Research Article

Received: 18 January 2023 Revised: 3 April 2023 Accepted article published: 14 April 2023 Published online in Wiley Online Library: 1 May 2023

(wileyonlinelibrary.com) DOI 10.1002/jsfa.12631

Varietal identification in pasta through an SSR-based approach: a case study

Valentina Fanelli,[a*] Maria Dellino,[a] Francesca Taranto,[b] Claudio De Giovanni,[a] Wilma Sabetta,[b,c] Pasquale De Vita[d] and Cinzia Montemurro[a,c,e*]

Abstract

BACKGROUND: Pasta is a worldwide popular Italian food made exclusively of durum wheat. The choice of variety to be used to produce pasta is at the discretion of the producer based on the peculiar characteristics of each cultivar. The availability of analytical approaches for the tracking of specific varieties along the productive chain is becoming increasingly important to authenticate the pasta products and distinguish between fraudulent activities and cross-contaminations during the production process. Among the different methods, molecular approaches based on DNA markers are the most used for these purposes because of their ease of use and high reproducibility.

RESULTS: In the present study, we used an easy simple sequence repeats-based method to identify the durum wheat varieties used to produce 25 samples of semolina and commercial pasta comparing their molecular profile with those of the four varieties declared by the producer and other 10 durum wheat cultivars commonly used in pasta production. All of the samples showed the expected molecular profile; however, most of them present also a foreign allele indicating a possible cross-contamination. Moreover, we evaluated the accuracy of the proposed approach through the analysis of 27 hand-made mixtures with increasing amounts of a specific contaminant variety, allowing the estimation of the limit of detection of 5% (w/w).

CONCLUSION: We demonstrated the feasibility of the proposed method and its effectiveness in the detection of not declared varieties when these are present in a percentage equal to or higher than 5%.

Supporting information may be found in the online version of this article.

Keywords: pasta; durum wheat; molecular traceability; SSR; varietal identification

INTRODUCTION

Durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.] is an economically and culturally staple crop for the Mediterranean countries, as a result of its large use in cereal-based food products, such as pasta, couscous, bulgur and bread. Among the European countries, Italy is the leading producer, with an annual production of about 4–4.5 million metric tonnes on average.[1] Pasta is a worldwide popular Italian food, and it is one of the most consumed products in the world. The Italian legislation foresees that pasta should be produced exclusively with durum wheat flour but a maximum level of 3% of common wheat is allowed as unintentional cross-contaminations.[2,3] Instead, no legal requirements are mandated for the use of specific varieties. The choice of variety to be used to produce pasta is at the discretion of the producer based on the peculiar characteristics of each cultivar. Some aspects, such as protein content, gluten composition, yellow index and browning, can significantly influence pasta quality and consumers' choice.[4-6] In the Southern regions of Italy, durum wheat is used for several types of local bread characterized by quality characteristics that are essentially or exclusively the result of a particular cultivation, transformation and elaboration phases, which all take place in the defined geographical area.[7,8] To

* *Correspondence to: V Fanelli or C Montemurro, Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, 70126, Italy. E-mail: valentina.fanelli@uniba.it (Fanelli); E-mail: cinzia.montemurro@uniba.it (Montemurro)*

Valentina Fanelli and Maria Dellino are contributed equally to this work.

a *Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy*

b *Institute of Biosciences and BioResources, National Research Council of Italy (IBBR-CNR), Bari, Italy*

c *Spin off Sinagri s.r.l., University of Bari Aldo Moro, Bari, Italy*

d *Research Centre for Cereal and Industrial Crops (CREA-CI), Foggia, Italy*

e *Institute for Sustainable Plant Protection–Support Unit Bari, National Research Council of Italy (IPSP-CNR), Bari, Italy*

encourage the maintenance and development of the territories and guarantee the authenticity and quality, the European Union labeled these local products as Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI).[9,10]

The increasing interest of consumers in high-quality products encourages producers in using high-quality raw material for pasta production and indicating the variety's name on the label. The price of a pasta package could be more variable depending on the kind of durum wheat and the exsiccation technique used in the production process. Some cultivars, such as Senatore Cappelli, have excellent organoleptic characteristics that are particularly appreciated by the consumer, who is willing to pay a large amount of money to buy that specific pasta.[11] The intentional addition of common wheat flour or durum wheat varieties, different from that declared on the product label, constitutes a fraudulent practice, as stated in the Italian Criminal Code (Article n. 515).[12] However, cross-contaminations are possible during the milling practices, leading to the presence of a low percentage of a contaminant variety or species in the final product. The availability of analytical approaches for tracking specific species or varieties along the productive chain, from seeds to transformed products, is becoming increasingly important to distinguish between intentional actions and cross contaminations during the pasta production process ensuring that consumer choices correspond to their expectations.

Many different methods are available for plant-based food traceability and authentication, which are mostly based on physico-chemical approaches.[13] Although they are highly efficient and reliable, the physico-chemical methods present remarkable limitations in the detection of varieties used to produce foodstuffs of plant origin because it is difficult to distinguish the physico-chemical profiles at a cultivar level. These limitations are overcome by the use of DNA-based approaches to food traceability because differences present in DNA sequence can allow distinguishing among the varieties.[14] Several methods are currently available for agri-food molecular traceability.[15,16] Among those, the approaches based on the use of molecular markers present several advantages, such as the high level of polymorphism and the possibility to analyze highly degraded DNA,[17] which often occurs in processed food products. In particular, techniques based on simple sequence repeats (SSR) are routinely used in food traceability because of their ease of use and high reproducibility. Well-established SSR-based protocols for varietal identification in processed agri-food products are available for wine, olive oil and jam.[14,18-23] An analytical approach aiming to trace the varieties used for Pasta production is necessary to properly valorize also this product. Although SSR-based traceability represents a traditional method compared to the most modern molecular techniques, it was already revealed to be a simple and effective approach in the authentication and traceability of some pasta products.[24,25] However, the principal aim of these works was the detection of common wheat contamination or adulteration to detect the 3% common wheat in durum wheat-based products. Over the years, some innovative DNA-based techniques were developed and applied to durum wheat-based product traceability with the same purpose.[26,27] Recently, a few studies have focused on pasta authentication at the varietal level through the use of some latest approaches, such as the digital PCR and LAMP (i.e. Loop mediated isothermal AMPlification).[15,28] In addition, gas chromatography–mass spectrometry and peptide based gas sensor array were used to evaluate the volatile organic compounds fraction of pasta.[29] However, such technologies are not available in all laboratories and require the development of *ad hoc* assays. Therefore, a simple and versatile approach may be necessary for commercial and routine purposes.

The present study aimed to: (i) apply an easy and reliable SSR-based method to identify wheat cultivars present in commercial pasta and evaluate its effectiveness and feasibility and (ii) estimate the limit of detection in the identification of the presence of a contaminant variety.

MATERIALS AND METHODS

Samples

Twenty-five commercial semolina and pasta samples (here named S1–S25) were provided from an Italian pasta factory and were indicated as composed of a blend of four different modern durum wheat varieties (Marco Aurelio, Pietrafitta, Quadrato, Redidenari; referred to here as 'References') by the producer. In addition, ten durum wheat varieties (Antalis, Aureo, Cappelli, Ciccio, Creso, Iride, Khorasan, Nadif, Saragolla and Timilia) were collected starting from a larger germplasm collection available at the Research Centre for Cereal and Industrial Crops (CREA-CI), Foggia (Italy), to select those showing polymorphic SSR patterns in comparison with the References. The seeds of all varieties were provided by CREA-CI for downstream analysis.

To test the robustness and the accuracy of SSR markers as an efficient method for contaminant detection in durum wheat semolina and pasta, 27 hand-made mixtures were prepared, at different concentrations, by mixing a blend of the above-mentioned four reference seeds in equal parts (25% each) and a growing amount of a fifth contaminant variety (Ciccio, Khorasan and Timilia) as described in Table 1. For each contaminant variety, different concentrations were tested: 0%, 1%, 3%, 4%, 5%, 6%, 12%, 24% and 48%. Mixtures were prepared by adding the corresponding amount of each semolina in a sterile tube and manually shaking the samples for several minutes before DNA extraction.

DNA isolation and PCR conditions

DNA was isolated from durum wheat seeds and commercial samples following a modified version of the protocol of Sharp *et al.*[30] The seeds and pasta were milled using a seed grinder to produce a fine powder before the DNA isolation. Briefly, the samples were incubated for 15 min at 65 °C with the extraction buffer (500 mmol L^{-1} NaCl, 100 mmol L^{-1} Tris pH 8.50, 50 mmol L^{-1} EDTA, pH 8.0, and SDS 20%). Then, samples were precipitated using potassium acetate and cold isopropanol. The precipitate was dissolved in 1 × Tris-EDTA and RNA was removed by digestion with RNase for 30 min at 37 °C. The DNA was again isopropanol precipitated and two washing steps were performed with 70% ethanol. The quantity and quality of extracted DNA were checked on 0.8% agarose gel and a Qubit 3.0 fluorometer (Life Technologies, Carlsbad, CA, USA).

Samples were genotyped using a set of 9 simple sequence repeat (SSR) markers: *DuPw004*, *DuPw115*, *DuPw167*, *DuPw205*, *DuPw217*, *Xgwm124*, *Xgwm155*, *Xgwm413* and *Xgwm663*.[31-33] (see Supporting information, Table S1). In addition, the D genome-specific SSR marker *Xgwm003* was used to check the presence of common wheat contamination.[31]

PCR mixtures contained 50 ng of genomic DNA, 1 × reaction buffer, 0.25 µmol L^{-1} forward and reverse primer mix, 0.08 mmol L^{-1} dNTP and 1 U of DreamTaq polymerase (Thermo Fisher, Waltham,

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Table 1. List of the 27 hand-made mixtures of durum wheat semolina

Blend	Reference sample %	Contaminant variety %	Contaminant variety
C1	100	0	Ciccio
C2	99	1	
C3	97	3	
C4	96	4	
C5	95	5	
C6	94	6	
C7	88	12	
C8	76	24	
C9	52	48	
K1	100	0	Khorasan
K2	99	1	
K3	97	3	
K4	96	4	
K5	95	5	
K6	94	6	
K7	88	12	
K8	76	24	
K9	52	48	
T1	100	0	Timilia
T2	99	1	
T3	97	3	
T4	96	4	
T5	95	5	
T6	94	6	
T7	88	12	
T8	76	24	
T9	52	48	

MA, USA) in a total volume of 25 µL. The forward primer was labeled at 5′ end with one of the FAM, HEX and NED fluorescent dyes (Sigma-Aldrich, St Louis, MO, USA). The amplification was carried out using the following protocol: 5 min of initial denaturation at 95 °C, followed by 35 cycles composed by denaturation at 95 °C for 30 s, annealing at the specific temperature for 45 s and extension at 72 °C for 40 s; the final extension was performed for 5 min.

Data analysis

The amplification products were analyzed by capillary electrophoresis (ABI PRISM 3100 Genetic Analyzer; Life Technologies) and allele sizes were assigned through the GeneMapper, version 3.7 (Life Technologies). The collected genetic data were used to construct a UPGMA (i.e. unweighted pair group method with arithmetic mean) tree via DARWIN, version 6.0.21 (https://darwin.cirad.fr) and principal coordinates analysis (PCoA) was performed using GenAlEx, version 6.5 (http://biology-assets.anu.edu.au/GenAlEx/Welcome.html).

GeneMapper profiles were used to calculate the limit of detection (LOD_6) according to Broeders *et al.*[34] A serial dilution was prepared for each contaminant variety as described in Table 1 and the analysis of each dilution was performed in hexaplicate ($n = 6$). The LOD_6 was defined as the lowest percentage of contaminant variety for which each of the six repeats provided a specific detectable peak.[34]

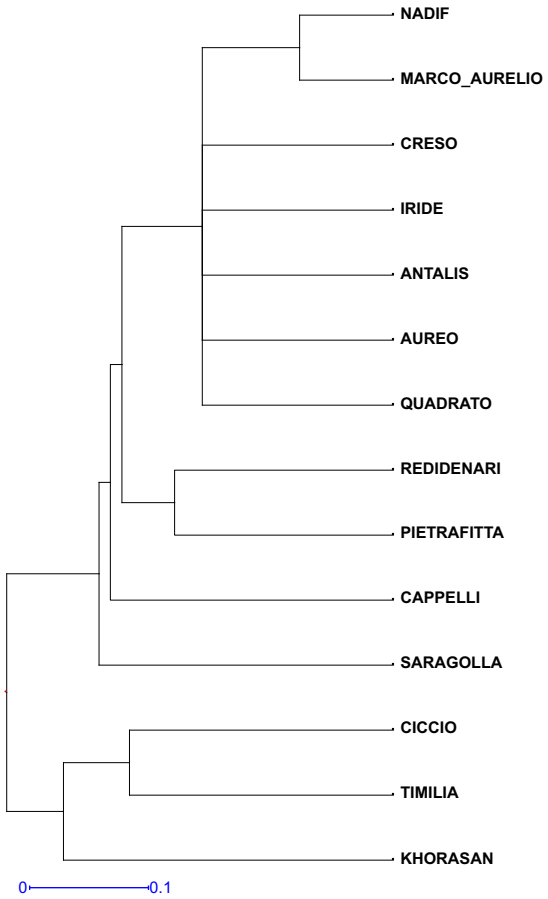

Figure 1. UPGMA dendrogram analysis of the 14 durum wheat varieties obtained through the amplification with the primers corresponding to *DuPw004*, *DuPw115*, *DuPw167*, *DuPw205*, *DuPw217*, *Xgwm124*, *Xgwm155*, *Xgwm413* and *Xgwm663* markers.

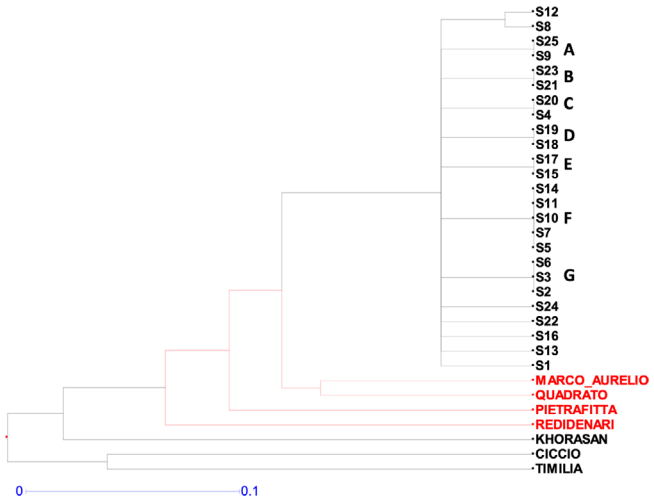

Figure 2. UPGMA dendrogram analysis of the 25 commercial samples and the 4 varieties declared by the producer (in red) obtained through the amplification with the primers corresponding to *DuPw004*, *DuPw115*, *DuPw167*, *DuPw205*, *DuPw217*, *Xgwm124*, *Xgwm155*, *Xgwm413* and *Xgwm663* markers. Khorasan, Ciccio and Timilia varieties were used as outgroups.

The contaminant variety percentage was estimated using the peak area obtained through the amplification of the hand-made mixtures with *DuPw004, DuPw205* and *Xgwm413*.

Table 2. Allelic patterns detected in the 25 commercial mixtures using 9 SSR markers

	DuPw004	*DuPw115*	*DuPw167*	*DuPw205*	*DuPw217*	*Xgwm155*	*Xgwm413*	*Xgwm124*	*Xgwm663*
Pietrafitta	a	a	c	b	c	b	b	a	a
Quadrato	a	a	b	b	a	b	a	a	b
Redidenari	a	a	a	b	b	b	b	b	a
Marco Aurelio	a	a	b	b	a	b	b	b	b
S1	1 a	1 a	2 ab	2 bx	2 ac	2 bx	2 ab	2 ab	1 b
S2	1 a	1 a	3 abc	2 by	2 ac	1 b	2 ab	2 ab	1 b
S3	1 a	1 a	3 abc	2 by	2 ac	1 b	2 ab	2 ab	1 b
S4	1 a	1 a	3 abc	3 bxy	2 ac	1 b	2 ab	2 ab	1 b
S5	1 a	1 a	3 abc	2 by	3 abc	1 b	2 ab	2 ab	1 b
S6	1 a	1 a	3 abc	2 by	2 ac	1 b	2 ab	2 ab	1 b
S7	1 a	1 a	3 abc	2 by	3 abc	1 b	2 ab	2 ab	1 b
S8	1 a	1 a	3 abc	3 bxy	3 abc	1 b	2 ab	2 ab	1 b
S9	1 a	1 a	3 abc	1 b	3 abc	1 b	2 ab	2 ab	1 b
S10	1 a	1 a	3 abc	2 by	3 abc	1 b	2 ab	2 ab	1 b
S11	1 a	1 a	3 abc	2 by	3 abc	1 b	2 ab	2 ab	1 b
S12	1 a	1 a	3 abc	3 bxy	3 abc	2 bx	2 ab	2 ab	1 b
S13	1 a	1 a	3 abc	2 by	1 a	1 b	2 ab	2 ab	1 b
S14	1 a	1 a	3 abc	2 by	3 abc	1 b	2 ab	2 ab	1 b
S15	1 a	1 a	2 ab	1 b	2 ab	1 b	2 ab	2 ab	1 b
S16	1 a	1 a	2 ab	1 b	3 abc	2 bx	2 ab	2 ab	1 b
S17	1 a	1 a	2 ab	1 b	2 ab	1 b	2 ab	2 ab	1 b
S18	1 a	1 a	3 abc	2 by	2 ac	1 b	2 ab	2 ab	2 ab
S19	1 a	1 a	3 abc	2 by	2 ac	1 b	2 ab	2 ab	2 ab
S20	1 a	1 a	3 abc	3 bxy	2 ac	1 b	2 ab	2 ab	1 b
S21	1 a	1 a	3 abc	1 b	2 ac	1 b	2 ab	2 ab	1 b
S22	1 a	1 a	2 ab	1 b	2 ac	1 b	2 ab	2 ab	1 b
S23	1 a	1 a	3 abc	1 b	2 ac	1 b	2 ab	2 ab	1 b
S24	1 a	1 a	3 abc	1 b	3 abc	2 bx	2 ab	2 ab	1 b
S25	1 a	1 a	3 abc	1 b	3 abc	1 b	2 ab	2 ab	1 b

Note: The number and composition of allelic combinations were indicated for each mixture and reference variety. Each letter represents a different allele.

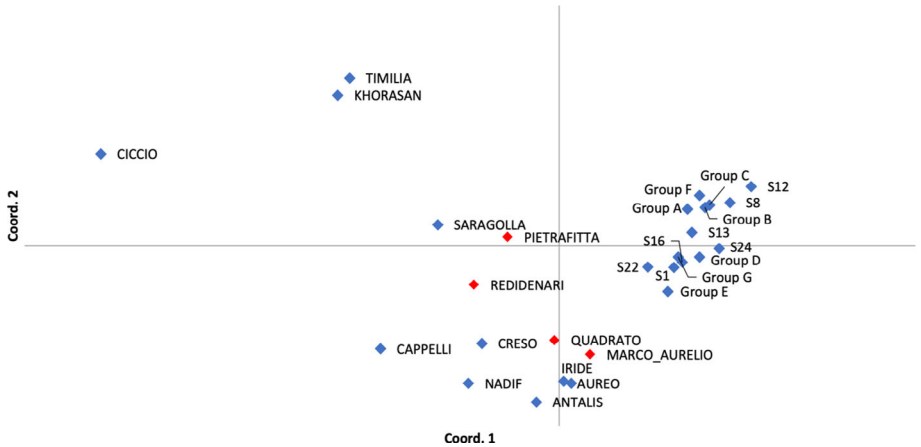

Figure 3. PCoA plot of the 14 durum wheat varieties and the 25 commercial mixtures based on the two principal coordinates (coordinate 1 = 44.37% and coordinate 2 = 15.71%).

For each percentage, the ratio between the area of the contaminant peak and the sum of the area of both the expected and the contaminant peaks was calculated. This value was compared with the actual percentage of the contaminant variety.

RESULTS AND DISCUSSION

Fingerprinting of durum wheat varieties and commercial samples

With the main purpose of verifying the feasibility of a simple and versatile SSR-based approach for pasta traceability, we analyzed

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

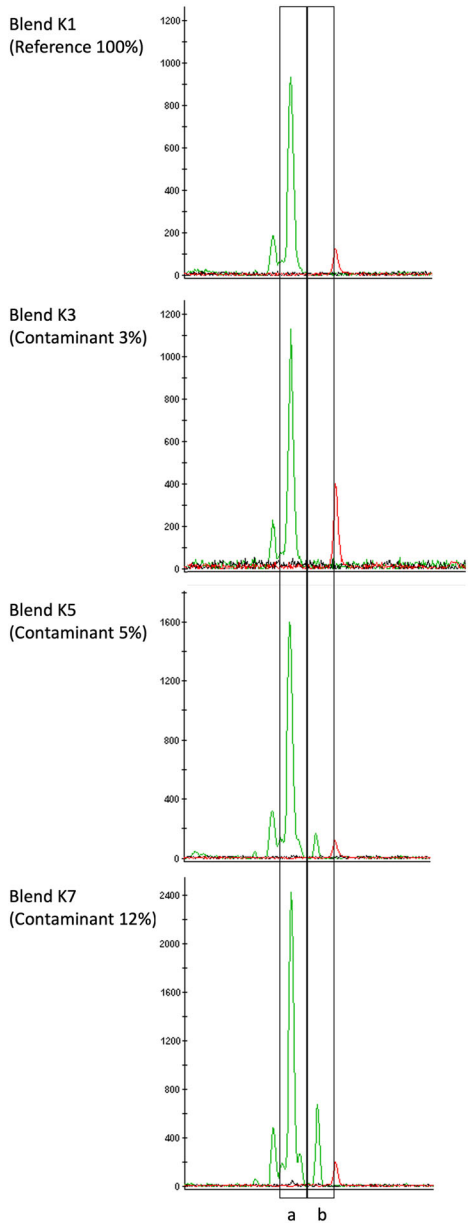

Figure 4. Comparative panel of the *DuPw004* profile in different hand-made blends. The alleles specific of the reference blend (a) and the contaminant variety (b) are indicated.

25 commercial semolina and pasta products by means of 10 SSR markers, out of which nine were for durum wheat and one was for bread wheat and compared the molecular profiles with those obtained from 14 certified durum wheat varieties. SSR markers are widely used to genotype agricultural species, including *Triticum* ones, and their effectiveness in discriminating the durum wheat varieties is consolidated.[35-38] All the markers amplified PCR fragments except the D genome-specific SSR marker *Xgwm003*, for which no amplification product was observed for any sample, indicating that common wheat is absent or present in a such low amount to be undetectable in the commercial samples. Considering all the other markers, a total of 30 alleles were detected, which ranged from 2 (*DuPw115*) to 6 (*Xgwm155*) (see Supporting information, Tables S1 and S2). *Xgwm155* resulted the most polymorphic marker, as also observed in previous studies.[37-39] Among the durum wheat varieties, Nadif and Marco Aurelio appeared to

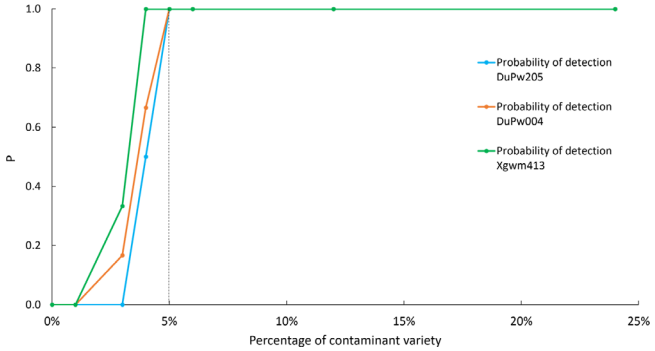

Figure 5. Probability of detection of a contaminant variety in a reference sample, constituted by a blend of four varieties using *DuPw004*, *DuPw205* and *Xgwm413* markers.

be the most similar (Fig. 1). This was rather predictable because these two varieties share a common parent in their pedigree (see Supporting information, Table S3). Instead, Ciccio, Timilia and Khorasan fell into the same cluster, clearly distinguishable from the other ones, indicating that their molecular profile is considerably different from the reference varieties (Fig. 1). Therefore, these cultivars were chosen as the contaminant varieties for the preparation of the hand-made mixtures.

The commercial mixtures presented similar molecular profiles. In particular, an identical profile was observed for S9 and S25 (Group A), S21 and S23 (Group B), S4 and S20 (Group C), S18 and S19 (group D), S15 and S17 (Group E), S5, S7, S10, S11 and S14 (Group F), and S2, S3 and S6 (Group G). The mixtures S1, S8, S12, S13, S16, S22 and S24 presented a unique profile (Fig. 2). All of the samples grouped with the References confirming the presence of these cultivars in the commercial samples.

The commercial samples showed the presence of multiple alleles for most of the SSR markers and the alleles of at least two of the four varieties declared by the producer were present, thus indicating that the mixtures were actually composed of these cultivars. Indeed, as specified by the producer, the pasta was produced with one or more of the four declared varieties. Groups C, D, F and G and the samples S1, S8, S12, S13, S16 and S24 also showed the presence of foreign alleles when amplified with primers corresponding to *DuPw205* and *Xgwm155* (Table 2). The peak height and the area of these alleles were much lower compared to those of the reference alleles, suggesting the presence of a cross-contamination rather than an intentional addition (see Supporting information, Fig. S1). Indeed, cross-contaminations are possible during growing, harvesting and semolina milling practices, leading to the presence of a low percentage of a contaminant variety in the final product.[40]

To visualize the genetic similarities among the 14 varieties and the commercial samples, PCoA was performed. The varieties were clearly distinct from each other's while the mixtures showed overlapping positions. The commercial samples were mainly grouped close to the four reference varieties in the two quadrants on the right of the graph (Fig. 3). The observed allelic pattern along with the results from PCoA suggested that all the commercial mixtures were composed of the same varieties, most likely comprising the References indicated by the producer; however, the presence of one or more contaminant variety was also detected.

Hand-made mixtures and limit of detection

With the purpose to test the utility of SSR markers in the detection of exogenous durum wheat varieties and to calculate the limit of

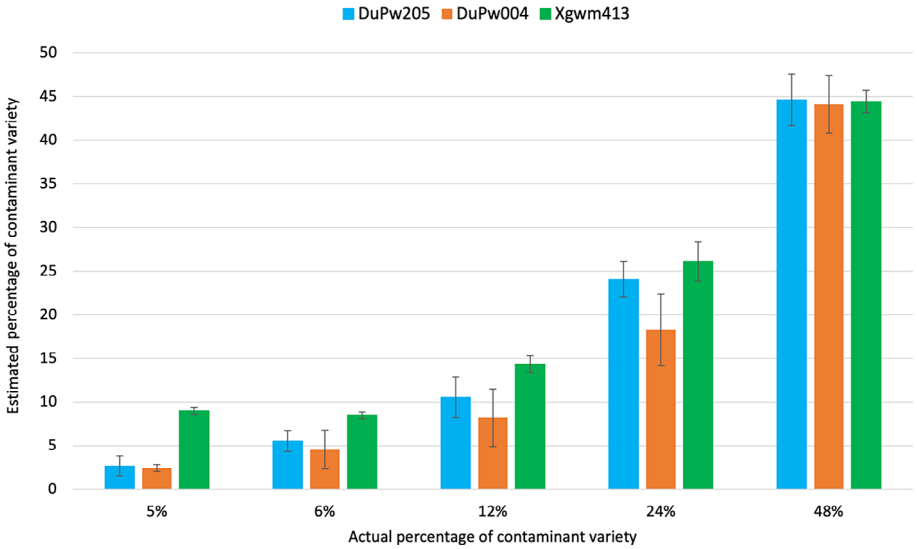

Figure 6. Actual contaminant variety percentages in comparison with those experimentally determined through SSR-based analysis in experimental mixtures.

detection (LOD_6), 27 hand-made semolina mixtures were prepared as described in Table 1 and tested with *DuPw004*, *DuPw205* and *Xgwm413* markers, which highlighted clear distinct allelic profiles between the References and the chosen contaminant varieties. For each marker, the contaminant peak was clearly distinguishable, and the peak height increased linearly with the concentration of the exogenous variety (Fig. 4).

The LOD_6 was calculated for each hand-made blend and the probability of detection was 1 when the percentage of contamination was equal to or greater than 5% (Fig. 5). This value is comparable to that reported in SSR-based traceability studies on other agri-food products, such as bread,[24,39] must[41,42] and olive oil.[43]

The contaminant variety percentage was estimated for each hand-made blend and compared with the actual percentage to estimate the accuracy of the proposed approach (Fig. 6). Using the *DuPw004* marker, the contaminant amount was marginally underestimated at all levels of contamination, whereas an overestimation was generally observed in the case of the *Xgwm413* marker. The *DuPw205* marker was shown to be the most accurate because a marginal underestimation was observed only at lower levels of contamination (up to 6%) (Fig.6; see also Supporting information, Table S4). This is supported by the percentage error or bias, which showed, for this marker, values lower than 13% for the levels of contaminations higher than 5%. For the *DuPw004* and *Xgwm413* markers, the bias decreased for increasing contamination values, indicating a higher accuracy for higher levels of contamination (see Supporting information, Table S4).

SSR-based methods are routinely used in food traceability because of their high level of polymorphism and the ability to be detected on a very small portion of DNA, which, in the case of fragmented DNA isolated from processed foods, may constitute an important advantage.[16,17] Although this approach was previously used for the detection of common wheat in pasta,[24,25] this is the first study in which its effectiveness was demonstrated also in the varietal identification. The proposed approach is simple and versatile, and it was shown to be reliable in the detection of contaminant variety in a percentage equal to or higher than 5%. SSR analysis was also effective in the preliminary estimation of the contaminant amount present in a sample and its sensitivity was shown to be consistent with those observed in traceability studies performed using more recent methods.[28]

CONCLUSIONS

Pasta is one of the most popular Italian foods in the world, and an analytical approach aiming to trace the varieties used for its production is necessary to reassure consumers in terms of transparency and to allow producers to adequately promote their product. Despite the remarkable advances made in molecular approaches, innovative techniques are only marginally used in agri-food authentication, whereas traditional methods, such as those based on SSR markers, are still the tools of choice.

In the present study, the feasibility of an easy SSR-based method to differentiate varieties used to produce commercial pasta was demonstrated. The proposed approach was useful to authenticate different samples of commercial pasta and semolina and detect the presence of a not declared variety. Moreover, the limit of detection of the proposed analytical procedure was estimated (5%) and its effectiveness in the preliminary estimation of the amount of a contaminant variety was demonstrated. Although this approach is not a quantitative method, it may represent a preliminary approach for the identification of the pasta composition and the detection of not declared varieties in a percentage equal to or higher than 5%.

AUTHOR CONTRIBUTIONS

VF, FT and CM were responsible for conceptualization. MD, CDG and WS were responsible for methodology. VF and MD were responsible for software. VF, MD and PDV were responsible for formal analysis. VF was responsible for writing the original draft. VF, MD, FT, CDG, WS, PDV and CM were responsible for reviewing and editing. CM was responsible for project administration.

ACKNOWLEDGEMENTS

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA

(PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This paper reflects only the authors' views and opinions, and neither the European Union nor the European Commission can be considered responsible for them.

Valentina Fanelli was supported by MIUR-PON Ricerca e Innovazione 2014–2020 (project AIM1809249-attività 2, linea 1).

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 De Vita P and Taranto F, Durum wheat (*Triticum turgidum* ssp. *durum*) breeding to meet the challenge of climate change, in *Advances in Plant Breeding Strategies: Cereals*, ed. by Al-Khayri J, Jain SM and Johnson DV. Springer Nature, Switzerland, Cham, pp. 471–524 (2019).
2 Italian Republic President Decree 9th February 2001, n. 187. Available: https://www.gazzettaufficiale.it/atto/serie_generale/caricaDettaglioAtto/originario?atto.dataPubblicazioneGazzetta=2001-0522&atto.codiceRedazionale=001A5636&elenco30giorni=false.
3 Italian Republic President Decree 5th March 2013, n. 41. Available: https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/6230.
4 Troccoli A, Borrelli GM, De Vita P, Fares C and Di Fonzo N, Durum wheat quality: a multidisciplinary concept. *J Cereal Sci* **32**:99–113 (2000).
5 Taranto F, Mangini G, Pasqualone A, Gadaleta A and Blanco A, Mapping and allelic variations of Ppo-B1 and Ppo-B2 gene-related polyphenol oxidase activity in durum wheat. *Mol Breed* **35**:1–10 (2015).
6 De Santis MA, Giuliani MM, Giuzio L, De Vita P, Lovegrove A, Shewry PR *et al.*, Differences in gluten protein composition between old and modern durum wheat genotypes in relation to 20th century breeding in Italy. *Eur J Agron* **87**:19–29 (2017).
7 Mefleh M, Conte P, Fadda C, Giunta F, Piga A, Hassoun G *et al.*, From ancient to old and modern durum wheat varieties: interaction among cultivar traits, management, and technological quality. *J Sci Food Agric* **99**:2059–2067 (2019).
8 Melini V, Melini F and Acquistucci R, Nutritional characterization of an Italian traditional bread from ancient grains: the case study of the durum wheat bread "Pane di Monreale". *Eur Food Res Technol* **247**:193–200 (2021).
9 Pasqualone A, Alba V, Mangini G, Blanco A and Montemurro C, Durum wheat cultivar traceability in PDO Altamura bread by analysis of DNA microsatellites. *Eur Food Res Technol* **230**:723–729 (2010).
10 Visioli G, Giannelli G, Agrimonti C, Spina A and Pasini G, Traceability of Sicilian durum wheat landraces and historical varieties by high molecular weight glutenins footprint. *Agronomy* **11**:143 (2021).
11 Ianiro G, Rizzatti G, Napoli M, Matteo MV, Rinninella E, Mora V *et al.*, A durum wheat variety-based product is effective in reducing symptoms in patients with non-celiac gluten sensitivity: a double-blind randomized cross-over trial. *Nutrients* **11**:712 (2019).
12 Italian Criminal Code, Article n. 515 (Frode nell'esercizio del commercio). Available: https://www.altalex.com/documents/codici-altalex/2014/10/30/codice-penale.
13 Wadood SA, Boli G, Xiaowen Z, Hussain I and Yimin W, Recent development in the application of analytical techniques for the traceability and authenticity of food of plant origin. *Microchem J* **152**:104295 (2020).
14 Pasqualone A, Di Rienzo V, Nasti R, Blanco A, Gomes T and Montemurro C, Traceability of Italian protected designation of origin (PDO) table olives by means of microsatellite molecular markers. *J Agric Food Chem* **61**:3068–3073 (2013).
15 Cibecchini G, Cecere P, Tumino G, Morcia C, Ghizzoni R, Carnevali P *et al.*, A fast, naked-eye assay for varietal traceability in the durum wheat production chain. *Foods* **9**:1691 (2020).
16 Fanelli V, Mascio I, Miazzi MM, Savoia MA, De Giovanni C and Montemurro C, Molecular approaches to agri-food traceability and authentication: an updated review. *Foods* **10**:1644 (2021).
17 Kumar P, Gupta V, Misra A, Modi D and Pandey B, Potential of molecular markers in plant biotechnology. *Plant Omics* **4**:141–162 (2009).
18 Ganopoulos I, Argiriou A and Tsaftaris A, Microsatellite high resolution melting (SSR-HRM) analysis for authenticity testing of protected designation of origin (PDO) sweet cherry products. *Food Control* **22**:532–541 (2011).
19 Di Rienzo V, Fanelli V, Miazzi MM, Savino V, Pasqualone A, Summo C *et al.*, A reliable analytical procedure to discover table grape DNA adulteration in industrial wines and musts. *Acta Hortic* **1188**:365–370 (2017).
20 Sabetta W, Miazzi MM, Di Rienzo V, Pasqualone A and Montemurro C, Development and application of protocols to certify the authenticity and traceability of Apulian typical products in olive sector. *Riv Ital Sostanze Grasse* **94**:37–43 (2017).
21 Gomes S, Breia R, Carvalho T, Carnide V and Martins-Lopes P, Microsatellite high-resolution melting (SSR-HRM) to track olive genotypes: from field to olive oil. *J Food Sci* **83**:2415–2423 (2018).
22 Crawford LM, Carrasquilla-Garcia N, Cook D and Wang SC, Analysis of microsatellites (SSRs) in processed olives as a means of cultivar traceability and authentication. *J Agric Food Chem* **68**:1110–1117 (2020).
23 Piarulli L, Savoia MA, Taranto F, D'Agostino N, Sardaro R, Girone S *et al.*, A robust DNA isolation protocol from filtered commercial olive oil for PCR-based fingerprinting. *Foods* **8**:462 (2019).
24 Pasqualone A, Montemurro C, Grinn-Gofron A, Sonnante G and Blanco A, Detection of soft wheat in semolina and durum wheat bread by analysis of DNA microsatellites. *J Agric Food Chem* **55**:3312–3318 (2007).
25 Sonnante G, Montemurro C, Morgese A, Sabetta W, Blanco A and Pasqualone A, DNA microsatellite region for a reliable quantification of soft wheat adulteration in durum wheat-based foodstuffs by real-time PCR. *J Agric Food Chem* **57**:10199–10204 (2009).
26 Carloni E, Amagliani G, Omiccioli E, Ceppetelli V, Del Mastro M, Rotundo L *et al.*, Validation and application of a quantitative real-time PCR assay to detect common wheat adulteration of durum wheat for pasta production. *Food Chem* **224**:86–91 (2017).
27 Morcia C, Bergami R, Scaramagli S, Ghizzoni R, Carnevali P and Terzi V, A Chip Digital PCR assay for quantification of common wheat contamination in pasta production chain. *Foods* **9**:911 (2020).
28 Morcia C, Terzi V, Ghizzoni R, Vaiuso C, Delogu C, Andreani L *et al.*, Digital PCR for genotype quantification: a case study in a pasta production chain. *Biology* **10**:419 (2021).
29 Gaggiotti S, Shkembi B, Sacchetti G and Compagnone D, Study on volatile markers of pasta quality using GC-MS and a peptide-based gas sensor array. *LWT–Food Sci Technol* **114**:108364 (2019).
30 Sharp PJ, Kreis M, Shewry PR and Gale MD, Location of β-amylases sequences in wheat and its relatives. *Theor Appl Genet* **75**:286–290 (1988).
31 Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P *et al.*, A microsatellite map of wheat. *Genetics* **149**:2007–2023 (1998).
32 Eujayl I, Sorrells ME, Baum M, Wolters P and Powell W, Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. *Theor Appl Genet* **104**:399–407 (2002).
33 Salina EA, Leonova IN, Efremova TT and Röder MS, Wheat genome structure: translocations during the course of polyploidization. *Funct Integr Genomics* **6**:71–80 (2006).
34 Broeders S, Huberb I, Grohmann L, Berben G, Taverniers I, Mazzara M *et al.*, Guidelines for validation of qualitative real-time PCR methods. *Trends Food Sci Technol* **37**:115–126 (2014).
35 Maccaferri M, Stefanelli S, Rotondo F, Tuberosa R and Sanguineti MC, Relationships among durum wheat accessions. I. Comparative analysis of SSR, AFLP, and phenotypic data. *Genome* **50**:373–384 (2007).
36 Laidò G, Mangini G, Taranto F, Gadaleta A, Blanco A, Cattivelli L *et al.*, Genetic diversity and population structure of tetraploid wheats

(*Triticum turgidum* L.) estimated by SSR, DArT and pedigree data. *PLoS One* **8**:e67280 (2013).
37 Uddin MS and Boerner A, Genetic diversity in hexaploid and tetraploid wheat genotypes using microsatellite markers. *Plant Tissue Cult Biotechnol* **18**:65–73 (2008).
38 El-Rawy MA, Assessment of genetic diversity for some Egyptian wheat varieties based on morphological characters and SSR markers. *Sci J Agric Sci* **2**:144–160 (2020).
39 Ramos-Cabrer AM, Fernández-Canto N, Almeida-García F, Gorostidi A, Lombardero-Fernández M, Romero-Rodríguez MÁ *et al.*, Traceability of the local cultivar 'Caaveiro' in flour mixtures used to produce Galician bread by simple sequence repeats and droplet digital polymerase chain reaction technology. *Int J Food Sci* **57**:7085–7098 (2022).
40 Kill RC and Turnbull K eds, *Pasta and Semolina Technology*. Wiley, Hoboken, NJ (2008).
41 Recupero M, Garino C, De Paolis A, Cereti E, Coisson JD, Travaglia F *et al.*, A method to check and discover adulteration of Nebbiolo-based Monovarietal musts: detection of Barbera and Dolcetto cv via SSR analysis coupled with lab-on-Chip® microcapillary electrophoresis. *Food Anal Methods* **6**:952–962 (2013).
42 Di Rienzo V, Miazzi MM, Fanelli V, Savino V, Pollastro S, Colucci F *et al.*, An enhanced analytical procedure to discover table grape DNA adulteration in industrial musts. *Food Control* **60**:124–130 (2016).
43 Chedid E, Rizou M and Kalaitzis P, Application of high-resolution melting combined with DNA-based markers for quantitative analysis of olive oil authenticity and adulteration. *Food Chem: X* **6**:100082 (2020).

Journal of The Science of Food and Agriculture published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.