# Rapid Detection of Goat Milk Mixed with Bovine Milk and Infant Goat Milk Formulas Mixed with Bovine Whey Powder by NIRS Fingerprints

**Yongji HE**
Northwest A&F University
**Wanjun ZENG**
Northwest A&F University
**Yuxuan ZHAO**
Northwest A&F University
**Xinpeng ZHU**
Ankang University
**Hongchang WAN**
Shaanxi Yatai Dairy Co., Ltd
**Meng ZHANG**
Shaanxi Yatai Dairy Co., Ltd
**Zhicheng LI** ( lizhicheng@nwsuaf.edu.cn )
Northwest A&F University

**Research Article**

**Keywords:** Goat milk, Infant goat milk formulas, Fingerprint, Near-infrared spectroscopy (NIRS), Principal component analysis (PCA)

**Posted Date:** April 18th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1509485/v1

# Abstract

The fingerprints of goat milk and infant goat milk formulas were established based on near-infrared spectroscopy (NIRS). In addition, the fingerprint-based principal component analysis (PCA) was used to identify infant goat milk formulas at different stages, whether goat milk was mixed with bovine milk, and whether goat milk formulas were mixed with desalted bovine whey powder. The results showed that there were six and nine characteristics of common maximum absorption intensity in goat milk and infant goat milk formulas, respectively. Among them, the NIR wavenumbers corresponding to the characteristic maximum absorption intensity of goat milk are: 10270.95, 8612.31, 8334.77, 6914.12, 5539.54, and 5202.52$cm^{-1}$, and the NIR wavenumbers corresponding to the characteristic maximum absorption intensity of infant goat milk formulas are: 8262.08, 6795.08, 6352.36, 5790.64, 5684.91, 5162.87, 4753.17, 4368.86, 4257.56 $cm^{-1}$. The method of combining NIRS and PCA was used to identify Stage 1, 2, and 3 of infant goat milk formulas. The use of the method combining NIRS and PCA enabled the discrimination of Stage 1, 2, and 3 of infant goat milk formulas. It was able to effectively identify adulterated desalted bovine whey powder from desalted goat whey powder and adulterated bovine milk from goat milk. The results suggested that different stages of infant goat milk formulas and goat milk and its infant formulas adulteration could be differentiated by NIRS combined with PCA, which provided convincing evidence for the identification and brand protection of goat milk and infant goat milk formulas.


# Introduction

The yield of goat milk is much lower compared to bovine milk, but it has incalculable significance for economic growth and provides many necessary nutrients for human beings (Nayik et al. 2021). The protein in goat milk has less immunogenicity, higher digestibility, and a ratio closer to human milk (Park et al. 2007). Goat milk and its yogurt, cheese, and powder products are of great significance to human nutrition. It can provide more nutrition than bovine milk for malnourished people in developing countries, and treat milk allergies and gastrointestinal diseases (Clark et al. 2017).

Due to abundant milk production, most infant formulas in the world are based on bovine milk protein ingredients. Nevertheless, $\alpha_{s1}$-casein is the main allergen, so choosing goat milk with less $\alpha_{s1}$-casein as the matrix of infant formulas can greatly reduce allergic reactions in infants and young children (Maathuis et al. 2017). In addition, Chen et al. (2022) had found that goat milk formulas have excellent properties, surface composition, and particle stability. In parallel, the digestive properties of a protein are critical as infant formula protein (Bourlieu et al. 2014). Goat milk acid-induced coagulation has a more open structure and finer protein chain (Wang et al. 2019; Ye et al. 2019). Therefore, infant goat milk formulas are attractive to consumers (Ahmed et al. 2015). Due to some factors such as supply and consumer demand, the market price of goat milk is generally higher than that of bovine milk in most parts of the world (Sen et al. 2021). Therefore, to make extra profits, some fraudsters mix goat milk with bovine milk to decrease cost, causing quality and safety issues of goat milk, and harming the health of

consumers and the image of manufacturers (Pinto et al. 2017). It is necessary to establish a simple and rapid identification method for the quality and safety control of goat milk and its infant formulas.

Fingerprinting techniques combine the concept of map technology with fingerprints and apply them to other levels (Chandra et al. 2011; Cai et al. 2006). Currently, fingerprinting technology is developing rapidly. The techniques applied to fingerprinting technology include chromatographic techniques such as liquid chromatography and gas chromatography (Gao et al. 2016; Xia et al. 2020; Kim et al. 2011), spectroscopy such as UV spectrum and near-infrared spectroscopy (NIRS) (Pierna et al. 2015; Mohamed et al. 2017), nuclear magnetic resonance, biochips and other technologies (Yu et al. 2020; Yi et al. 2017). Fingerprints usually realize their functions through discriminant analysis and methods that mainly include cluster analysis, similarity analysis, principal component analysis (PCA), etc. (Shi et al. 2021). He et al. (2019) used HPLC combined with diode array detection (DAD) and MS to establish an accurate, economic, and rapid chromatographic fingerprint, which was successfully used to identify flavonoids and polyphenols in mulberry leaves, which is expected to provide a basis for the development of mulberry leaves. Shawky et al. (2019) applied NIRS combined with PCA and hierarchical clustering analysis (HCA) to establish fingerprints for the identification of different citrus fruit peels and their prediction of bioactive compounds.

NIRS is a fast, high-throughput, and non-destructive analysis method, which can analyze almost any matrix. In recent years, it had many applications in food certification and adulteration detection (Cozzolino 2021; Ma et al. 2017). It covers the wavelength range adjacent to the mid-infrared and extends to the visible light region (Lohumi et al. 2015; Pasquini 2018). The American Society for Testing and Materials (ASTM) defines the NIR region of the electromagnetic spectrum as corresponding to the wavenumber range of 12820-3959cm$^{-1}$. In the NIR region, the most prominent absorption band is related to the vibration combination of functional groups such as -CH, -NH, -OH (and -SH) (Liang et al. 2020). The NIRS technique measures the absorption of photons in the wavenumber range by hydrogen-containing functional groups (Williams et al. 2010). Zhang et al. (2017) established the oil content of the camelina seeds model with the help of NIRS technology and compared the prediction performance of partial least squares (PLS) regression and principal component regression (PCR). Qin et al. (2020) used portable NIRS technology to predict the content of total volatile basic nitrogen (TVB-N) in frozen pork samples without thawing. Pieters et al. (2012) used NIRS as an online process analyzer to monitor protein unfolding and hydrogen bond interactions with protein freeze-drying protectors during freeze-drying, showing the early detection of protein defects and obtaining prospective views of mechanical process information of the freeze-drying protective agent. The study used NIRS technology to establish fingerprints of goat milk and its infant goat milk formulas. Comparing adulterated spectra and combining PCA, the NIRS fingerprint distinguished infant goat milk formulas from different objects and provided a basis for the quality control of raw goat milk and the safety of infant goat milk formulas as well.

# 1 Materials And Methods

## 1.1 Materials

Eleven batches of 110 goat milk samples were collected from the bulk tanks of five dairy farms in Shaanxi province, China (every month from January to November, 2020). The temperature of the goat milk was maintained at 4 ± 2°C during transport and storage. Eleven batches of Y brand infant goat milk formulas were from a sponsor. All of them were regular milk-based types and came from different seasons and different batches. Three different stages of infant goat milk formulas are included and they are Stage 1 for infants aged between 0 and 6 months, Stage 2 for infants aged between 6 and 12 months, and Stage 3 for toddlers aged between 12 and 36 months. These infant goat milk formulas were stored at room temperature before opening the external packages. Desalted goat whey powder was purchased from Emroserum Company of France, and desalted bovine whey powder was purchased from Friesland Campina Company of Netherlands. All other reagents were of analytical grade purity in this research.

## 1.2 Methods

### 1.2.1 Spectra collection

NIR spectra were acquired with an MPA Multi-Purpose FT-NIR Analyzer (Bruker, Germany). The spectra were acquired at room temperature (20-23 ℃). The spectral range between 12000 and 4000 $cm^{-1}$ (833-2500 nm) was scanned with a resolution of 8 $cm^{-1}$. Each sample of goat milk and infant goat milk formula was scanned for 64 seconds.

### 1.2.2 Preparation of adulterated samples

Goat milk mixed with bovine milk: 2%, 5%, 10%, 15%, 25%, 50%, 80%, 100% (w/w) of bovine milk were mixed into goat milk.

Desalted goat whey powder mixed with desalted bovine whey powder: based on the formula of Stage 2 infant goat milk formulas provided by Shaanxi Y Dairy Co., Ltd., keeping other ingredients unchanged, desalted goat whey powder mixed with 2%, 5%, 10%, 15%, 25%, 50%, 80%, and 100% (w/w) desalted bovine whey powder was added to the Stage 2 infant goat milk formulas.

### 1.2.3 Validation of the NIRS method

To validate the method, stability, precision, and reproducibility tests were carried out using the sample of Stage 2 formulas. For the stability test, the NIR spectra of the same sample were scanned at 0, 4, 8, 12, 20, and 24 h, respectively. For the precision test, six samples were prepared and tested independently. For the reproducibility test, the same sample was tested continuously 6 times under the same conditions. For each test, the absorbance of features was used to calculate the relative standard deviation (RSD).

## 1.3 Data analysis

The collected NIRS of goat milk and infant goat milk formulas were analyzed by OMNIC 2.0 spectral analysis software (Thermo Electron Corp., USA), and the characteristic common maximum absorption intensity was determined according to the overall profile similarity of the spectral map, and the NIR

spectral fingerprint of goat milk and infant goat milk formulas was established. PCA was carried out by Minitab 16.2.3 analysis software (Six Sigma Academy International, L.L.C., USA) to analyze the scores of infant goat milk formulas at different stages, the adulteration of goat milk, and desalted goat whey powder. The statistical differences in the results were analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL) software. The data were analyzed by one-way ANOVA test and Duncan's test, $P < 0.05$ was considered to be significant.

# 2 Results And Discussion

# 2.1 Establishment of fingerprint atlas

2.1.1 Establishment of shared maximum absorption intensity of goat milk

Fingerprints are based on the inherent quality characteristics of foods and can reflect the internal characteristics of the food detected by modern instruments such as spectroscopy and chromatography. They are integral, systematic, characteristic, and stable (Fang et al. 2006).

As a fast and non-destructive detection technique, NIRS is often used for qualitative and quantitative analysis of chemical components, with a wavelength range of 780 ~ 2526nm. Frequency multiplication and combined frequency absorption of H-containing groups (CH, OH, NH, and SH) take charge to generate the region. It has the advantages of simplicity, speed, and non-destructiveness, but exists poor anti-interference, low sensitivity, and requires a large number of samples. During the experiments, these methods can be combined to take advantage of their strengths and circumvent their weaknesses to obtain more accurate experimental data (Chavan et al. 2017).

Figure 1 presented the spectra of goat milk from 11 different batches. The absorption bands of goat milk NIR spectra resembled those found by Andueza et al. (2013). The 6 maximum absorption intensities identified as the main common absorption maximum in the figure were shared by 11 batches of samples. The corresponding NIR wave values of the common maximum absorption intensity were 10270.95, 8612.31, 8334.77, 6914.02, 5539.54, and 5202.52 $cm^{-1}$, which meant that there were 6 groups of similar major substances in these 11 different batches.

Observing the obtained spectra (Fig. 1), the peaks at 8612.31, 8334.77 $cm^{-1}$ are from the C-H stretch and the second overtone, which may be related to fat and fatty acids in goat milk (Temizkan et al. 2020; Nunez-Sanchez et al. 2016); whereas that at 5202.52 $cm^{-1}$ is associated with the stretching and bending of the O-H groups of water in the samples (Vasafi et al. 2021). Also, the combination of bending of the O-H group and stretching of the C-O group in lactose leads to the emergence of wave peak 5539.54 $cm^{-1}$ (Mohamed et al. 2021).

2.1.2 Establishment of shared maximum absorption intensity of infant goat milk formulas

The spectra of 11 batches of samples from three types of Stage 1, 2, and 3 infants goat milk formulas of Y company showed the 9 maximum absorption intensities shared by these samples and were the main common maximum absorption intensities (Fig. 2). The corresponding NIR wave values of the common maximum absorption intensity were 8262.08, 6795.08, 6352.36, 5790.64, 5684.91, 5162.87, 4753.17, 4336.86, and 4257.56 $cm^{-1}$, which indicated that there were 9 groups of samples with similar main substances in these 11 different batches.

Analyzing these received spectra (Fig. 2), secondary overtones of C-H stretching vibrations in saturated fat structures cause the band at 8262.08 $cm^{-1}$ (Huang et al. 2016). The first overtone of the NH stretching and the first overtone of the OH stretching result in peaks of 6795.08 and 6352.36 $cm^{-1}$, respectively (Henn et al. 2017). The bands at 5790.64, 5684.91 $cm^{-1}$ could be attributed to the first overtone of the CH, $CH_2$, and $CH_3$ vibration while the band at 5612.87 $cm^{-1}$ is related to the secondary overtones of OH stretching and CH stretching (Kang et al. 2006; Ferreira et al. 2014). The band at 4753.17 $cm^{-1}$ is connected to the N – H symmetric stretching vibration (Wu et al. 2009). The combination of CH stretching and $CH_2$ deformation determines the band at 4336.86 $cm^{-1}$. The band at 4257.56 $cm^{-1}$ depends on $CH_2$ stretching and $=CH_2$ bending of the C□C alkene group (Hourant et al. 2000).

Goat milk, an opaque medium, has multiple light dispersion effects because of opaque. Diffuse reflection adopted in the experiment minimizes signal interference from surface reflections, thus obtaining sufficient spectral information (Melfsen et al. 2012). In addition, although NIR is widely used in adulteration identification of bovine milk, goat milk, camel milk, or their milk products, the corresponding wavelength range set by NIR is short, and the sample common peaks measured are few. Kasemsumran et al. (2007) used NIR to quantify the feasibility of milk adulteration. The spectral range opted was 1100 to 2500 nm, and the obtained samples had two common peaks. By contrast, the NIRS of the experiment has a wide scanning range and more common peaks are obtained. Therefore, the identification of more organic groups can improve the accuracy of subsequent identification of adulteration.

### 2.1.3 Fingerprint methodology verification results

It can be seen from Table 1 that the absorbance value corresponding to the characteristic common maximum absorption intensity in goat milk was calculated, the range of relative standard deviation (RSD) of the stability test was 0.11%~0.55%; the range of RSD of the precision test was 0.46%~0.94%; the RSD of the reproducibility of the test ranges from 2.95–4.62%; the RSD values of both precision and reproducibility were less than 5%, the stability RSD value is less than 1%, which proved that the reproducibility, stability, and precision of this NIR fingerprinting method in goat milk were validated.

Table 1
Method validation of near-infrared spectroscopy fingerprint of goat milk

| Wavenumber | Reproducibility | | Stability | | Precision | |
|---|---|---|---|---|---|---|
| ($cm^{-1}$) | Mean value | RSD[α]/% | Mean value | RSD[α]/% | Mean value | RSD[α]/% |
| 10270.95 | 0.47 | 3.49 | 0.51 | 0.45 | 0.50 | 0.46 |
| 8612.31 | 0.64 | 4.46 | 0.69 | 0.55 | 0.68 | 0.63 |
| 8334.77 | 0.67 | 4.20 | 0.72 | 0.42 | 0.71 | 0.54 |
| 6914.02 | 1.70 | 4.48 | 1.75 | 0.11 | 1.77 | 0.47 |
| 5539.54 | 1.28 | 4.62 | 1.45 | 0.52 | 1.43 | 0.94 |
| 5202.52 | 1.90 | 2.95 | 1.91 | 0.20 | 1.95 | 0.50 |

[α] RSD, relative standard deviation

The results of the validation experiments of the NIRS of infant goat milk formulas were shown in Table 2. For the reproducibility test, RSDs of the absorbances of the nine features ranged from 0.33–1.43%. For the stability test, the RSD ranged from 0.39–2.71%. The RSD for the precision test ranged from 0.19–1.11%. All of the RSD values were below 5.0%. Therefore, the reproducibility, stability, and precision of this NIR fingerprinting method in infant goat milk formulas were validated.

Table 2
Method validation of near-infrared spectroscopy fingerprint of infant goat milk formulas

| Wavenumber | Reproducibility | | Stability | | Precision | |
|---|---|---|---|---|---|---|
| ($cm^{-1}$) | Mean value | RSD[α]/% | Mean value | RSD[α]/% | Mean value | RSD[α]/% |
| 8262.08 | 0.37 | 0.33 | 0.37 | 0.39 | 0.37 | 1.11 |
| 6795.08 | 0.60 | 0.69 | 0.63 | 1.79 | 0.61 | 0.34 |
| 6352.36 | 0.58 | 0.79 | 0.60 | 1.74 | 0.58 | 0.56 |
| 5790.64 | 0.66 | 0.85 | 0.67 | 0.96 | 0.66 | 0.53 |
| 5684.91 | 0.62 | 0.85 | 0.64 | 1.11 | 0.62 | 0.96 |
| 5162.87 | 0.68 | 0.93 | 0.74 | 1.49 | 0.68 | 0.49 |
| 4753.17 | 0.91 | 1.10 | 0.94 | 2.71 | 0.90 | 0.42 |
| 4336.86 | 1.07 | 1.43 | 1.10 | 1.56 | 1.07 | 0.48 |
| 4257.56 | 1.06 | 1.40 | 1.09 | 1.61 | 1.06 | 0.19 |

[α] RSD, relative standard deviation

# 2.2 Detection Of Sample Adulteration Based On Fingerprint Atlas

PCA is a multivariate statistical analysis method that extracts a few comprehensive indicators from multiple indicators that have correlations. It can simplify multiple variables into a few comprehensive variables through orthogonal transformation, then explain the prominent relationship among the elements in the original variables. The process of extracting principal components is to filter out the principal component factors through matrix calculation after deriving the principal components (Cozzolino et al. 2019).

2.2.1 PCA of the difference in infant goat milk formulas at different stages

Figure 3 was obtained by combining the NIRS and PCA of infant goat milk formulas with three different stages. There existed two principal components (PC1 and PC2). Principal component 1 contained 76.7% of sample information, and principal component 2 contained 13.2% of sample information. PC1 and PC2 explained 89.9% of sample information together. The main location of PCA score points for Stage 1, 2, and 3 of infant goat milk formulas samples were respectively in the third quadrant, the first quadrant, and the fourth quadrant, which can be explained by the differences in their main nutritional ingredients (Table 3). The results of the PCA scores map proved that the use of NIRS technology to distinguish and identify infant goat milk formulas with three different objects was applicable.

Table 3

The main chemical composition of Stage 1, 2, and 3 infant goat milk formulas of Y brand[α]

| Stage No. | Dry matter (g/100g, means ± SD, n = 11) | | | | |
|---|---|---|---|---|---|
| | Protein | Fat | Carbohydrate | Moisture | Ash |
| Stage 1 | $13.10 \pm 0.20^{c}$ | $26.05 \pm 0.25^{a}$ | $55.03 \pm 0.13^{a}$ | $2.69 \pm 0.11^{b}$ | $3.13 \pm 0.05^{c}$ |
| Stage 2 | $18.35 \pm 0.10^{b}$ | $20.93 \pm 1.25^{c}$ | $53.25 \pm 1.27^{b}$ | $2.92 \pm 0.06^{a}$ | $4.58 \pm 0.15^{b}$ |
| Stage 3 | $19.06 \pm 0.27^{a}$ | $21.94 \pm 0.51^{b}$ | $51.62 \pm 0.37^{c}$ | $2.92 \pm 0.18^{a}$ | $4.94 \pm 0.11^{a}$ |

[α] Different superscript letters within a column denote statistically significant differences according to Duncan's test ($P < 0.05$)

2.2.2 PCA of goat milk blended with bovine milk

The PCA score chart of principal component regression analysis was shown in Fig. 4. Two principal components (PC1 and PC2) were in the figure. PC1 represented 74.8% of sample information and PC2 contained 21.6% of sample information. PC1 and PC2 jointly explained 96.3% of sample information together.

It was clear from Fig. 4 that goat milk samples were separated from adulterated samples on the PCA score chart, indicating that the coupling of PCA with the NIRS fingerprinting method can effectively identify adulterated goat milk from pure goat milk.

The main chemical composition of goat milk and bovine milk were shown in Table 4. Although there was no significant difference in protein content between goat milk and bovine milk ($P > 0.05$), the structure of the casein micelles, various biologically active peptides, and non-protein nitrogen compounds such as amino acids, nucleotides, and nucleosides are different between the two (Teixeira et al. 2022). Furthermore, the levels and proportion of $\alpha_{S1}$-casein, $\alpha_{S2}$-casein, and β-casein + κ-casein varied in goat milk and bovine milk (Ceballios et al. 2009). Samples with subtle differences can be differentiated using NIRS fingerprint technology combined with PCA according to their ingredients and the proportion relationships among ingredients because the main characteristics of fingerprint technology lie in its “integrity” and “fuzziness” (Chang et al. 2020). It was also easy to obtain that the fat content of goat milk and bovine milk was significantly different ($P < 0.05$) from Table 4. The difference in the structure and content of organic matter in two types of milk is the reason why PCA combined with the NIRS fingerprinting method can well distinguish goat milk and adulterated goat milk mixed with different proportions of bovine milk.

Table 4

Comparison of main chemical composition of between goat milk and bovine milk[α]

| Type of milk | Dry matter (g/100g, means ± SD, n = 11) | | | | |
|---|---|---|---|---|---|
| | Protein | Fat | Lactose | Moisture | Ash |
| Goat milk | 3.56 ± 0.04[a] | 4.07 ± 0.11[a] | 4.58 ± 0.01[a] | 87.01 ± 0.08[b] | 0.70 ± 0.01[a] |
| Bovine milk | 3.04 ± 0.16[a] | 3.26 ± 0.10[b] | 4.41 ± 0.13[a] | 88.37 ± 0.18[a] | 0.71 ± 0.03[a] |

[α] Different superscript letters within a column denote statistically significant differences according to Duncan’s test ($P < 0.05$)

2.2.3 Principal component analysis of desalted goat whey powder mixed with desalted bovine whey powder

The NIRS fingerprints of desalted goat whey powder mixed with desalted bovine whey powder in different proportions to apply the established NIRS fingerprinting method. The correlations of two principal components (PC1 and PC2) with desalted goat whey powder blended with different proportions of desalted bovine whey powder were shown in Fig. 5.

The cumulative variance contribution rate was 99.8%. The first component PC1 accounted for 99.5% of the total variance while the second component PC2 contained 0.3% of the total variance. It can be seen from Fig. 5 that the PCA score points of Y brand Stage 2 infant goat milk formulas samples were mainly located on the right half of the score map, while the PCA score points of adulterated milk powder samples

were located on the left side of the score map. They were perfectly separated. The main component results showed that the use of NIRS technology can validly identify the adulteration of infant goat milk formulas blending desalted bovine whey powder.

It is known the main chemical composition in desalted goat whey powder and desalted bovine whey powder from Table 5. Among them, the protein content of desalted goat whey powder and desalted bovine whey powder was not significantly different ($P > 0.05$). However, Whey powder is composed of β-lactoglobulin (β-LG), α-lactoglobulin (α-LA), serum albumin (SA), lactoferrin, immunoglobulin, and other components (Kerasioti et al. 2019). Bovine whey powder is made up of 50–63% β-LG, 20% α-LA, 6–8% SA, and 1% immunoglobulin G (lg G) while goat whey powder consists of 40% β-LG, 30% α-LA, 10% SA, and 10% immunoglobulin GH (lg G-H) composition (Saxton et al. 2021; Zhao et al. 2020). Furthermore, the fat and lactose content of the two kinds of desalted whey powder was significantly different ($P < 0.05$). Differences in the content of various types of protein and other organic components lead to NIRS fingerprints can well identify adulteration of varying proportions of desalted bovine whey powder in infant goat milk powder.

Table 5
Comparison of main chemical composition of between desalted goat whey powder and desalted bovine whey powder[α]

| Type of whey powder | Dry matter (g/100g, means ± SD, n = 3) | | | | |
|---|---|---|---|---|---|
| | Protein | Fat | Lactose | Moisture | Ash |
| Desalted goat whey powder | 13.96 ± 0.15[a] | 0.77 ± 0.02[b] | 83.94 ± 0.02[a] | 1.51 ± 0.14[b] | 0.95 ± 0.05[a] |
| Desalted bovine whey powder | 13.10 ± 0.17[a] | 1.30 ± 0.17[a] | 83.00 ± 0.01[b] | 2.00 ± 0.00[a] | 0.93 ± 0.12[a] |

[α] Different superscript letters within a column denote statistically significant differences according to Duncan's test ($P < 0.05$)

# 3 Conclusions

In this study, the fingerprints of goat milk and infant goat milk formulas were established based on NIRS technology. By comparing the established NIR fingerprint of goat milk with the NIRS of adulterated goat milk and combining with the PCA, it was found that they were completely separated on the PCA score map, demonstrating that the NIR fingerprint could effectively identify the situation of adulterated goat milk; by comparing the established fingerprint of Y brand Stage 2 infant goat milk formulas with adulterated desalted bovine whey powder with the help of PCA, it was found that they were completely separated on the PCA score chart, which meant that the NIR fingerprint can effectively identify infant formulas adulteration of desalted goat whey powder. Given all of that, the NIRS fingerprint combined with the PCA method can distinguish different stages of infant goat milk formulas and adulteration, then achieve quality control.

# 4 Declarations

**Author Contribution** HE Yongji: investigation, wrote and edited the manuscript; ZENG Wanjun: envisaged the concept, investigation, methodology, wrote original draft, edited the manuscript; ZHAO Yuxuan: investigation, wrote and edited the manuscript; ZHU Xinpeng: envisaged the concept, project administration, supervision, resources; WAN Hongchang: envisaged the concept, resources, funding acquisition; ZHANG Meng: envisaged the concept, funding acquisition; LI Zhicheng: envisaged the concept, project administration, supervision, resources, wrote and edited the manuscript. All authors have read and approved the final version of the manuscript.

**Funding** This study was partly supported by Shaanxi Provincial Department of Science and Technology Project (No. 2021ZDLNY02-10) and the Science and Technology Program (No. 20193037YF025NS025) of Xi'an City, the Government of PR China.

**Data Availability** The datasets generated during the current study are included in this published article, or they are available from the corresponding author on reasonable request.

**Ethics Approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Informed Consent** Not applicable.

**Conflicts of interest** All the authors declare no competing interests regarding the publication of this paper.

# References

1. Ahmed AS, El-Bassiony T, Elmalt LM, Ibrahim HR (2015) Identification of potent antioxidant bioactive peptides from goat milk proteins. Food Res Int 74:80–88.
2. Andueza D, Rouel J, Chilliard Y, Leroux C, Ferlay A (2013) Prediction of the goat milk fatty acids by near infrared reflectance spectroscopy. 115(6):612–620.
3. Bourlieu C, Menard O, Bouzerzour K, Mandalari G, Macierzanka A, Mackie AR, Dupont D (2014) Specificity of infant digestive conditions: Some clues for developing relevant in vitro models. Crit Rev Food Sci Nutr 54(11):142–1457.
4. Cai M, Zhou Y, Gesang S, Bianba C, Ding L (2006) Chemical fingerprint analysis of rhizomes of gymnadenia conopsea by HPLC-DAD-MSn. J Chromatogr B 844(2):301–307.
5. Ceballos LS, Morales ER, Adarve GDLT, Castro JD, Martinez LP, Sampelayo MRS (2009) Composition of goat and cow milk produced under similar conditions and analyzed by identical methodology. J Food Compos Anal 22(4):322–329.
6. Chandra A, LI YQ, Rana J, Persons K, Hyun C, Shen S, Mulder T (2011) Qualitative categorization of supplement grade ginkgo biloba leaf extracts for authenticity. J Funct Foods 3(2):107–114.

7. Chang S, Yin C, Liang S, Lu M, Wang P, Li Z (2020) Confirmation of brand identification in infant formulas by using near-infrared spectroscopy fingerprints. Anal. Methods 12:2469–2475.
8. Chavan RB, Bhargavi N, Lodageskar A, Shastri NR (2017) Near infrared spectroscopy: a tool for solid state characterization. Drug Discov Today 22(12):1835–1843.
9. Chen Q, Yin Q, Xie Q, Evivie SE, Zhao X, Li B, Huo G (2022) Elucidating the physicochemical properties and surface composition of goat milk-based infant formula powders. Food Chem 377:131936.
10. Clark S, Garcia MBM (2017) A 100-year review: Advances in goat milk research. J Dairy Sci 100(12):10026–10044.
11. Cozzolino D, Power A, Chapman J (2019) Interpreting and reporting principal component analysis in food science analysis and beyond. Food Anal Methods 12:2469–2473.
12. Cozzolino D (2021) The ability of near infrared (NIR) spectroscopy to predict functional properties in foods: challenges and opportunities. Molecules 26:6981.
13. Fang KT, Liang YZ, Yin XL, Chan K, Lu GH (2006) Critical value determination on similarity of fingerprints. Chemometr Intell Lab Syst 82(1–2):236–240.
14. Ferreira DS, Galao OF, Pallone JAL, Poppi RJ (2014) Comparison and application of near-infrared (NIR) and mid-infrared (MIR) spectroscopy for determination of quality parameters in soybean samples. Food Control 35(1):227–232.
15. Gao P, Li J, Li Z, Hao J, Zan L (2016) Establishment and application of milk fingerprint by gel filtration chromatography. J Dairy Sci 99(12):9493–9501.
16. He X, Chen X, Qu X, Ma L, Xu W, Huang K (2019) Evaluation of flavonoid and polyphenol constituents in mulberry leaves using HPLC fingerprint analysis. Int J Food Sci Tech 55(2):526–533.
17. Henn R, Kirchler CG, Grossgut M, Huck CW (2017) Comparison of sensitivity to artificial spectral errors and multivariate LOD in NIR spectroscopy – Determining the performance of miniaturizations on melamine in milk powder. Talanta 166:109–118.
18. Hourant P, Baeten V, Morales MT, Meurens M, Aparicio R (2000) Oil and fat classification by selected bands of near-infrared spectroscopy. Appl Spectrosc 54(8):1168–1174.
19. Huang M, Kim MS, Chao KL, Qin JW, Mo CY, Esquerre C, Delwiche S, Zhu QB (2016) Penetration depth measurement of near-infrared hyperspectral imaging light for milk powder. Sensors 16(4):441.
20. Kang N, Kasemsumran S, Woo Y, Kim H, Ozaki Y (2006) Optimization of informative spectral regions for the quantification of cholesterol, glucose and urea in control serum solutions using searching combination moving window partial least squares regression method with near infrared spectroscopy. Chemometr Intell Lab Syst 82(1):90–96.
21. Kasemsumran S, Thanapase W, Kiatsoonthon A (2007) Feasibility of near-infrared spectroscopy to detect and to quantify adulterants in cow milk. Anal Sci 23(7):907–910.
22. Kerasioti E, Veskoukis A, Virgiliou C, Theodoridis G, Taitzoglou I, Kouretas D (2019) The strong antioxidant sheep/goat whey protein protects against mTOR overactivation in rats: a mode of action

mimicking fasting. Antioxidants 8(3):71.

23. Kim H, Shin H (2011) Simple and automatic determination of aldehydes and acetone in water by headspace solid-phase microextraction and gas chromatography-mass spectrometry. J Sep Sci 34(6):693–699.
24. Liang W, Wei Y, Gao M, Yan X, Zhu X, Guo W (2020) Detection of melamine adulteration in milk powder by using optical spectroscopy technologies in the last decade – a review. Food Anal Methods 13:2059–2069.
25. Lohumi S, Lee S, Lee H, Cho B (2015) A review of vibrational spectroscopic techniques for the detection of food authenticity and adulteration. Trends Food Sci Tech 46(1):85–98.
26. Ma HL, Wang JW, Chen YJ, Cheng JL, Lai ZT (2017) Rapid authentication of starch adulterations in ultrafine granular powder of Shanyao by near-infrared spectroscopy coupled with chemometric methods. Food Chem 215:108–115.
27. Maathuis A, Havenaar R, He S, Bellmann S (2017) Protein digestion and goat and cow milk infant formula and human milk under simulated infant conditions. J Pediatr Gastr Nutr 65(6):661–666.
28. Melfsen A, Hartung E, Haeussermann A (2012) Accuracy of milk composition analysis with near infrared spectroscopy in diffuse reflection mode. Biosyst Eng 112(3):210–217.
29. Mohamed AF, Amal AM, Anja E, Sherifa F, Ludger AW (2017) Assessment of sensory metabolites distribution in 3 cactus Opuntia ficus-indica fruit cultivars using UV fingerprinting and GC/MS profiling techniques. Lwt-food Sci Technol 80:145–154.
30. Mohamed H, Nagy P, Agbaba J, Kamai-Eldin A (2021) Use of near and mid infra-red spectroscopy for analysis of protein, fat, lactose and total solids in raw cow and camel milk. Food Chem 334:127436.
31. Nayik GA, Jagdale YD, Gaikwad SA, Devkatte AN, Dar AH, Dezmirean DS, Bobis O, Ranjha MMAN, Ansari MJ, Hemeg HA., Alotaibi SS (2021) Recent insights into processing approaches and potential health benefits of goat milk and its products: a review. Front Nutr 8:789117.
32. Nunez-Sanchez N, Martinez-Marin AL, Polvillo O, Fernandez-Cabanas VM, Carrizosa J, Urrutia B, Serradilla JM (2016) Near infrared spectroscopy (NIRS) for the determination of the milk fat fatty acid profile of goats. Food Chem 190:244–252.
33. Park YW, Juárez M, Ramos M, Haenlein GFW (2007) Physico-chemical characteristics of goat and sheep milk. Small Rumin Res 68(1):88–113.
34. Pasquini C (2018) Near Infrared Spectroscopy: a mature analytical technique with new perspectives – a review. Anal Chim Acta 1026: 8–36.
35. Pierna JAF, Abbas O, Lecler B, Hogrel P, Dardenne P, Baeten V (2015) NIR fingerprint screening for early control of non-conformity at feed mills. Food Chem 189:2–12.
36. Pieters S, Beer TD, Kasper JC, Bouipaep D, Waszkiewicz O, Goodarzi M, Tistaert C, Friess W, Remon J, Vervaet C, Heyden YV (2012) Near-infrared spectroscopy for in-line monitoring of protein unfolding and its interactions with lyoprotectants during freeze-drying. Anal Chem 84(2):947–955.

37. Pinto AD, Terio V, Marchetti P, Bottaro M, Mottola A, Bozzo G, Bonerba E, Ceci E, Tantillo G (2017) DNA-based approach for species identification of goat-milk products. Food Chem 229:93–97.
38. Qin OY, Wang L, Zareef M, Chen QS, Guo ZM, Li HH (2020) A feasibility of nondestructive rapid detection of total volatile basic nitrogen content in frozen pork based on portable near-infrared spectroscopy. Microchem J 157:105020.
39. Saxton R, McDougal OM (2021) Whey protein powder analysis by mid-infrared spectroscopy. Foods 10(5):1033.
40. Sen S, Dundar Z, Uncu O, Ozen B (2021) Potential of Fourier-transform infrared spectroscopy in adulteration detection and quality assessment in buffalo and goat milks. Microchem J 166:106207.
41. Shawky E, Selim DA (2019) NIR spectroscopy-multivariate analysis for discrimination and bioactive compounds prediction of different Citrus species peels. Spectrochim Acta A 219:1–7.
42. Shi T, Wu G, Jin Q, Wang X (2021) Detection of camellia oil adulteration using chemometrics based on fatty acids GC fingerprints and phytosterols GC–MS fingerprints. Food Chem 352:129422.
43. Teixeira JLD, Carames ETDS, Baptista DP, Gigate ML, Pallone JAL (2022) Adulteration detection in goat dairy beverage through NIR spectroscopy and DD-SIMCA.Food Anal Methods 15:783–791.
44. Temizkan R, Can A, Dogan MA, Mortas M, Ayvaz H (2020) Rapid detection of milk fat adulteration in yoghurts using near and mid-infrared spectroscopy. Int Dairy J 110:104795.
45. Vasafi PS, Paquet-Durand O, Brettschneider K, Hinrichs J, Hitzmann B (2021) Anomaly detection during milk processing by autoencoder neural network based on near-infrared spectroscopy. 299:110510.
46. Wang Y, Eastwood B, Yang Z, Campo LD, Knott R, Prosser, Carpenter E, Hemar Y (2019) Rheological and structural characterization of acidified skim milks and infant formulae made from cow and goat milk. Food Hydrocoll 96:161–170.
47. Williams P, Manley M, Fox G, Geladi P (2010) Indirect detection of fusarium verticillioides in maize (Zea maize L.) kernels by NIR hyperspectral imaging. J Near Infrared Spec 18(1):49–58.
48. Wu D, He Y, Shi JH, Feng SJ (2009) Exploring near and midinfrared spectroscopy to predict trace iron and zinc contents in powdered milk. J Agric Food Chem 57(5):1697–1704.
49. Xia Y, Yu S, Liang J, Yang B, Kuang H (2020) Chemical fingerprinting techniques for the differentiation of polysaccharides from genus Astragalus. J Pharmaceut Biomed 178:112898.
50. Ye A, Cui J, Carpenter E, Prosser C, Singh H (2019) Dynamic in vitro gastric digestion of infant formulae made with goat milk and cow milk: Influence of protein composition. Int Dairy J 97:76–85.
51. Yi T, Gu X, Li W, Wu H, Cai B, Zhang B (2017) Techniques for biological fingerprinting of traditional Chinese medicine. Trends Analyt Chem 97:272–282.
52. Yu Z, Madelin G, Sodickson DK, Cloos MA (2020) Simultaneous proton magnetic resonance fingerprinting and sodium MRI. Magn Reson Med 83(6):2232–2242.
53. Zhang K, Tan Z, Chen C, Sun XS, Wang D (2017) Rapid prediction of camelia seed oil content using near-infrared spectroscopy. Energ Fuel 31(5):5629–5634.

54. Zhao X, Cheng M, Zhang X, Li X, Chen D, Qin Y, Wang J, Wang C (2020) The effect of heat treatment on the microstructure and functional properties of whey protein from goat milk. J Dairy Sci 103(2):1289–1302.

# Figures

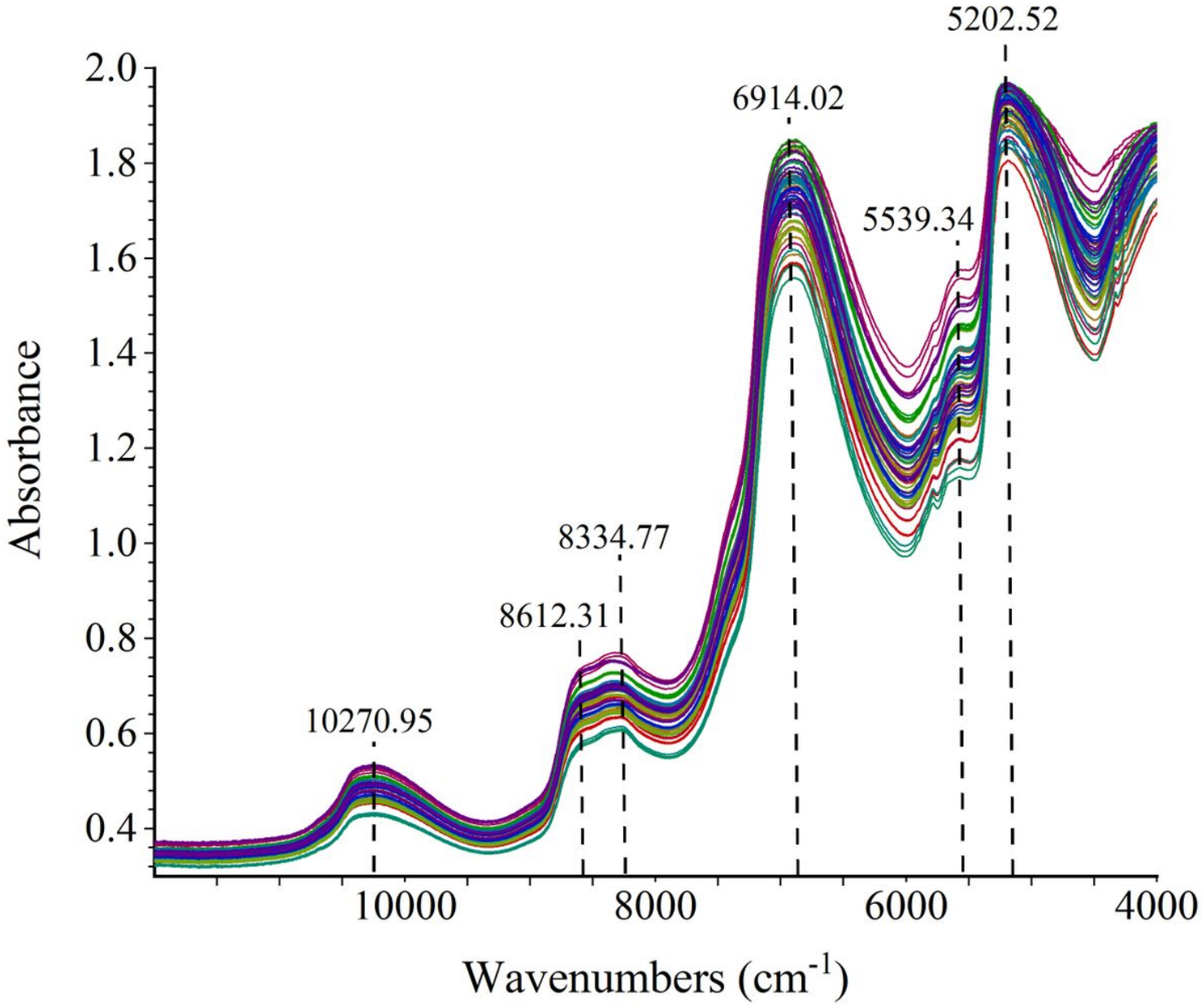



**Figure 1**

Near-infrared spectroscopy fingerprint of goat milk

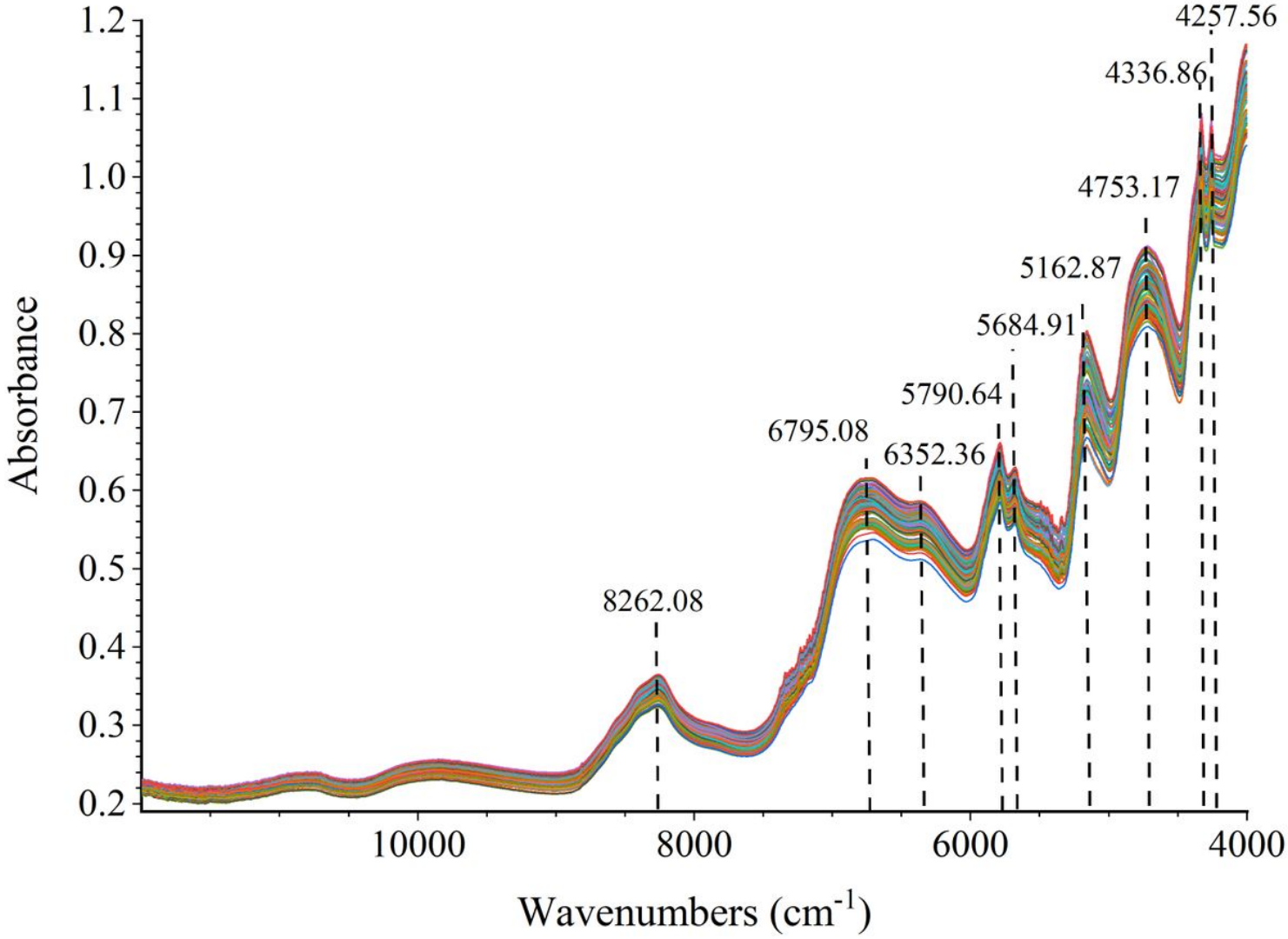
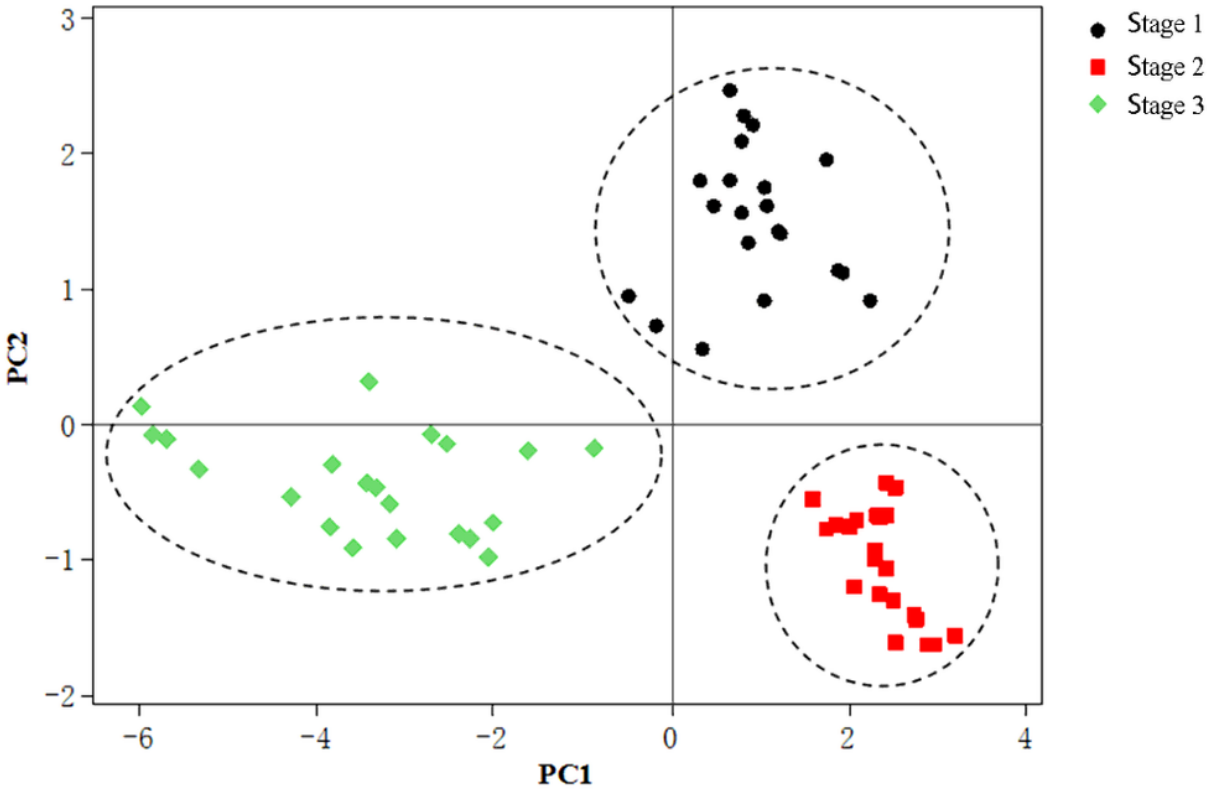



**Figure 2**

Near-infrared spectroscopy fingerprint of infant goat milk formulas of Y brand




## Figure 3

PCA score map of infant goat milk formulas (Stage 1 for infants aged between 0-6 months, Stage 2 for infants aged between 6-12 months, Stage 3 for infants aged between 12- 36 months)

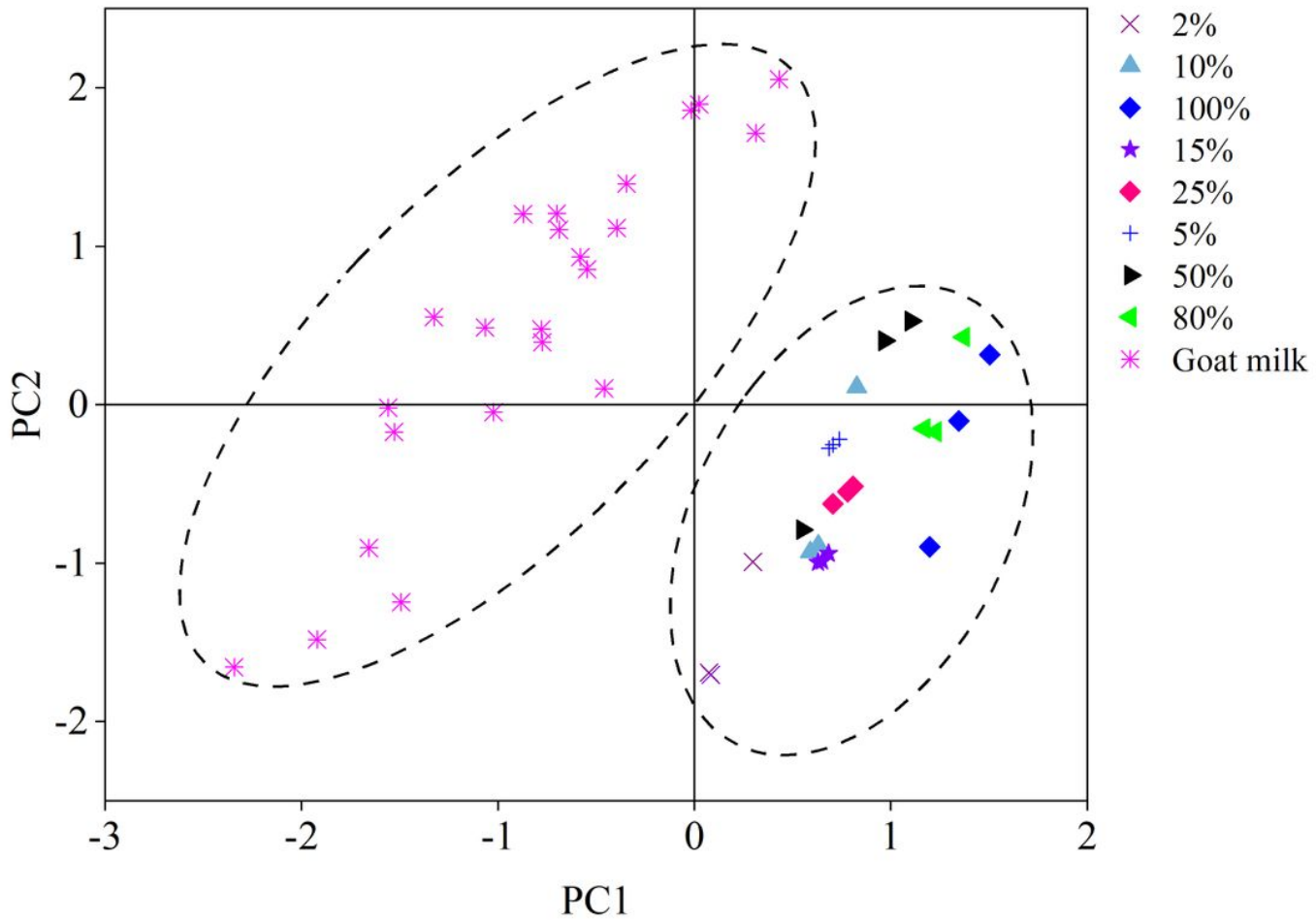



**Figure 4**

PCA score map of goat milk blended with different proportions of bovine milk (2%, 10%, 15%, 25%, 50%, 80%, 100% represent different proportions of bovine milk are mixed into goat milk)

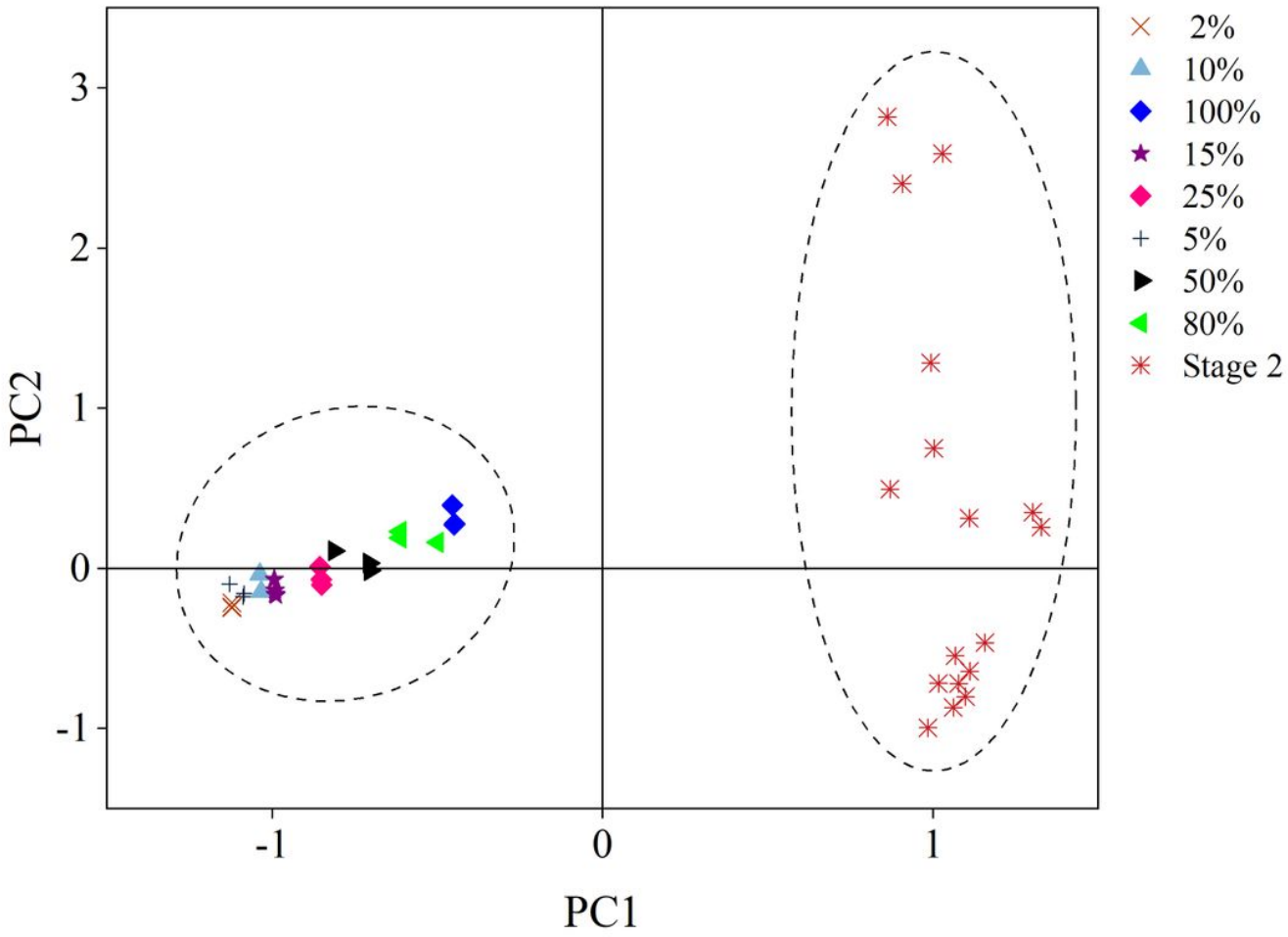



**Figure 5**

PCA score map of desalted goat whey powder blended with different proportions of desalted bovine whey powder (2%, 10%, 15%, 25%, 50%, 80%, 100% represent different proportions of desalted bovine whey powder were added to the Stage 2 infant goat milk formulas processed with desalted goat whey powder)