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ORIGINAL PAPER

Systematic multi-year surveillance of honey compliance and fraud in North Macedonia, 2020–2024

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Abstract

Honey adulteration represents a significant food safety and economic concern globally, yet comprehensive data from North Macedonia remains lacking. This study evaluated 538 honey samples (391 domestic, 147 imported) collected between 2020 and 2024 for compliance with national and EU quality standards. Overall, 9.48% of samples failed at least one quality parameter, with domestic honey showing higher non-compliance (12.53%) than imported (1.4%). The primary causes of non-compliance were elevated hydroxymethylfurfural (64.7%) and reduced diastase activity (43.1%), indicating heat-related exposure. Among 16 samples failing C4 sugar analysis, 7 (43.8%) met all other regulatory requirements, suggesting sophisticated adulteration methods that evade routine testing. Statistical analysis revealed very strong associations between heat-related parameters. Temporal analysis showed declining overall non-compliance but increasing prevalence of diastase-related failures. These findings, representing the first systematic assessment of honey adulteration in North Macedonia, indicate that while basic market surveillance appears effective, current testing protocols may miss economically motivated adulteration. Implementation of routine C4 sugar analysis, particularly for samples passing conventional parameters, is essential for comprehensive fraud detection and consumer protection.

Keywords Honey authentication · Physicochemical compliance · Hydroxymethylfurfural · Diastase activity · Food fraud surveillance · C4 sugar adulteration

Introduction

Food fraud, defined as the deliberate adulteration or misrepresentation of food products, presents persistent public health and economic concerns, particularly for high-value commodities [1–3]. Evidence indicates a rise in such activities, notably during the COVID-19 pandemic, prompting evolving preventative measures from regulatory bodies worldwide [3–7].

Among food products susceptible to fraud, honey has emerged as a particularly attractive target due to its high market value, consumer preference for natural products, and the substantial profit margins achievable through adulteration [8–12]. Consumers often choose honey as a natural sweetener, recognizing it for more than just its taste. It contains a mix of antioxidants, small amounts of vitamins, and minerals, which contribute to its traditional use as an anti-inflammatory and antibacterial agent [13–17]. The vulnerability of honey to fraud is further exacerbated by challenges facing legitimate production. Environmental degradation,

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intensive agricultural practices, climate change, and increasing bee diseases have significantly constrained authentic honey supply, widening the gap between consumer demand and available product [18]. This supply-demand imbalance has created favorable conditions for fraudulent practices, with global trade data and laboratory analyses confirming a significant presence of adulterated or non-compliant honey in international markets [9, 19, 20].

Honey adulteration typically manifests through the addition of C3 and C4 sugar syrups, the harvesting of immature honey requiring artificial thermal dehydration, and the masking of geographical origin via transshipment or resin technology [20–24]. While traditional quality parameters such as hydroxymethylfurfural (HMF) levels, diastase activity, electrical conductivity, and sugar profiles serve as established indicators of freshness, and processing history, the detection of sophisticated fraud increasingly relies on advanced techniques like Liquid Chromatography-Isotope Ratio Mass Spectrometry (LC-IRMS) and Nuclear Magnetic Resonance (NMR) to mitigate public health risks [9, 21, 25, 26].

Recognizing the scale of this problem, the European Commission has implemented Coordinated Action Plans in 2015 and 2021 to assess honey compliance with regulations such as the EU Honey Directive (2001/110/EC) [21, 22]. These initiatives have revealed concerning levels of non-compliance across member states, highlighting the need for enhanced surveillance at national levels. While regional studies in neighboring Serbia and earlier preliminary work in North Macedonia have investigated honey quality based on regional characteristics, according to the National rulebook for standards and determination of bee products quality, comprehensive and ongoing assessment remains vital for consumer protection and market integrity [27–29].

North Macedonia presents a unique context for honey quality assessment. The country has experienced growth in beekeeping operations, driven by strong cultural traditions and steady domestic demand for honey for both medicinal and culinary purposes [30]. Honey production plays a crucial role in rural livelihoods and agricultural sustainability. Despite this economic significance, current knowledge is limited to sporadic, cross-sectional assessments that fail to capture the evolving nature of food fraud. To date, no longitudinal analysis has evaluated how honey adulteration patterns in the region respond to changing regulatory pressures over time. This absence of systematic, multi-year data hinders the development of predictive risk models essential for alignment with EU food safety protocols. The Food and Veterinary Agency has an annual monitoring program for honey quality that includes laboratory testing of compliance with the national legislation that is harmonized with the EU Honey Directive (2001/110/EC). The laboratories performing these analyses must be ISO 17,025 accredited and participate in proficiency tests.

The aim of this study was to conduct a multi-year assessment of the quality and authenticity of honey in North Macedonia (2020–2024), in accordance with national and European regulations, with particular emphasis on the frequency of non-compliance and potential indicators of adulteration as well as to propose evidence-based surveillance strategies for future monitoring.

Materials and methods

Honey samples

All samples in this study ($n=538$) were provided to the Faculty of Veterinary Medicine-Skopje, from 2020 to 2024, by the Food and Veterinary Agency as part of the national monitoring program and included inspector targeted, by beekeepers and private citizens as voluntary submissions. All samples were stored at 4 °C and handled according to the internal SOP within the ISO 17,025 standard. Although botanical metadata was not available for every sample, this limitation does not preclude the assessment of market compliance. Consequently, the study applied the universal regulatory baselines established in the National Rulebook and EU Directive 2001/110/EC, which define the legality of honey regardless of its specific floral source.

Determination of physicochemical parameters

The determination of the physicochemical parameters followed methods described by the International Honey Commission [31], to check the compliance with the National and European legislative values, referred later as regulatory values (RV). All physicochemical assessments utilized analytical-grade reagents (purity ≥ 99%). The methods are accredited under ISO 17025 standard and have undergone full validation to ensure analytical reliability. For moisture content determination, the refractive index of the samples was measured using a KRÜSS Optronic GmbH (Germany). The conductometer used for measuring the electrical conductivity of the samples was InoLab Cond 7110 (Germany). A Binder drying oven (model 53, Germany), with a temperature of 135 °C, was used to determine insoluble matter. Hydroxymethylfurfural and diastase activity were determined using an ultraviolet/visible (UV/Vis) spectrophotometer (model Cary 60, Agilent Technologies, USA). High-Performance Liquid Chromatography (HPLC) - with a Refractive Index Detector (RID) 1260 Infinity II series, Agilent Technologies, USA) was used to analyze fructose, glucose, and sucrose. The sugars were separated on an

amino column (4.6 × 250 mm, 5 µm particle size, Zorbax Carbohydrate, Agilent Technologies, USA). C4 sugar analysis was carried out using 13 C/12 C EA/LC-IRMS (Detection of C4/C3 sugars) ICS SOP 520 − 13 (2021-03) method [32]. Due to the high analytical costs and specialized infrastructure required for Isotope Ratio Mass Spectrometry, C4 sugar analysis was performed on a subset of samples (n = 49) provided by the Food and Veterinary Agency.

Data collection and Preparation

Quality parameters were categorized according to established standards as outlined in the National and European legislation [27, 33]. For HMF, diastase activity, and C4 sugar content, additional subcategories were created to better distinguish values as indicators of honey age, heat treatment, sugar feeding to bees, and the association between these parameters (Table 1). Import status was categorized as "Yes" (imported) or "No" (domestic).

Statistical analysis

Descriptive statistics

Frequency distributions (counts and percentages) were calculated for all quality parameters to identify the most common causes of non-compliance. Cross-tabulations were performed to examine relationships between parameters.

Table 1 Subcategorization of HMF, diastase activity and C4 sugar values

Parameters	Reference values	Subcategorization	Categorization
HMF	< 10 mg/kg	Fresh honey	Compliant
	10–30 mg/kg	Compliant aged or heat-treated honey	Compliant
	30–40 mg/kg	Compliant aged or heat-treated honey with higher content	Compliant
	> 40–200 mg/kg	Non-compliant values	Non-compliant
	200.00 mg/kg	Extremely high non-compliant values	Non-compliant
Diastase activity	> 20.00 DN	Fresh honey	Compliant
	8.00–20.00 DN	Compliant aged or heat-treated honey	Compliant
	< 8.00 DN	Non-compliant value	Non-compliant
C4 sugar	< 7.00%	Compliant	Compliant
	7.00–15.00%	Indicates potential bee feeding with sugar during nectar flow	Non-compliant
	> 15.00%	Indicates blending with a larger amount of sugar syrup	Non-compliant

Association analysis

Chi-square tests of independence were conducted to examine associations between pairs of non-compliance parameters. For each pair, a 2 × 2 contingency table was created, and the chi-square statistic (χ^2) was calculated. The strength of association was quantified using Cramer's V coefficient, with values interpreted as: < 0.1 (negligible), 0.1–0.2 (weak), 0.2–0.4 (moderate), 0.4–0.6 (relatively strong), 0.6–0.8 (strong), and 0.8-1.0 (very strong). Odds ratios (OR) with 95% confidence intervals (CI) were calculated to quantify the magnitude of associations. The OR represents how likely a sample is to be non-compliant for one parameter when it is non-compliant for another. For contingency tables with expected cell frequencies less than 5 (particularly analyses involving import status), Fisher's exact test was used instead of the chi-square test.

Temporal trend analysis

The samples were grouped by year (2020–2024) based on their identification codes. For each year, we calculated the total number and percentage of non-compliant samples, as well as the percentage of non-compliant samples that failed on each quality parameter.

Simple linear regression was used to assess temporal trends in non-compliance rates for each parameter. The slope coefficient (β) indicates the average annual percentage point change, while the coefficient of determination (R^2) quantifies the proportion of variance explained by the linear trend.

Multi-parameter analysis

We analyzed the number of parameters for each non-compliant sample failed (ranging from one to five or more) to understand the complexity of quality issues. Co-occurrence analysis was performed to identify which parameters most frequently failed together, expressed as counts and percentages of the total non-compliant samples.

Statistical analyses were performed using R statistical software (version 4.2.0; R Foundation for Statistical Computing, Vienna, Austria). All p-values < 0.05 were considered statistically significant.

Results

Physicochemical characterization

Of the 538 samples analyzed, 147 (27%) were imported and 391 (73%) were domestically produced. In total, 51

Table 2 Annual distribution of non-compliant samples and parameter-specific non-compliance

Year	Total samples	Non-compliant samples (%)	HMF (%)	Diastase activity (%)	Reducing sugars (%)	Sucrose (%)	C4 sugar (%)
2020	104	11 (10.6%)	63.6	27.3	45.5	27.3	0.0
2021	117	20 (17.1%)	50.0	35.0	25.0	20.0	35.0
2022	78	8 (10.3%)	87.5	50.0	0.0	12.5	50.0
2023	123	7 (5.7%)	71.4	57.1	57.1	0.0	28.6
2024	116	5 (4.3%)	80.0	80.0	80.0	20.0	60.0

Table 3 Compressed table of Raw data showcasing the annual and total number of samples, with parameter compliance according to the National and EU honey directive (2001/110/EC) [27, 33]

Parameter and Compliance	Year (number of samples)					
HMF	2020 (104)	2021 (117)	2022 (78)	2023 (123)	2024 (116)	Total (538)
< 10 mg/kg	58	59	54	99	94	364
10–30 mg/kg	31	34	16	16	13	110
30–40 mg/kg	8	14	1	3	5	31
40–200 mg/kg	**7**	**8**	**6**	**4**	**4**	**29**
> 200 mg/kg	**0**	**2**	**1**	**1**	**0**	**4**
Reducing sugars (fructose + glucose)						
Compliant	100	112	78	119	112	521
Noncompliant	**5**	**5**	**0**	**4**	**4**	**18**
Sucrose						
Compliant	101	113	77	123	115	529
Noncompliant	**3**	**4**	**1**	**0**	**1**	**9**
Water content						
Compliant	104	117	78	123	116	538
Noncompliant	**0**	**0**	**0**	**0**	**0**	**0**
Electrical conductivity						
Compliant	104	117	78	123	116	538
Noncompliant	**0**	**0**	**0**	**0**	**0**	**0**
Free acid						
Compliant	104	117	78	123	116	538
Noncompliant	**0**	**0**	**0**	**0**	**0**	**0**
Water-insoluble mater						
Compliant	104	116	78	123	116	537
Noncompliant	**0**	**1**	**0**	**0**	**0**	**1**
Diastase activity	2020 (9)	2021 (52)	2022 (25)	2023 (67)	2024 (60)	Total (213)
> 20.00 DN	0	25	12	50	56	143
8.00–20.00 DN	6	20	9	13	0	48
< 8.00 DN	**3**	**7**	**4**	**4**	**4**	**22**
C4 sugar	2020 (0)	2021 (17)	2022 (13)	2023 (13)	2024 (4)	Total (49)
< 7.00%	0	12	9	11	1	33
7.00–15.00%	**0**	**3**	**3**	**2**	**0**	**8**
> 15.00%	**0**	**4**	**1**	**0**	**3**	**8**

Non-compliant categories and samples are in bold

samples (9.48%) were identified as non-compliant for at least one parameter according to the RV of National and International Regulations [27, 33]. The annual distribution showed: 11 (10.6%) non-ompliant samples out of 104 in 2020, 20 (17.1%) non-ompliant samples out of 117 in 2021, 8 (10.25%) non-ompliant samples out of 78 in 2022, 7 (5.7%) non-ompliant samples out of 123 in 2023, and 5 (4.3%) non-ompliant samples out of 116 in 2024 (Table 2). Of the imported honey, only 2 samples (1.4%) out f 147 were non-compliant, whereas 49 (12.53%) out f 391 domestically produced honey samples were non-compliant. Compressed data of all the analyzed honey samples for their regulatory compliance, with additional subcategorization, is presented in Table 3.

Table 4 Contingency table for HMF and diastase activity for all samples

	Low diastase activity	Normal diastase activity	Total
High HMF	21 (9.9%)*	1 (0.5%)	22 (10.3%)
Normal HMF	1 (0.5%)	190 (89.2%)*	191 (89.7%)
Total	22 (10.3%)	191 (89.7%)	213 (100%)

*Statistically significant result

Parameter specific compliance

Out of the 538 samples tested for HMF, there were 364 (67.7%) samples that were classified as fresh and properly stored honey with value under 10 mg/kg, 110 (20.4%) were aged or heat-treated honey samples with value between 10 and 30 mg/kg, 31 (5.8%) were aged or heat-treated honey samples on the upper limit of acceptance between 30 and 40 mg/kg, 29 (5.40%) were non-compliant samples with value between 40 and 200 mg/kg and 4 (0.7%) were samples with extremely high values over 200 mg/kg (Table 3). Nine (1.67%) of the 538 samples tested failed to comply with the sucrose standard. Of the 18 (3.35%) non-compliant samples for reducing sugars, 3 samples had exceeded the sucrose values, indicating adulteration (RV < 5.0%) (Table 3). Out of the 213 samples tested from 2020 to 2024, 22 (10.33%) were non-compliant, 47 (22.07%) had values between 8 and 20 DN, and 143 (67.13%) had high diastase activity (above 20 DN) (Table 3). Out of 49 samples tested, 16 (32.7%) were non-compliant. Eight samples (16.33%) had C4 values between 7 and 15%, and 8 samples (16.33%) had values above 15% (Table 3). Only 1 sample was found to be non-compliant, exceeding the maximum value. All 538 tested samples were compliant with the prescribed RV of water content, electrical conductivity, and free acid for honey (Table 3).

Statistical analysis

Association analysis: HMF vs. diastase activity

A significant and very strong association was found between HMF levels and diastase activity (χ^2 = 192.11, df = 1, $p < 0.0001$, Cramer's V = 0.95). Among non-compliant samples with high HMF levels and tested for diastase activity, 95.5% also had low diastase activity. Among non-compliant samples with low diastase activity, 95.5% also had high HMF levels (95% CI [181.5, 18248.7]) (Table 4).

Table 5 Contingency table for HMF and reducing sugars for all samples

	Low reducing sugars	Normal reducing sugars	Total
High HMF	13 (2.4%)*	20 (3.7%)	33 (6.1%)
Normal HMF	5 (0.9%)	500 (92.9%)*	505 (93.9%)
Total	18 (3.3%)	520 (96.7%)	538 (100%)

*Statistically significant result

Table 6 Contingency table for diastase activity and reducing sugars for all samples

	Low reducing sugars	Normal reducing sugars	Total
Low diastase activity	12 (5.6%)*	10 (4.7%)	22 (10.3%)
Normal diastase activity	0 (0%)	191 (89.7%)*	191 (89.7%)
Total	12 (5.6%)	201 (94.4%)	213 (100%)

*Statistically significant result

Association analysis: HMF vs. Reducing sugars

A relatively strong association was found between HMF levels and reducing sugars (χ^2 = 141.85, df = 1, $p > 0.0001$, Cramer's V = 0.51). The odds ratio analysis indicated that samples with non-compliant reducing sugars were 65 times more likely to have non-compliant HMF levels (95% CI [21.14, 199.82]) (Table 5).

Association analysis: diastase activity vs. reducing sugars

A strong association was found between diastase activity levels and reducing sugars (χ^2 = 110.35, df = 1, $p > 0.0001$, Cramer's V = 0.72). The odds ratio analysis indicated that samples with non-compliant reducing sugars were 456 times more likely to have non-compliant diastase activity (95% CI [25.2, 8279.5]) (Table 6).

Temporal trends in quality parameters

The yearly pattern of parameter-specific non-compliance is shown in Fig. 1. Within the non-compliant samples, the percentage with specific parameter issues showed distinct trends over time. The percentage of non-compliant samples with high HMF levels followed a variable pattern: in 2020 (63.6%), 2021 (50.0%), 2022 (87.5%), 2023 (71.4%), and 2024 (80.0%). Linear regression analysis indicated an annual increase of 5.42% points (R^2 = 0.35), suggesting a moderate increasing trend in the proportion of HMF-related non-compliance among non-compliant samples. Low diastase activity showed a strong and consistent increasing trend: in

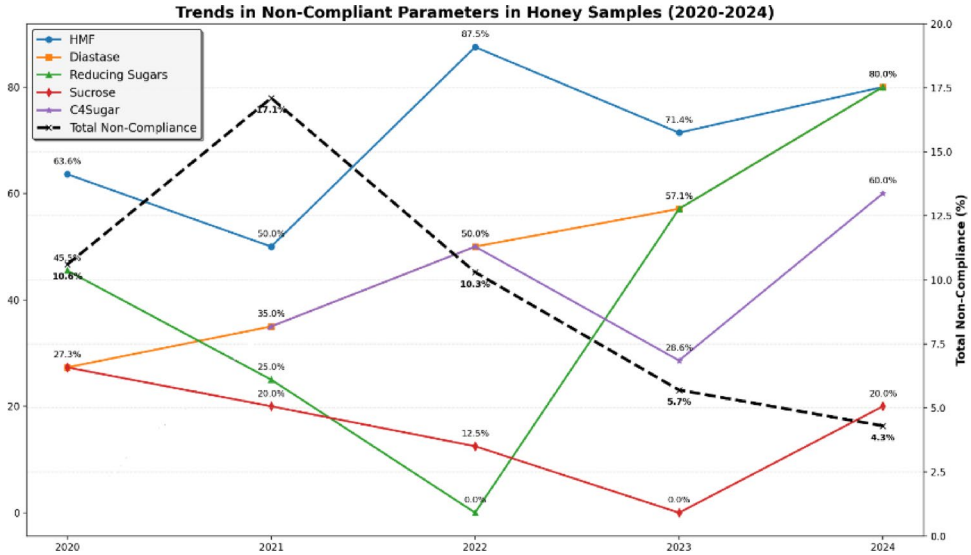

Fig. 1 Temporal trends in parameter-specific non-compliance (2020–2024). The solid lines indicate the percentage of non-compliant samples that fail on each parameter by year. The dashed black line represents the overall non-compliance rate (the percentage of all samples tested that year). Note that there is a strong increasing trend in diastase activity non-compliance (R^2 = 0.96), in contrast to the decreasing overall non-compliance rate

Table 7 Distribution of non-compliant samples by number of failed parameters

Number of non-compliant parameters	Number of samples	Percentage
1 parameter	23	45.1
2 parameters	13	25.5
3 parameters	11	21.6
4 parameters	3	5.9
5 + parameters	1	2.0

2020 (27.3%), 2021 (35.0%), 2022 (50.0%), 2023 (57.1%), and 2024 (80.0%). Linear regression analysis revealed an annual increase of 12.75% points (R^2 = 0.96), indicating a highly significant upward trend in diastase activity problems. Reducing Sugars non-compliance showed a volatile pattern: in 2020 (45.5%), 2021 (25.0%), 2022 (0.0%), 2023 (57.1%), and 2024 (80.0%). Despite the variability, linear regression analysis indicated an overall increasing trend of 10.11% points per year (R^2 = 0.27). Non-compliant sucrose levels showed a generally improving trend: in 2020 (27.3%), 2021 (20.0%), 2022 (12.5%), 2023 (0.0%), and 2024 (20.0%). This low R^2 (0.28) strongly supports that the pattern is highly volatile and suggest the trend is weak and may be unstable over longer periods. Linear regression analysis confirmed a decreasing trend of 3.46% points annually (R^2 = 0.28). C4 Sugar levels non-compliance showed substantial variation: in 2021 (35.0%), 2022 (50.0%), 2023 (28.6%), and 2024 (60.0%). Linear regression revealed a moderate increasing trend of 5.36% points annually (R^2 = 0.24).

Multi-parameter non-compliance

The number of samples found non-compliant for one or more parameters is shown in Table 7, while the distribution of non-compliant parameters throughout the years is presented in Fig. 2.

Co-occurrence analysis

The co-occurrence patterns of non-compliance parameters are presented as a heatmap in Fig. 3. The most frequent co-occurrence was between high HMF levels and low diastase activity, found in 21 samples (41.2% of all non-compliant samples). Other notable co-occurrences included HMF and reducing sugars (25.5%), diastase activity and reducing sugars (23.5%), HMF and C4 sugar (15.7%), and diastase activity and C4 sugar (11.8%). The heatmap visualization reveals clusters of related quality issues centered on HMF and diastase activity parameters.

Discussion

This study provides the first comprehensive assessment of honey quality compliance in North Macedonia, revealing a 9.48% non-compliance rate among tested samples over 2020–2024, with marked differences between domestic (12.53%) and imported (1.4%) honey. This is lower than the first coordinated action plan (14%) [22], second coordinated action plan (46%) arried out in the EU [21], the study in Serbia (14.1%) [28] and study in North Macedonia that tested 45 samples of forest honey obtained directly from local beekeepers [34]. Because only 2 (1.4%) of 147 impored samples were non-compliant, the number of imported samples sent for analysis decreased yearly from 45, 41, 27, 19, and 15, respectively. However, the stark disparity between domestic failure rates and imported honey suggests that North Macedonia's quality challenges may be driven by localized processing inconsistencies or "amateur"

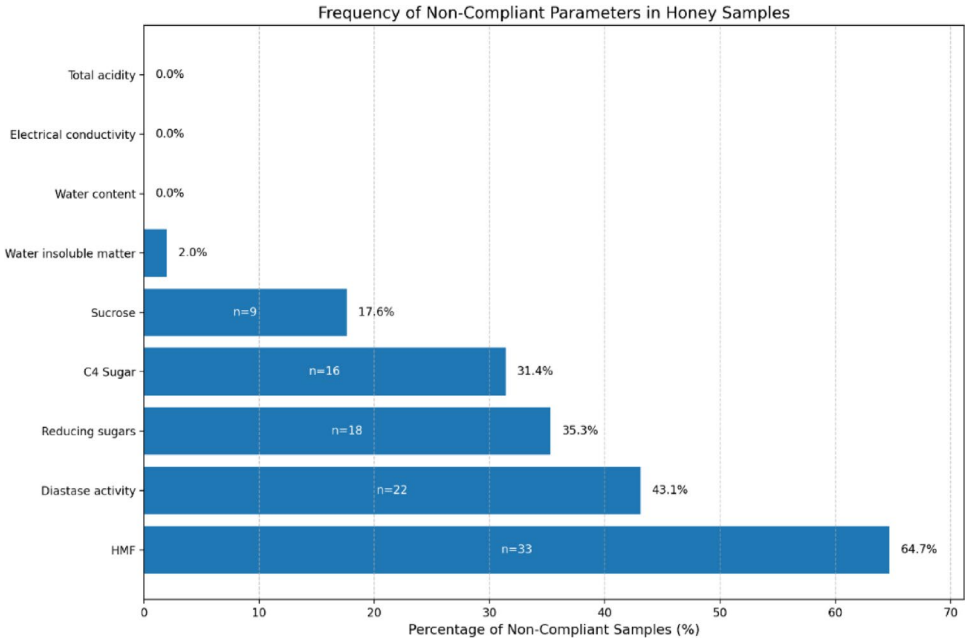

Fig. 2 Distribution of non-compliance parameters among 51 non-compliant honey samples (2020–2024)

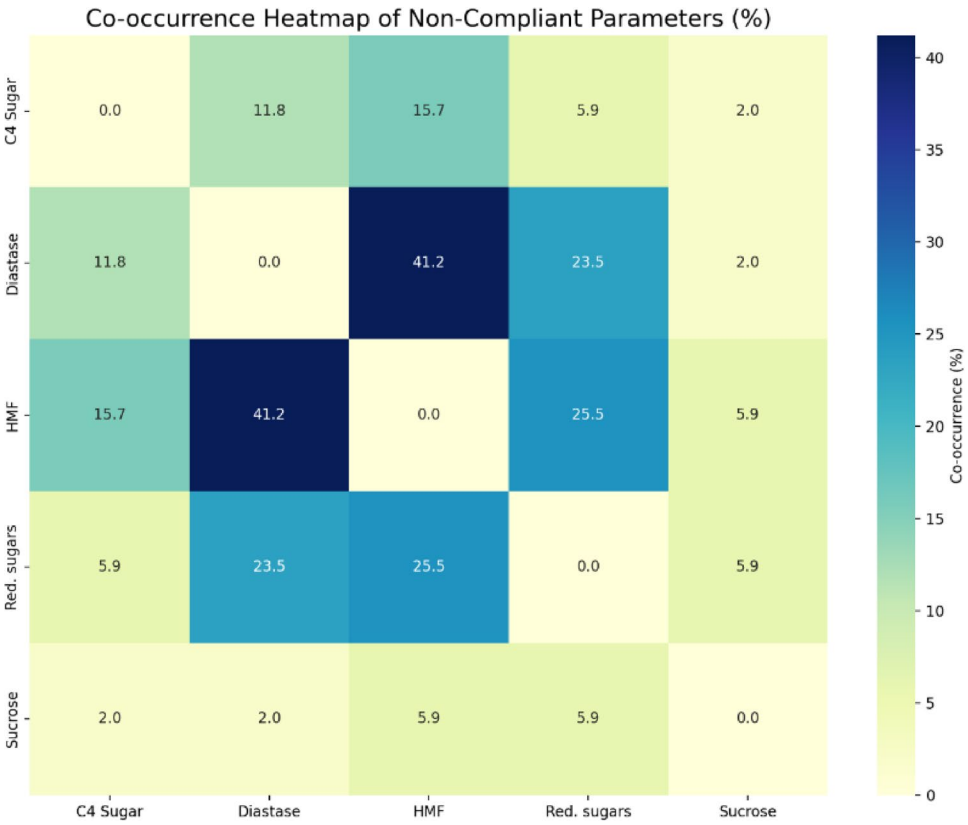

Fig. 3 Co-occurrence heatmap of the percentage of non-compliant samples exhibiting pairs of non-compliant parameters

adulteration rather than the highly sophisticated, large-scale fraud often found in global supply chains. The sudden rise of non-compliant samples in 2021 (17.1%) is in line with the European data in the second CAP in 2021, which had a sudden surge of adulterated samples (46%) [21], compared to the first CAP in (14%) [22]. National limittions on movement and working restrictions most likely played key roles, shifting honey sales towards online platforms where oversight was weaker and consumers could not verify authenticity. Heightened demand and significant economic gain for honey as a perceived health product during COVID-19 potentially fueled fraud.

Hydroxymethylfurfural was the most frequent cause of non-compliance (64.7%) (Fig. 2), with variable percentage

patterns throughout the years (Fig. 1). By structure a cyclic aldehyde, HMF is formed from sugar degradation through the Maillard reaction during heat processing or extended storage [35, 36]. The presence of glucose, fructose, acids, and minerals in honey accelerates its formation [37]. The RV of honey for HMF is set at a maximum value of 40 mg/kg. What stands out from the results in Table 3 are the 4 samples with extremely high values exceeding 200 mg/kg, which indicate that they may not be honey, but be composed of sugar products likely created by hydrolysis with excessive thermal treatment [19] .

Diastase activity is widely recognized as a key indicator of honey freshness and quality because it is a thermolabile enzyme naturally present in honey that decreases significantly upon aging or excessive thermal processing [26, 28]. The assigned RV for diastase activity is above 8 DN. The extent of diastase activity depends on the types of flowers, the timing of nectar collection, environmental factors, and the bees themselves [38]. Reduced diastase activity was the second most frequent cause of non-compliance, with 43.1% (Fig. 2. Low values suggest lower nectar content, which may indicate heat treatment or adulteration [39]. Out of the 213 samples tested, 47 (22.07%) had values btween 8 and 20 DN, suggesting they were aged or heat-treated but compliant, and 143 (67.14%) were classifed as fresh honey with high diastase activity (above 20 DN) (Table 3).

Reducing sugars (35.3%) shown in Fig. 2 were the third most frequent non-compliant parameter. Glucose and fructose, the primary components of honey, typically account for over 65% of its content [22, 26]. According to RV, nectar honey should have a glucose and fructose sum of at least 60%, whil honeydew honey (or its blends with nectar honey) should not fall below 45% of toal sugars. However, 18 samples failed to meet these standards. Factors like adulteration or dilution with water, syrups, or other sugars can reduce monosaccharide levels. Additionally, heating may caramelize sugars, breaking down monosaccharides into other compounds [28]. Another study reported similarly low glucose and fructose levels (19.6–59.1%) in non-cmpliant samples, attributing this to early harvesting due to the bee enzymes not having enough time to process the oligosaccharide content from the nectar or honey dew [40].

The C4 sugar content is highly valued when testing the authenticity of honey. Honey adulteration can occur by directly adding sucrose syrups from sugar beet, mixing in industrial sugar (glucose and fructose) syrups derived from starch through heat, enzyme, or acid processing, or by overfeeding bee colonies with these syrups during peak nectar flow [41, 42]. Syrups used to adulterate honey, which are much cheaper than honey, may come from C3 or C4 plants, categorized by their carbon metabolism. C3 plants, using the Calvin (C3) cycle to fix CO_2, have a lower $^{13}C/^{12}C$ ratio ($\delta^{13}C$) compared to C4 plants, which use the Hatch-Slack (C4) cycle. Most plants contributing to honey are C3, while corn and sugarcane used for adulteration and bee feeding are C4. It is widely agreed that the difference in $\delta^{13}C$ values between honey protein and honey ($\Delta\delta^{13}C$) should not exceed 1‰, as values above this indicate at least 7% adultration, which is the maximum accepted value for honey [41, 43]. The C4 sugar content was the fourth most frequent non-compliant parameter (31.4%) (Fig. 2). ight samples had C4 values between 7 and 15%, indicating ptential bee feeding with sugar during nectar flow, and 8 samples with values above 15%, which indicaes blending with a larger amount of sugar syrup (Table 3). Critically, 7 (43.8%) (Table 3) saples failing C4 sugar analysis met al.l other regulatory requirements, indicating that current routine testing may miss sophisticated adulteration with sugar syrups. This finding strongly supports implementing regular C4 sugar analysis, particularly for samples that pass conventional physicochemical tests.

Unlike monosaccharides, oligosaccharides are found in smaller quantities in honey [44]. Among them, the disaccharide sucrose is notable, as elevated levels may suggest adulteration, particularly from added sugar syrup [45]. Sucrose content was the fifth most frequent cause for non-compliance (17.6%) (Figre 2), and its concentration should not exceed 5 g/100 g. During honey ripening, sucrose interacts with enzymes and is converted by hydrolysis to glucose and fructose [38]. Osaili et al. [40] noted that high sucrose paired with low monosaccharide levels could result from overfeeding bees or harvesting honey too early, before sucrose fully converts to glucose and fructose.

Our findings of a significant and very strong association between HMF levels and diastase activity confirm that samples having non-compliant values for HMF or diastase activity are more than 95% likely to be non-compliant for the other parameter. Only two samples (0.9%) showed exceptions to this association (Table 4). The odds ratio analysis indicated that samples with non-compliant diastase activity were 3990 times more likely to have non-compliant HMF. The relatively strong association found between HMF levels and reducing sugars (Table 5) and the strong association found between diastase activity levels and reducing sugars (Table 6) are also likely because all three parameters are temperature-influenced. While diastase activity degradation and HMF formation are more susceptible to heat exposure, the caramelizing process of the sugars needs more time [28]. The results confirmed no non-compliant samples with low reducing sugars and regular diastase activity, while there were non-compliant samples with normal reducing sugars and low diastase activity (Table 6). These findings suggest that the reducing sugar content, HMF, and diastase activity is interrelated, with the strongest association being between

HMF content and diastase activity. This was also confirmed by the co-occurrence patterns of non-compliance parameters shown in Fig. 3, where the most frequent co-occurrence is observed with high HMF levels and low diastase activity, followed by HMF and reducing sugars, and diastase activity and reducing sugars.

The modes of honey adulteration are constantly evolving. The temptation for adulteration has increased due to the high price of honey compared to adulterants and the limitations of official detection methods [9, 18]. Usage of tailor-made syrups designed to mimic the sugar composition of natural honey makes detection as well as indirect adulteration, where bees are fed sugar syrups during the main nectar flow period, also exists and is considered extremely difficult to detect [20, 22]. Environmental factors such as humidity, combined with beekeeper practices during harvest, handling, storage and the degree of maturity reached in the hive are documented to have a negative impact on the water content of honey and may influence noncompliance rates in some regions [26, 28]. The samples in our study were all compliant for water content. On the other hand, storage duration and temperature as well as thermal processing to prevent crystallization can have direct impact on the freshness parameters like Hydroxymethylfurfural and diastase activity. Prolonged storage or storage at high temperatures leads to a significant increase in HMF and a decrease in enzymatic activity, causing the honey to exceed permitted limits [19, 46]. Training is crucial to avoid these mistakes that might have led to the noncompliance of some of the samples tested. Beyond adding sugars, other forms of fraud include mislabeling the geographical and/or botanical origin of honey, using ion exchange resins to remove residues and lighten color, as well as masking the true country of origin, often by removing pollen [18, 22]. These modes of adulteration constantly vary, requiring different detection strategies [20]. Traditional methods for assessing honey authenticity are often time-consuming, subjective, or not sensitive enough for sophisticated adulteration [24, 38]. The need for effective control and detection methods has grown as honey consumption and imports have increased, and the development of new analytical methods has been a major focus [20, 22, 47]. Isotope Ratio Mass Spectrometry (IRMS) combined with Liquid Chromatography (LC) has been developed as an improvement, enabling the determination of δ13C isotopic ratios of individual sugars. This method offers better sensitivity, reportedly detecting adulteration with C4 sugars at 1% and C3 sugars at 10% [20]. Despite these advancements, no single universal method exits that can detect all types of honey adulterants with sufficient sensitivity and robustness. This means several complementary methods must often be applied. The rapid development of new adulteration modes and materials contrasts with the slow pace of establishing official methods [20, 22]. The fact that sophisticated adulteration was detected in 32.7% of the subset tested for C4 sugars, with nearly half of those passing coventional tests, confirms that traditional parameters are no longer sufficient as a sole line of defense. While the selective nature of this testing is a limitation of current national monitoring resources, it provides a statistically significant 'proof of concept' for the inadequacy of current regulatory frameworks in detecting economically motivated fraud.

The study's limitations, particularly the non-random sampling based on inspector suspicion, suggest that the actual prevalence of non-compliance in the general market may differ from our findings. Based on these results, we recommend: (1) mandatory C4 sugar testing for at least 20% of samples passing routine tests, (2) establishment of a random sampling protocol alongside targeted enforcement, (3) enhanced focus on storage, handling and processing practices to prevent enzyme heat degradation and HMF formation, and (4) continued annual monitoring with expanded sample sizes to confirm temporal trends. These measures will protect both consumers from fraudulent products and legitimate beekeepers from unfair market competition, while supporting North Macedonia's alignment with EU food safety standards.

Conclusions

Our study, as the first comprehensive assessment of honey quality in North Macedonia successfully identified critical vulnerabilities in the honey market integrity, specifically within domestic production which exhibited a 12.53% noncompliance rate compared to only 1.4% in imported samples. The findings demonstrate that while most failures stem from heat-related degradation evidenced by the very strong association between elevated HMF and reduced diastase activity, a significant portion of sophisticated fraud remains undetected by conventional testing. Specifically, nearly half of the samples failing C4 sugar analysis would have passed as compliant under standard physicochemical screening, highlighting a clear gap in current surveillance protocols.

To strengthen consumer protection and ensure the economic viability of legitimate beekeepers, we propose several evidence-based policy shifts. First, the Food and Veterinary Agency should implement mandatory C4 sugar testing for a minimum of 20% of samples that pass routine parameters to detect economically motivated adulteration. Second, the current suspicion-based monitoring should be augmented with randomized sampling protocols to obtain a more accurate prevalence of market-wide non-compliance. Third, targeted educational initiatives and stricter enforcement of storage and handling practices are necessary to

mitigate enzymatic degradation and HMF formation during processing. These measures are essential for aligning North Macedonia's honey sector with the rigorous standards of the EU Honey Directive (2001/110/EC) and ensuring a transparent, competitive market environment.

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Declarations

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