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Detection of Durum Wheat Pasta Adulteration in the Jordanian Market by Polymerase Chain Reaction Technology

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ABSTRACT

Taking into account the impact of monitoring food adulteration on the quality of food products, the aim of this study was to use polymerase chain reaction technology to detect possible adulteration of durum wheat pasta products in the Jordanian market. Cetyltrimethyl ammonium bromide method was applied for extracting genomic DNA from twenty six randomly collected pasta products, the results suggested the suitability of this method for DNA extraction from pasta products. Specific primers were used to ascertain the presence or absence of Dgas44 sequence in the extracted genomic DNA from pasta products, this sequence is known to be present in the genome of common bread wheat and absent in the genome of durum wheat. The obtained results showed that 65.4% of tested pasta products sold in the Jordanian markets are not made solely from durum wheat as indicated by manufacturers.

Key words: Food adulteration, durum wheat, Dgas44 sequence, pasta, polymerase chain reaction

INTRODUCTION

Nowadays ascertaining food authenticity is a subject of serious concern to the consumers and food authorities, where correct and adequate labeling of food composition has become crucial. Besides the composition of specific foods is always a key factor in the quality of the final product (Aktas *et al.*, 2009; Gupta and Panchal, 2009; Terzi *et al.*, 2003). There are reports about adulteration of various types of food and the necessity for authenticity testing of raw materials and final products for example adulteration of high quality rice with low quality products (Ren *et al.*, 2006), meat varieties (Rodriguez *et al.*, 2003), wheat flour (Von Buren *et al.*, 2001) and milk (Abdel-Rahman and Ahmed, 2007).

One of popular wheat products worldwide is pasta. This food product is manufactured from special type of wheat known as durum wheat, which gives the required quality of the pasta (Sissons, 2008). Considering the possible adulteration of pasta products with common bread wheat, several methods had been invented to investigate adulteration of durum wheat pasta, these included near infrared spectroscopy (Cocchi *et al.*, 2006), immunoassays (Stevenson *et al.*, 1994) and Polymerase Chain Reaction (PCR) method (Alary *et al.*, 2002; Bryan *et al.*, 1998; Tilley, 2003; Terzi *et al.*, 2003, 2005; Wiseman *et al.*, 1998). The PCR technique is considered more reliable, rapid and readily replicable for accuracy than other methods based on the analysis of proteins. The protein based methods are problematical owing to the wide range of processing conditions employed

throughout the pasta production (Bryan *et al*., 1998). It is worth noting that PCR methods were developed to distinguish pure durum wheat products from adulterated products without ambiguity by using specific primers. Investigators were able to design specific primers, which were reported in the literatures to detect adulteration in durum wheat products. One of PCR studies had utilized two types of primers, first one to identify part of a durum wheat gene encoding a lipid transfer protein and second type of primers to detect common wheat by amplifying part of the puroindoline-b gene (Alary *et al*., 2002). Second PCR approach used primers which can amplify Dgas44 sequence present in all *Triticum aestivum* cultivars (wheat bread) but absent in all of the *T. durum* cultivars (Tilley, 2003; Wiseman *et al*., 1998). In this investigation detection of pasta adulteration in products obtained from the Jordanian market was ascertained by molecular PCR analysis for the detection of Dgas44 sequence.

MATERIALS AND METHODS

Pasta products: Twenty six presumed durum wheat pasta products were obtained during the period from March 2010 and June 2010 from the Jordanian Food and Drug Testing Administration, the Royal Scientific Society Testing Laboratories and supermarkets in Amman, Jordan.

Genomic DNA extraction: Pasta samples were ground then the genomic DNA was extracted by cetyltrimethyl ammonium bromide (CTAB) method (Querci *et al*., 2006). Five hundred microliter of CTAB was added to 100 mg of homogeneous sample already mixed with 300 μL of sterile deionized water. The mixture was then subjected to protinase K (20 mg mL^{-1}) and RNase (10 mg mL^{-1}) treatments at 65°C for 30 and 10 min, respectively, centrifuged for ten minutes. The supernatant was mixed with 500 μL chloroform and centrifuged for 15 min, this step was repeated and the mixture was centrifuged for 5 min. Two volumes of CTAB precipitation solution were added to the supernatant, mixed gently by pipetting and incubated at room temperature for 60 min then centrifuged for 5 min. The supernatant was discarded and the precipitate was dissolved in 350 μL NaCl (1.2 M), 350 μL of chloroform was added and mixed for 30 sec followed by centrifugation for 10 min. The upper layer was transferred to a new microcentrifuge tube and 0.6 volume of isopropanol was added, mixed gently by inversion then centrifuged for 10 min, the supernatant was discarded. Five hunderd microliter of 70% ethanol was added to the precipitate, followed by brief gentle mixing and centrifuged. The supernatant was discarded, the DNA precipitate was allowed to dry, then the DNA pellet was re-dissolved in 100 μL sterile deionized water. The prepared DNA solution was divided into aliquots of 10 μL then stored at -20°C for up to six months. Centrifugation was carried out at 16000 rpm for the aforementioned indicated periods.

Determination of concentration and purity of extracted DNA: The concentration of extracted DNA is determined by measuring at 260 nm against a blank. The ratio A260/A280 is used to estimate the purity of extracted DNA (Querci *et al*., 2006). The measurements were performed using Jenway spectrophotometer (Genova/UK).

DNA amplification: Amplification reactions were performed according to the reported method (Tilley, 2003). PCR reactions were carried out in a total volume of 50 μL. Each reaction mixture contained 100 ng of prepared DNA, 1X PCR buffer, 2.5 U TopTaq DNA polymerase, 15 μM $MgCl_2$ obtained as TopTaq TM PCR kit (Qiagen/Germany), 50 μM dNTPs (Promega/USA), 2.5 μL of 10 pmol primer stocks (Alpha DNA/Canada), the final volume was completed to 50 μL with nuclease

free water. The amplification of Dgas 44 sequence was performed in the Applied Biosystem Thrmocycler 9902 with heating lid. The amplification program was started for min at 95°C for initial denaturation followed by 30 cycles of 94°C for 1 min (denaturation), 65°C for 45 sec (annealing) and 72°C for 30 sec (extension). The final extension cycle was at 72°C for 5 min. Each run included a negative control, composed of all PCR mix components expect DNA.

Primers: The forward and reverse primers specific to Dgas44 sequence in the D genome of wheat were used in the amplifications (Table 1). The primers were obtained from Alpha DNA, Canada.

Gel electrophoresis: The amplification products in parallel with DNA marker ladder of 100 bp (Qiagen) were separated on 1.5% agarose gel and visualized under UV light after staining with ethidium bromide for molecular size determinations in base pair (bp) of DNA fragments (Sambrook and Russell, 2001).

RESULTS

Sources of pasta products: A total of twenty six pasta products available in Amman markets were obtained during the period March and June 2010. These were collected from the Jordanian Food and Drug Testing Administration, the Royal Scientific Society Testing Laboratories and supermarkets in Amman, Jordan.

Twenty two pastas were products of nine countries and four of the pasta products were of unknown origin (Table 2).

DNA concentration and purity of extracted DNA: The purity and concentration of genomic DNA extracted by CTAB methods from pasta samples were investigated. The obtained results showed noticeable variations in the concentrations and purities of extracted genomic DNA from various samples of pasta products. It was possible in this study to identify five categories which were recognized according to the yield of extracted DNA. The lowest yield's range of extracted DNA was 2-10 ng μL^{-1} and the highest yield's range was 53-70 ng μL^{-1} (Table 3). The results of purity

Table 1: Sequences of primer pair for Dgas44 sequence used for detection of adulterated pasta

Primer pair of Dgas44 sequence	Primer sequence (5' - 3')	PCR product size	References
Forward	CTTCTACGGGTCAGGGCAC	287 base pairs	Bryan *et al.* (1998)
Reverse	CTAATGCCCCTGCGGCTTAA		Tilley (2003)

Table 2: Country origin of analyzed pasta products in this study, numbers between brackets indicated number of adulterated pasta products

Country of origin	No. of samples
Turkey	6 (6)
France	1 (0)
Italy	7 (2)
Jordan	1 (0)
KSA	1 (0)
Tunisia	2 (2)
Kuwait	1 (1)
UAE	1 (1)
Egypt	2 (1)
Unknown	4 (4)

Table 3: Concentration of DNA obtained from various pasta samples following extraction by CTAB method

No. of samples	Range of DNA concentration (ng μL^{-1})
3	2-10
3	12-18
6	20-29
10	35-49
4	53-70

Table 4: DNA purity indicated by $_{260}/_{280}$ values following DNA extraction by CTAB method from pasta food samples

No. of samples	Range of DNA purity
1	1.45
2	1.56-1.59
5	1.62-1.67
18	1.70-1.79

Fig. 1: Agarose gel electrophoresis of extracted DNA by CTAB method from pasta products, electrophoresis was performed on 1% agarose gel and run with 3 volt cm^{-1}. Lanes 1-4 indicated the pasta products

measurements of extracted DNA varied between 1.45 and 1.79 (Table 4). According to the obtained purity values four groups were identified The largest group showed the highest DNA purity and represented eighteen pasta products. These results suggested the suitability of CTAB method for DNA extraction from pasta products. Agarose gel analysis of extracted DNA from various pasta products had shown that the isolated DNA fragments by CTAB method composed of high molecular weight and a smear of lower molecular weight degraded DNA (Fig. 1).

Dgas44 sequence detection in pasta products: In this investigation PCR technique was used for the amplification of Dgas44 sequence by the specific primers (Table 1). The results showed that 17 out of 26 presumed wheat durum pasta products in the Jordanian markets were adulterated with common bread wheat (Table 2). This is equivalent to 65.4% adulteration of pasta products with common bread wheat. The amplified Dgas44 sequence in the adulterated pasta products gave size of 287 bp (Fig. 2); this amplicon was not detected in the non adulterated pasta products. All pasta products used in this study were labeled by the manufacturers as being made from durum wheat.

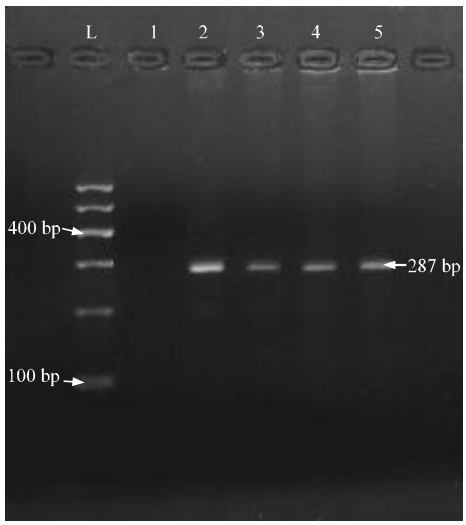

Fig. 2: Detection of Dgas44 sequence of 287 bp in extracted DNA from pasta products on agarose gel, electrophoresis was performed on 2% agarose gel and run with 3 volt cm^{-1}. Lane 1 represents negative control. Lanes 2, 3, 4 and 5 represent adulterated pasta, lane L indicated the 100 bp ladder

DISCUSSION

In recent years authenticity testing of food composition for ascertaining food adulteration is becoming an important issue and considered of vital importance for reasons related food quality, allergy and religious restrictions (Branciari *et al.,* 2000; Dalmasso *et al.*, 2004; Lopez-Calleja *et al.,* 2005).

In the present investigation pasta adulteration was ascertained and the approach was based on detection of bread (common) wheat (*T. aestivum*) in products which are made from durum wheat. There are several methods used for detection pasta adulteration. One of these methods is based on detection of specific variants of protein found in one wheat species but not found in other species. McCarthy *et al.* (2006) had reported that when common wheat (*Triticum aestivum* L.) gliadins were separated by Reverse Phase-HPLC, a major doublet peak eluted at 47.20 and 47.94 min. This peak was consistently found to be absent in durum wheat (*Triticum durum* Desf) gliadins separated under identical conditions. It was observed in durum wheat gliadins; a characteristic small peak eluted at 48.30 min followed at 50.47, 51.37, 52.80 min by larger peaks. The peak area ratio of the peaks eluting at 50.47 and 51.37 min was found to be 2.18 (±0.14). This ratio was found to decrease proportionally on contamination of durum wheat flour with flour from some common wheat varieties (McCarthy *et al.*, 2006). Other method utilized immunoassays for detection of common wheat in pasta products made from durum wheat (Stevenson *et al.*, 1994). It is worth noting that one of earliest investigations carried out on detection of adulteration of food pasta reported the adulteration by addition of animal proteins, this type of adulteration was detected by serum precipitation (Mazzaracchio *et al.*, 1965). These methods gave uncertain results due to possible denaturation of proteins during the processing conditions employed during pasta production (Bryan *et al.*, 1998).

On the other hand several investigators have demonstrated the practicability of PCR technology for detecting common wheat adulteration of pasta products (Alary *et al.*, 2002; Bryan *et al.*, 1998;

Tilley, 2003; Terzi *et al*., 2003, 2005). Our investigation provided further evidence of the potential of PCR technology for the detection of pasta products adulteration by hexaploid common bread wheat, it showed powerful molecular approach for ascertaining pasta products depicted as being manufactured exclusively from durum wheat. The obtained results in this study showed the possibility of detection of common bread wheat adulteration of durum wheat pasta sold in the Jordanian markets; this was achievable by using PCR technology with aid of specific primers for Dgas44 sequence. The results demonstrated that pure durum wheat pastas were distinguished from adulterated pastas without ambiguity. Furthermore, the results of this study made it obvious and support the earlier studies about possibility of using Dgas44 sequence as a suitable molecular marker for the detection of common wheat adulteration of pasta. It had been reported that this sequence is a repetitive sequence that occurs at a high copy number (200-300) within the D genome of common bread wheat and appears to be absent from the genome of durum wheat (McNeil *et al.,* 1994). Other investigators were able to detect pasta adulteration by amplifying part of a durum wheat gene encoding a lipid transfer protein where a common bread wheat DNA was detected by amplifying part of the puroindoline-b gene (Alary *et al*., 2002). It is worth noting that the size of amplicons of amplified DNA sequence is important in the molecular investigation aimed for detection of adulteration of processed food adulteration, it is suggested that amplified PCR products of less than 300 base pairs in length are suitable for such analysis of processed wheat product (Tilley, 2003). The size of amplified DNA fragment obtained by specific primers for Dgas44 sequence is 287 base pairs in length and was detected in 65.4% of pastas used in the present investigation.

It is interesting to note that the study revealed that two out of seven pasta products purchased from Italy were adulterated according to Dgas44 PCR assay (Table 2). In this respect, Wiseman *et al*. (1998) indicated in his study that Italian law prohibits the manufacture of pasta containing *T. aestivum* for sale in Italy but not for subsequent export. Further Wiseman and coworkers stated that several European countries including Italy, Spain and France take the firm view that the inclusion of common wheat in pasta is effectively adulteration. Thus, considering the consumer right , food safety and the food labeling regulations, it is important to emphasize on the fact that product offered for sale in a misleading way, must be considered adulterated, hence, the presence of a common bread wheat in durum wheat pasta products should be declared on the product label. Our aim is the detection and authentication of products in an adequate manner.

CONCLUSION

The majority of DNA extracted from pasta products by CTAB method showed high purity, this indicated the suitability of CTAB method for genomic DNA extraction from pasta products. The specific primers used in this study showed the possible use of Dgas44 sequence and PCR assay for the identification of common bread wheat species used in the production of pastas. Application of this molecular diagnostic method revealed that 65.4% of pasta products were adulterated with bread wheat.

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