Investigating the Presence of GMO Markers P35S and CP4 EPSPS in Popular Soy Based Formulas

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ABSTRACT

During the late 20th century, genetically modified organisms (GMOs) were introduced, and the first GMO drug was approved by the United States Food and Drug Administration. Since then, GMO crops have been created in order to increase crop yield, enhance nutrition and composition, food quality, and provide greater food security for corporations. However, GMOs became a main point of concern for some parents as many believe GMOs pose health risks to children. Therefore, the creation of non-genetically modified organisms (non – GMOs) has become a popular demand. Soy has been around for several centuries, first originating from China, and has been consumed throughout many Asian nations ever since. Soy was only introduced within the United States in the 1960s among many food products and became a renowned ingredient within the American diet. Therefore, because of soy's recognition as an ingredient, 93 % of soy products have been identified to be GMO within the United States. Five labeled soy-based baby formulas were tested for the presence of Roundup Ready (RR) and P35s. The samples tested were Mama Bear, Parent's Choice, Similac, Enfamil, and Gerber. Positive results for the presence of P35s in four out of the five samples demonstrated a potential presence of genetic modification on labeled soy–based formulas. As a result, this experiment will bring more public awareness relative to the misleading and inaccurate labeling of food products. Products consisting of inaccurate labeling should be addressed as they could lead to developmental issues within children at a young age and impact the nation's population.

INTRODUCTION

- Genetically modified organisms (GMO) are defined as organisms that have been transformed from their original state by having one or more genes being inserted into them to enhance and promote their viability (2)
- Around the mid 1990's, the United States began to utilize genetically modified soybeans and corn in commonly consumed products (2)
- Polls found that around 50% of Americans are afraid that GMOs are harmful to the population due to their negative connotation and could lead to several health risks (5)
- By 2015, 179.7 million hectares of genetically modified (GM) crops were identified to be GMOs, totaling to 10% of the world's arable land (3)
- Previous studies have found that in areas such as Italy, GM soybeans were apparent in several species of goats, noted by the expression of both the 35S promoter and the CP4 EPSPS gene in their blood and milk samples (6)
- The 35S promotor specific to the cauliflower mosaic virus (CaMV) is one of the most commonly found transgenic constructs in most GMO crops grown commercially due to its strong nature as a constitutive promoter (7)
- CP4 EPSPS in GM soybeans relates to the *Roundup Ready* group of herbicides that are commonly found in GM crops and allows for a tolerance to glyphosate and are therefore herbicide-resistant (4)

MATERIALS AND METHODS

SAMPLES: Mama Bear Soy-Based Powder Infant formula, Parent's Choice Soy Baby Formula Powder, Similac Soy Isomil Lactose-Free Powder Baby Formula, Enfamil Plant-Based Soy Powder Infant Formula, and Gerber Soy Powder Infant Formula

RESULTS

Samples	260/280 ratio	Std. Dev	Concentration (ng/uL)	Std. Dev
Mama Bear (MB)	1.62	0.02828427	5.1	2.82842712
Gerber (GB)	1.585	0.0212132	7.05	1.6263456
Similac (SM)	1.705	0.03535534	9.45	0.91923882
Parent's Choice (PC)	1.66	0.07071068	6.5	1.27279221
Enfamil (EF)	1.71	0.02828427	8.7	0.14142136
Laura Soybean (LSB)	1.7	0.04242641	8.75	1.76776695
Roundup Ready (RR)	1.71	0.11313708	5.4	1.83847763
Corn (CRN)	1.735	0.10606602	2.55	0.6363961

Table 1. Nanodrop Analysis of DNA Extracted From Soy-Based Baby Formulas: DNA was extracted three times for each sample using the GeneJet DNA extraction kit (n = 3). Purity readings of DNA and concentrations of DNA were obtained using Nanodrop. The data displayed are the purity averages and standard deviations of the two samples used within the experiment.

Primers	Sequences	Target Gene	Amplicon Size (BP)	Annealing Temperature
GMO 3 GMO 4	GCCCTCTACTCCACCCCCATCC GCCCATCTGCAAGCCTTTTTGTG	Lectin Gene	188	63
P35s-cf3 P35s-cf4	CCACGTCTTCAAAGCAAGTGG TCCTCTCCAAATGAAATGAACTTCC	P35S Promoter	123	60

Table 2: The primer pairs that were used, the amplicon sizes, and the annealing temperatures for each targeted gene. *Source:* Noor et al. (1)

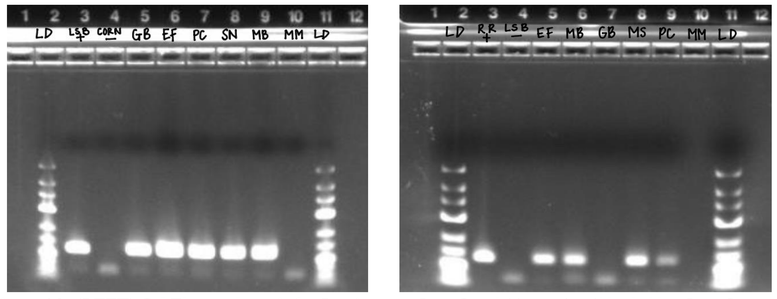

Figure 1A: (LEFT) Gel Electrophoresis for Lectin gene. *Lane 2 and 11* contains 1kb+ ladder. *Lane 3* contains positive control (LSB). *Lane 4* contains negative control (Corn). *Lane 5* -9 contains all samples: Internal position, Gerber (1 GB), Enfamil (2 EF), Parent's choice (2 PC), Similac (1 SM), and Mama bear (1MB). Lane 10 contains master mix as a control. **Figure 1B: (RIGHT) Gel electrophoresis for 35S Promoter** *Lane 2 and 11* contains 1kb+ ladder. *Lane 3* contains positive control Roundup Ready (RR). *Lane 4* contains negative control Laura Soy-bean (LSB). *Lane 5* -9 contains all samples: Internal Position, Enfamil (2 EF), Mama Bear (1 MB), Gerber (1 GB), Similac (1 SM), Parent's choice (2 PC), Mama bear (1MB) *Lane 10* contain master mix as a control.

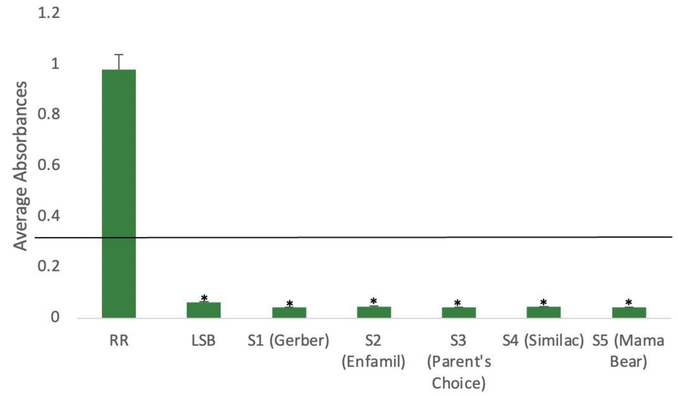

Figure 2. ELISA shows no evidence of CP4-EPSPS protein in tested baby formula: Graph of ELISA analysis comparing the average absorbance between the Roundup Ready positive control, Laura Soybean negative control, and the five soy-based baby formula samples.

	Roundup Ready (RR)	Laura Soybean (LSB)	Gerber (GB)	Enfamil (EF)	Parent's Choice (PC)	Similac (SM)	Mama Bear (MB)
Average Absorbance (630 nm)	0.98075	0.061	0.04275	0.04375	0.04175	0.04375	0.043
Standard Deviation	0.060340009	0.004272	0.001258306	0.004573474	0.000957427	0.000957427	0.001414214

Table 3. ELISA shows no evidence of CP4-EPSPS protein in tested baby formula: Quantitative data of the two ELISA assays. The average absorbances and standard deviations were compared for the samples (n = 4).

Summary Chart

Samples / Primer Set	MB	GB	SM	PC	EF	LSB	CORN	RR
P35S	✓	X	✓	✓	✓	X	X	✓
Lectin	✓	✓	✓	✓	✓	✓	X	✓
ELISA								
CP4 EPSPS	X	X	X	X	X	X	X	✓

Table 4: Summary chart of overall data. Checks represent a positive result with a band at the perspective location while an X represents a negative result with no bands at the location.

DISCUSSION AND FUTURE DIRECTIONS

- With the current data collected from Gel electrophoresis at approximately 118 bp, it suggests that Lectin gene is present within all samples include Mama Bear, Parent's Choice, Similac, Enfamil, and Gerber
- The ELISA assay data displayed a clear negative result for EPSPS protein in the baby formula samples.
- The PCR displayed that the DNA of the samples are amplifiable while looking for specific genes.
- The gel electrophoresis displayed the confirmation of the soy-based ingredients for the samples and positive results of the P35S gene for Enfamil, Mama Bear, Similac, and Parent's choice.
- Future directions will include performing PCR and gel electrophoresis to further confirm the ELISA assay result and detect if there is a presence of the EPSPS gene in the baby formula samples.

ACKNOWLEDGEMENT

We would like to give special thanks to Georgia Gwinnett College for providing the necessary materials to accomplish our research. In addition, many thanks to Dr. Ernest Ricks Jr. PhD for his continued support during our research project in BIOL 4300.

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