Journal of Food and Drug Analysis, Vol. 11, No. 1, 2003, Pages 16-21 藥物食品分析 第十一卷 第一期

Qualitative and Quantitative Evaluation of Rice Component in Cereal Products by the DNA-based Technology

LIRNG-YIR YEH, HSIAO-HUA CHEN, CHIEN-CHI CHEN, CHI-CHANG CHOU AND WEIGUANG FU*

Food Industry Research & Development Institute, P.O. Box 246 Hsinchu.300 Taiwan, R.O.C

(Received: October 31, 2001; Accepted: December 27, 2001)

ABSTRACT

A method based upon the polymerase chain reaction (PCR) has been developed for distinguishing rice species from other cereals. Two specific primer sets designed from 5S ribosomal RNA genes and promoter of rice glutelin genes were able to identify even small amount of DNA fragments in rice. DNA isolated from grain, flour and such products gave similar results. Furthermore, evaluation of quantitative analysis of rice in the samples was accomplished by hybridization with probe designed from promoter of rice glutelin genes. The analysis of a lab-made sample (a mixture of TCW70 rice flour and cassava starch) with nonradioactive labeling probe was performed. The R^2 of calibration curve was 0.98 and the calculated amount of rice flour in the sample was very close to the actual value with CV < 3% (n = 2). However, this method is only proper for rice flour, because it is difficult, but necessary, to select a suitable standard that can exhibit rice species and broken situation of DNA about the samples.

Key words: rice, polymerase chain reaction (PCR), hybridization

INTRODUCTION

In order to comply with government's regulation of the imported rice and rice related products after entering WTO, it is necessary to establish a set of methods to analyze the quantitative percentage of rice in rice-related products. Rice related quantitative studies were rare in the past reports. If samples were raw powder of mixed rice flour containing only waxy rice and maize or waxy rice and cassava, crude amount of waxy rice flour in samples can be evaluated by microscopic examination after staining and measurement of starch[(1)]. If, however, samples were multi-component cereals other than waxy rice, maize, and cassava or samples were processed products, they cannot be analyzed by this method. In addition, the rice may be grounded and heated during the process of production, making protein denatured and starch mashed. Sometimes, the ratio of salts in samples is altered due to additives for flavor and preservation. Among them, DNA is the only component that will not be affected by the process of production. Although to DNA molecule breakage happen during the process of grounding and heating, their specificity will not be altered. Therefore, a method of DNA analysis was developed based on this concept to qualitatively analyze rice and rice-related products. Also, the practicability was evaluated using quantitative analysis of DNA to calculate the percentage of rice in rice related products.

DNA identification technology is commonly considered as the most authentic technology for species identification. Since the technology of polymerase chain reaction (PCR) has been published in 1985, reports using PCR for DNA analysis increased incredibly like chain reaction. PCR consists of three steps at different temperatures: denaturation, annealing of extension primer, and primer extension. Based on the three-step cycle, huge amount of amplified target DNA fragments can be accumulated after multiple cycles for further analysis. Before using PCR to analyze rice-related products, DNA of fine quality has to be extracted as the template. Because the interference caused by starch in cereal flour samples was significant, this research were divided to three parts:

1. The discussion of method to extract DNA from cereal flour and processed products: Most published reports used seedling or plants leaves as material for analysis. Applying to cereal sample was quite a challenge. Rice, formed mainly by albumen, contains more than 70% of starch, which will certainly cause interferences. Processed products, whose starch has been mashed completely, has the problem of adhesion caused by the spiral structure of mashed starch. After reviewing references, the extraction procedures of DNA were nonetheless the same. Thus, the report, basing on extracting samples with large amount of starch, by Allmann et al. in 1993[(2)] was adopted for further test and modification.

2. Qualitative analysis by PCR: It is necessary to have a proper primer before proceeding with PCR. Many primers have been published for species identification in cereal samples. Two different primer sets were applied in this research: the primer based on 5S RNA genes and the primer based on promoter of rice glutelin genes. The major application of the primer based on 5S RNA genes is identification of cereal species. This primer is reactive to samples of many cereal species due to major PCR products of different lengths. Thus, cereal species in samples can be identified by analyzing the lengths of PCR products. However, analyzing samples of mixed cereals has some defects.

* Author for correspondence. Tel:886-3-5223191 Ext. 303; Fax:886-3-5214016; E-mail:fwg@firdi.org.tw

According to the report by Ko et al. in 1994[3], when there were more than three cereal species in the sample, the results of gel electrophoresis after PCR were inconsistent with the results from samples containing single cereal species. The most significant interference come from wheat and rye. Samples in this research are rice and rice-related products, so that the PCR results using primers of 5S RNA genes are more complicated. Interferences were highly expected. Therefore, the primer based on promoter of rice glutelin genes, published by Suzuki et al. in 1997[4], is also applied. In their report, such primer has been applied to analyze rice and its plant tissues, but not to samples of other cereals. It is necessary for this research to test the primer on DNA of other common cereals by PCR.

3. Practicability evaluation of quantitative DNA analysis: If a DNA fragment specific to rice can be identified, the practicability of quantitative analysis can be further evaluated. Common techniques for quantitative analysis on DNA are quantitative PCR and hybridization. Because quantitative PCR has not been well developed and it has limitations on instruments, hybridization is adopted in this research. The basic principle of hybridization is to hybridize a so-called probe, which is a fragment of radioactive or antibody-labeled genes specific to rice, to the DNA in samples. Since this probe is reactive to rice DNA only, the amount of rice DNA in samples can be estimated by detecting the amount of annealed probes. Thus, the practicability of this theory to calculate the amount of rice in samples by analyzing the amount of rice DNA in samples can be evaluated.

MATERIALS AND METHODS

I. *Sample preparation*

1. Waxy rice flour: TCW70 (provided by Prof. Lu, S., Department of Food Science, National Chung-Hsing University, Taiwan)
2. Indica rice flour (provided by Prof. Lu, S., Department of Food Science, National Chung-Hsing University, Taiwan)
3. Japonica rice and Indica rice TCS10 flour: raw rice purchased from traditional market and grounded to flour in lab.
4. Corn starch and cassava starch (provided by Dr. Wu, J.Y., Food Industry Research and Development Institute, Taiwan)
5. Rice flour imported from Thailand (provided by Dr. Wu, J.Y., Food Industry Research and Development Institute, Taiwan)
6. Wheat flour (provided by Dr. Wu, J.Y., Food Industry Research and Development Institute, Taiwan)
7. Wheat, maize, radish, and sweet potato: purchased from ordinary supermarket.
8. Commercial waxy rice flour (A), commercial waxy rice flour (B), rice noodle, radish cake, and slice rice noodle: purchased from ordinary supermarket.

II. *DNA Extraction*

A modified procedure from Allmann et al. in 1993[2] was followed for extraction. After treated with liquid nitrogen and grinded by mortar (processed products containing mashed starch were first grinded by homogenizer), 1 g of sample was weighed and mixed with 10 mL of extraction buffer (0.1 M Tris-HCl, pH 7.5, with 0.01 M EDTA, 0.1 M NaCl, 2% SDS, and 1% N-cetyl-N,N,N-trimethyl-ammonium bromide, CTAB) in a 50 mL centrifuge tube, and then mixed with 200 μL of 10 mg/mL RNase (CLONTECH, USA) and 200 μL of α-amylase (Sigma, A-3306, USA), followed by gentle vortex at 60˚C for 1.5 hrs. Another incubation at 50˚C for 2.5 hrs was done after adding 200 μL of 10 mg/mL proteinase K (CLONTECH, USA). This sample was gently mixed with 5 mL (1/2 volume) of chloroform solution (chloroform : isoamyl alcohol = 24 : 1) forming a homogeneous layer of emulsion. After centrifuged at 15000 rpm for 15 mins, aqueous layer (upper layer) was transferred to another 50 mL centrifuge tube. The same extraction procedure was repeated with 5 mL (1/2 volume) of saturated phenol solution (phenol: chloroform : isoamyl alcohol = 25 : 24 : 1, purchased from BDH, UK). The final aqueous layer was gently mixed with 2 volume of absolute ethyl alcohol and 1/10 volume of 4 M LiCl, and then stored overnight at -20˚C. After centrifuged at 16500 rpm for 20 mins, the upper layer was discarded. The precipitate of DNA was then washed with 5 mL of 70% ethyl alcohol and centrifuged at 16500 rpm for 20 mins. With the upper layer discarded, the tube containing the precipitate of DNA was dried by vacuum to remove ethyl alcohol. The precipitate of DNA was dissolved completely in 0.5 mL of sterile H_2O in 37˚C water bath, and then stored at -20˚C for further application. (All reagents were prepared according to the book of Molecular Cloning[5])

III. *Qualitative PCR of Rice DNA*

In a PCR tube, 50 μL of reaction mixture (containing sample DNA (about 1 ng of DNA), 0.1 unit of Tag DNA polymerase, 1 μM primer R(n), 1 μM primer F(n), 0.4 mM dNTP, 0.01 M Tris -HCl, 2mM $MgCl_2$, 0.05 M KCL, and 0.1 mg/mL gelatin, pH 8.3) was added. Two sets of primer in this research were tested for PCR respectively: designed from 5S RNA genes, R1: 5'-TGGGAAGTCCTCGTGTTGCA-3', F1: 5'-TTTAGTGCTGGTATGATCGC-3'[3], and designed from promoter of rice glutelin genes, R2: 5'-AGCAATAGCGATGACGAGTC-3', F2: 5'-CTTTAGGTTAGGCCTCAGATG-3'[4] (both synthesized by PROTECH, Taiwan). PCR was carried out by the GeneAmp PCR System 9600 (PE Biosystems, USA). The temperature program was as follows: After DNA was denatured at 94˚C /5mins, there were 35 temperature cycles (94˚C/30 secs; 56˚C/30 secs; and 72˚C/1 mins) followed by holding at 72˚C/5 mins, and then cooled to room temperature[3]. The PCR product was separated by electrophoresis using

1.5% agarose gel (with 0.5X TBE buffer, containing 0.045 M Tris-borate and 0.001 M EDTA, pH8.0, dissolved under heat then cooled), which was then photographed after stained by ethidium bromide.

IV. *Probe Synthesis by PCR and Labeling*

It was carried out by the non-radioactive PCR DIG Probe Synthesis Kit (B. M. Biochemica, Germany), which is able to label DIG-11-dUTP while the probe is synthesized by PCR. The primer in this research is self-designed based on promoter of rice glutelin genes, R: 5'-GGA-GAAGCTGACCAATAATCA-3' and F: 5'-TTACCTT-TAGTATCCAACTTG-3'. Using DNA of waxy rice flour-TCW70 as template, PCR reagent in the kit was added for PCR. The temperature program was as follows: after DNA was denatured at 94˚C/5mins, 35 temperature cycles (94˚C/30 secs; 56˚C/1 min; and 72˚C/2 mins), hold at 72˚C/5 mins, and then cooled to room temperature[3]. The PCR product was separated by electrophoresis using 1.0% agarose gel (with 1X TAE buffer, containing 0.04 M Tris-acetate and 0.001 M EDTA, pH8.0, dissolved under heat then cooled). After staining, the major fragment of 1200 bp on gel was sliced under UV light. In a 2 mL centrifuge tube, this piece of gel combined with 3 volumes of 6 M NaCl was melted completely in 55˚C water bath (about 5 mins). Subsequently, 5 μL of Glass Milk (BIO 101, France, 1 μL of Glass Milk can absorb 1 μg of DNA.) was gently mixed with it to emulsion. This mixture was washed 3 times with 0.5 mL of New Wash solution (BIO 101, France) with centrifuge of 7000 rpm for 10 secs in between. Fragments of probe were dissolved at 55˚C for 5 mins after 5 μL of sterile water was added to the dried lower layer. The precipitate was removed by centrifuging at 7000 rpm for 10 secs, so that the clear supernatant of probe solution was preserved in aliquots at 4˚C for further application[6].

V. *Quantitative Analysis (Hybridization) of Rice DNA*

Protocols in the User's Guide published by B. M. Biomedica (Germany) were the following[6]: In a 2.5 mL centrifuge tube, 15 μL of dissolved DNA sample solution was heated in 100˚C boiling water for 10 mins, making double strand DNA to become single strand, then cooled down in ice bath immediately. From this solution, 10 μL was blotted on the nylon membrane. After exposed to UV light for DNA cross linking, the membrane was put into a hybridization bag (This can be substituted by a well-sealed plastic bag.) with 30 mL of hybridization solution (from DIG Wash and Block Buffer Set Kit, B. M. Biomedica, Germany) containing 5X SSC solution (diluted from 20X SSC, 20X SSC solution contained 3.0 M NaCl and 300 mM sodium citrate, pH 7), 0.1% N-lauroylsarcosine, 0.2% SDS, and 1% blocking solution[6]. This bag was put in 68˚C water bath for 2-hr pre-hybridization (The bag must be immersed completely), and then the solution was discarded. In addition, excess probe was well mixed with a new hybridization solution. This mixture was heated at 100˚C boiling water for 10 mins making double strand DNA of probe to become single then cooled down in ice bath immediately. This probe-containing hybridization solution was poured into the hybridization bag described above and incubated overnight in 68˚C water bath after all air bubbles in bag have been removed. Afterwards, this probe-containing hybridization solution was stored at -20˚C in a 10 mL sterile centrifuge tube (It can be used repeatedly for half year). The excess probe on nylon membrane was washed with 30 mL of 2X, 0.5X and 0.1X SSC solution containing 0.1% SDS, respectively, in 68˚C water bath for 15 mins. After rinsed with washing solution (10 mM malic acid, 15 mM NaCl, and 0.03% Tween20, pH 7.5) for 1 min, the membrane was transferred to a clean shallow plate. 30 mL of blocking solution is added to the membrane, which must be covered by the liquid completely. Shake gently and horizontally for 30 to 60 mins (It is fine to be longer). With this blocking solution discarded, 30 mL of blocking solution containing 10000-time diluted anti-Digoxigenin-AP (750 units/mL of alkaline phosphatase,) was added then shaken gently and horizontally for 30 mins. Subsequently, this nylon membrane was washed twice with the same washing buffer for 15 mins each to remove excess antibody. After rinsing with 30 mL of detection buffer (10 mM Tris-HCl and 10 mM NaCl, pH 9.5) for 2 mins, the membrane was sealed in a plastic bag with 100-time detection buffer diluted CSPD® (25 mM Disodium 3-(4-methoxyspiro {1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3,3,1,1^{3,7}] decan} 4-yl) phenyl phosphate) with air bubbles removed. This bag was incubated in 37˚C oven for 15 mins, and then the intensity of fluoresce was detected by exposure to the film. Quantitative analysis was carried out by densitometer scanning to the exposed film after development. Regression was calculated using the logarithm of rice amount as X-axis and the logarithm of scanned density value with background value subtracted as Y-axis[7].

RESULTS AND DISCUSSION

I. *Extraction of Rice DNA*

Extraction of DNA from samples is to be discussed first. Using Tris buffer (pH7.5) to extract DNA from cereal samples, this research repeated the protocol published by Allmann et al. in 1993[2]. It was found that the interference caused by starch could not be eliminated. Especially while starch was mashed completely in products, the DNA solution became adhesive due to the spiral structure of mashed starch so that subsequent procedures could not be continued. For modification, 1% of CTBA, which is able to dissolve the cell membrane and form complexes with DNA molecules so that they can be separated from starch to improve their precipitation and purification, was added. Meanwhile, α-amylase was also added to digest mashed starch so that the problem caused by adhesive sample solution can be solved. The agarose gel electrophoresis of DNA

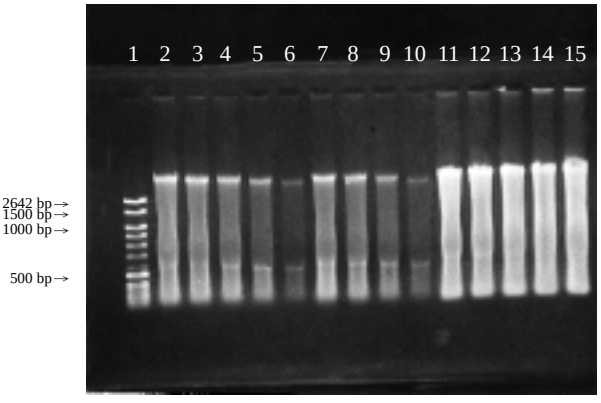

Figure 1. Agarose gel electrophoresis of DNA extracted from waxy rice flour. (0.4% agarose gel). The samples included 1.0 g, 0.7 g, 0.5 g, 0.3 g, 0.1 g waxy rice flour (lane 2~ 6), 0.7~0.1 g waxy rice flour added with 0.3~0.9 g cassava flour (lane 7~10), 0.7~0.1 g waxy rice flour added with 0.3~0.9 g wheat flour (lane 11~14), and 1.0 g wheat flour (lane 15). DNA size markers XIV (B.M. Biochemica) were shown in lane 1.

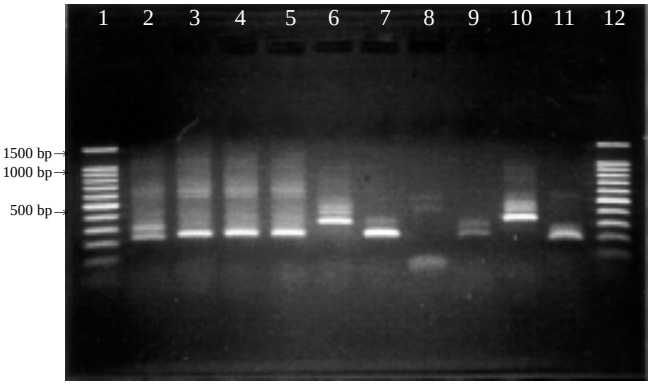

Figure 2. Agarose gel electrophoresis of the PCR products amplified from the DNA of cereals using the primer based on 5S ribosomal RNA genes[3] (1.5% agarose gel). The samples included Indica rice (lane 2), Indica rice TCS10 (lane3), Japonica rice (lane4), waxy rice (lane 5), wheat (lane 6), maize (lane 7), radish (lane 8), commercial waxy rice flour A (lane 9), wheat flour (lane 10) and sweet potato (lane 11) . DNA size markers (BDH) were shown in lanes 1 and 12.

extracted and purified from samples is shown in Figure 1, in which some preliminary results were observed. Taking waxy rice flour (TCW70) for example, the result of 0.4 % agarose gel electrophoresis of DNA extracted from different amount (1.0 g, 0.7 g, 0.5 g, 0.3 g, and 0.1 g, Lane 2~6) of samples is similar to the result of DNA extracted from different amount of mixed waxy rice flour (TCW70) and cassava starch (0.7+0.3 g, 0.5+0.5 g, 0.3+0.7 g, and 0.1+0.9 g, Lane 7~10). It appeared that the interference caused by starch is decreased to an acceptable range. Regarding the experiment on mixture of waxy rice flour and wheat flour, no trend tendency appeared on the agarose gel electrophoresis because the gene group of wheat is much larger than rice.

For further analysis, a clear DNA solution was also obtained from samples of processed products, such as radish cake, rice noodle, and slice rice noodle, which contain a great amount of mashed starch. However, the DNA was severely broken during the process of production so that no clear signal appeared on the agarose gel electrophoresis (data not shown).

II. *Qualitative PCR of Rice DNA*

Two primer sets were applied in this research to screen the presence of rice in samples: the primer set designed based on 5S RNA genes[3] and the primer set designed based on promoter of rice glutelin genes[4]. The primer set designed based on 5S RNA genes is reactive to samples of many cereal species resulting major PCR fragment of different lengths, which can be applied to differentiate cereal species. In the report by Henry et al. in 1997[8], the lengths of PCR major fragment from DNA of different cereal samples were listed. They were 320 bp from barley, 280 bp from maize, 235 bp from oat, 255 bp from rice, 400 bp from rye, 230 bp from sorghum, and 315 bp from wheat.

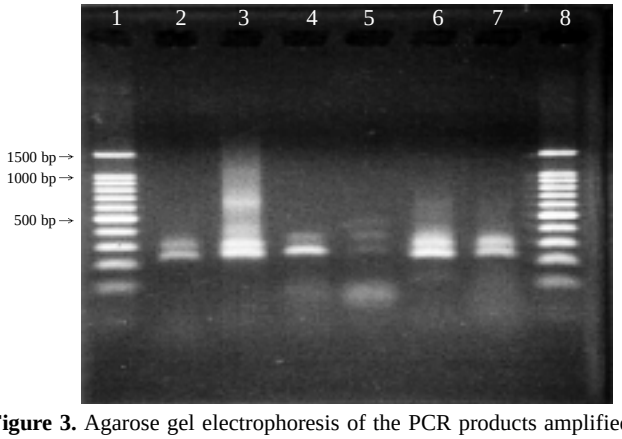

Figure 3. Agarose gel electrophoresis of the PCR products amplified from the DNA of cereals products using the primer based on 5S ribosomal RNA genes[3] (1.5% agarose gel). The samples included commercial waxy rice flour B (lane 2), rice flour imported from Thailand (lane 3), corn starch (lane 4), rice noodle (lane 5), radish cake (lane 6) and slice rice noodle (lane 7). DNA size markers (BDH) were shown in lanes 1 and 8.

The objective of this research is to identify the presence of rice in samples by this primer set, and to assure that other common material in rice products will not cause interference with the reading of experiment results. Figure 2 and Figure 3 showed the agarose gel electrophoresis of the PCR products amplified from the DNA of cereals and from the DNA of cereal products using the primer based on 5S RNA genes, respectively. It was found in Figure 2 that the result of Indica rice is different from other species. There are two DNA fragments after PCR with similar intensity. One is larger than 255 bp, and another is smaller than 255 bp. The PCR major product amplified from waxy rice, Japonica rice, and Indica rice TCS10 is the same at about 255 bp. Since Japonica rice and Indica rice TCS10 are usually cooked as steamed rice for food but not used for processed products, whereas waxy rice and Indica rice are frequently

used for rice-related products, the rice species in samples can be preliminarily differentiated according to these results. That is, the PCR results from this primer set are able to provide information on rice species in samples. In the result of other cereal samples in Figure 2, it was found that the DNA of wheat and radish (Lane 6 and 8) does not cause interference with the reading of results. However, the length of PCR amplified fragments from the DNA of maize and sweet potato using the primer set based on 5S RNA genes is similar to that from DNA of waxy rice, Japonica rice, and Indica rice TCS10 (Lane 7, 11 and Lane 3~5). They can not be differentiated by the resolution of conventional electrophoresis because interference caused misreading. This result was shown in Figure 3, which is an agarose gel electrophoresis of the PCR products amplified from the DNA of cereal products using the primer set based on 5S ribosomal RNA genes. It was found that Indica rice flour is the raw material for both radish cake and slice rice noodle (Lane 6 and 7). The most interesting finding is that although the sample of commercial waxy rice flour B was labeled as waxy rice flour, likely contains Indica rice (Lane 2). The rice flour imported from Thailand contains huge amount of Indica rice as well (Lane 3). The rice noodle sample may contain corn starch, because their results on electrophoresis are similar (Lane 5 and 4). Due to the similar major fragment from PCR as waxy rice flour's signal, waxy rice flour also may be included as raw material. Thus the result of Figure 3 was not able to clearly determine whether rice is the raw material for the sample of rice noodle. That is, results from other experiment will be needed for further determination.

Since maize, sweet potato, waxy rice, Japonica rice, and Indica rice TCS10 can not be differentiated by ordinary electrophoresis when the qualitative analysis was carried out by PCR using the primer set based on 5S RNA genes, the method, which used the primer set based on promoter of rice glutelin genes, published by Suzuki et al. in 1997[4] was adopted for PCR analysis. The electrophoresis result in Figure 4 showed that this primer set is reactive only to rice DNA synthesizing a major fragment of 234 bp, whereas it is not reactive to DNA of other cereal samples. Combined with the result from Figure 5, it was confirmed that rice is added as the raw material in the previous (Figure 3) rice noodle sample but this primer set could not confirm the rice species present in the sample. Furthermore, different rice species have the same PCR results from this primer set. That is, their products are simple with major fragment of the same length. Therefore, this primer set should be very suitable for making a simple measurement to the amount of rice in samples while quantitative PCR is going to be considered in the future.

Overall, qualitative analysis by DNA is able to sensitively and accurately determine whether rice DNA is present in samples or not. Although microscopic examination is able to identify the presence of rice more rapidly[1], it cannot identify the starch granule when samples were completely mashed starch products. Thus, qualitative analysis by DNA is needed. Because of its extremely high sensitivity, qualitative analysis by DNA still can be carried out in samples that contained purified starch or were highly processed, such as rice noodle, even if their DNA was severely broken.

III. *Practicability Evaluation of Quantitative Analysis for the Amount of Rice in Samples*

The ultimate goal of this research is to roughly estimate the amount of rice in samples based on the amount of rice DNA in samples. Hybridization is the most common method of quantitative analysis of DNA. Hybridization uses labeled and specific DNA fragment or randomly synthesized oligonucleotide fragment as a probe to quantitate DNA molecules in samples. Factors that will affect the reaction process to success include reaction temperature,

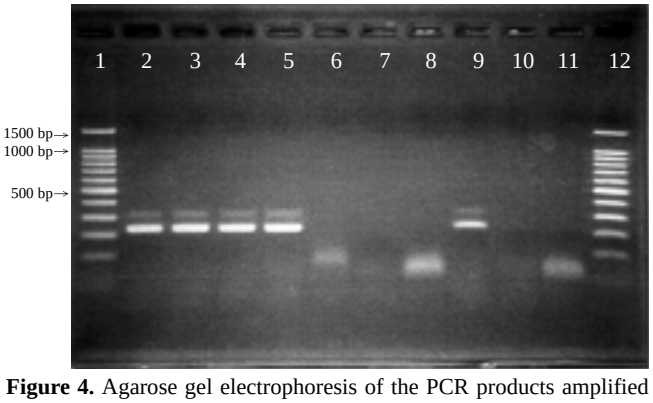

Figure 4. Agarose gel electrophoresis of the PCR products amplified from the DNA of cereals using the primer based on promoter of rice glutelin genes[4] (1.5% agarose gel). The samples included Indica rice (lane 2), Indica rice TCS10 (lane3), Japonica rice (lane4), waxy rice (lane 5), wheat (lane 6), maize (lane 7), radish (lane 8), commercial waxy rice flour A (lane 9), wheat flour (lane 10) and sweet potato (lane 11) .DNA size markers were shown in lanes 1 and 12.

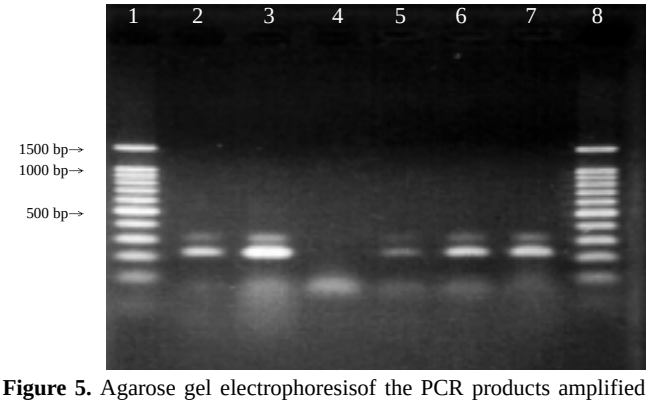

Figure 5. Agarose gel electrophoresisof the PCR products amplified from the DNA of cereals products using the primer based on promoter of rice glutelin genes[4] (1.5% agarose gel). The samples included commercial waxy rice flour B (lane 2), rice flour imported from Thailand (lane 3), corn starch (lane 4), rice noodle (lane 5) , radish cake (lane 6) and slice rice noodle (lane 7). DNA size markers were shown in lanes 1 and 8.

salt concentration, pH value, probe selection, probe concentration, and probe labeling. Probe selection is the most important key factor.

Basically, if the probe is longer, the specificity and selectivity will be better, but the reaction time is extend to about 16 hrs. On the other hand, although a shorter probe, has a better sensitivity and shorter reaction time of perhaps 30 mins short-chain probes are more difficult to be labeled. As the result, it is less sensitive and easier to cause false positive reaction due to less specificity. In this research the sequence, listed in the report published by Suzuki et al. in 1997[4], from promoter of rice glutelin genes was undergone matching analysis in the public database to design two fragments of DNA, which are more specific to rice than other cereals for testing. Results showed that the probe of 180 bp is less specific and causes false positive results and poor reproducibility, and so it was abandoned. However, the probe of 1200 bp is only reactive to rice DNA (data not shown). This result fulfilled the need for this research, so this probe was used for analysis in all experiments below. The concentration of probe was controlled at excess.

Taking TCW70 waxy rice as an example, the practicability, (including accuracy, repeatability, and reproducibility,) was evaluated using quantitative analysis of DNA to estimate the percentage of rice in samples. Different amount of cassava starch was mixed with 100% of TCW70 waxy rice flour to make 6 samples that contain 100, 70, 50, 30, 10, and 0% of waxy rice flour, respectively, for testing the linearity by hybridization. Regression was calculated using the logarithm of rice amount as X-axis and the logarithm of scanned density value with background value subtracted as Y-axis[7]. The linear coefficient of correlation (R^2 value) reaches 0.98 after regression from two trials at different time. It illustrated that the linearity of analytical results is in an acceptable range and the reproducibility is good. Thus a cereal sample of known ratio was prepared for testing the accuracy and repeatability of this analytical method. The sample was 0.4 g of 100% TCW70 waxy rice flour combined with 0.6 g of cassava starch. After two repeated analysis, the logarithm of scanned density value from samples subtracted by the background value yield to the equation $y = 0.6118x + 3.5695$. The antilogarithm of the X value is the percentage of rice in samples.

Self-prepared samples theoretically contains 40% waxy rice flour. The actual result is 40.85% ± 1.10% which is extremely close to the amount of waxy rice flour in the self-prepared samples. The C.V. value of two repeated analysis is 2.7%. It appeared that it is practicable to estimate the amount of rice in samples by this qualitative analysis of DNA in samples, given the standard samples were selected appropriately and accuracy and repeatability both lie in the acceptable range. However, the amount of DNA in samples is affected greatly by the rice species and production process. If this quantitative analysis is applied to actual samples, the effect from different rice species and different process of production has to be considered while selecting the standard samples. The most important factor that will affect the accuracy of this analytical method is finding a standard sample, representative and descriptive of the status of DNA in samples.

ACKNOWLEDGEMENTS

This study was supported by the Council of Agriculture, Executive Yuan, Republic of China. We thank Prof. Shin Lu and Dr. Jiing-Yang Wu for providing us pure cereal products. We thank Mr. Shu-Meng Lin for his translation.

REFERENCES

1. Heng, M. C. and Lu, S. 1997. Studies on the purity identification of waxy rice and cassava flour. Bulletin of Taichung District Agricultural Improvement Station 56: 11-21.
2. Allmann, M., Candrian, U., Hofelein, C. and Luthy, J. 1993. Polymerase chain reaction(PCR): a possible alternative to immunochemical methods assuring safety and quality of food. Z. Lebensm. Unters. Forsch. 196: 248-251.
3. Ko, H. L., Henry, R. J.,Graham, G. C., Fox, G. P., Chadbone, D. A. and Haak, I. C. 1994. Identification of cereals using the polymerase chain reaction.J.Cereal Sci. 19: 101-106.
4. Suzuki, A., Suzuki, T., Tanabe, F., Toki, S., Washida, H., Wu, C. Y. and Takaiwa, F. 1997. Cloning and expression of five myb-related genes from rice seed.Gene 198: 393-398.
5. Maniatis, T., Fritsch, E. and Sambrook, J.1989.Appendix B: Preparation of reagents and buffer used in molecular cloning. In “Molecular Cloning 3”. 2nd ed. pp.B1~E7. Irwin, N. ed. Cold Spring Harbor Lab.,USA.
6. Miltenburg, R., Ruger, B., Grunewald-Janho, S., Leons, M. and Schroder, C. 1995. The DIG System User’s Guide for Filter Hybridization. Boehringer Mannheim GmbH. Biochemica, Germany.
7. Hill, J. M., Halford, W. P., Wen, R., Engel, L. S., Green, L. C. and Gebhardt, B. M. 1996. Quantitative analysis of polymerase chain reaction products by Dot Blot. Anal. Biochem. 235: 44-48.
8. Henry, R. J., Ko, H. L. and Weining, S. 1997. Identification of cereals using DNA-based technology. Cereal Food World 42: 26-29.