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(54) Method for identifying cow milk and horse milk using Mid-infrared spectrum MIR

(57) The present disclosure relates to a method for quickly identifying cow milk and horse milk, which is related to mid-infrared spectroscopy analysis. The method comprises the following steps: (1) selecting fresh cow milk and fresh horse milk samples; (2) scanning the samples in the mid-infrared spectrum range to obtain mid-infrared spectrum data; (3) preprocessing the original mid-infrared spectrum to remove outliers; (4) dividing the preprocessed data set into a training set and a test set according to the principle of stratified sampling; (5) reducing the dimension of the training set to improve the speed of model training; (6) using K Nearest Neighbor (KNN) algorithm and other algorithms on the training set to build models through 10-fold cross-validation that are able to distinguish between cow milk and horse milk, and accuracy, specificity, sensitivity and AUC were used to evaluate and filter the models; (7) predicting the generalization ability of the optimal model.

Method for identifying cow milk and horse milk using Mid-infrared spectrum MIR

Field

The invention belongs to the field of milk product analysis technology, and in particular, relates to a rapid method for identifying cow milk and horse milk. The present invention is related to the field of analyzing the components of dairy products using infrared spectrum.

Background

The contents and proportions of protein, amino acids, lactose, minerals and other ingredients in horse milk are close to those in breast milk, which is easier to be absorbed by infants and young children and causes reduced allergic symptoms [1,4,8]. Further, in the horse milk, abundant vitamins and minerals are involved in human metabolism, and have the effects of regulating human physiological functions, improving human immunity and preventing and treating diseases. Unsaturated fatty acids and low-molecular fatty acids are effective in preventing hypercholesterolemia and arteriosclerosis. Free glycans in horse milk have a wide range of biological effects, with potential prebiotics, antimicrobial, anti-adhesion and immunomodulatory activities. In particular, free glycans are able to promote the growth of beneficial microorganisms in intestinal tract, which are extremely valuable to human health [6]. Studies have found that horse milk can regulate the expression of Salmonella typhimurium pathogenic genes and has anti-reproductive effects on Caco-2 cells, which provides new evidence to demonstrate horse milk can promote gastrointestinal health [5].

At present, the methods for determining the components of cow milk and horse milk include high performance liquid chromatography (HPLC), gas chromatography (GC), Coomassie brilliant blue-ultraviolet detection method [2], near infrared (NIR) spectroscopy [3] and Mid-infrared (MIR) spectroscopy, etc. Based on the D-LOOP gene of horse mitochondria and the 16S-RNA gene of bovine mitochondria, Lu Deng et al. designed specific primers for double-stranded PCR which can detect 0.1% cow milk mixed in horse milk [7]. But there is no method to quickly and accurately identify a batch of cow milk and horse milk.

Mid-infrared spectroscopy is a rapid, non-destructive, non-polluted, modern technology that can analyze multiple ingredients at the same time, which has been quickly developed in recent years and widely used in the quality inspection of agricultural products and food. However, there are no related research and literature reports on the identification of cow milk and horse milk by mid-infrared spectrum.

Summary

To overcome the defects in the conventional art, the present closure provides a method for quickly identifying a batch of cow milk and horse milk based on Fourier transform mid-infrared spectrum, which features rapid, high precision, low cost, simple operation, batch identification and strong practicability.

The present closure is implemented by the solutions as follows.

A method for quickly identifying cow milk and horse milk includes the following steps:

Step 1 collecting samples of fresh cow milk and fresh horse milk, respectively;

Step 2 collecting mid-infrared spectrum of samples in Step 1, the samples of cow milk and horse milk are respectively poured into a cylindrical sampling tube with a diameter of 3.5 cm and a height of 9 cm, the height of the liquid level is ensured greater than 6cm, after heating in a water bath at 42°C for 15-20 minutes, a solid optical fiber probe is extended into the liquid to absorb samples for detection, a mid-infrared spectrometer is used to scan the samples of cow milk and horse milk in the wave number range of 4000-400cm^{-1}, and the corresponding light transmittance of the samples is output through a connected computer to obtain a sample spectrum;

Step 3, preprocessing the collected original mid-infrared spectrum data by converting the spectrum data from transmittance (T) to absorbance (A) according to $A=\log_{10}(1/T)$ at first, then removing the absorption area of water, and detecting spectrum anomaly using LOF (Local Outlier Factor) to remove outliers at last;

Step 4, dividing the data set into a training set and a test set;

Step 5, reducing dimension of principal components of the training set by selecting the number of principal components when the cumulative explained variance ratio is greater than 99.9%;

Step 6, building and filtering models: with the mid-infrared spectrum of the milk samples in the training set as the input value, and the categories of cow milk and horse milk as the output value, K Nearest Neighbor (KNN) algorithm, BP neural network algorithm, random forest (RF) algorithm and support vector machine (SVM) algorithm are followed to build models on the training set through 10-fold cross-validation for comparison, and accuracy, specificity, sensitivity and AUC indicators are used to evaluate and filter the models;

Step 7, evaluating the generalization ability of the optimal model by predicting the samples in test set, the corresponding evaluation indicators are used to evaluate the model's performance on the test set, and a confusion matrix is used to refine the performance of the model on the test set;

Step 8, realizing the above-mentioned preprocessing the mid-infrared spectrum data, building and validating models, and outputting confusion matrix using Python 3.8.3.

Brief description of the drawings

FIG 1. is a mid-infrared spectrum of horse milk after anomaly detection processing.

FIG 2. is a mid-infrared spectrum of cow milk after anomaly detection processing.

FIG 3. is a comparison diagram of mid-infrared spectrums of cow milk and horse milk after anomaly detection processing.

FIG 4. is a TSNE visualization diagram of the preprocessed data.

FIG 5. is a ROC curve of the model on the test set.

FIG 6. is a confusion matrix diagram of the model on the test set.

Detailed description

The present disclosure will be further described in detail below in conjunction with the drawing and the embodiment.

The present disclosure provides a method for quickly identifying cow milk and horse milk, which includes the following specific steps. The experimental methods mentioned in the examples without specifying specific conditions shall be carried out in accordance with conventional methods and conditions, or in accordance with the conditions recommended by the manufacturer (such as product instruction manual).

Example 1

Instruments and equipment: MilkoScan^{TM}7RM milk composition detector produced by FOSS Analytical (according to the product instruction manual). The operation steps were provided as follows:

1. 60 samples of fresh horse milk and 60 samples of fresh cow milk were collected, respectively;

2. The samples of cow milk and horse milk were respectively poured into a cylindrical sampling tube with a diameter of 3.5 cm and a height of 9 cm, the height of the liquid level was ensured greater than 6cm, after heating in a water bath at 42°C for 15-20 minutes, a MilkoScan^{TM}7RM milk composition

detector produced by FOSS Analytical was extended into the liquid to absorb samples for detection, a mid-infrared spectrometer was used to scan the samples of cow milk and horse milk in the wave number range of 4000-400cm^{-1}, and the corresponding light transmittance of the samples was output through a connected computer to obtain a sample spectrum.

3. The collected original mid-infrared spectrum data were preprocessed by converting the spectrum data from transmittance (T) to absorbance (A) according to $A=\log_{10}(1/T)$ at first, then removing the absorption area of water, and detecting spectrum anomaly using LOF (Local Outlier Factor) to remove outliers at last;

4. The data set was divided into training set and test set by stratified sampling method. The training set comprising mid-infrared spectrum data of 92 samples was used for building a qualitative discrimination model, and the test set comprising mid-infrared spectrum data of 24 samples was used to evaluate the prediction effect of the qualitative discrimination model.

5. The dimension of principal components of the training set was reduced: the number of principal components was selected when the cumulative explained variance ratio was greater than 99.9% to obtain 16 principal components.

6. With the mid-infrared spectrum of the milk samples in the training set as the input value, and the categories of cow milk and horse milk as the output value, K Nearest Neighbor (KNN) algorithm, BP neural network algorithm, random forest (RF) algorithm and support vector machine (SVM) algorithm are followed to build models on the training set through 10-fold cross-validation; The accuracy, specificity, sensitivity and AUC of the models built by the four algorithms were all 1 on the training set, indicating that these four algorithms had good modeling effects on the identification of cow milk and horse milk, and all four models were able to accurately identifying cow milk and horse milk. Generally speaking, the SVM algorithm had a good performance in various classification problems. The SVM

algorithm was based on nonlinear mapping and was a novel small-sample learning method; in the SVM classification decision, a small number of support vectors played a decisive role, which avoided the possibility of "dimensional disaster" to a certain extent and make the training model have better stability. Therefore, the models built by the SVM algorithm was chosen for the identification of cow milk and horse milk.

7. The generalization ability of the optimal model was evaluated by predicting 24 samples in test set. The results represented by accuracy, specificity, sensitivity and AUC indicate the predicted value of the mid-infrared spectrum obtained was consistent with the actual value. The accuracy, specificity, sensitivity and AUC of the model on the test set were all 1, indicating that the SVM algorithm had a high learning ability for classifying cow milk and horse milk, and the built model can accurately identify between cow milk and horse milk. FIG 6 used a confusion matrix to represent the performance of the model on the test set. Comparing the classification results with the actual values, no error classification can be seen from the figure, showing that the model can correctly classify cow milk and horse milk with high accuracy.

Table 1. The performance of the models on the training set and test set

Algorithm	Data set	Model evaluation indicators			
		Accuracy	sensitivity	specificity	AUC
KNN	training set	1	1	1	1
	test set	1	1	1	1
BP	training set	1	1	1	1
	test set	1	1	1	1
RF	training set	1	1	1	1
	test set	1	1	1	1
SVM	training set	1	1	1	1
	test set	1	1	1	1

The invention has high learning ability, and the built model can accurately identify cow milk and horse milk. FIG 6 uses the confusion matrix to represent the performance of the model on the test set, comparing the classification results with the actual value, no error classification can be seen from the figure, showing that the model can accurately classify cow milk and horse milk with high accuracy.

Main references

[1] Liu Zhian. Study of fresh mare’s milk nutritional quality of grazing Yili horse [D]. Xinjiang Agricultural University, 2014;

[2] Wang Shuyang, etc., Determination and analysis of fatty acids in fresh camel milk [J]. Journal of Gansu Agricultural University, 2011, 46(01): 127-132;

[3] Yang Jinhui, Characteristics of near-infrared spectrum, fatty acids and protein composition of milk from different dairy animals [D]. Chinese Academy of Agricultural Sciences, 2013.

[4] AKBAR NIKKHAH. Equidae milk promises substitutes for cow and human breast milk[J]. turkish journal of veterinary & animal sciences, 2012, 36(5):470-475;

[5] Guri Anilda,Paligot Michele,Crèvecoeur Sebastien, et al. In vitro screening of mare's milk antimicrobial effect and antiproliverative activity.[J]. FEMS microbiology letters, 2016,363(2);

[6] Karav S , Salcedo J , Frese S A , et al. Thoroughbred mare's milk exhibits a unique and diverse free oligosaccharide profile[J]. FEBS Open Bio, 2018, 8;

[7] Lu Deng,Aili Li,Yang Gao,et,al. Detection of the Bovine Milk Adulterated in Camel, Horse, and Goat Milk Using Duplex PCR[J]. Springer US,2020,13(2);

[8] Malacarne M , Martuzzi F , Summer A , et al. Protein and fat composition of mare's milk: Some nutritional remarks with reference to human and cow's milk[J].

International Dairy Journal, 2002, 12(11):869-877。

CONCLUSIES

1. Werkwijze voor het snel identificeren van koeienmelk en paardenmelk, gekenmerkt door het omvatten van de volgende stappen:

Stap 1. het verzamelen van monsters van respectievelijk verse koeienmelk en verse paardenmelk;

Stap 2. het verzamelen van midden-infraroodspectrum van monsters in Stap 1, waarbij monsters van respectievelijk koeienmelk en paardenmelk in een cylindrische verzamelbuis met een diameter van 3,5 cm en een hoogte van 9 cm worden gegoten, waarbij de hoogte van het vloeistofniveau op hoger dan 6 cm gewaarborgd wordt, na het verwarmen in een waterbad bij 42 °C gedurende 15-20 minuten een vaste optische vezelsonde in de vloeistof gestoken wordt om monsters te absorberen voor detectie, een midden-infraroodspectrometer wordt gebruikt om de monsters van koeienmelk en paardenmelk te scannen in het golflengtebereik van 4000 - 400 cm^{-1}, en de overeenkomstige lichttransmissie van de monsters wordt uitgevoerd door een verbonden computer om een monsterspectrum te verkrijgen;

Stap 3. Het voorverwerken van de verzamelde oorspronkelijke midden-infraroodspectrumgegevens door het eerst omzetten van de spectrumgegevens van transmissie (T) naar absorptie (A) volgens $A = \log_{10}(1/T)$, vervolgens het verwijderen van het absorptiegebied van water, en het detecteren van spectrumanomalie bij gebruik van LOF (Lokale uitschieterfactor - Local Outlier Factor) om ten slotte uitschieters te verwijderen;

Stap 4. het verdelen van de gegevens in een trainingsset en een testset;

Stap 5. het reduceren van grootte van hoofdcomponenten van de trainingsset door het kiezen van het aantal hoofdcomponenten wanneer de cumulatieve verklaarde variantieverhouding groter is dan 99,9 %;

Stap 6. het bouwen en filteren van modellen: met het midden-infraroodspectrum van de melkmonsters in de trainingsset als de invoerwaarde, en de categorieën van koeienmelk en paardenmelk als de uitvoerwaarde, worden K Nearest Neighbor (KNN)-algoritme, BP neuraal netwerkalgoritme, random forest (RF)-algoritme en support vectoralgoritme gevolgd om modellen te bouwen op de trainingsset door 10-voudige kruisvalidatie voor vergelijking, en worden nauwkeurigheids-, specificiteits-, gevoeligheids- en AUC-indicatoren gebruikt om de modellen te evalueren en te filteren;

Stap 7. het evalueren van het generalisatievermogen van het optimale model door het voorspellen van de monsters in de testset, waarbij de overeenkomstige evaluatie-indicatoren worden gebruikt om de prestatie van het model op de testset te evalueren, en een verwarringsmatrix wordt gebruikt om de prestatie van het model op de testset te verfijnen.

2. Werkwijze voor het snel identificeren van koeienmelk en paardenmelk, waarbij in Stap 2 de monsters worden gescand met een melkbestanddelendetector.

3. Werkwijze voor het snel identificeren van koeienmelk en paardenmelk, waarbij in Stap 3 de werkwijze voor het voorverwerken van gegevens is om:

(1) de spectrumgegevens om te zetten van transmissie (T) naar absorptie (A) volgens $A = \log_{10}(1/T)$;

(2) het absorptiegebied van water te verwijderen;

(3) spectrumanomalie te detecteren: bij gebruik van Local Outlier Factor-algoritme om de uitschieters te verwijderen.

4. Werkwijze voor het snel identificeren van koeienmelk en paardenmelk, waarbij in Stap 4 de voorverwerkte gegevensset verdeeld wordt in een trainingsset en een testset volgens het principe van gelaagd monsteren, met percentages van respectievelijk 80 % en 20% van de gegevensset.

-o-o-o-

FIG 1.

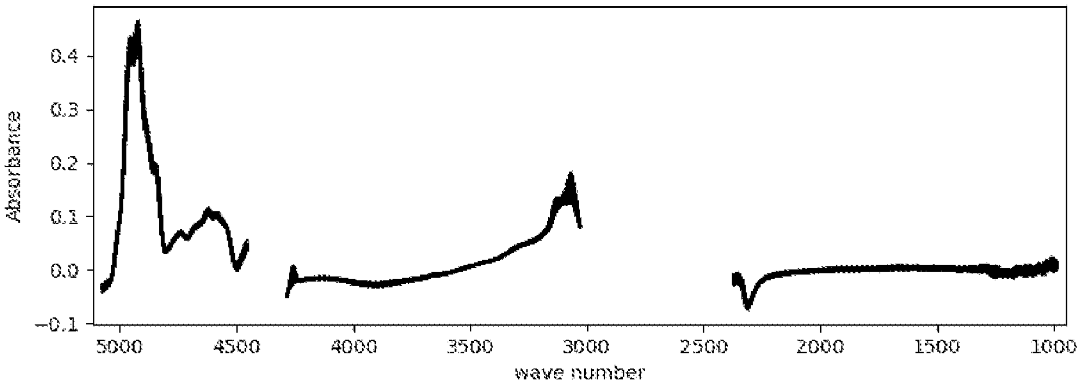


FIG 2.

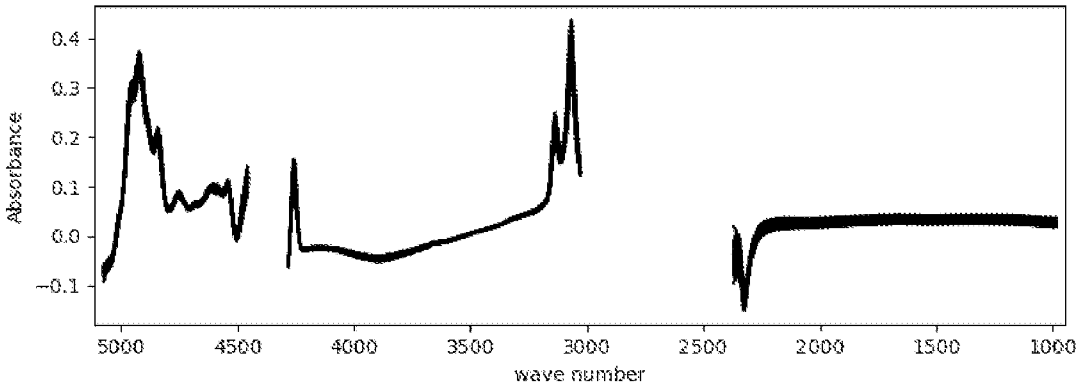


FIG 3.

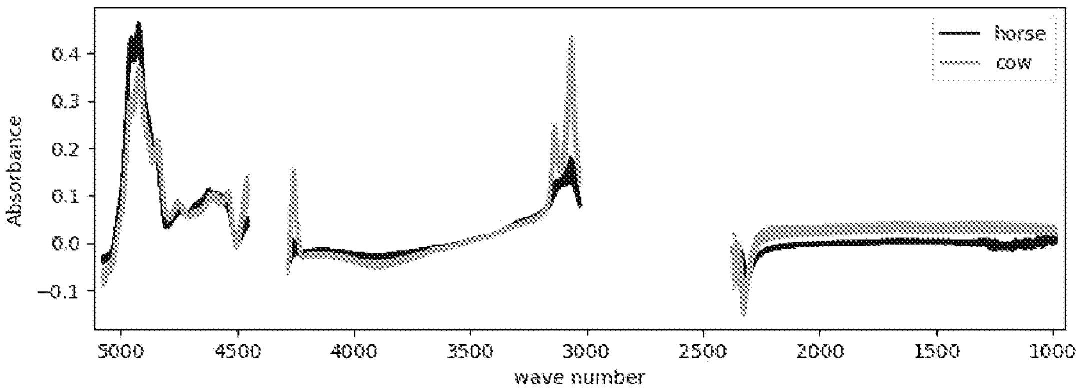


FIG 4.

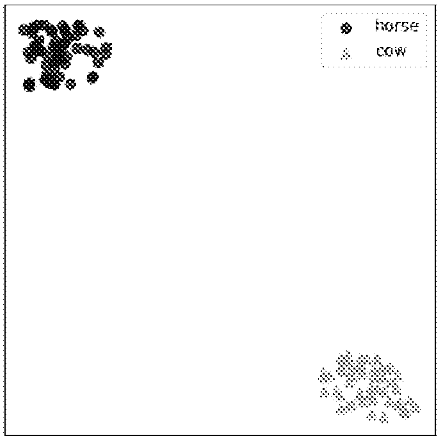


FIG 5.

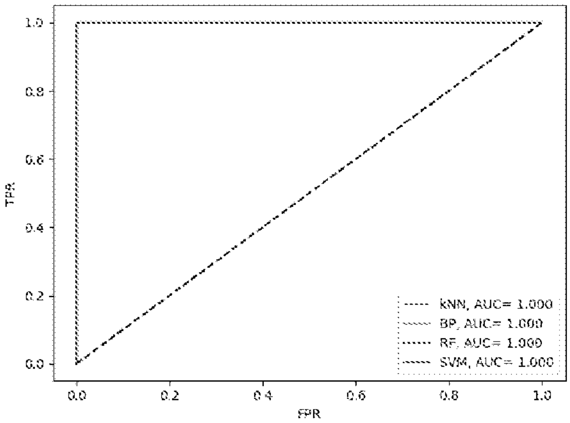
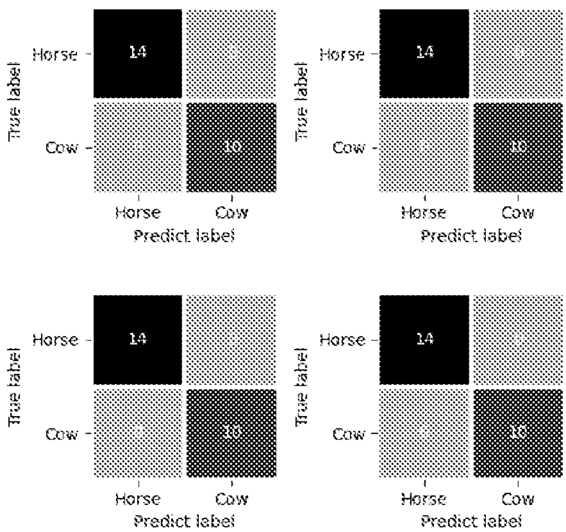


FIG 6.



Rijksdienst voor Ondernemend Nederland

OCTROOIAANVRAAG NR.:

NO 141584
NL 2029011

ONDERZOEKSRAPPORT

BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK

RELEVANTE LITERATUUR

Categorie[1]	Literatuur met, voor zover nodig, aanduiding van speciaal van belang zijnde tekstgedeelten of figuren.	Van belang voor conclusie(s) nr:	Classificatie(IPC)
A	KUMAR NAVJOT ET AL: "Rapid classification of different types of ghee using mid infrared spectroscopy", 2017 TRENDS IN INDUSTRIAL MEASUREMENT AND AUTOMATION (TIMA), IEEE, 6 januari 2017 (2017-01-06), bladzijden 1-5, XP033163909, DOI: 10.1109/TIMA.2017.8064802 [gevonden op 2022-04-25] * het gehele document *	1-4	INV. G01N21/3577 G01N33/04
A	ETZION Y ET AL: "Determination of Protein Concentration in Raw Milk by Mid-Infrared Fourier Transform Infrared/Attenuated Total Reflectance Spectroscopy", JOURNAL OF DAIRY SCIENCE, AMERICAN DAIRY SCIENCE ASSOCIATION, US, deel 87, nr. 9, 1 september 2004 (2004-09-01), bladzijden 2779-2788, XP026970124, ISSN: 0022-0302 [gevonden op 2022-04-25] * het gehele document *	1-4	Onderzochte gebieden van de techniek: G01N
X,P	CN 112 666 111 A (UNIV HUAZHONG AGRICULTURAL) 16 april 2021 (2021-04-16) * het gehele document *	1-4	
X,P	CN 112 525 850 A (UNIV HUAZHONG AGRICULTURAL) 19 maart 2021 (2021-03-19) * het gehele document *	1-4	

Indien gewijzigde conclusies zijn ingediend, heeft dit rapport betrekking op de conclusies ingediend op:

Plaats van onderzoek:	Datum waarop het onderzoek werd voltooid:	Bevoegd ambtenaar:
München	27 april 2022	Passier, Martinus

[1] NDERLINCATEGORIE VAN DE VERMELDE LITERATUUR

X: de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur
Y: de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht
A: niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft
O: niet-schriftelijke stand van de techniek
P: tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur
T: na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvrage, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding
E: eerdere octrooi(aanvrage), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven
D: in de octrooiaanvrage vermeld
L: om andere redenen vermelde literatuur
&: lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

EOB FORM 02.83 (P0414B)

AANHANGSEL BEHORENDE BIJ HET RAPPORT BETREFFENDE HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK, UITGEVOERD IN DE OCTROOIAANVRAGE NR.

NO 141584
NL 2029011

Het aanhangsel bevat een opgave van elders gepubliceerde octrooiaanvragen of octrooien (zogenaamde leden van dezelfde octrooifamilie), die overeenkomen met octrooischriften genoemd in het rapport.
De opgave is samengesteld aan de hand van gegevens uit het computerbestand van het Europees Octrooibureau per
De juistheid en volledigheid van deze opgave wordt noch door het Europees Octrooibureau, noch door het Bureau voor de Industriële eigendom gegarandeerd;; de gegevens worden verstrekt voor informatiedoeleinden.

27-04-2022

In het rapport genoemd octrooigeschrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
CN 112666111 A	16-04-2021	GEEN	
CN 112525850 A	19-03-2021	GEEN	

EPO FORM P0466

SCHRIFTELIJKE OPINIE

DOSSIER NUMMER	INDIENINGSDATUM	VOORRANGSDATUM	AANVRAAGNUMMER
NO141584	20.08.2021	01.10.2020	NL2029011

CLASSIFICATIE
INV. G01N21/3577 G01N33/04

AANVRAGER
HUAZHONG AGRICULTURAL UNIVERSITY

Deze schriftelijke opinie bevat een toelichting op de volgende onderdelen:

- ☒ Onderdeel I Basis van de schriftelijke opinie
- ☐ Onderdeel II Voorrang
- ☐ Onderdeel III Vaststelling nieuwheid, inventiviteit en industriële toepasbaarheid niet mogelijk
- ☐ Onderdeel IV De aanvraag heeft betrekking op meer dan één uitvinding
- ☒ Onderdeel V Gemotiveerde verklaring ten aanzien van nieuwheid, inventiviteit en industriële toepasbaarheid
- ☒ Onderdeel VI Andere geciteerde documenten
- ☐ Onderdeel VII Overige gebreken
- ☐ Onderdeel VIII Overige opmerkingen

DE BEVOEGDE AMBTENAAR

Passier, Martinus

SCHRIFTELIJKE OPINIE

Onderdeel I Basis van de Schriftelijke Opinie

1. Deze schriftelijke opinie is opgesteld op basis van de meest recente conclusies ingediend voor aanvang van het onderzoek.

2. Met betrekking tot **nucleotide en/of aminozuur sequenties** die genoemd worden in de aanvraag en relevant zijn voor de uitvinding zoals beschreven in de conclusies, is dit onderzoek gedaan op basis van:

   a. type materiaal:

   ☐ sequentie opsomming

   ☐ tabel met betrekking tot de sequentie lijst

   b. vorm van het materiaal:

   ☐ op papier

   ☐ in elektronische vorm

   c. moment van indiening/aanlevering:

   ☐ opgenomen in de aanvraag zoals ingediend

   ☐ samen met de aanvraag elektronisch ingediend

   ☐ later aangeleverd voor het onderzoek

3. ☐ In geval er meer dan één versie of kopie van een sequentie opsomming of tabel met betrekking op een sequentie is ingediend of aangeleverd, zijn de benodigde verklaringen ingediend dat de informatie in de latere of additionele kopieën identiek is aan de aanvraag zoals ingediend of niet meer informatie bevatten dan de aanvraag zoals oorspronkelijk werd ingediend.

4. Overige opmerkingen:

SCHRIFTELIJKE OPINIE

Onderdeel V Gemotiveerde verklaring ten aanzien van nieuwheid, inventiviteit en industriële toepasbaarheid

1. Verklaring

Nieuwheid	Ja:	Conclusies 1-4
	Nee:	Conclusies
Inventiviteit	Ja:	Conclusies 1-4
	Nee:	Conclusies
Industriële toepasbaarheid	Ja:	Conclusies 1-4
	Nee:	Conclusies

2. Citaties en toelichting:

Zie aparte bladzijde

Onderdeel VI Andere geciteerde documenten

☒ Andere geciteerde openbaarmakingen

Zie aparte bladzijde

☐ Niet schriftelijke openbaarmakingen

Re Item V

Prior art

1 Reference is made to the following documents cited in the search report:

D1 KUMAR NAVJOT ET AL: "Rapid classification of different types of ghee using mid infrared spectroscopy"

D2 ETZION Y ET AL: "Determination of Protein Concentration in Raw Milk by Mid-Infrared Fourier Transform Infrared/Attenuated Total Reflectance Spectroscopy"

D3 CN 112 666 111 A

D4 CN 112 525 850 A

Technical field

2 The application relates to the field of spectrally analysing dairy samples.

Novelty and inventive step

independent claim

3 The subject-matter of **claim 1** is novel, because **D1** and **D2** do not disclose an identification of cow milk and horse milk. **D1** is about classifying different types of ghee and **D2** is about determining protein concentration in cow milk. As a matter of fact, neither **D1** nor **D2** mention horse milk.

4 The subject-matter of **claim 1** involves an inventive step.

4.1 **D1** can be considered as closest prior art. It discloses a rapid classification of different types of ghee using mid-infrared (4000 - 400 cm^{-1} range) ATR (Attenuated Total Reflectance) spectroscopy. The spectra are submitted to a Principle Component Analysis (PCA) and subsequent pattern recognition to identify different types of ghee. A confusion matrix is used to represent the performance of the identification method.

4.2 **D1** does not disclose:

(i) horse milk samples being collected and analysed as in step 1. of **claim 1**;

(ii) the specific dimensional and heating parameters during the sample preparation as defined in step 2. of **claim 1**;

(iii) obtaining the spectra by detecting light transmittance and converting the transmittance spectra to absorbance spectra as defined in step 3. of **claim 1**;

(iv) the machine learning using a training and a test set and having the spectra of the training set as input value and the categories of cow milk and horse milk as output values as well as predicting the test samples as defined in steps 4.-7. of **claim 1**.

4.3 These features (i)-(iv) result in a method of quickly and accurately identifying cow milk and horse milk. Neither **D1** nor **D2** disclose or hint at such a method.

4.4 Although **D1** is about the classification of different types of ghee, it does not provide the skilled person with an indication that an identification of horse and cow milk could be done.

D2 is about determining protein concentration in cow milk using reflectance sprectroscopy and machine learning (neural network), but is silent about absorption spectrometry, horse milk, or any type of identification of milk types.

Furthermore, the specific dimensional and heating parameters during the sample preparation as defined in step 2. of **claim 1** ensure an optimal preparation of both horse and cow milk samples simultaneously and is nowhere disclosed or hinted in **D1** or **D2**.

dependent claims

5 Assuming that **claims 2-4** are dependent on **claim 1** (see point 8 below), their subject-matter is also novel and inventive through this dependency.

Industrial applicability

6 The subject-matter of **claims 1-4** is industrially applicable, for example in medical or agricultural applications when a fast and reliable identification of a milk type is required.

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7 **D3** and **D4** are earlier disclosures of the subject-matter of the current application and unequivocally disclose all features of **claims 1-4** of the current application.

Other

8 Although **claims 2-4** do not refer back to **claim 1**, it has been assumed for the purpose of this Written Opinion that they all depend on **claim 1**, i.e. "Werkwijze voor het snel identificeren van koeienmelk en paardenmelk *volgens conclusie 1*, waarbij ...".

9 It appears that **claim 3** is redundant, as its subject-matter is already present in **claim 1**.