Authentication test of seasoning food materials made from turmeric using real-time PCR

Alfi Sophian[1*], Ratna Purwaningsih[2]

[1]National Food and Drug Testing Development Center, Food and Drug Monitoring Agency, Indonesia
[2]Directorate of Processed Food Production Supervision, Food and Drug Supervisory Agency, Indonesia
[*]Corresponding author: alfi.sophian@pom.go.id

ARTICLE INFO

DOI:10.46223/HCMCOUJS.tech.en.13.2.2862.2023

Received: July 22nd, 2023

Revised: September 05th, 2023

Accepted: September 07th, 2023

Keywords:

amplification; FDA; PCR; seasoning; turmeric

ABSTRACT

The authentication test for spices made from turmeric using real-time. This study aimed to determine whether the spice ingredients claimed to be made from turmeric actually contain turmeric, as asserted by the product. The sample set consisted of 10 types of spices based on turmeric, which were obtained from various supermarkets. The research employed real-time PCR analysis using the SYBR Green method as the chosen approach. Data analysis was performed based on Ct analysis and Tm analysis. The measurement of DNA purity and concentration resulted in a DNA concentration value of 56.205 and a DNA purity value of 2.302, measured at a wavelength of A260/A280. The real-time PCR analysis yielded Ct values ranging from 15.20 to 15.83 with an average of 15.50, and Tm values ranging from 78.70 to 89.00 with an average of 78.83. Based on these data, it can be concluded that the ready-to-use spice products, claimed to be made from genuine turmeric, indeed contain turmeric, as supported by the results of real-time PCR amplification.

1. Introduction

The challenge of product authentication, driven by the rapid development of science and technology, opens up new dimensions in the world of food product testing. Authentication poses a new problem that arises when the supply of raw materials is insufficient. Several reasons can lead to the occurrence of raw material substitution, including the limited availability of certain raw materials and their relatively high prices, prompting the pursuit of profits by using cheaper substitutes. However, such substitutions can be detrimental to the community, particularly if the substituted ingredients have negative impacts, such as causing allergies due to certain ingredients. Consequently, consumers may remain unaware of the actual content of a product, which might not align with their individual needs.

For this reason, a test is needed to determine the authenticity of a product circulating in the community, ensuring its genuine claim. Several authentication testing techniques have been developed in the world of science, including chromatographic, isotopic, elemental, sensory, non-chromatographic, and molecular-based testing. The commonly used molecular analysis technique is PCR (Polymerase Chain Reaction). PCR analysis was first developed by Mullis in 1986 and has since become very popular in the world of molecular biology (Mullis et al., 1986). DNA-based molecular analysis techniques are currently the most reliable methods for species identification in molecular assays.

The authentication test technique using the nucleic acid identification method was carried out by Lanzilao, Burgalassi, Fancelli, Settimelli, and Fani (2005) using PCR-RFLP, a technique for identifying species based on the length of the DNA fragments. Additionally, other nucleic acid-based techniques have been utilized, namely single-strand polymorphism hybridization (SSCP) (Rehbein, Kress, & Schmidt, 1997), DNA hybridization techniques (Chikuni, Ozutsumi, Koishikawa, & Kato, 1990), and real-time PCR techniques (Fajardo et al., 2008). Real-time PCR has been employed in authentication tests to identify species in various commodities, such as raw meat (Chung, 2018), food products (Danezis, Tsagkaris, Camin, Brusic, & Georgiou, 2016), and medicinal plant samples.

Based on the foregoing, this study was conducted to test whether the sample used authentically contained turmeric as stated on the packaging label. Furthermore, this research is a reference for similar research and can be a reference in test methods for testing product authentication.

2. Materials and methods

2.1. Materials

The ingredients in this study were 10 packs of turmeric-based ready-to-use seasoning purchased in Gorontalo province, Food lysis buffer [Qiagen] lot. 157036830, Proteinase K Solution [Qiagen] lot. 157038187, Buffer VB [iNtRON] paint. 17154.100, Buffer RW1 [iNtRON] paint. 17154.100, Buffer RW2 [iNtRON] paint. 17154.100, and Buffer EB [iNtRON] paint. 17154,100. Master mix RealQ Plus 2x Master Mix Green Without Rox. Paint. No. A323402 [Ampliqon].

2.2. DNA isolation

Weigh 0.5g sample into a 2ml centrifuge tube, add 1mL lysis buffer and 20µL Proteinase K, vortex, and incubate at 70^{o}C for 02 hours. The sample was then centrifuged at 14,000rpm for 10 minutes. Transfer the supernatant into a 2mL centrifuge tube, add 750µL chloroform, then centrifuge for 20 minutes at 14,000rpm, transfer the supernatant into a new 2mL centrifuge tube and add 750µL VB and incubate for 02 minutes at room temperature. Vortex then put all the liquid into a 2ml centrifuge tube and centrifuge again for 02 minutes. Transfer the spin column into a collection tube then add 500µL RW1 and centrifuge again for 01 minute. Transfer the spin column to a collection tube, add 700µL RW2, and then centrifuge again for 01 minute. Transfer the spin column to a 1.5mL centrifuge tube, then add 70µL of elution buffer (EB), centrifuge again for 01 minute. Discard the spin column and store the results of DNA isolation in the refrigerator (-20^{o}C) if it has not been used immediately (Sophian 2021a; Sophian, Purwaningsih, Igirisa, et al., 2021).

2.3. Purity and concentration analysis

Analysis of purity and concentration was carried out using a nano photometer (Sophian, 2021d; Sophian, Purwaningsih, Igirisa, et al., 2021; Sophian & Syukur, 2021; Utaminingsih & Sophian, 2022; Utami, Utaminingsih, & Sophian, 2023).

2.4. Primers

The primers used were sequences designed from the MATK gene designed from the NCBI site with forwarding primary sequences: 5' GGA CCG ATT TAG CGG ATT GTG 3' and reverse primary sequences: 5' TCT GGC ACA TGA AAG TCG AAG T 3'. The total length of the primes sequence is 137bp.

2.5. Master mix setup

The master mix setup is done according to the procedure contained in the RealQ Plus 2x Master Mix Green Without Rox Master mix manual (Sophian, 2021b).

2.6. Real-Time PCR setup

Metode setup real-time PCR: Pre-denaturation at 95^{o}C for 15 (01 cycle), denaturation at 95^{o}C for 30 seconds, and annealing at 60^{o}C for 60 seconds (40 cycles). The melt curve analysis: ramp from 50^{o}C to 90^{o}C, hold for 90 seconds (1st step), and hold for 05 seconds (next step) (Sophian, Purwaningsih, Igirisa, et al., 2021).

2.7. Interpretation of results

The interpretation of the results is based on whether there is an increase in the CT (Cycle Threshold) and Tm (melting temperature) values (Sophian, Purwaningsih, Lukita, & Ningsih, 2020; Sophian, Purwaningsih, Muindar, Igirisa, & Amirullah, 2021; Sophian, Sri, & Sofia, 2022).

4. Result and discussion

Results from The results of concentration measurements and purity analysis of isolated DNA are presented in (Table 1). In the table, it can be seen that the average value of the isolated DNA concentration is at a value of 56.205, and the average value of purity analysis at wavelength A260/280 is at a value of 2.302.

Table 1

DNA isolation data samples

Sample	Concentration	A260/280
1	41.60	2.384
2	41.60	2.384
3	53.65	2.318
4	55.50	2.223
5	65.00	2.211
6	68.40	2.331
7	72.30	2.332
8	71.60	2.344
9	45.80	2.244
10	46.60	2.251
Average	56.205	2.302

Based on Table 1 above, it can be seen that the purity value of the extracted DNA is at an average value of 2.302. Good DNA purity values are in the range of 1.7 - 2.1 (Sophian, 2021c; Sophian, Purwaningsih, Igirisa, et al., 2021; Sophian et al., 2022; Utaminingsih, Utami, & Sophian, 2022). If the result of DNA isolation has a purity below 1.7, then it is suspected that the isolation result is contaminated with protein, and vice versa; if the result of DNA isolation has a purity value above 2.1, it is suspected that the isolation result is contaminated with RNA. Of the 5 nucleotide acid constituents that make up DNA or RNA, if you read the absorbance at wavelength A260/A280, it will show different values for each nucleotide component, namely: guanine (1.15), adenine (4.50), cytosine (1.51), uracil (4.00) and thymine (1.47). The results of the purity analysis

resulting from the absorbance readings are then averaged and will be the absorbance value. This is the basis for determining the purity values generally for DNA analysis in the range (1.8 - 2.0).

The results of real-time PCR amplification are presented in (Table 2). In the table, it can be seen that the average value of Ct is at a value of 15.50, and the average value of the Tm analysis is at a value of 78.83.

Table 2

Data from Real-Time PCR amplification

Sample	Ct	Tm
1	15.77	78.70
2	15.83	78.70
3	15.71	78.70
4	15.25	78.80
5	15.20	79.00
6	15.37	78.80
7	15.40	78.80
8	15.25	79.00
9	15.83	78.80
10	15.40	79.00
Average	15.50	78.83

The results of the Ct or Tm analysis can be used as the result in assessing the success of the amplification process carried out by real-time PCR. This method has a different system between Ct and Tm, where Ct is used to see the number of cycles compared to the threshold, while Tm uses the melting point of DNA bonds better known as the melt curve (Sophian, Utaminingsih, Bhakti, & Rahmawati, 2023; Sophian, Purwaningsih, Igirisa, et al., 2021; Sophian et al., 2020).

The cycle value is generated when the fluorescence signal is detected and captured by the detector, where the greater the CT value, the smaller the concentration, and vice versa. The CT value will be calculated when the threshold is placed as the baseline, where the threshold is placed on the exponential part of the curve formed (World Health Organization, 2016).

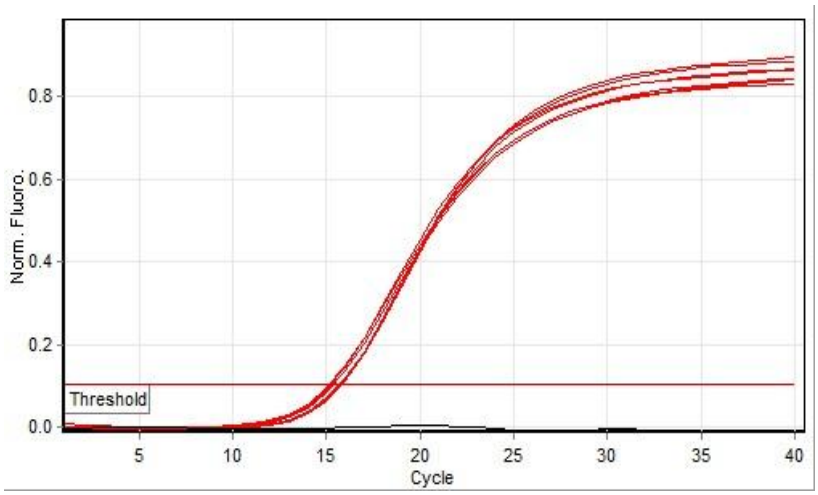

Figure 1. Analysis of Cycling (Ct)

The results of cycling analysis by looking at the Ct value showed that the amplification using real-time PCR was successfully carried out with an average Ct value of 15.50. The Ct value in the cycling analysis is influenced by the amount of template DNA concentration used. The higher the concentration, the smaller the Ct value, and vice versa (Sophian et al., 2020).

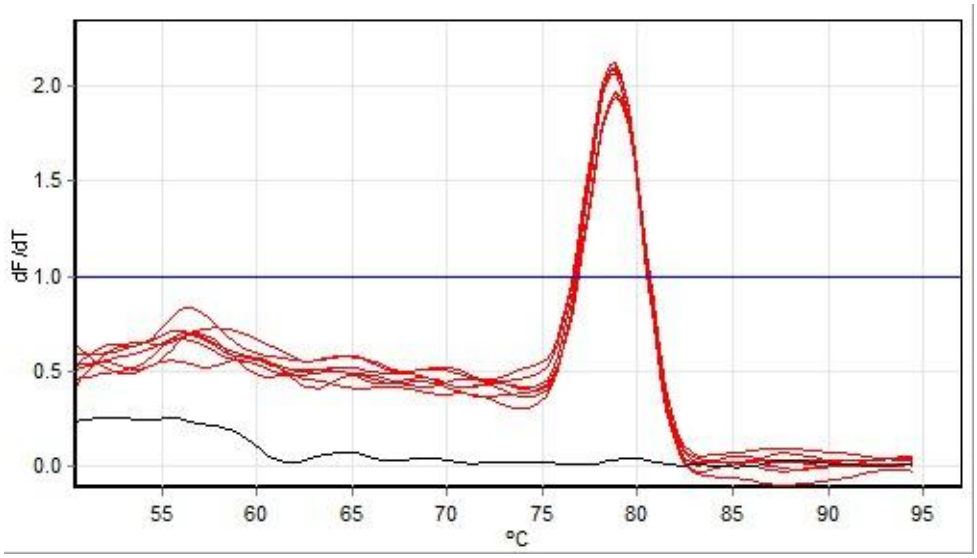

Figure 2. Analysis of melt curve (Tm)

The results of melt curve analysis by looking at the Tm value show that the real-time PCR amplification shows the Tm value is at an average value of 78.83. This value is influenced by the nucleic acid content of Guanine (G) and Cytosine (C), so the use of different primers with the same target gene will cause different Tm values. Melt curve works on the principle of producing a curve in the form of a specific single peak on each detected band or on the species whose nucleotide component is amplified. An error that often occurs in melt curve analysis is the presence of double peaks that appear after the amplification analysis process.

The use of real-time PCR in conducting species identification studies is beginning to develop towards product identification. One application of this technique is the identification of certain plant or animal species in a product on the market better known as the authentication test. The application of this technique is also supported by the ability to design specific primers of the target DNA. This ability is important because mistakes in determining the target gene or selecting a primary will cause the identified target to be non-specific, such as the species being identified as the target.

The primer used in this study was the plant DNA barcode plastids gene, namely the maturase K gene (MATK). This gene has the advantage of differentiating intraspecific variation (Hollingsworth, Graham, & Little, 2011). The primer was designed from the NCBI website with a length of 137bp. The primary length selection is adjusted to the primary length commonly used in real-time PCR analysis, which ranges from 80 - 250bp. The selection of the MATK gene as a primer is because MATK is a DNA barcode gene that is specific for plants.

The sample used in this study is a food spice that is claimed to contain turmeric as raw material. The authentication test is carried out to see whether the testing technique and primer used can detect the presence of the turmeric claimed in the sample package. Sample characteristics also influence the extraction technique used to produce good DNA isolation. In samples containing curcumin, such as turmeric, the presence of curcumin is an inhibitor when amplified, so that the

choice of the DNA extraction method is also important. In the extraction stage, lysis was carried out using food lysis buffer, which was added with proteinase K and then incubated at 70^{o}C for 02 hours. The function of heating is to activate the proteinase K enzyme so that the lysis process can run well. One of the other functions of proteinase K is to eliminate interference with the isolation of DNA extracted from proteins.

The results of cycling analysis on real-time PCR using the SYBR green kit showed that the MATK gene identified in the spice samples claimed to be made from turmeric, which means that there was a turmeric content in the sample tested. Likewise, the melt curve (Tm) analysis by looking at the melting point shows the identified MATK gene. The MATK gene is a gene coding for maturase K proteins found in chloroplasts. This gene is often used in identifying plant species because it has a better level of accuracy. This gene has been the reference gene for the standard DNA barcode coding since 2003, which has been validated through several studies. This gene was first identified from the tobacco plant (Nicotiana tabacum) by Sugita, Shinozaki, and Sugiura (1985). The MATK gene is a gene encoding for the K sub-unit maturase enzyme contained in chloroplasts in plants. According to Soltis, Soltis, and Doyle (1998), in the MATK gene, the nucleotide sequence of the MATK gene can produce about 500bp.

5. Conclusions

As a conclusion in this study, the MATK gene was detected in the sample, which means that the samples claimed to be made from turmeric were identified as containing turmeric.

ACKNOWLEDGEMENTS

The authors would like to thank the head of the National Agency of Drug and Food Control (NADFC) Indonesia/FDA Indonesia for laboratory support.

References

Chikuni, K., Ozutsumi, K., Koishikawa, T., & Kato, S. (1990). Species identification of cooked meats by DNA hybridization assay. *Meat Science, 27*(2), 119-128.

Chung, H. H. (2018). Real-time polymerase chain reaction (RT-PCR) for the authentication of raw meats. *International Food Research Journal, 25*(2), 632-638.

Danezis, G. P., Tsagkaris, A. S., Camin, F., Brusic, V., & Georgiou, C. A. (2016). Food authentication: Techniques, trends & emerging approaches. *TrAC Trends in Analytical Chemistry, 85*, 123-132.

Fajardo, V., González, I., Martín, I., Rojas, M., Hernández, P. E., García, T., & Martín, R. (2008). Real-time PCR for detection and quantification of red deer (Cervus elaphus), fallow deer (Dama dama), and roe deer (Capreolus capreolus) in meat mixtures. *Meat Science, 79*(2), 289-298.

Hollingsworth, P. M., Graham, S. W., & Little, D. P. (2011). Choosing and using a plant DNA barcode. *PLoS ONE, 6*(5), Article e19254. doi:10.1371/journal.pone.0019254

Lanzilao, I., Burgalassi, F., Fancelli, S., Settimelli, M., & Fani, R. (2005). Polymerase chain reaction-restriction fragment length polymorphism analysis of mitochondrial cytb gene from species of dairy interest. *Journal of Aoac International, 88*(1), 128-135. doi:10.1093/jaoac/88.1.128

Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G., & Erlich, H. (1986). Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. *Cold Spring Harbor Symposia on Quantitative Biology, 51*, 263-273. doi:10.1101/sqb.1986.051.01.032

Rehbein, H., Kress, G., & Schmidt, T. (1997). Application of PCR-SSCP to species identification of fishery products. *Journal of the Science of Food and Agriculture, 74*(1), 35-41. doi:10.1002/(sici)1097-0010(199705)74:1<35::aid-jsfa765>3.0.co;2-2

Rodríguez, M. A., García, T., González, I., Hernández, P. E., & Martín, R. (2005). TaqMan real-time PCR for the detection and quantitation of pork in meat mixtures. *Meat Science, 70*(1), 113-120. doi:10.1016/j.meatsci.2004.12.005

Soltis, P. S., Soltis, D. E., & Doyle, J. J. (1998). *Molecular systematics of plants*. New York, NY: International Thomson Publishing.

Sophian, A. (2021a). Detection of species DNA in chicken meatball products using NGF genes as molecular markers. *BiosciED: Journal of Biological Science and Education, 2*(2), 47-51. doi:10.37304/bed.v2i2.3422

Sophian, A. (2021b). DNA isolation of chicken feathers from the base of the young feathers, the base of the old feathers, and the tip of the feathers. *Bioeduscience, 5*(2), 104-108. doi:10.22236/j.bes/526211

Sophian, A. (2021c). Short communication: Analysis of purity and concentration of extracted DNA on salted fish processed food products. *Asian Journal of Natural Product Biochemistry, 19*(1), 21-24. doi:10.13057/biofar/f190104

Sophian, A. (2021d). Species DNA detection using PGR gene genetic markers in chicken nuggets. *Indonesian Food Science & Technology Journal, 5*(1), 17-20. doi:10.22437/ifstj.v5i1.14618

Sophian, A., & Syukur, A. (2021). Analysis of purity and concentration of isolated DNA in making raw DNA of rat species. *Journal of Food and Drug Safety, 1*(2), 1-5. doi:10.54384/eruditio.v1i2.75

Sophian, A., & Yustina, Y. (2023). Analisis Nilai Kemurnian DNA Menggunakan Nano Fotometer pada Rasio 260/230 yang Diisolasi dari Produk Nugget. *Muhammadiyah Journal of Nutrition and Food Science (MJNF), 3*(2), 82-86.

Sophian, A., Purwaningsih, R., Igirisa, E. P. J., Amirullah, M. L., Lukita, B. L., & Fitri, R. A. (2021). Short Communication: Detection of Salmonella typhimurium ATCC 14028 and Listeria monocytogenes ATCC 7644 in processed meat products using Real-Time PCR Multiplex Method. *Asian Journal of Natural Product Biochemistry, 19*(1), 17-20. doi:10.13057/biofar/f190103

Sophian, A., Purwaningsih, R., Lukita, B. L., & Ningsih, E. C. (2020). Detection of Salmonella typhimurium ATCC 14028 in supplement health product liquid preparation using Real-Time PCR (qPCR). *Biofarmasi Journal of Natural Product Biochemistry, 18*(2), 65-69. doi:10.13057/biofar/f180202

Sophian, A., Purwaningsih, R., Muindar, M., Igirisa, E. P. J., & Amirullah, M. L. (2021). Detection of Salmonella typhimurium ATCC 14028 in powder prepared traditional medicines using Real-Time PCR. *Borneo Journal of Pharmacy, 4*(3), 178-183. doi:10.33084/bjop.v4i3.1838

Sophian, A., Sri, U., & Sofia, U. D. (2022). DNA isolation in processed chicken meat products (nugget) using modified DNeasy Mericon Food kit (Qiagen). *Ho Chi Minh City Open University Journal of Science-Engineering and Technology*, *12*(2), 15-21.

Sophian, A., Utaminingsih, S., Bhakti, T. S., & Rahmawati, D. (2023). Real-time PCR application in confirmation test of Salmonella Typhimurium on instant noodle. *Ho Chi Minh City Open University Journal of Science-Engineering and Technology*, *13*(1), 3-9.

Sugita, M., Shinozaki, K., & Sugiura, M. (1985). Tobacco Chloroplast tRNALys(UUU) Gene Contains a 2,5-kilobase-pair Intron. *Proceeding of the National Academy of Sciences, 82*(11), 3557- 3561.

Utami, S. D., Utaminingsih, S., & Sophian, A. (2023). Analisis DNA Hasil Isolasi Pada Produk Pangan Olahan Ikan (Surimi Ikan) Menggunakan Nano Photometer. *JRST (Journal Riset Sains dan Teknologi), 7*(1), 9-13.

Utaminingsih, S., & Sophian, A. (2022). Analysis of purity and concentration of DNA isolation results on chondroitin samples. *BiosciED: Journal of Biological Science and Education*, *3*(2), 56-61.

Utaminingsih, S., Utami, S. D., & Sophian, A. (2022). Isolasi DNA pada Produk Otak-Otak Ikan Bandeng. *Muhammadiyah Journal of Nutrition and Food Science (MJNF)*, *3*(1), 36-41.

World Health Organization. (2016). *Establishment of PCR laboratory in developing countries* (2nd ed.). Retrieved October 10, 2022, from https://www.medbox.org/pdf/5e148832db60a2044c2d4cab