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A COMPARATIVE ANALYSIS OF MILK-CLOTTING COAGULANTS IN CAMEL MILK CHEESEMAKING

Abstract: Camels (*Camelus dromedarius*), which are highly resilient to extreme climatic conditions, play a vital role in ensuring food security and the sustainability of pastoral farming in Kazakhstan. Enzymatic coagulation is a key stage in cheese production, determining the structure, yield and quality characteristics of the product. The efficiency of coagulation and the intensity of proteolysis depend on the nature of the coagulant and its rennet and proteolytic activity, which is particularly important when processing camel milk, given its specific protein composition.

This study aimed to conduct a comparative analysis of camel's milk cheeses produced using microbial and animal-derived coagulants. The study assessed dry matter content, yield, and textural characteristics, and evaluated the effect of coagulant type on the formation of the cheese's peptide profile. The results obtained show that, under various temperature conditions, the Kalase coagulant exhibited a longer coagulation time, ranging from 224,8 s at 34 °C to 95,6 s at 40 °C, compared with the Chy-Max coagulant, which ranged from 180 s at 34 °C to 88 s at 40 °C. In contrast, at higher temperatures (38-40 °C), the difference narrowed to 7,6-9 s. Syneresis showed that when using the Chy-Max coagulant, the curd released an average of 55,6% whey, whilst cheese samples using the Kalase coagulant showed a lower syneresis level of 45,6%. The cheese yield

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using the Chy-Max coagulant was 20,9%, whilst that using the coagulant Kalase was 18,7%. The total solid content in the cheese samples using the coagulant Chy-Max and Kalase was $48,4\pm0,05$ and $47,3\pm0,5$, respectively. The textural parameters of the cheeses indicated a denser texture when the microbial coagulant was used. Biologically active peptides were also identified in both cheese samples, which possess antihypertensive, antioxidant, antimicrobial, and immunomodulatory properties.

Key words: camel milk, cheese, coagulation, microbial coagulant, animal coagulant, peptide composition.

Introduction

Milk coagulation is a key stage in cheese production. It is achieved by coagulant preparations that break down kappa-casein, leading to the aggregation of casein micelles and the formation of a protein network [1].

The rate and extent of syneresis are influenced by temperature, holding time, pH, structural and mechanical properties, and the proteolytic activity of the coagulating [2,3].

Coagulants of animal and microbial origin differ in proteolytic specificity, resulting in distinct peptide profiles. These peptides can exhibit biological activity and functional properties that influence cheese flavour, aroma, and texture [4].

In recent years, interest in bioactive peptides has grown due to their health-promoting properties, which depend on specific amino acid sequences. Bioactive peptides are chains of 2–20 amino acids linked by amide or peptide bonds, which are released when proteolytic enzymes cleave them from inactive protein forms. They have been reported to exhibit antimicrobial, antioxidant, antidiabetic, and antihypertensive activities [5].

Most studies on bioactive peptides have focused on cow's milk and, to a lesser extent, on sheep's and goat's milk [6]. In contrast, information on peptides derived from camel milk remains limited, highlighting the need for further research.

This study aimed to conduct a comparative analysis of camel's milk cheeses produced using coagulants of microbial and animal origin.

Materials and Methods

Fresh whole camel milk from *C. dromedarius* from a private herd in the local region was obtained.

Soft camel milk cheeses were produced using two types of coagulants:

Microbial coagulant Chy-Max M 1000 (Chr. Hansen, Denmark) is a purified solution of chymosin obtained by fermentation of *Aspergillus niger* var. *awamori*.

Kalase (CSK Food Enrichment, The Netherlands) is an animal-derived rennet containing approximately 80% chymosin and 20% pepsin.

Cheesemaking. Milk was pasteurised to 63-65°C. It was cooled to the coagulation temperature of 34-40°C. The initial pH of the milk was 6,3. Milk-clotting coagulants were added in accordance with the manufacturer's instructions: 50 µL/L for Chy-Max and approximately 9 drops/L for Kalase. Coagulation was performed under controlled conditions at temperatures ranging from 34 to 40°C. Coagulation was carried out under controlled conditions and monitored by recording time until firm curd formation was achieved. After coagulation, the curd was cut, moulded and salted. All cheesemaking trials were performed in triplicate and the cheese samples underwent the following analyses. Both types of cheese were produced under identical processing conditions, differing only in the type of rennet used.

The coagulation time of camel milk under different conditions was determined using a modified method based on the International Dairy Federation standard (IDF 157:2007) [7].

Syneresis was evaluated based on the volume of whey released from the curd.

Total solids of the cheese samples were determined by the oven-drying method in accordance with AOAC (1997), method 926.08 [8]. Samples were dried to a constant weight in a forced-air oven at $102 \pm 2^\circ\text{C}$. Total solids were calculated gravimetrically as the percentage loss in mass after drying.

Yield of camel milk cheese was calculated using the following equation:

$$Yield (\%) = \frac{W_{ch}}{W_m} \times 100, \quad (1)$$

where «Wch» – weight of cheese after whey drainage(g).

Wm – weight of raw milk used for cheese production(g).

Texture profile analysis. Texture profile analysis was carried out on all samples using a texture analyser (TX-700 Texture Analyser, France).

The biologically active peptides were identified using nano-flow reverse-phase liquid chromatography coupled with tandem mass spectrometry (nanoLC-MS/MS). Mass spectra were recorded on an Impact II ESI-Q-TOF instrument (Bruker Daltonics, Germany). Biological activity was identified using the database of biologically active milk peptides [5].

Statistical analysis. All experiments were conducted in triplicate ($n=3$), and the results are presented as the mean and standard deviation (SD). Statistical differences between samples were evaluated using an independent Student's t-test. Differences were considered significant at $p<0,05$. The data analysis was performed using IBM SPSS Statistics 23.0 and MS Excel.

Results and discussion. In this study, the effect of two proteolytic coagulants, Chy-Max and Kalase, on the coagulation time of camel milk was investigated. Coagulation time was used as an indicator of rennet efficacy and clot formation kinetics. The effect of temperature on milk coagulation time was assessed for Chy-Max and Kalase in the range of 34-40°C (Fig. 1). As the temperature increased, a progressive reduction in coagulation time was observed for both rennets.

The rate of syneresis determines the rate of whey separation and, consequently, the residence time of the curd in the cheese vat, during which fermentation takes place (Fig. 2).

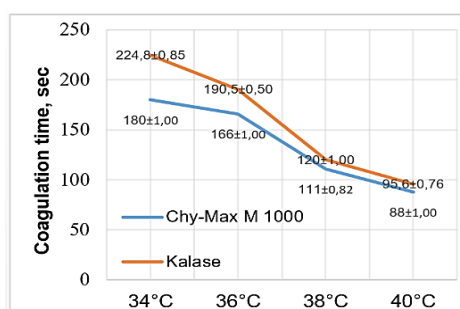


Figure 1 – Coagulation time of camel milk as a function of coagulant and temperature. Values are means $\pm$ SD; significant differences at $p < 0,05$; $n = 3$

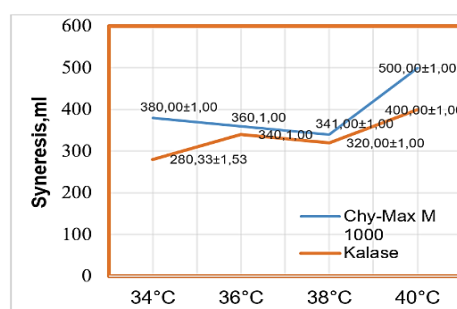


Figure 2 – Syneresis rate as a function of coagulant and temperature. Values are means $\pm$ SD; significant differences at $p < 0,05$; $n = 3$

At all tested temperatures, Kalase exhibited a longer coagulation time compared to Chy-Max ($p < 0.05$). The difference between the two coagulants was more pronounced at lower temperatures (34-36°C). At 34°C, the coagulation time was 180 s and 224,8 for Chy-Max and Kalase, respectively, whereas at higher temperatures (38-40°C) the difference decreased to 7,6-9 s.

Temperature and coagulant type significantly influenced whey separation from the camel milk curd. On average, the use of Chy-Max resulted in higher syneresis (55,6%) compared to Kalase 45,6 % ($p < 0,05$). In cheese produced with Chy-Max, syneresis decreased slightly between 34 and 38 °C, followed by a marked increase at 40 °C. In contrast, Kalase showed a moderate increase in syneresis with increasing temperature, while the total whey yield remained lower than that of Chy-Max under all tested conditions.

The physicochemical and textural properties of soft camel's milk cheeses produced with various coagulants are presented in Table 1.

Table 1 Physicochemical and textural properties of soft camel's milk cheeses

Parameters	Chy-Max	Kalase
Yield, g	88,13 $\pm$ 0,30	75,00 $\pm$ 0,50
Total solids, %	48,4 $\pm$ 0,33	47,3 $\pm$ 0,45
Hardness(N)	22,53 $\pm$ 0,46	17,26 $\pm$ 0,76
Cohesiveness*	0,34 $\pm$ 0,006	0,37 $\pm$ 0,009
Springiness*	0,81 $\pm$ 0,005	0,75 $\pm$ 0,037
Gumminess (N)	7,63 $\pm$ 0,32	6,46 $\pm$ 0,43
Chewiness(N)	6,19 $\pm$ 0,30	4,85 $\pm$ 0,54
Resilience*	0,15 $\pm$ 0,006	0,19 $\pm$ 0,015

Values are means $\pm$ SD; significant differences at $p < 0,05$; $n = 3$.

*Cohesiveness, springiness, and resilience are dimensionless parameters

According to Table 1, the cheese yield from camel's milk significantly depends on the type of coagulant used ($p < 0,05$). When the Chy-Max was used, the cheese yield was 88,13g, whereas the use of the Kalase resulted in a slightly lower yield 75,00 g. This corresponds to yield values of approximately 20,9% and 18,7% respectively.

The total solids content was also significantly higher in camel milk cheese produced using the Chy-Max ($48,4\% \pm 0,33\%$), compared to cheese produced using Kalase ($47,3\% \pm 0,05$) (Fig. 3).



Figure 3 – Camel milk cheese produced with
a) microbial coagulant ChyMax and b) animal coagulant Kalase

Texture profile analysis revealed that cheese produced using Chy-Max exhibited a firmer and more structured texture, as indicated by significantly higher hardness ($22,53 \pm 0,46$ N) and gumminess ($7,63 \pm 0,32$ N) compared to Kalase ($17,26 \pm 0,76$ N and $6,46 \pm 0,43$ N, respectively). In contrast, cheeses produced with Kalase demonstrated slightly higher cohesiveness and resilience values, suggesting a more flexible internal structure and improved ability to recover after deformation. Springiness values were higher for cheeses produced with Chy-Max, indicating greater elasticity.

Based on the peptide profiles presented in Tables 2 and 3, the majority of peptide fragments were derived from β -CN. This indicates that β -CN is more susceptible to proteolytic cleavage than α s1-CN regardless of the type of coagulant used.

Table 2 – Identified peptides in camel milk cheese with animal-derived coagulant of Kalase

Mass observed (Da)	Mr(calc)2 (Da)	Score	Sequence	Biology properties
β-CN-derived peptides				
654.8690	1307.7312	40	LPPLQPAVMVPF	(ACE)-inhibitory DPP-IV inhibitory activity
428.7641	855.5178	41	ETIIPKR	(ACE)-inhibitory activity, anti-cancer and anti-inflammatory properties
593.9983	1778.9865	24	KVLPVPQQMVPYPQR	DPP-IV inhibitory activity ($IC_{50} = 41,45$ M)
518.7698	1035.5349	38	QEPVPDPVR	(ACE)-inhibitory activity
718.9294	1435.8551	68	GLHPVPQPLVPVIA	antioxidant, antimicrobial and immunomodulatory properties
Alfa-casein αs1-CN-derived peptides				
954.4441	1906.8836	48	YPEVFQNEPDSIEEVL	not found
508.7949	1015.5815	68	AVVSPIQFR	anti-cancer and anti-inflammatory properties

According to Tables 2 and 3, fifteen peptides with biological activity were identified in both samples. In particular, the sequences LPPLQPAVMVPF and KVLPVPQQMVPYPQR have been previously associated with angiotensin-converting enzyme (ACE) inhibitory and dipeptidyl peptidase-IV (DPP-IV) inhibitory activity, suggesting potential antihypertensive and antidiabetic properties. In addition, the peptide GLHPVPQPLVPVIA, detected in both samples, has been reported to exhibit antioxidant, antimicrobial, and immunomodulatory activity [17].

At the same time, differences in the peptide profile were observed depending on the coagulant. In cheeses produced using Kalase, peptides such as ETIIPKR and QEPVPDPVR were identified, which have been reported to potentially exhibit ACE-inhibitory activity [15]. In contrast, cheeses produced using Chy-Max contained longer peptide sequences (e.g VYSHTEPIYPILPQNF and AMPVQAVLPFQEPVPDPVR), indicating a higher degree of proteolysis due to differences in enzymatic specificity.

Table 3 – Identified peptides in camel milk cheese with microbial coagulant of Chy-Max

Mass observed (Da)	Mr(calc)2 (Da)	Score	Sequence	Biology properties
β-CN-derived peptides				
1008.0168	2014.0200	28	VYSHTEPIYPILPQNF	ACE-inhibitory
654.8687	1307.7312	56	LPPLQPAVMVPF	(ACE)-inhibitory activity and antidiabetic (DPP-IV inhibitor)
593.9951	1778.9865	57	KVLPVPQQMVYPQR	DPP-IV inhibitory activity (IC ₅₀ = 41,45 мкМ)
1045.5560	2089.1030	17	AMPVQAVLPFQEPVPD PVR	DPP-IV c IC ₅₀
718.9279	1435.8551	28	GLHPVPQPLVPVIA	antioxidant, antimicrobial and immunomodulatory properties
αs1-CNderived peptides				
657.8789	1313.7595	30	ILDLAVVSPIQF	anti-cancer and anti-inflammatory properties
537.2618	1072.5189	53	SSHPYLEQL	ACE-inhibitory
515.3223	1028.6634	58	GPLPLPLPLL	DPP-IV Inhibitory

Analysis of αs1-casein-derived peptides also revealed differences between samples. The peptide AVVSPIQFR, detected in cheese with Kalase, has been characterized with anti-inflammatory and anticancer activity. In Chy-Max samples, the peptide ILDLAVVSPIQF with similar biological properties was also detected. Peptide SSHPYLEQL has been associated with angiotensin-converting enzyme (ACE) inhibitory [18], while GPLPLPLPLL has been reported as a dipeptidyl peptidase-IV (DPP-IV) inhibitory peptide [19].

Discussion

A comparative analysis of coagulants showed that the preparations are characterized by different kinetic parameters of milk coagulation, which can be used to optimize technological modes and increase the yield of cheese from camel milk. According to previous studies, differences between animal and microbial coagulants are manifested in the coagulation rate, gel formation and strength, which directly affect the technological properties of the clot [9].

In the conducted studies, the microbial enzyme Chy-Max demonstrated higher coagulation activity and shorter clotting time in camel milk. At the same time, the animal coagulant Kalase, with the correct dosage, can also ensure stable curd formation and the formation of a pronounced flavor profile of the cheese. However, the use of animal coagulants may be limited by modern requirements for production ethics and animal welfare, which increases interest in microbial alternatives [10].

The differences in cheese yield can be explained by the specificity of the enzyme, which effectively hydrolyzes k-casein and promotes the formation of a denser clot with less loss of whey [11].

Similar trends have been reported, where cheeses produced using animal rennet were characterised by a higher dry matter content [12]. Dry matter content varied between 65.2% and 68.8% depending on the type of rennet and milk pre-treatment, with microbial coagulant slightly reducing the concentration of solids due to higher proteolytic activity [13]. It has also been noted that microbial coagulants may lead to the formation of a softer curd and a moderate reduction in yield compared to animal rennets[14].

A comparison of the results shows that the use of Chy-Max promotes the formation of more structurally flexible and potentially bioactive peptide fragments compared with the Kalase, which may be due to differences in the specificity of enzymatic cleavage [16]. The amino acid composition of peptide sequences and the stage of cheese ripening are key factors influencing the biological activity of peptides [20]. It has been reported that higher levels of bioactive activity are often observed during the ripening period, particularly in cheeses produced with starter cultures [5]. A study [21] investigated six cheese types (Karish, Feta-Type, Domoati, RAS, Gouda, and Edam) produced under different technological conditions, and reported anti-hypertensive peptides in all types of cheeses. Furthermore, the release of antioxidant peptides generated by pepsin hydrolysis of whey and casein fractions from goat's milk has been reported [22]. The antioxidant activity of cheese is influenced by the type of coagulants exhibit higher in vitro antioxidant activity compared to those obtained using animal coagulants [23].

Thus, the choice of coagulant has a complex effect on the coagulation, texture, yield, and peptide profile of camel milk cheese, requiring a comprehensive technological justification.

Conclusions

The results of the present study demonstrate that the type of coagulant significantly affects the coagulation process, physicochemical properties, texture, and peptide profile of camel milk cheese.

At all tested temperatures, animal coagulant Kalase exhibited a significantly longer coagulation time compared to microbial coagulant Chy-Max with most pronounced differences observed at lower temperature (34-36 °C), where the coagulation time reached 224,8 s for Kalase and 180 s for Chy-Max at 34 °C. At higher temperatures (38-40 °C), this difference decreased to 7,6-9 s.

Cheese with was significantly higher in samples with Chy-Max (88,13±0,30, approximately 20,9%) compared to Kalase (75,00±0,50), approximately 18,7%. Chy-Max also resulted in higher total solids content (48,4 ± 0,33%) and a firmer texture, by hardness (22,53±0,46 N) and gumminess (7,63±0,32 N).

The use of different types of coagulants affects the peptide profile of camel milk cheese. A total of fifteen bioactive peptides were identified in both cheese samples, including sequences associated with angiotensin-converting enzyme (ACE) inhibitory and dipeptidyl peptidase-IV (DPP-IV) inhibitory activities.

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ТҮЙЕ СҮТІНЕН ІРІМШІК ӨНДІРУ КЕЗІНДЕ СҮТТІ ҰЙЫТАТЫН ФЕРМЕНТТЕРДІ САЛЫСТЫРМАЛЫ ТАЛДАУ

Түйелер (Camelus dromedarius) экстремалды климаттық жағдайларға өте төзімді, азық-түлік қауіпсіздігін және Қазақстандағы жайылымдық шаруашылықтардың тұрақтылығын қамтамасыз етуде маңызды рөл атқарады. Ферменттік коагуляция ірімшік жасаудағы маңызды кезең болып табылады, өнімнің құрылымын, өнімділігін және сапалық сипаттамаларын анықтайды. Коагуляцияның тиімділігі және протеолиздің қарқындылығы коагулянттың табиғатына және оның сүтті ұйыту және протеолитикалық белсенділігіне байланысты, бұл әсіресе түйе сүтін өзінің ерекше ақуыз құрамымен өңдеу кезінде маңызды.

Бұл зерттеудің мақсаты микробтық және жануарлардан алынатын ферменттерді пайдаланып өндірілген түйе сүтінен жасалған ірімшіктерді салыстыру болды. Құрғақ заттардың мөлшері, өнімділігі, құрылымдық сипаттамалары және ірімшіктің пептидтік профилінің қалыптасуына коагулянт түрінің әсері бағаланды. Нәтижелер әртүрлі температуралық жағдайларда Kalase ферментінің ұзағырақ коагуляция уақытын көрсеткенін, 34°C температурада 224,8 секундтан 95,6 секундқа дейін өзгергенін көрсетеді. 40 градуста, Чу-Мах ферментімен салыстырғанда 34°C температурада 180 с-тан 40°C температурада 88 с-қа дейін, ал жоғары температурада (38-40°C)

айырмашылық 7,6-9 с-қа дейін азайды. Синерез *Chy-Max* ферментін қолданған кезде сүзбе орта есеппен 55,6% сарысу бөлетінін көрсетті, ал *Kalase* ферментін қолданатын ірімшік үлгілерінде синерезис деңгейі 45,6% төмен болды. *Chy-Max* ферментін қолданатын ірімшік өнімділігі 20,9%, ал жануар текті мәйектен жасалған *Kalase* ферментімен 18,7% болды. *Chy-Max* және *Kalase* қолданатын ірімшік үлгілеріндегі құрғақ зат сәйкесінше $48,4\% \pm 0,05$ және $47,3\% \pm 0,05$ көрсетті. Ірімшіктердің текстуралық көрсеткіштері микробтық ферментпен тығыздық құрылымды көрсетті. Екі ірімшік үлгісінде де биологиялық белсенді пептидтер анықталды, олар гипертонияға қарсы, антиоксидантты, микробқа қарсы және иммуномодуляциялық қасиеттерге байланысты.

Түйін сөздер: түйе сүті, ірімшік, ұю, микробтық фермент, жануарлардан алынатын фермент, пептидтік композиция.

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СРАВНИТЕЛЬНЫЙ АНАЛИЗ МОЛОКОСВЕРТЫВАЮЩИХ ФЕРМЕНТОВ ПРИ ПРОИЗВОДСТВЕ СЫРА ИЗ ВЕРБЛЮЖЬЕГО МОЛОКА

Верблюды (Camelus dromedarius), обладающие высокой устойчивостью к экстремальным климатическим условиям, играют важную роль в обеспечении продовольственной безопасности и устойчивости пастбищных хозяйств Казахстана. Ферментативное свертывание является ключевым этапом сыроделия, определяющим структуру, выход и качественные характеристики продукта. Эффективность коагуляции и интенсивность протеолиза зависят от природы коагулянта, его молокосвертывающей и протеолитической активности, что особенно важно при переработке верблюжьего молока с его специфическим белковым составом.

*Целью настоящего исследования явился сравнительный анализ сыров из верблюжьего молока, полученных с использованием ферментов микробного и животного происхождения. Оценены показатели сухих веществ, выход, текстурные характеристики, а также влияние типа коагулянта на формирование пептидного профиля сыра. Полученные результаты демонстрируют, что при различных температурных режимах фермент *Kalase* демонстрировал более длительное время свертывания от 224,8 с. при 34 °C до 95,6 с. при 40, по сравнению с ферментом *Chy-Max* с 180 с при 34 °C до 88 с при 40 °C..тогда как при более высоких температурах (38-40°C) разница сократилась до 7,6-9 с. Синерезис показал, что при применении фермента *Chy-Max* сгусток выделял в среднем 55,6% сыворотки, в то время как образцы сыра, с использованием фермента *Kalase*, показали более низкий уровень синерезиса 45,6%. Выход сыра с использованием фермента *Chy-Max* составил 20,9%, в то время как с использованием сычужного фермента животного происхождения *Kalase* составил 18,7%. Сухие вещества в образцах сыра с использованием *Chy-Max* и *Kalase* показали $48,4\% \pm 0,05$ и $47,3\% \pm 0,05$ соответственно. Текстурные показатели сыров показали более плотную текстуру с использованием микробного фермента. Также были идентифицированы биологически активные пептиды у обоих образцов сыра, которые относятся к антигипертензивным, антиоксидантным, антимикробным и иммуномодулирующим свойствам.*

Ключевые слова: верблюжье молоко, сыр, свертывание, микробный фермент, фермент животного происхождения, пептидная композиция.

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ИНТЕГРИРОВАННАЯ СИСТЕМА ГЕОИНФОРМАЦИОННОГО МОНИТОРИНГА, ЦИФРОВОЙ ПАСПОРТИЗАЦИИ И ПОДГОТОВКИ ОБРАЗЦОВ *HALYOMORPHA* *HALYS STÅL* В ЮЖНОМ КАЗАХСТАНЕ

Аннотация: В статье предложена и апробирована интегрированная схема раннего выявления, цифровой паспортизации и подготовки образцов *Halyomorpha halys Stål* к последующей молекулярно-генетической верификации в условиях юга Казахстана. Целью исследования являлась разработка воспроизводимой системы, объединяющей полевой мониторинг, GPS-привязку очагов, стандартизированный учет образцов и формирование коллекции, пригодной для последующего анализа по маркерам COI и ITS1. Полевую апробацию системы выполнили в Ботаническом саду г. Алматы с использованием феромонных ловушек и ловчих поясов на яблоне и груше. В ходе наблюдений было зарегистрировано 215 особей на разных стадиях развития. Наибольшая численность выявлена в центральной части обследованной территории; максимальные показатели зафиксированы в точках D1 и D2. Установлено, что феромонные ловушки более эффективно выявляют имаго, тогда как ловчие пояса повышают обнаруживаемость нимфальных стадий. Предложен унифицированный цифровой паспорт образца, обеспечивающий трассируемость данных от момента отбора до этапа лабораторной обработки и пространственного анализа. Разработанная система может служить основой для дальнейших диссертационных исследований по молекулярной структуре инвазивных популяций *H. halys* и региональных программ фитосанитарного мониторинга.

Ключевые слова: *Halyomorpha halys*; молекулярно-генетическая идентификация; COI; ITS1; геоинформационный мониторинг; феромонные ловушки; цифровой паспорт образца; Южный Казахстан.

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