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DNA authentication of brewery products: basic principles and methodological approaches

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Abstract: Beer DNA authentication is the process of authentication by identification of barley malt *Hordeum vulgare* or its substitutes, as well as hops and yeast. The method is based on molecular genetic analysis of residual quantities of nucleic acids extracted from the cellular debris of the final product. The aim of the study was to analyse scientific and methodical approaches to extraction of residual quantities of beer raw materials nucleic acids and beer DNA authentication for their later application in determining brewing products authenticity. The technological level discloses the method of DNA extraction from wines, modified for extraction of nucleic acids from beer samples. The method includes the following characteristic peculiarities: stage enzymatic hydrolysis of polysaccharides and polypeptides of dissolved lyophilisate, multiple sedimentation and resursuspension of nucleoproteid complex, RNA removal followed by DNA extraction by organic solvents, and additional DNA purification by magnetic particle adsorption. This review presents the analysis of genetic targets used as molecular markers for gene identification of malting barley varieties and beer DNA authentication. We also provided the interpretation of PCR analysis of *Hordeum vulgare* varieties and samples of commercial beer. Data on SSR- and SNP-markers of *Hordeum vulgare* nuclear DNA, used for barley varieties identification and potentially suitable for beer DNA authentication, are also presented. We also analysed genetic targets used in malting barley substitute detection, as well as hops and yeast identification in beer. Data on correlation of amplified DNA targets with beer quality indicators were systematised.

Keywords: Alcoholic beverages, malting barley, *Hordeum vulgare*, DNA, authentication, identification, marker, PCR

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INTRODUCTION

Wide assortment of brewery products and their multicomponent composition refers them to the segment of difficult-to-identify goods. Their authentication is aimed at protecting consumers and manufacturers' rights [1].

One of the strategically important tasks achievable by multidisciplinary science-intensive approaches is the search for objective identification criteria with a high degree of authenticity assessment of brewery products [2].

Molecular and genetic research methods can provide the technological process of DNA authentication of beer brands [3], thereby expanding the complex scheme of brewery products identification, traditionally based on documentary, visual, sensory and physical and chemical analyses [4].

Beer brands DNA authentication is a technological process of the authenticity verification by the gene identification of *Hordeum vulgare* barley malt, or its substitutes, as well as its key ingredients – hops and yeast, by molecular genetic analysis of residual quantities of nucleic acids extracted from the cellular debris of the products [3].

The analysis of scientific and methodological approaches points to the applicability of DNA technologies for detecting counterfeit and falsified brewery products.

RESULTS AND DISCUSSION

Extraction of DNA residues of beer raw materials. The technological level discloses a method for DNA extraction from wines [5, 6]. It was later

modified for extraction of nucleic acids from beer samples [3]. The method includes the following characteristic peculiarities: stage enzymatic hydrolysis of polysaccharides and polypeptides of dissolved lyophilisate, multiple sedimentation and resursuspension of nucleoproteid complex, RNA removal followed by DNA extraction by organic solvents, and additional DNA purification by magnetic particle adsorption.

Figure 1 demonstrates stages of DNA extraction according to the modified method. In particular, enzymatic hydrolysis of polysaccharides by α-amylase (*Bacillus licheniformis*) takes 3 h instead of 1 h, when DNA is extracted from wines [3, 5]. The time of enzymatic hydrolysis of polypeptides by proteinase K (*Tritirachium album*) is also increased up to 3 h. The sedimentation time of non-hydrolysed cellular debris by centrifugation at 8000 g is reduced to 1 min instead of 15 min when DNA is extracted from wines. At the stage of DNA extraction from the lyophilised beer powder, the sedimentation of the nucleoprotein complex is carried out by mixing the supernatant with two volumes of cold absolute ethanol instead of two volumes of cold isopropanol. At the next stage we mixed a solution of unpurified DNA with an equal volume of 70% ethanol. The maturing of the mixture at 0°C takes 3 min instead of 10 min, as with wines. During the subsequent nucleoprotein complex sedimentation, along with the stepwise addition of 10 μL of 3M sodium acetate and two volumes of cold isopropanol to the pre-transferred transparent supernatant, 3 μL of Ethachinmate linear polyacrylamide is added. After RNA removal and deproteinisation, the sedimentation of purified DNA is carried out without adding 70% ethanol. (Cf. DNA extraction from wines involves in the nucleic acids sedimentation in 0.2 M NaCl and two volumes of cold ethanol, followed by washing with 70% ethanol). Later, nucleic acids precipitate, resuspended in the elution buffer, undergoes an additional purification by adsorption on magnetic particles, which is one of the key modification elements of the method for extracting residual DNA of beer raw materials [3].

The ability of magnetic particles to bind DNA reversibly and easily be deposited from the suspension in the magnetic field ensures high quality of nucleic acids purification and their preservation. Magnetic particles, as a rule, are a paramagnetic core with a highly developed surface covered with a polymer film with exposed covalent-bond carboxylic groups. Magnetic tripods, used in manual and automated modes, are made of neodymium magnets resistant to demagnetisation.

The additional purification by adsorption on magnetic particles of the modified method of extraction of nucleic acids from beer samples actually took the place of polymer polyvinylpyrrolidone widely used to reduce the inhibitory effect of polyphenols on PCR [3, 7–10].

Approaches to beer DNA authentication. Genetic targets, used as molecular markers for malting barley varieties identification, can also be analysed for commercial beer DNA authentication (Table 1) [3].

Polygalacturonase is an enzyme that performs hydrolytic cleavage of α-1,4-glycoside bonds in pectin. The DNA target was the locus of its gene (*HvPG1*) eamplified by a corresponding pair of primers constructed by Pulido *et al.* based on the analysis of expressed sequence tag (EST) deposited in GenBank (A/N: EF427919) [11]. The generated PCR products *a* and *b* of the *HvPG1* gene locus detected in the barley and beer samples were 89% and 79% identical to the previously deposited nucleotide sequence mRNA polygalacturonase *Hordeum vulgare*. Among the studied Japanese barley varieties, only the high quality 'Ryofu', recommended for brewing, generated two discrete fragments (*a*, *b*), like most American and Australian barley varieties, except for Stimling (Table 2). All the beer samples were marked only by the country of manufacture. They generated the PCR product *b* and more than half of the samples generated the additional fragment *a* (Table 2). The analysed DNA target was included in the group of DNA markers of identification and differentiation of beer samples, but did not correlate with the indicators of beer quality [3].

Hordeins are polymorphic proteins of barley grain coded by 7 *HrdA-G loci* which are localised in the short arm of the 5th *Hordeum vulgare* chromosome [12, 13]. Due to the established connection of the hordein-coding loci alleles with brewing qualities of barley grain, this block of targets is a priority for molecular and genetic analysis [14, 15]. From the three analysed loci (*HrdA*, *HrdB* and *HrdC*) only one (*HrdC*) was able to identify a single sample of beer out of 22 investigated by the presence of a specific PCR product *e* (Table 2) [3]. However, high variability of *HrdA* locus (up to 90% identity of nucleotide sequences of compared barley varieties with corresponding reference sequence (GenBank A/N: AF474373) indicates a certain potential of DNA authentication of beer on the analysed target by sequencing the amplified locus. The block of DNA targets under study also did not correlate with the indicators of beer quality [3].

Amylosis content in barley starch influences the quality of malt barley. Therefore, waxy-barley varieties may be a preferred option for their malting in brewing because starch with low amylosis content is more susceptible to enzymatic hydrolysis [18]. Molecular mechanism is embedded *in Hordeum vulgare* waxy-genes located on 7 HS chromosome. They lead to the elimination of granule-bound starch synthase (GBSS) [18, 19]. Primers selected for *Waxy*-locus amplification had the positive control status due to generation of specific PCR product in all the samples of barley and beer [3]. Their sequeneed nucleotide DNA sequences were identical to each other and showed 98% identity to the corresponding reference *Hordeum vulgare subsp. Vulgare* sequence, previously deposited to GenBank (A/N: X07931) [20].

1. LIOPHILISED BEER POWDER RESUSPENDING

Lyophilisate dissolving in 500 µL of resuspending buffer (0.1M Tris-HCl (pH 8.0), 0.1 MNaCl)

2. POLYSACCHARIDES ENZYMATIC HYDROLYSIS

Beer suspension processing with 100 µL of thermostable α-amylase (*Bacillus licheniformis*)

Incubating the produced mixture at 80°C for 3 h

3. POLYPEPTIDES ENZYMATIC HYDROLYSIS

Suspension processing with 100 µL of proteinase K (*Tritirachium album*) with 0.2% SDS

Incubating the produced mixture at 55°C for 3 h

4. NON-HYDROLYZED CELL DEBRIS SEDIMENTATION

Centrifugation at 8000 g for 1 min at 40°C

Supernatant transfer to a new tube

5. NUCLEOPROTEID COMPLEX SEDIMENTATION

Mixing the supernatant with two volumes of cold absolute ethanol and holding the mixture at 0°C (on ice) for 15 min

Centrifugation at 8000 g for 15 min at 4°C

6. NUCLEOPROTEID COMPLEX RESUSPENDING

Sediment resuspending in 300 µL of elution buffer (0.1M Tris-HCl (pH 8.0), 0.1M EDTA)

7. NUCLEOPROTEID COMPLEX SEDIMENTATION

Mixing the crude DNA solution with an equal volume of cold 70% ethanol and holding the mixture at 0°C (on ice) for 3 min

Transfer the clear supernatant to a new tube and stepwise addition of 10 µL of 3M sodium acetate, 3 µL of linear polyacrylamide Ethachinmate and 2 volumes of cold isopropanol

Centrifugation at 8000 g for 15 min at 4°C

8. NUCLEOPROTEID COMPLEX RESUSPENDING

Sediment resuspending in 300 µL of elution buffer (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA)

9. RNA REMOVAL

Suspension Treatment with RNAse A at 55°C for 30 min

10. DEPROTEINISATION

Extraction with equal volume of neutral phenol

Recovery of the aqueous phase by centrifugation at 8000 g for 15 min (4°C)

Extraction with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1)

Recovery of the aqueous phase by centrifugation at 8000 g for 15 min at 4°C

11. SEDIMENTATION OF PURIFIED DNA

Repeat stage 7 without adding 70% ethanol

12. RESUSPENDING OF SEDIMENTAL DNA

Sediment resuspending in 125 µL of elution buffer (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA)

13. ADDITIONAL STAGE OF DNA PURIFICATION BY ADSORPTION ON MAGNETIC PARTICLES

Figure 1 Stages of DNA extraction from lyophilised beer powder

Hemicelluloses are vegetable homo- and heteropolysaccharides, which are an integral part of the endosperm cell walls. The highest content of xylanes was reported to be among the main components of hemicellulose [21]. The malt barley softens as a result of the decomposition of the cell wall. Xylanase is involved in the degradation of xylanes to xylooligosarachides, whose gene locus was used as a target for primers originally designed for DNA analysis of rice samples [3]. It is noteworthy that among the 16 varieties of barley, only three varieties (Metcalfe, Nishinohoshi and Ryofu) showed a positive amplification signal (Table 2). At the same time, due to possible obtaining inconclusive data, the authors [3] presented neither the results of PCR of beer samples, nor data on amplification of the *HrdB* locus.

Barley Z proteins are the main beer protein which influence beer quality, especially foam stability [22–24]. In addition, Z4 and Z7 proteins can be used as positive and negative markers of foam stability [25]. DNA-markers of foam stability developed by Limure *et al.* were also used in by Nakamura *et al.* for barley varieties identification and beer DNA-authentication [3, 25]. Identifying and differentiating barley and beer samples procedure by the gene locus, encoding proteins Z4 and Z7 differ. In the first case PCR analysis is performed by interpreting three discrete PCR products (*h, i-a, i-b*), and in the second –by the presence or absence of a specific fragment *j*.

Based on the analysis, the authors recommended the further use of the tested primers for amplification of the analysed gene loci [3]. In addition, a negative correlation of the amplified PCR product *h* gene locus encoding Z7 protein with beer bitterness, as well as a positive correlation of PCR product *i-a* similar locus with foam stability (Table 1) were revealed.

Many enzymes, incl. α-amylase and β-amylase, are activated in the malting process [26, 27]. Their substrates are amylosis and amylopectin or products

Table 1 Genetic targets used as molecular markers for brewing barley varieties identification and beer DNA authentication

Target	PCR product	Primer sequence	Correlation (+/-)	Source
Polygalacturonase (*HvPG1*)	*a*	F: 5′-GACAGAATGGCGTTCAAGAACAT-3′	N/A	[3, 11]
	b	R: 5′-AGCAAGTTGCCTTCCAGCTTGAT-3′	N/A	
Hordein A (*HrdA*)	c	F: 5′-AGATAGCGTTTTGAAGGTCAC-3′ R: 5′-TAGACCTGCAATAATTTCCA-3′	N/A	[3, 16]
Hordein B (*HrdB*)	*d-1*	F: 5′-TCACACATAAGGTTGTGTGAC-3′	N/A	[3, 17]
	d-2	R: 5′-CAAGCTTTCCCACAACAACCA-3′	N/A	
Hordein C (*HrdC*)	*e*	F: 5′-AATTTAAACAACTAGTTTCGGGTGG-3′ R: 5′-CAAGCTTTCCCACAACAACCACCAT-3′	N/A	[3, 16]
Barley starch synthase (*waxy*)	*f*	F: 5′-CAATTCATCCGATCACTCAATCAT-3′ R: 5′- CAGGCCGACAAGGTGCTG -3′	N/A	[3, 16]
Xylanase	*g*	F: 5′-GGTACAACGTCGCGTCGG-3′ R: 5′-CGTGTACCAGACGGTCCAGATACAGC-3′	N/A	[3, 21]
Protein Z7	*h*	F: 5′-GGTCACATGACGTGTATTAATCTCC-3′	–*	[3, 24]
	i-a	R: 5′-CGTTGGTGGCAGCAGACTCGGGG-3′	+**	
	i-b		N/A	
Protein Z4	*j*	F: 5′-GAGACGTGTAGTAATCTTCG-3′ R: 5′-GCGAGCACAAATTGCACCACC-3′	–***	[3, 24]
α-amylase	*k*	F: 5′-AAGGTCTCGTGTCGATCCCAAGGAGGC-3′ R: 5′-CTAAGCCTCGTCTTCGTCCCC-3′	N/A	[3]
Barley lipoxygenase (*LOX1*)	*l*	F: 5′-GCAACGGAGGGAGTAAAACA-3′ R: 5′-CGATGGCTTGGACCAATTAC-3′	+****	[3, 34]
Barley yellow mosaic virus (*rym5*)	*m*	F: 5′-GAGTCGTCACAACGTACCTTGC-3′ R: 5′-GTGGCTGTAAATAGGCTAAGGCC-3′	N/A	[3, 34]
Barley powdery mildew (*mlo*)	*n*	F: 5′-TAGCAATCACGGTCACGTCAAC-3′	N/A	[3, 34]
	o	R: 5′-CCGCAAGGCTGCTATGAAAAGGG-3′	N/A	
Barley trypsin inhibitor (*Itr1*)	p	F: 5′-CAACTAACAGAAAGTCAGAAAGCAC-3′ R: 5′-CACAATACTGAAAATACTCTGATGC-3′	–*****	[3, 37]
Barley β-glucanase (*HvCslF6*)	*s*	F: 5′-GCCAAGACCAAGTACGAGAAGC-3′ R: 5′-TGTTCTTGGAGAAGAAGATCTCG-3′	N/A	[3, 40]

–* a negative correlation of the amplified PCR product *h* of the gene locus encoding the protein Z7 with beer bitterness
+** a positive correlation of the amplified PCR product *i-a* of the gene locus encoding the protein Z7 with foam stability
–*** a negative correlation of the amplified PCR product *j* of the gene locus encoding the protein Z4 with the detectable PCR product *h* of the gene locus encoding the protein Z7
+**** a positive correlation of the amplified DNA target with beer taste saturation
–***** a negative correlation of the detected DNA matrix with the saturation of beer taste
N/A not applicable

Table 2 Interpreted results of PCR analysis of brewing barley varieties and beer samples

Barley varieties	PCR products																		
	a	*b*	*c*	*d–1*	*d–1*	*e*	*f*	*g*	*h*	*i–a*	*i–b*	*j*	*k*	*l*	*m*	*n*	*o*	*p*	*s*
Vlamingh	+	+	–	+	+	–	+	–	–	–	+	–	+	–	–	–	–	–	–
Hamelin	+	+	–	+	+	+	+	–	+	+	+	–	+	–	–	–	–	+	–
Stimling	–	+	–	–	+	+	+	–	–	+	–	–	–	–	–	–	–	–	+
Bardin	+	+	–	–	+	+	+	–	–	+	–	+	+	–	–	–	–	+	+
Salute	+	+	+	–	+	–	+	–	–	–	+	–	+	–	–	–	–	–	+
Schouner	+	+	–	+	+	–	+	–	–	+	–	+	–	–	–	–	–	+	–
Maritime	+	+	–	–	+	+	+	–	–	–	+	+	+	–	–	+	–	+	+
Flag ship	+	+	–	–	+	–	+	–	–	+	–	–	–	–	–	–	–	+	+
Metkafe	+	+	–	+	+	–	+	+	+	+	+	+	–	+	+	+	–	+	+
Harushizuku	–	+	+	+	–	–	+	–	–	+	–	+	+	–	–	–	+	–	–
Houshun	+	–	+	+	–	–	+	–	–	+	–	–	–	–	+	+	–	–	–
Mikamogolden	–	–	+	+	–	–	+	–	–	–	+	+	–	–	+	–	+	–	–
Skygolden	–	+	+	+	–	+	+	–	–	–	+	+	+	–	+	–	+	–	–
Nishinohoshi	–	–	+	+	–	+	+	+	–	+	–	+	–	+	+	+	–	–	+
Nishinochikara	–	–	–	+	–	+	+	–	–	+	–	+	+	+	+	–	+	+	+
Ryofu	+	+	+	+	–	+	+	+	–	–	+	+	+	+	+	+	–	+	+
Samples of beer	PCR products																		
	a	*b*	*c*	*d–1*	*d–1*	*e*	*f*	*g*	*h*	*i–a*	*i–b*	*j*	*k*	*l*	*m*	*n*	*o*	*p*	*s*
Czechoslovakia–a	+	+	+	n/a	n/a	–	+	n/a	+	+	+	–	+	+	n/a	n/a	n/a	+	+
USA–a	–	+	+	n/a	n/a	–	+	n/a	–	+	–	–	+	+	n/a	n/a	n/a	+	+
Belgium–a	–	+	+	n/a	n/a	–	+	n/a	+	+	+	–	–	+	n/a	n/a	n/a	–	+
USA–b	+	+	+	n/a	n/a	–	+	n/a	+	+	+	+	+	+	n/a	n/a	n/a	–	+
Netherlands–a	+	+	+	n/a	n/a	+	+	n/a	–	+	+	–	+	+	n/a	n/a	n/a	+	+
Thailand–a	+	+	+	n/a	n/a	–	+	n/a	–	+	+	–	+	+	n/a	n/a	n/a	–	+
Denmark–a	+	+	+	n/a	n/a	–	+	n/a	–	+	+	–	+	–	n/a	n/a	n/a	–	+
England–a	–	+	+	n/a	n/a	–	+	n/a	–	+	+	–	+	+	n/a	n/a	n/a	–	+
Germany–a	–	+	+	n/a	n/a	–	+	n/a	–	+	–	–	+	+	n/a	n/a	n/a	–	+
Australia–a	–	+	+	n/a	n/a	–	+	n/a	–	+	–	–	+	–	n/a	n/a	n/a	+	+
Mexico–a	–	+	+	n/a	n/a	–	+	n/a	–	+	–	–	+	+	n/a	n/a	n/a	–	+
USA–c	+	+	+	n/a	n/a	–	+	n/a	+	+	+	–	+	+	n/a	n/a	n/a	+	+
Germany–b	+	+	+	n/a	n/a	–	+	n/a	+	+	+	–	+	+	n/a	n/a	n/a	–	+
England–b	–	+	+	n/a	n/a	–	+	n/a	–	+	+	–	+	+	n/a	n/a	n/a	+	+
Peru–a	–	+	+	n/a	n/a	–	+	n/a	–	+	–	–	+	+	n/a	n/a	n/a	–	+
England–c	+	+	+	n/a	n/a	–	+	n/a	+	+	–	+	–	–	n/a	n/a	n/a	+	+
Germany–c	+	+	+	n/a	n/a	–	+	n/a	+	+	–	+	–	–	n/a	n/a	n/a	+	–
Italy–a	+	+	+	n/a	n/a	–	+	n/a	+	–	–	+	+	–	n/a	n/a	n/a	+	+
Japan–a	+	+	+	n/a	n/a	–	+	n/a	+	–	–	+	+	–	n/a	n/a	n/a	+	–
Japan–b	+	+	+	n/a	n/a	–	+	n/a	+	+	–	–	+	–	n/a	n/a	n/a	+	–
Japan–c	+	+	+	n/a	n/a	–	+	n/a	–	+	–	–	+	+	n/a	n/a	n/a	–	+
Japan–d	+	+	+	n/a	n/a	–	+	n/a	+	–	–	–	+	–	n/a	n/a	n/a	+	+

+ a positive amplification signal
– a negative amplification signal
n/a not applicable

of their hydrolysis. Primers developed on the basis of nucleotide sequence of the gene locus encoding α-amylase initiated the amplification of PCR product *k* in most of the barley varieties and beer samples (Table 2) [3, 28]. It is noteworthy that the amino acid sequence of the target had 69% identity with *Mla*-locus of resistance to powdery mildew *Hordeum vulgare* (GenBank A/N: AF427791) [29]. The used set of primers was included in the group of molecular labeling systems of barley varieties, and therefore has a certain potential of practical application for beer authentication, although the authors did not mention it [3].

Lipoxygenase-deficient barley varieties with reduced or lost activity of *LOX* genes have a positive impact on quality indicators such as beer taste and foam stability [30–33]. The set of primers constructed by Nagamine *et al.* resulted in amplification of the specific PCR product *l* in a small number of studied barley varieties and in more than half of beer samples, whose sequenced nucleotide sequences had 99% identity with the reference sequence

of locus *LoxA*-gene *Hordeum vulgare* (GenBank A/N: L35931) [3, 34, 35]. The tested set of primers was recommended for further use in the amplification of the analysed gene locus for barley varieties identification and beer brands differentiation. It should also be noted that the authors [3] additionally revealed a positive correlation between the amplified DNA target and beer taste saturation (Table 1).

The selection of barley varieties with genetic resistance to viral, bacterial and fungal diseases is aimed at high-quality grain production [36]. A number of DNA markers of resistance of barley to yellow mosaic virus (*rym5*-locus) and powdery mildew (*mlo*-locus) [34] integrated into breeding programs can also be used in molecular labelling of brewing barley varieties, which is clearly demonstrated in the work [3]. The authors interpreted the PCR analysis data of barley samples taking into account the presence or absence of specific PCR products *m* (*rym*), *n* and *o* (*mlo*) recorded on the corresponding electrophoregrams. But the results of the PCR analysis of beer samples and their correlation with quality indicators were not provided [3].

Protein inhibitors of proteolytic enzymes play an important role both in formation of homeostatic reactions in plants and in the process of seed maturation and germination. Selected primers to the trypsin inhibitor (*Itr1*) gene locus led to the amplification of the specific PCR product *p* in half of the tested barley varieties and beer samples [37]. Thus, the DNA marker was concluded to be highly informative [3]. Additionally, the DNA sequences of the *Itr1*-gene locus of the material had 94% identity with the same locus of the *Hordeum vulgare subsp. vulgare gene* (GenBank A/N: (X65875) [38]. Also, in the study [3] a negative correlation of the detected DNA matrix with beer taste saturation was revealed (Table 1).

The content (1–3, 1–4) of β-D-glucan in barley grain, which determines its hardness, is much higher compared to other cereals [39]. However, for barley varieties used in brewing, a lower the content of this polysaccharide in the grain is desirable in order to achieve a more effective flow of the malting process [40]. The amplification procedure of the locus of the *HvCslF6* gene with a selected primer pair led to the production of a specific PCR product *s* in a number of American, Australian and Japanese brewing barley varieties [3, 40]. The most of the beer samples also gave a positive amplification signal (Table 2). The obtained amino acid sequence of the target had 83% identity with *Hordeum vulgare CslF6*-gene (GenBank A/N: EU267181) [41]. The used primer set was also included in the group of systems of barley varieties molecular labelling and beer DNA-authentication [3].

Microsatellites are widely used molecular markers which are suitable for identification of *Hordeum vulgare*. A wide variety of SSR-markers are being used [42–44]. Tomka *et al*. described a high potential of the five SSR-markers for brewing barley varieties identification [45].

Table 3 shows the sequence of oligonucleotide primers of the corresponding *Hordeum vulgare* SSR-markers of nuclear DNA, as well as the range of lengths of detected alleles and their number. The genetic identification procedure includes PCR method with subsequent data interpretation by horizontal or vertical gel electrophoresis and DNA fragmentary analysis of capillary gel electrophoresis. The SSR-markers, potentially suitable for beer DNA authentication, are advisable to test in the formulation of single PCR, with a set of primers of a single SSR-marker to achieve a reproducible result.

Alongside with SSR-markers, SNP-markers, used for barley varieties identification, including brewing ones, also have high identification capacity [46–48].

Table 4 shows oligonucleotide primers sequences of the corresponding SNP markers of *Hordeum vulgare* nuclear DNA, as well as the size of amplified loci of discriminated alleles [46]. The procedure of gene identification is carried out by the Amplification Refractory Mutation System (ARSM-PCR), followed by data interpretation by horizontal gel electrophoresis or by high resolution melting curves (HRM) analysis on PCR platforms in real time. It should be mentioned that we selected five SNP-markers (out of nine described by Chiapparino *et al.* [46] as potentially suitable for beer DNA authentication due to generation of relatively small allele-specific PCR products, whose size was not more than 200 bp (Table 4).

Table 3 SSR-markers of nuclear DNA *Hordeum vulgare* used for genetic identification of brewing barley varieties, potentially suitable for beer DNA authentication

No.	SSR marker	The sequence of oligonucleotide primers	Allel lenghts, bp	Number of alleles
1	*Bmac 0040*	F: 5′-AGCCCGATCAGATTTACG-3′ R: 5′-TTCTCCCTTTGGTCCTTG-3′	196-226 bp (196/200/208/214/220/226)	6
2	*Bmac 0134*	F: 5′-CCAACTGAGTCGATCTCG-3′ R: 5′-CTTCGTTGCTTCTCTACCTT-3′	140-174 bp (140/144/162/168/174)	5
3	*Bmag 0125*	F: 5′-AATTAGCGAGAACAAAATCAC-3′ R: 5′-AGATAACGATGCACCACC-3′	128-148 bp (128/132/138/144/148)	5
4	*Bmag 0211*	F: 5′-ATTCATCGATCTTGTATTAGTCC-3′ R: 5′-ACATCATGTCGATCAAAGC-3′	150-170 bp (150/154/162/170	4
5	*Bmag 0222*	F: 5′-ATGCTACTCTGGAGTGGAGTA-3′ R: 5′-GACCTTCAACTTTGCCTTATA-3′	140-178 bp (140/144/162/168/170/174/178)	7

Table 4 SSR-markers of *Hordeum vulgare* nuclear DNA used for brewing barley varieties identification, potentially suitable for beer DNA authentication

No.	Locus (position)	The sequence of oligonucleotide primers	PCR product, bp
1	MWG2062 (325 A-G)	FOP: 5′-GTTGTGTCAAGCATATCGGTTGCTCTT-3′ ROP: 5′-CAGCACGTTCGAAAACAATAGGATCC-3′	198 bp
		FIP: 5′-AAGAATTATGCCAATTATTGGCGTGTCA-3′	101 bp (A allele)
		RIP: 5′-CACACTGCATGTCATCAAACAAGCAC-3′	151 bp (G allele)
2	ABC465 (254 C-T)	FOP: 5′-CAGGTACACCTGGAAGCTCTACTCAGAG-3′ ROP: 5′-CAGCAGCCTGAATTCAACAAAACATAC-3′	236 bp
		FIP: 5′-TGGAGATGTTCTACGCTCTCAAGTACAGT-3′	130 bp (T allele)
		RIP: 5′-CTGTTGGTCAGATAACCTACCAGGATG-3′	162 bp (C allele)
3	MWG2218 (175 G-C)	FOP: 5′-CTCTCCGACATCGACCGCTTCCTCTTCG-3′ ROP: 5′-GCCGCATCATCCCTGGTGTCATCACCT-3′	215 bp
		FIP: 5′-GGGGACGTCATCCACGTCTGTCGACC-3′	127 bp (C allele)
		RIP: 5′-GTTCCCGCGGTGGGCTTTGTTTCCTC-3′	140 bp (G allele)
4	ABC156 (231 T-G)	FOP: 5′-CTTGGTCCATATAGGTCTCTCTTTTC-3′ ROP: 5′-CCTCCTGATATACTTGAGAGACTCAATA	74 bp
		FIP: 5′-TCCATATAGGTCTCTCTTTTCTTATTATG-3′	70 bp (G allele)
		RIP: 5′-TGAGAGACTCAATACTCATGAATTTCA-3′	60 bp (T allele)
5	MWG801 (344 G-A)	FOP: 5′- CAACAACCCCAATACCAGGCCAGCTCCACA-3′ ROP: 5′-AACCCTCGACTGCTCAAGGCAGAGCCGC-3′	256 bp
		FIP: 5′-GAAGCATGCTCGCACGACACCCATCC-3′	175 bp (C allele)
		RIP: 5′-CGGCAGCGGAGGGGAAGGGGAGCAGT-3′	133 bp (A allele)

FOP is a forward outer primer; ROP is a reverse outer primer; FIP is a forward inner primer; and RIP is a reverse inner primer

The detection of brewing barley substitutes in beer, which is often used as a cheap source of starch, makes it possible to evaluate the products sold for qualitative, quantitative, information and complex falsification. Table 5 demonstrates primer sequences targeting genetic targets used in the detection of brewing barley substitutes in beer, such as granule-bound starch synthase of rice, β-conglycinin of soya, and zein of maize [49, 50]. Nevertheless, other PCR systems developed for the identification of cereals in food products can also be suitable for beer DNA authentication [51].

The effect of hops and yeast on beer quality is well-known. Thus, hop has a bactericidal effect on beer as well as provides its bitterness, aroma and foam stability [52]. Yeast is used in beer fermentation and

Table 5 Genetic targets used in detecting brewing barley substitutes and identifying hops and yeast in beer

Target	PCR product	Primer sequence	Correlation (+/–)	Source
GBSS (rice)	*t*	F: 5′-GGATGAAGGCCGGAATCCTG R: 5′-CTTGCCCGGATACTTCTCCT	missing	[3, 49]
B-conglycinin	*u*	F: 5′-TTTGGCATTGCTTACTGGGAAAAAGAG R: 5′-TCTGTAGGAGTCTCTGTCGTCGTTG	missing	[3, 50]
Zein	v	F: 5′-CACATGTGTAAAGGTGAAGCGAT R: 5′-GCTCGCCGCAAGCGCTTGTTG	missing	[3, 49]
Hop-a	*w*	F: 5′-GGAACCGTTGCCTAATCCTAAGATT R: 5′-GTGTTTTCCGTATCTACGCGCTGGG	missing	[3]
Hop-b	*x*	F: 5′-AATTAGGGCATGCCATGAATATT R: 5′-TGGCATAGTTAAATTATTTCG	–* –**	[3]
Hop-c	*y*	F: 5′-AAATAAAACTTTACATGTGATA R: 5′-CTGAATTGTCGGCGT	missing	[3]
Yeast-a (*S. cerevisiae*)	*z-a*	F: 5′-GTTTTGCGCTCATTAAAACCTAGTGGGAG R: 5′-GTCATTTTTTTTAGTGGTGCTAATC	+*** –****	[3]
Yeast-b (thioredoxin)	*z-b*	F: 5′-ATGGTCACTCAATTAAAATCCGCTTCT R: 5′-CTATACGTTGGAAGCAATAGCTTGCTTG	missing	[3]

–* a negative correlation of the amplified PCR product *t* of the corresponding locus of the hop gene (Hop-b) with beer bitterness
–** a negative correlation of the amplified PCR product *t* of the corresponding hop gene locus (Hop-b) with beer astringency
+*** a positive correlation of the amplified PCR product *z-a* of the corresponding yeast gene locus with beer acidity
–*** a negative correlation of the amplified PCR product *z-a* of corresponding locus of the *S. cerevisiae* gene yeast with beer umami
N/A is not applicable

impacts its character and taste [53]. Table 5 also presents sets of primers which initiate the amplification of specific PCR products of the corresponding loci of hops and yeast genes. They also allow the identifying or differentiating of commercial beer samples [3]. In addition, a negative correlation of the amplified PCR product *t* of the corresponding locus of the hop gene (Hop-b) with beer bitterness and astringency was revealed. The amplified PCR product *z-a* of the corresponding locus of the yeast gene *S. cerevisiae* showed a positive correlation with beer acidity and a negative correlation with beer umami [3]. Taking into account the rapid development of genomic and bioinformation technologies, metagenomic analysis, which allows determining yeast species diversity in beer samples without microorganisms allocating and cultivating, is one of the promising approaches to beer DNA authentication [54, 55].

CONCLUSION

Analysis of scientific and methodical approaches to extraction of residual quantities of nucleic acids of beer raw materials and beer DNA-authentication indicates the applicability of molecular and genetic analysis in detecting counterfeit and falsified brewery products. The use of DNA technologies helps determine the authenticity and origin of the brewery industry products. Molecular labelling systems suitable for identification of *Hordeum vulgare* barley malt, or its substitutes, as well as hops and yeast, can ensure traceability of the product life cycle. Systematic data on correlation of amplified DNA targets with beer quality indicators can be of practical importance when choosing raw materials for brewery production.

CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

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