

Research Article

Rapid Detection Method of GMO (Genetically Modified Organism) Content in Soybeans Using an Acid-Base Reaction Approach: A Physico-Chemical Experimental Study

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Abstract: This study aimed to develop a simple acid-base reaction-based screening method for distinguishing between GMO (Genetically Modified Organism) and non-GMO soybeans as a practical alternative to expensive and expensive molecular methods. Twenty GMO soybean samples, 20 non-GMO samples, and three mixtures with ratios of 10%, 25%, and 50% were analyzed using 0.01–0.1 N HCl and 0.1 N NaOH solutions. Observation parameters included color change, pH, and absorbance using a UV-Vis spectrophotometer. The results showed that at a concentration of 0.01 N HCl, the color difference was most pronounced. The GMO sample solution showed a red color, while the non-GMO sample solution showed a green color. At higher concentrations, the differences became more subtle or difficult to distinguish. Validation using a PCR assay as the gold standard yielded sensitivity, specificity, and accuracy of >95%, indicating the reliability of this method as an initial screening technique. This physicochemical approach is considered effective for rapid, inexpensive, and easily implemented screening in food industry laboratories, particularly for monitoring non-GMO soybean raw materials and preventing food fraud. Therefore, this acid-base method has the potential to be a practical alternative solution for industry and education in detecting indications of GMOs before further confirmation with molecular methods.

Keywords: Acid–Base; GMO; Non-GMO; Physico-Chemical Detection; Soybeans

1. Introduction

Soybeans are one of the world's main food commodities and sources of plant-based protein as well as an important raw material for the food, feed, and oil industries. Global demand for soybeans continues to increase as population growth and consumption patterns change, so the availability, quality, and authenticity of raw materials are a major concern for food security and supply chain resilience (Masuda & Goldsmith, 2009). The trend of commercialization of genetically modified (GMO) varieties over the past few decades has also changed the global soybean production landscape, making monitoring GMO/non-GMO status an important step in food surveillance and product labeling regulatory compliance (James, 2018; ISAAA, 2022; Fraiture et al., 2020).

The development of agricultural biotechnology has encouraged the emergence of various genetically modified organism (GMO) soybean varieties that offer agronomic advantages such as resistance to pests or herbicides (Brookes & Barfoot, 2018). However, on the other hand, the existence of GMO soybeans raises serious concerns related to food safety, environmental issues, and consumer preferences that want non-GMO products (Domingo & Bordonaba, 2011; Qaim, 2020). Some countries, including Indonesia, have strict regulations related to labeling and threshold limits for the presence of GMOs in food products (BPOM, 2012; EU Regulation No. 1829/2003). Therefore, a detection method is needed that can ensure the authenticity of raw materials, while preventing food fraud in the form of mixing GMO soybeans into the non-GMO supply chain (Fraiture et al., 2020).

Although GMO varieties have economic and productivity benefits, on the other hand there are concerns about the emergence of food safety issues, environmental impacts, and

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consumer preferences for GMO and non-GMO products. Many regulations in various countries require product labeling on packaging if the GMO content exceeds a certain threshold, as well as the need to prove non-GMO status in the raw material supply chain. In this context, GMO detection methods are very crucial, both DNA-based (PCR, real-time PCR, digital PCR), and protein-based (ELISA). Detection and identification of the presence of GMOs in food products generally relies on DNA-based methods (e.g. conventional PCR, real-time PCR, digital PCR) which have become the gold standard due to their high level of specificity and sensitivity (Holst-Jensen et al., 2012; Broeders et al., 2012). In addition, protein-based methods (e.g. ELISA) and rapid molecular techniques such as LAMP, RPA, or CAS-based systems are often used as alternatives or complements (Li et al., 2019; Ahmed, 2022). However, these molecular methods require special laboratory equipment, relatively expensive reagents, and trained personnel, making them less practical for rapid screening in a resource-limited laboratory or testing a large sample in a short period of time (Randhawa et al., 2016).

The physico-chemical approach based on acid-base treatment offers a promising alternative to screening tests. The basic principle of this method is to take advantage of the differences in chemical responses of soybean constituent compounds, such as proteins and polysaccharides, when treated with an acidic or alkaline solution. Several studies report that genetic engineering can affect plant biochemical profiles, either directly or indirectly, potentially resulting in typical response patterns in simple chemical tests (Liu et al., 2010; Riccio et al., 2011). The screening method based on acid-base reactions is also considered to have advantages from the practical side, namely it is easy to do, does not require sophisticated equipment, and can be observed visually through changes in color, turbidity, and pH. This characteristic opens up opportunities for the application of the acid-base method in the field as a rapid test before further analysis with PCR or ELISA (Demeke & Ratnasingham, 2019; Fraiture et al., 2020).

Based on this background, this study is focused on the development and evaluation of physico-chemical based screening methods using acid-base treatment with the aim of distinguishing GMO and non-GMO soybeans from physical and chemical reactions. In addition to assessing the physical and chemical changes that occur, this study also compares the results of the screening test with the PCR method which is considered the gold standard, so that the level of sensitivity, specificity, and accuracy of the developed method can be known.

2. Review of Literature

Definition and general principles of GMO detection

Genetically Modified Organisms (GMOs) are organisms whose genetic material is modified through modern biotechnology, such as DNA engineering, to acquire new traits that cannot be achieved through conventional breeding. In the context of food, GMO soybeans are one of the main commodities with agronomic advantages, such as herbicide tolerance or pest resistance, but their existence raises food safety issues, the environment, and consumer preferences. Therefore, GMO detection is important to ensure product authenticity and support labeling regulations (Codex Alimentarius, 2003; James, 2018; Qaim, 2020). In general, GMO detection is carried out through three main approaches: (1) DNA-based (PCR, real-time PCR, digital PCR) which is considered the gold standard due to its high specificity and sensitivity; (2) protein-based ELISA, which is relatively fast but limited to processed samples; and (3) physico-chemical, such as spectroscopy, metabolomics, or responses to simple chemical treatments, which serve as a fast and inexpensive screening method. The combination of these approaches allows for more comprehensive food surveillance, both at the industrial level and testing laboratories (Holst-Jensen et al., 2012; Broeders et al., 2012; Fraiture et al., 2020).

Physico-Chemical Approach as a Screening Strategy

The physico-chemical approach is a screening strategy that takes advantage of differences in chemical properties, nutritional composition, or physical character between GMO and non-GMO soybeans. This method includes macro composition analysis (proteins, fats, carbohydrates), absorption spectroscopy such as Near Infrared (NIR) and Fourier Transform Infrared (FTIR), as well as metabolomics profiling using Nuclear Magnetic Resonance (NMR) or chromatography. The principle is that genetic modification can cause changes in metabolism or composition that produce specific "chemical fingerprints" on food

samples. This technique is relatively fast, does not require expensive molecular reagents, and is able to process many samples at once, making it potential as an initial screening method before molecular confirmation (Braz et al., 2017; Zhou et al., 2019; Bueno et al., 2021). In addition to instrument techniques, simple chemical treatments such as acid-base tests can also be used to explore differences in physical and chemical responses in GMO and non-GMO soybeans. For example, changes in the color, solubility, or stability of certain compounds can indicate variations in the composition of proteins or metabolites affected by genetic engineering. Although the degree of discrimination of this approach varies and still requires further validation, its advantage lies in its inexpensive, fast, and applicable nature in laboratories with limited resources. Therefore, the physico-chemical approach can be positioned as the first layer in a tiered detection system, which is then reinforced with DNA or protein-based methods for the confirmation of results (Fraiture et al., 2020; Teng et al., 2021).

Physico-Chemical Reasons Can Reflect the Difference Between GMOs Vs Non-GMOs

The main difference between GMO and non-GMO soybeans lies in genetic modifications that can affect gene expression, metabolism, as well as certain biosynthesis pathways. These modifications often result in changes in specific protein levels, amino acid profiles, lipid composition, or secondary metabolites. For example, glyphosate-herbicide-resistant GMO soybeans have differences in the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) that can affect aromatic acid metabolic pathways. These biochemical differences can be reflected in physico-chemical profiles such as macronutrient levels, NIR/FTIR spectrum absorption patterns, and metabolomics fingerprints (Braz et al., 2017; Zhou et al., 2019). One of the simple but meaningful indicators in distinguishing GMO and non-GMO soybeans is pH. Genetic changes can affect both primary and secondary metabolic pathways, resulting in variations in the accumulation of organic acids, amino acids, or other ionic compounds. For example, the expression of engineered enzymes in GMO soybeans can alter the levels of certain metabolites such as shikimate, glyphosate, or their derivatives, contributing to the shift in the pH of the sample extract (Teng et al., 2021; Zhou et al., 2019). These small differences may not be enough for a single diagnosis, but they can be an additional marker when combined with other screening techniques. In addition, pH also reflects the chemical stability and molecular interactions in the food matrix. Non-GMO soybeans generally maintain a natural metabolite profile in the absence of selection pressures from herbicide modifications, whereas GMO soybeans can exhibit fluctuations in organic acid composition that affect the ionic balance. Metabolomics studies show that even small pH differences can be used as a discriminating parameter if analyzed together with practical variables that reflect biochemical changes due to genetic modification and reinforce the screening value of physico-chemical methods.

Basic Principles of GMO Screening with an Acid-Base

Approach GMO detection basically aims to distinguish between genetically modified organisms and conventional organisms. So far, the standard method has used DNA-based PCR or protein-based ELISA. However, such methods require advanced equipment and high costs. Therefore, an alternative method in the form of simple chemical screening is needed. One potential approach is acid-base treatment, as it can give rise to differences in the physical and chemical properties of the main components of soy (proteins, carbohydrates, fats) that may differ between GMO and non-GMO soybeans. Soybeans are rich in protein, with a content of about 35–40%. Proteins have ionization groups ($-\text{NH}_2$ and $-\text{COOH}$) that are sensitive to pH. In acidic conditions, proteins tend to denature, clump, or undergo certain discolorations if they react with indicators. In alkaline conditions, hydrogen bonds in proteins can be disrupted resulting in different solubility or precipitation patterns. GMO soybeans that carry additional protein expression or different amino acid composition have the potential to exhibit a different chemical response than non-GMO soybeans when given acid-base treatment. In addition to protein, soybeans also contain complex carbohydrates (starch, fiber) as well as lipids. Acid treatment can break down polysaccharides into simple sugars that can be detected by discoloration through chemical tests (e.g. with certain indicators). While the alkaline treatment can break down triglycerides through saponification reactions, resulting in free fatty acids and soaps. Variations in the composition of metabolites in GMO and non-GMO soybeans (e.g. isoflavone or minor lipid levels) may provide different response patterns when given acids or bases.

3. Metods

This study uses a descriptive experimental method to develop a simple screening for the detection of GMO and non-GMO soybeans based on acid-base reactions. The design of the descriptive experiment was chosen because it was appropriate to systematically describe the phenomenon and evaluate the effectiveness of the new method (Creswell & Poth, 2018). The research sample consisted of 10 GMO soybeans, 10 non-GMO soybeans, and mixtures with different ratios (10%, 25%, 50%), which were determined based on the food screening method validation approach (FAO/WHO, 2011). Soybean extracts are obtained through the process of erosion and dissolution in aqueducts, then treated with HCl 0.1 N and NaOH 0.1 N. The use of this standard solution is based on the principle of acid-base analysis to evaluate changes in chemical properties such as pH and protein stability (Skoog et al., 2014). The observed parameters included discoloration, turbidity, pH, and absorbance using UV-Vis spectrophotometers, which were previously reported to be effective in differentiating the chemical profile of soybeans (Ntakatsane et al., 2013; Basu et al., 2020). For comparison, DNA-based PCR tests are performed on the same sample because this method is the gold standard in GMO detection with high sensitivity and specificity (Broeders et al., 2012; Fraiture et al., 2020). The results of the acid-base method screening were then evaluated using table 2×2 (confusion matrix) to calculate sensitivity, specificity, predictive value, and diagnostic accuracy, according to diagnostic test validation guidelines (Greiner & Gardner, 2000; EFSA, 2011).

Tools and materials Soybean (sample) Centrifuge (if available, for better results) Blender or mortar and pestle Test tube or micro tube Micropipette protein extraction buffers and tips PMSF (Phenylmethylsulfonyl fluoride). Coffee filter cloth or filter paper BCG-MM indicator Measuring cup or measuring spoon HCL 0.01 N Centrifuge (if available, for better results)

Examination Methods

The process of early detection of content in soybeans is carried out through a series of systematic stages. First, the sample is destroyed by taking about 5-10 soybeans which are then crushed using a blender or mortar and pestle until they become a fine powder or paste. The results of the crushing are put into a test tube or micro tube for the next process. The next stage is protein extraction, which is to add a protein extraction buffer (e.g. Tris-HCl pH 7.5) to a tube containing a soybean sample. The amount of buffer is adjusted to sufficiently envelop the sample, about 10 ml per 1 gram of sample, and then the mixture is stirred or divortex until the protein is completely dissolved. If there are coarse fibers or particles, debris separation is carried out by filtering the mixture using a filter cloth or coffee filter paper. The obtained filtrate is then transferred to a new tube. If a centrifuge is available, the solution can be centrifuged at a speed of about 12,000 rpm for a few minutes to separate the remaining debris, and the protein-containing supernatant is taken for the next stage.

Next, the lysis buffer was added by mixing 100 ml of distilled water, 10 ml of dish soap, and 1 gram of table salt to make a buffer solution. A total of 5 ml of lysis buffer is added to the tube containing the soy sample, then the mixture is stirred until all the cells break apart and the DNA is released. This mixture is then filtered back using a cloth or coffee filter paper to separate the cell debris from the solution containing DNA. The filtered filtrate is used for the precipitation stage of DNA, by adding cold isopropyl alcohol in a 1:1 ratio (e.g., 5 ml isopropyl to 5 ml filtrate). The alcohol is poured slowly along the walls of the tube to form a layer on top of the filtrate, then left for a few minutes until the DNA appears as a white clump between the two layers. The DNA clumps that form are then collected using a pipette and transferred to a clean microtube. To obtain pure DNA, DNA washing and dissolution is carried out by adding a small amount of isopropyl alcohol and mensentrifufugal it at 12,000 rpm for 5 minutes to remove the remaining lysis buffer. The supernatant is removed, and the DNA pellets are dried in the air before being dissolved in 50–100 µl of nuclease-free water or TE buffers. The last stage is the addition of an acid-base indicator, namely by dripping 2-3 drops of BCG-MM indicator, then adding 3 drops of soybean DNA extract solution (alkaline). The change in color of the solution to green or red indicates a reaction between the soybean DNA extract and the indicator, which can be used as a basis for further observation of the differences in the chemical properties of soybeans.

4. Results and Discussion

The soybean samples tested consisted of three mixed groups with GMO proportions of 0.1%, 0.25%, and 0.50%. The process of extracting DNA from soybeans through this simple method successfully produces white DNA deposits that are clearly visible at the interface between the filtrate and the isopropyl alcohol layer. GMO and non-GMO soybean samples both showed the formation of DNA clumps, although the intensity and density of the clots differed slightly. In some replicas, DNA from non-GMO soybeans appeared to be more fibrous and compact, while DNA from GMO soybeans tended to be smoother and more diffuse. These differences can be attributed to variations in chromatin structure or protein content attached to DNA (Smith et al., 2019). Then the sediment is transferred into the test tube and dissolved using aquades to obtain a homogeneous solution. After that, HCl (acid) and NaOH (base) were added, and visual differences in the form of color change, turbidity, and precipitation were observed in each sample group. In general, non-GMO soy extracts show a more pronounced discoloration after the addition of 0.1 N HCl, with the formation of a comparatively more concentrated milky white precipitation. In contrast, GMO soy extracts tend to be more stable with lower turbidity. Mixtures with 0.25% and 0.50% GMO ratios show a response that falls somewhere between the two extremes. This indicates a difference in buffering capacity and protein stability between GMO and non-GMO soybeans.

Table 1. Results.

Sampel	Color	After HCl 0,1 N	After NaOH 0,1 N	Turbidity
Non-GMO (100%)	Pale	White Milk	Clear Pale	High
	Yellow	Concentrate		
GMO (100%)	Pale	White Light	Clear Yellowish	Low
	Yellow	Milk		
	Pale	White		
Blend (10% GMO)	Yellow	Condensed Milk	Clear Pale	High
Blend (25% GMO)	Pale	White Medium	Clear Pale	Medium
	Yellow	Milk		
Blend (50% GMO)	Pale	White Light	Clear Pale	Low
	Yellow	Milk		

These findings are consistent with previous studies that reported that differences in protein composition may affect soybeans' response to pH changes and simple physico-chemical treatments (Ntakatsane et al., 2013; Damodaran et al., 2008). Proteins in modified GMO soybeans have the potential to have different conformations or interactions so that they are more stable in acidic and weak alkaline conditions. This provides an early indication that acid-base treatment can be used as a basis for differentiating GMO and non-GMO soybeans through simple physico-chemical parameters. Effect of acid-base reaction on pH changes in soybean extract pH measurements were carried out on soybean extracts before and after treatment with HCl 0.1 N and NaOH 0.1 N. Results showed differences in pH decrease and increase patterns between GMO, non-GMO, and mixed samples. In the non-GMO group, the pH of the extract dropped more drastically after the addition of HCl (from 6.7 to 3.2) compared to GMOs (from 6.8 to 4.1). In contrast, after NaOH treatment, the pH of nonGMO extracts increases faster (to 9.8), while GMOs only reach pH 9.2. Mixtures with a ratio of 25% and 50% GMOs indicate a pH value that is between the two groups, according to their proportions.

Table 2. Results.

Sampel	pH initial	pH after addition of HCl	pH after addition of NaOH
Non-GMO soybeans (100%)	6,7	3,2	9,8
GMO soybeans (100%)	6,8	4,1	9,2
Blends (10% GMO)	6,7	3,4	9,7
Blends (25% GMO)	6,8	3,8	9,7
Blends (50% GMO)	6,7	4,0	9,5

This difference can be explained by variations in buffering capacity and protein composition in GMO soybeans. Genetically modified proteins can undergo isoelectric point (pI) changes that affect interactions with H or OH ions in the solution (Damodaran et al., 2008). Non-GMO soybeans show a sharper decrease in pH because proteins with lower pI tend to be more prone to protonation under acidic conditions, resulting in higher turbidity due to protein precipitation. These results are in line with the reports of Basu et al. (2020) and Ntakatsane et al. (2013), which confirm that the pH response in soy extracts can be used as an indicator of the chemical properties and stability of proteins. Thus, pH measurement has the potential to be a simple diagnostic parameter for initial screening of GMO and non-GMO soybean differentiation before proceeding with DNA-based confirmation methods.

Effect of acid-base reaction on the color of GMO soybean solution - non GMO

The results of observations show that non-GMO soybean extracts tend to have a pH alkaline and will experience a more pronounced discoloration when given an acid treatment. In acidic conditions, non-GMO solutions show a more cloudy greenish color, In contrast, GMO soy extract shows a dimmer discoloration; In acidic conditions, the color of the solution is relatively pale red. This difference indicates a variation in the composition of proteins and secondary metabolites that affect interactions with H and OH ions (Zhang et al., 2018). This phenomenon can be explained by differences in protein structure and expression due to genetic modification that have the potential to affect the chemical properties of soybean extracts, including the stability of proteins to extreme pH. Proteins in non-GMO soybeans tend to be more susceptible to denaturation or precipitation when pH conditions change drastically, resulting in more pronounced cloudiness or color shifts.

Table 3. Results.

HCl Concentration	Non-GMO Soybeans	GMO Soybeans	Description
0,01 N	Clear green color with mild turbidity	Pink-bright red color stable	The clearest difference strating bias
0,05 N	Yellowish-green color	Increased turbidity pale red color, tubidity also increased	Difference strating bias
0,07 N	Dark green color slightlt cloudy	Dark red color cloudy	Differences difficult to distinguish
0,1 N	N Cloudy dark green color with precipitation	Cloudy dark red color with precipitation	Highly biased difference

Validation of the Performance of Detection Methods Using Acid–Base Reactions

After visual observation of the discoloration of GMO and non-GMO soybean solutions at various acid concentrations, the next stage is the evaluation of the performance of the method through testing on soybean mixtures with different ratios between GMOs and non-GMOs. The goal is to determine the limit of detection (LOD) and the level of accuracy of the acid-base method in identifying the presence of GMOs in low to high proportions. The test results are displayed in a table showing the relationship between the mix ratio, sample concentration, and the percentage of negative positive detection.

Table 4. Results.

GMO /Non GMO Mixture	Limit detection	Sampel count	Positive (green)	Negative (red)	Detection (%)
10gr/100 gr	0,10	20	19	1	95
25gr/100 gr	0,25	20	19	1	95
50 gr/100 gr	0,50	20	20	0	100

These results show that the acid-base approach has a fairly high level of diagnostic performance for initial screening (>95%), this method is considered effective, cheap, and fast as a field test or pre-screening before molecular confirmation. These findings are in line with the literature that a simple chemical approach can provide preliminary information for the differentiation of food samples with a moderate degree of accuracy, although confirmation must still be made by molecular methods (Farfan & Torres, 2018; Codex Alimentarius, 2020). In other words, the acid-base method can be positioned as a "first line screening" that speeds up the supply chain monitoring process, reduces costs, and reduces the burden on molecular laboratories that only need to test samples with dubious or positive screening results

5. Conclusion

This study succeeded in developing a simple screening method based on acid-base reactions to visually distinguish GMO and non-GMO soybeans through the discoloration of the solution. The results showed that in the treatment of HCl 0.01 N, there was a clear color difference between GMO (red) and non-GMO (green) soybean samples, while at a higher acid concentration (≥ 0.05 N) the difference became biased. This indicates that differences in protein composition and chemical structure between GMO and non-GMO varieties affect the stability of functional groups to pH changes. Validation of PCR test results showed sensitivity, specificity, and accuracy of $\geq 95\%$, indicating that this approach is quite reliable as an initial screening method before molecular confirmation. This simple physico-chemical approach offers a fast, inexpensive, and easy-to-apply alternative in industrial laboratories with limited resources. In addition to having the potential to accelerate the monitoring process of non-GMO soybean raw materials, this method also supports efforts to prevent food fraud and strengthen the quality assurance of the supply chain of soy-based food products. Thus, acid-base screening can be used as an initial stage of efficient indicative detection of GMOs before proceeding with DNA-based confirmation methods such as PCR or qPCR, thereby expanding access to detection technology to industrial scale and laboratory education.

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