from cold pressed sunflower oil

PCRs specific to 18S ribosomal RNA gene
Comparison standard and modified CTAB methods



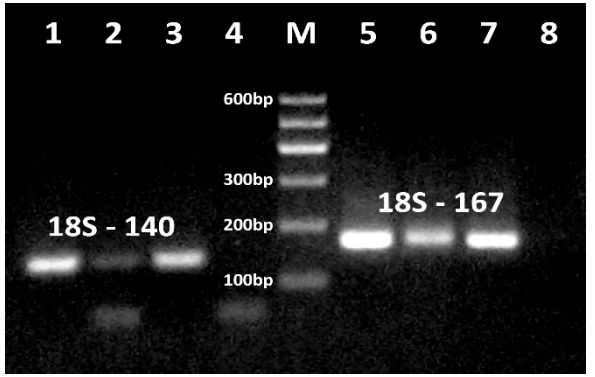
Amplicon 18S-302

Samples:
1. Sunflower seeds, **standard CTAB method**
2.cold-pressed sunflower oil, **standard CTAB method**
3. cold-pressed sunflower oil, **modified CTAB method**
4. Water -negative control



Amplicons 18S-140 (1-5) and 18S – 167(6-10)

Samples:
1, 6. Sunflower seeds, **standard CTAB method**
2, 7.cold-pressed sunflower oil, **standard CTAB method**
3-4, 8-9. cold-pressed sunflower oil, **modified CTAB method**
5.10. Water negative control



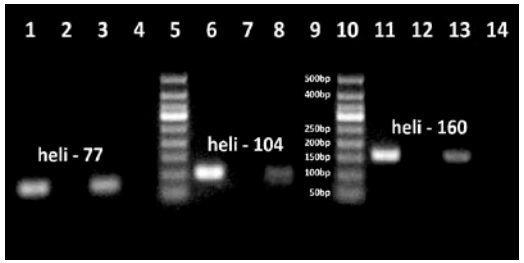
Amplicons 18S-140 (1-4) and 18S – 167 (5-8)

Samples:
1, 5. maize seeds, standard CTAB method
2, 6. refined maize oil, modified CTAB method
3, 7. cold-pressed sunflower oil, modified CTAB method
4, 8. Water negative control

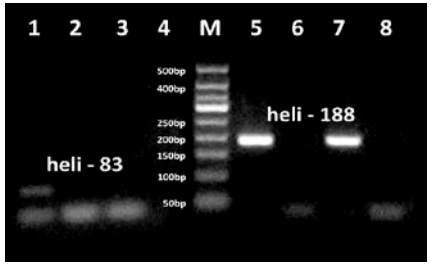
Results

- The modified CTAB method gave amplifiable DNA from oils, but standard CTAB method did not give genomic DNA from oils
- Standard CTAB method gave amplifiable DNA from Sunflower and maize seeds
- The expected amplicons were amplified in both seeds and oil samples by PCRs targeting 18S RNA gene
- 18S302 amplicon-specific PCR is a more efficient method than 18S167 and PCR 18S140 amplicon-specific PCRs

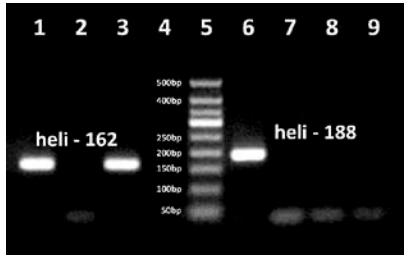
Sunflower detection in oils by PCR
Comparison of standard and modified CTAB methods



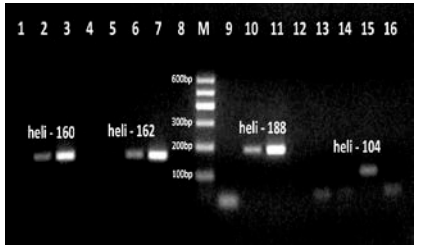
PCR amplicons
heli-77 (1-4); heli-104 (6-9; heli-160 (11-14)



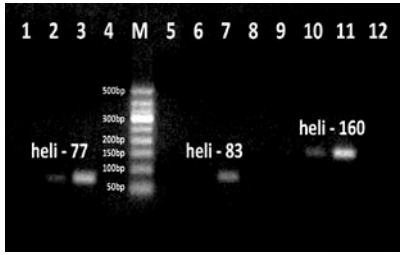
PCR amplicons
heli -83 (1-4); heli-188 (6-9)



PCR amplicons
heli -162 (1-4); heli-188 (6-9;



PCR amplicons
Heli-160 (1-4), heli -162 (5-8);
heli-188 (9-12); heli-104 (13-16)



PCR amplicons
Heli-77 (1-4), heli -83 (5-8);
heli-160 (9-12)

Samples

1, 6, 11. Sunflower seeds, standard CTAB method
2, 7, 12. 2.cold-pressed sunflower oil, **standard CTAB method**
3, 8, 13. cold-pressed sunflower oil, **modified CTAB method**
4. Water negative control

Samples

1, 5, 9, 13. cold-pressed sunflower oil, **Olive Oil kit**
2, 6, 10, 14. cold-pressed sunflower oil, **NucleoSpin Food kit**
3, 7, 11, 15**.** Sunflower seeds, plant mini kit
4, 8, 12, 16. Water negative control

Results

- A modified CTAB method was found to be the best method for extracting amplifiable DNA from sunflower oil
- The standard CTAB method was found to be the best method for extracting amplifiable DNA from sunflower seeds
- DNA obtained by the NucleoSpin Food Mini Kit is useful for PCR detection of sunflower in oil
- DNA obtained with the Olive Oil DNA Isolation Kit failed to detect sunflower by PCR in the oil

Conclusions

- The oil type (cold-pressed, refined), DNA extraction and amplification methods are important for successful PCR analysis
- Centrifugation of the oils at 18,000 g, at 4 ºC was the best method for DNA enrichment from the oils
- The modified CTAB method was found to be the best DNA extraction method for PCR analysis of sunflower and maize oils
- In addition, a PCR system specific for the 18S-302 amplicon of the 18S ribosomal RNA gene was identified as the best method for DNA traceability in oils

Acknowledgments

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Thank You for Your Attention!

Ilia State University
Nelly Datukishvili
Kakha Karchkhadze
Tata Ninidze
Tamar Koberidze

I.Beritashvili Center of Experimental Biomedicine
Kakha Bitskinashvili
Tamar Kutateladze
Boris Vishnepolsky