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INVESTIGATION OF REGULAR AND HYPOALLERGENIC INFANT MILK FORMULAS BY ATR-FTIR SPECTROSCOPY COMBINED WITH MULTIVARIATE ANALYSIS METHODS

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ABSTRACT
Infant formulas contain nutrients and bioactive ingredients designed to meet the nutrition requirements of infants. The majority of infant formulas are cow's milk (CM) based regular ones. Since CM is one of the most important allergen source, there are specialized formulas such as partially and extensively hydrolyzed, aminoacid-based for CM-allergic infants. In the current study, attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) in combination with multivariate analysis was used to identify the molecular differences between commercially available hypoallergenic (HF) and regular formulas (RF) in Turkey. Higher saturated fatty acid (FA) content and qualitatively longer acyl chained FAs were observed in hypoallergenic formulations. The results of hierarchical cluster analysis (HCA) and principal component analysis (PCA) showed that there were differences between the infrared spectra of the two groups. The HF and RF samples were clearly separated from each other in 1200-800 cm^{-1} spectral region with 83% variation and 100% accuracy.
Keywords: ATR-FTIR spectroscopy, hypoallergenic formula, infant milk formula, multivariate analysis methods

STANDART VE HİPOALERJENİK BEBEK SÜTÜ FORMÜLLERİNİN ATR-FTIR SPEKTROSKOPİSİ VE ÇOK DEĞİŞKENLİ ANALİZ YÖNTEMLERİYLE İNCELENMESİ

ÖZ
Bebek formülleri, bebeklerin beslenme gereksinimlerini karşılamak için tasarlanmış besinler ve biyoaktif bileşenler içerir. Bebek formüllerinin çoğu inek sütü (CM) bazlı normal formüllerdir. CM en önemli alerjen kaynaklarından biri olduğu için CM alerjisi olan bebekler için kısmen ve yoğun hidrolize, aminoasit bazlı gibi özel formüller bulunmaktadır. Bu çalışmada, Türkiye'de ticari olarak satılan standart ve hipoalerjenik bebek sütü formüllerinin moleküler farklılıklarının ayırt edilebilmesi

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amacıyla zayıflatılmış toplam yansıma (ATR)- Fourier Dönüşümlü Kızılötesi (FTIR) spektroskopisi, kemometrik yöntemlerle birlikte kullanılmıştır. Hipoalerjenik formül (HF) örneklerinde standart formüllere (RF) göre önemli derecede daha yüksek doymuş yağ asidi miktarı ve niteliksel olarak daha uzun zincirli yağ asidi gruplarının olduğu bulunmuştur. Hiyerarşik kümele analizi (HCA) ve temel bileşen analizi (PCA) sonuçlarına göre, %100 duyarlılık ve %83 varyasyon ile en belirgin ayrımın olduğu spektral bölge 1200-800 cm^{-1} olarak belirlenmiştir.
Anahtar kelimeler: ATR-FTIR spektroskopisi, bebek sütü formülü, hipoalerjenik formül, çok değişkenli analiz yöntemleri.

INTRODUCTION

Although breast milk is the optimal source of nutrition for infants, today, the use of ready-made infant formula has increased due to reasons such as insufficient breastfeeding or cutting off breastfeeding of babies from women who have to work.

Infant formula contains nutrients and bioactive ingredients designed to meet the essential nutrition to maintain and support adequate growth and optimal health for infants and as a suitable alternative to breast milk when a mother is unable to breastfeed (Joeckel and Phillips, 2009; Blanchard et al., 2013; Green Corkins and Shurley, 2016). The most common infant formulas are regular ones based on cow's milk. In infant formulas, cow's milk, whey protein concentrate, demineralized whey, carbohydrates such as lactose, maltodextrin, or sucrose and vegetable oils were used. Vitamins and minerals might be added to the formulas (Food and Agriculture Organization, 1981). It is necessary to adjust the whey/casein proteins ratio to support better digestibility and the nutritional needs of the baby (Blanchard et al., 2013). There are also specialized formulas for infants showing signs and symptoms of intolerance and with specific medical conditions, including allergies (Heine et al., 2002; Andres Martinez and Ballew, 2011; Martin et al., 2016; Osborn et al., 2017).

Hypoallergenic infant formulas are advised for infants who cannot be breastfed and have cow's milk allergy (CMA), affecting 2-3% of all babies (Dias et al., 2022). These formulas have shorter, easier digestible and potentially less allergenic proteins obtained by hydrolysis of larger protein chains. There are 3 main varieties of hypoallergenic formulas; partially, extensively hydrolyzed, and free amino acid-based. The partial or extensive cleavage of cow's milk proteins obtained by peptidases is one of the most effective methods of reducing allergic reactions to these proteins. The American Academy of Pediatrics (AAP) propounds the use of extensively hypoallergenic formulas in babies and children with milk allergies (American Academy of Pediatrics, Committee on Nutrition, 2000). The more extensively the formula hydrolyzes, the less potentially allergenic compounds remain, and the better an allergic infant can tolerate it. Moreover, free amino acid-based formula is formulated with 100% free amino acids for infants whose symptoms of hypersensitivity persist on extensively hydrolyzed cow's milk protein (Nocerino et al., 2021). There is a research about the negative effects of the cow milk processing into the hypoallergenic formula. This research aimed to decide the transforming growth factor-beta (TGF-β) and the anti-inflammatory activity of hypoallergenic and regular formulas. The authors proposed that hypoallergenic formulas do not have *in vitro* TGF-β activity and have lower anti-inflammatory activity in comparison with regular ones (Panahipour et al., 2020).

Infrared (IR) spectroscopy is a technique that has been applied (mid-infrared (MIR) and near-infrared (NIR)) in many fields such as food, agriculture, medicine, biomedical applications, and chemistry (Linker, 2011; Gok et al., 2015; Bayari et al., 2020, Baltacioglu, 2022; Yonar et al., 2022).

Infrared spectroscopic analysis is highly sensitive and quick method to obtain high quality spectrum of organic and inorganic compounds. It is a non-destructive technique and can identify even small amount of samples without the requirement of sample preparation by its attenuated total reflectance unit (ATR). Combined with multivariate analysis such as hierarchical cluster

analysis (HCA) and principal component analysis (PCA), FTIR has been useful for additive detection in a variety of food products and routinely used in the dairy industry to determine milk components such as fat, protein, and lactose (Wang et al., 2009; Gok et al., 2015; Granato et al., 2018; Rovere et al., 2021). FTIR spectroscopy has been used to measure the amount of melamine and cyanuric acid in milk and infant formula powders (Mauer et al., 2009; Lu et al., 2009; Jawaid et al., 2014; Huang et al., 2014; García-Miguel et al., 2018). It can be concluded that FTIR spectroscopy coupled with multivariate classification allows for rapid and robust identification and quantification of adulterants in food even if their concentrations are very low. The analysis of whey protein powder intentionally adulterated with either protein or amino acids was carried out by mid-infrared spectroscopy. This study provided evidence that product tampering can be easily monitored with IR method (Saxton and McDougal, 2021). Another study which aimed to observe iron-induced oxidation in liquid infant formula using IR spectroscopy claimed that IR spectroscopy was able to identify the spectral changes specific to lipid oxidation (Daoud et al, 2020). Milk fat and its fatty acid (FA) composition are important in dairy production and human health. Rovere et al. used infrared spectral data to predict FA composition in milk (Rovere et al., 2021).

There are no any other studies to demonstrate the spectral differences between regular and hypoallergenic infant formulas. The aim of the present study was to develop a fast, accurate and reagent free analytical method for determining the spectral differences in fat, protein, carbohydrate of hypoallergenic and regular formulas commercially available in the Turkish markets by using ATR-FTIR spectroscopy in combination with multivariate data analyses.

MATERIALS AND METHODS

Sampling

Different random commercial regular (n=6) and hypoallergenic (n=5) infant formula powder samples for babies up to 6 months of age were purchased from markets in Turkey.

ATR-FTIR spectral data collection and data analysis

ATR-FTIR spectra were recorded on a Perkin Elmer Frontier model FTIR spectrometer (Perkin Elmer Inc., USA) equipped with a Quest single reflection attenuated total reflectance (ATR) accessory (Specac Ltd., UK). A small quantity of the infant formula samples was placed on the diamond ATR crystal plate. To check the reproducibility of the identical spectra, three spectra for individual formula samples were collected in an absorbance mode over the range of 4000–450 cm^{-1} at 4 cm^{-1} and 64 co-added scans at room temperature. Prior to sample measurement, a background spectrum of the empty, clean ATR crystal was recorded which is automatically subtracted from the recorded spectra of the samples by using Perkin Elmer Spectrum software (version 10.03.06). The average spectrum was taken for each sample and the baseline corrected average spectra were normalized with respect to ν(OH) band (3650-3030 cm^{-1}).

The area values under the spectral bands are proportional to the concentration of the respective molecules /the functional groups in the samples. Information about the relative concentration of the respective molecules in the samples can be obtained by the band area/band intensity analysis (Bayarı et al., 2020; Akgun et al., 2021; Yonar et al., 2022). The infrared integrated band area ratios to evaluate spectroscopic data and their suggestions are listed in Table 1.

Multivariate analysis methods

HCA and PCA were performed to discriminate the HF groups from RF groups based on their spectral differences using Unscrambler X 10.3 software (Camo Software Inc., Norway). The average baseline corrected vector normalized spectra were used for HCA. The similarities between the spectra are determined by using distance calculation and classification algorithms. The results are displayed as dendrograms constructed using Ward's algorithm. Heterogeneity values in the dendrograms represent the magnitude of the similarity and its higher values indicate higher dissimilarity between the groups.

Table 1. The integrated spectral regions and interpretation for the infrared bands used in this study

Integrated band area ratios	Indication
CH_2 as stretching / CH_3 as stretching	aliphatic acyl chain length of fatty acids
CH_2 as stretching / (CH_2 as stretching +CH_2 s stretching)	saturated lipids content
HC=CH olefinic band/ (CH_2 as stretching +CH_2 s stretching)	unsaturation index
ester C=O stretching / (CH_2 as stretching +CH_2 s stretching)	carbonyl amount

PCA is a mathematical tool that aims to represent the variation present in the dataset (Nieuwoudt et al., 2004; Granato et al., 2018;) and used to reduce the dimension of datasets by transforming the old coordinate system (peaks) into the new coordinate system (PCs). In PCA, each spectrum is characterized with a single point on scores plot with axes called principal components (PCs). Loadings plot that displays the variables make the greatest contribution to the principal components is calculated for each PC (Wold et al., 1987).

Prior to analysis, FTIR spectra were pre-processed by performing baseline correction and vector normalization without doing smoothing or any additional pre-processing (Esbensen, 2010). Initially, mean-centered PCA was conducted over whole region, 3050-2800 and 1800-800 cm^{-1} spectral regions for the studied groups and then, the best spectral regions as 1700-1500 and 1200-800 cm^{-1} were chosen from the loading plots for the analyses. PCA results are presented as score and loading plots.

Statistics

The results were expressed as mean ± standard error of mean (SEM). The data were evaluated using an unpaired (independent) t-test in GraphPad Prism 6 (GraphPad Software, Inc.) to compare the means of the studied groups (two independent groups- regular and hypoallergenic infant formulas) for determining the significance of the difference between them. $P<0.05$ was considered as statistically significant. The degree of significance for the comparison of the hypoallergenic formulas with respect to regular ones was denoted as $*P<0.05$, $**P<0.01$, $***P<0.001$, $****P<0.0001$.

RESULTS AND DISCUSSION

ATR-FTIR Spectra

Figs. 1A and B show the average ATR-FTIR spectra of the regular (RF) and hypoallergenic infant formula (HF) samples, respectively. Table 2 presents the band assignment of the main bands in agreement with the literature (Sjaunja 1984; Guillén and Cabo, 1997; Kaylegian et al., 2009; Santos et al., 2013; Grelet et al. 2015; Botelho et al., 2015; Ye et al., 2017; Wiercigroch et al., 2017; Balan et al., 2020).

The mid-infrared spectrum contains specific and characteristic absorption bands arising from the functional groups of various biomolecules such as fats, proteins, and carbohydrates. FTIR is widely used for qualitative and quantitative determination of food components.

Whole cow's milk contains 3.2-4.7% fat depending on particularly feeding strategies. Milk fat contains a complex mixture of various lipid substances. These are primarily triglycerides and fatty acids (FAs). Milk fat contains saturated FA and monounsaturated FA, approximately 60-70%, 20-35% and 5%, respectively (Djordjevic et al., 2019).

To obtain information about spectral parameters such as the amount of unsaturation level in fat and aliphatic acyl chain length of FA and the amount of carbonyl group in samples, the integrated area ratios of some specific bands were calculated (Table 1, Fig. 2A-D).

The band area ratios related to saturated FA content and aliphatic acyl chain length in FAs were found to be significantly higher in HF group compared to RF group (Fig. 2A, B) indicating the higher saturated FA content and the presence of

qualitatively longer acyl chained FAs. In addition, there is a unique vibrational band of unsaturated lipids approximately at 3008 cm^{-1} which is assigned as olefinic C=CH stretching vibration and can be employed for the content of unsaturated FAs. The molecular fingerprint region also contains some considerable lipid-associated spectral bands such as carbonyl (C=O) stretching mode of the lipids located at 1744 cm^{-1}. The band area ratios related to the carbonyl amount and unsaturation index (Table 1) were found to be lower in HF group compared to RF group (Fig. 2C, D). The unsaturation index was obtained by taking the ratio of olefinic band area to total saturated FA areas (Table 1). For hypoallergenic infant formulas, increases in the areas of all saturated or unsaturated fatty acids are observed. So the decrease observed in unsaturation index, considering not significant, demonstrates the more increase of saturated FAs.

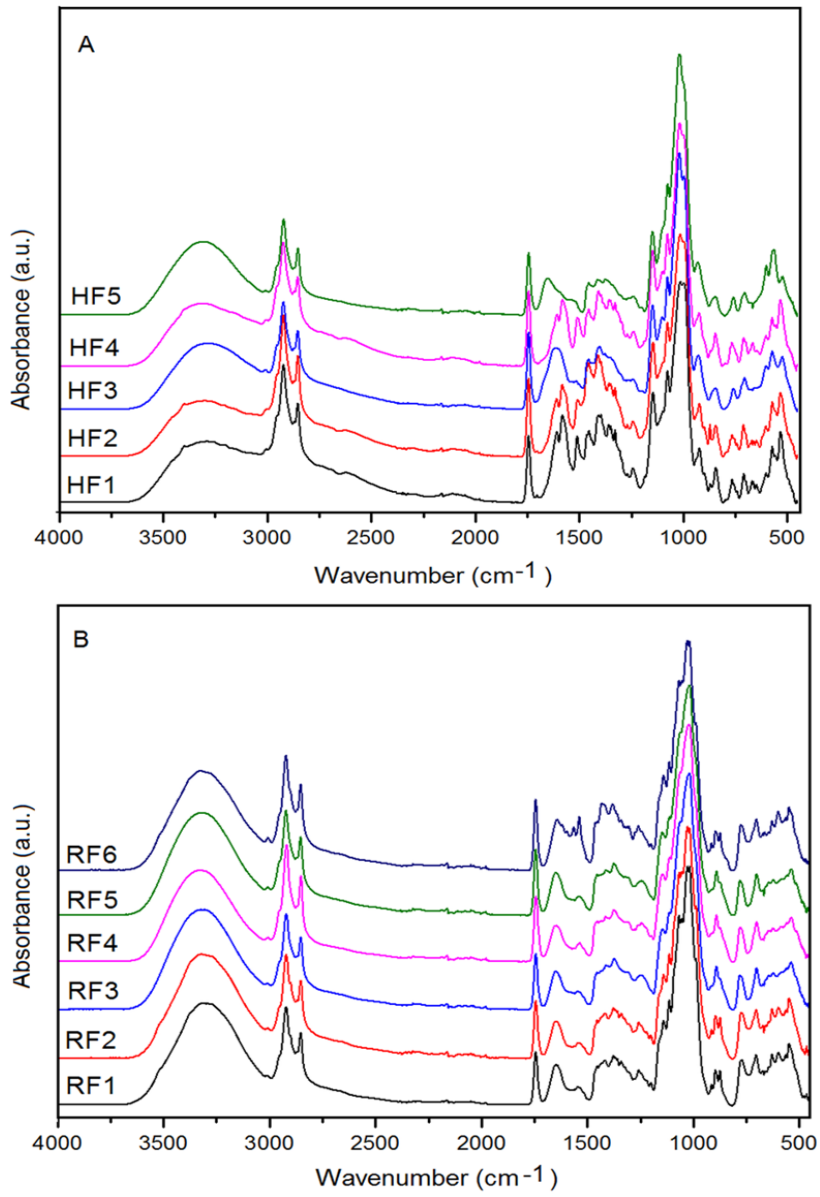

Fig. 1. Comparative ATR-FTIR spectra of A) hypoallergenic (HF) and B) regular infant formula (RF) samples in the 4000-450 cm^{-1} region.

Table 2. Assignments for the main bands in the FTIR spectrum of infant formula[a]

Wavenumber (cm^{-1})	Band Assignment	Presence status
3300	O–H stretching vibrations of water	HF and RF
3008	Olefinic =CH stretching vibration	HF and RF
2954	CH_3 group: C-H antisymmetric stretching	HF and RF
2923	Aliphatic CH_2 group :C-H antisymmetric stretching	HF and RF
2901	C- H stretching	RF
2872	CH_3 group: C-H symmetric stretching	HF and RF
2853	Aliphatic CH_2 group: C-H symmetric stretching	HF and RF
1744	Carbonyl C=O stretching -triglyceride esters	HF and RF
1648	Amide I: C=O and C-N stretching vibration	RF
1608	N-H bending of a primary amine	HF (available in free amino acid-based HFs)
1584	Carboxylic acid: COO^- antisymmetric stretching	HF (available in free amino acid-based HFs)
1538	Amide II: N−H in plane bending and C−N stretching	RF
1512	N-terminal amino group: NH_3^+ scissoring	HF
1467	CH_2 group (C-H bending), fat	HF and RF
1455	CH_2 , CH_3 groups (C-H asymmetric bending)	HF and RF
1396	Carboxylic acid: COO^- symmetric stretching	HF (available in free amino acid-based HFs)
1378	CH_3 group (C–H bending)	RF
1350	C-O stretching, deformation CH,	HF
1270	Amino acid side-chain vibrations	HF (free amino acid-based HFs)
1260	Amide III C-N stretching	RF
1244	C-O-C stretching-carbohydrate	HF and RF
1148	C-O-C ether stretching-carbohydrate	HF and RF (shift towards higher wavenumbers in HF)
1115	C-O-C stretching- carbohydrate	RF
1102	O=P–O or phosphate group of the casein proteins / C-O-C stretching of carbohydrate	HF
1076	C-O, C-C, C-H stretching -carbohydrate	HF and RF
1030	C-O stretching –carbohydrate (polysaccharide skeleton)	RF
1018	Phenylalanine-protein/C-O stretching vibrations of glucose	HF and RF
988	C-H out-of-plane bending	HF and RF
896	C-H out-of-plane bending	RF
846	Carbohydrate	HF

[a] The assignments are according to the literature [(Sjaunja 1984; Guiilen and Cabo, 1997, Kaylegian et al., 2009, Santos et al. 2013; Grelet et al. 2015; Botelho et al., 2015; Ye et al., 2017; Wiercigroch et al., 2017; Balan et al., 2020]. RF, Regular infant formula, HF, hypoallergenic infant formula

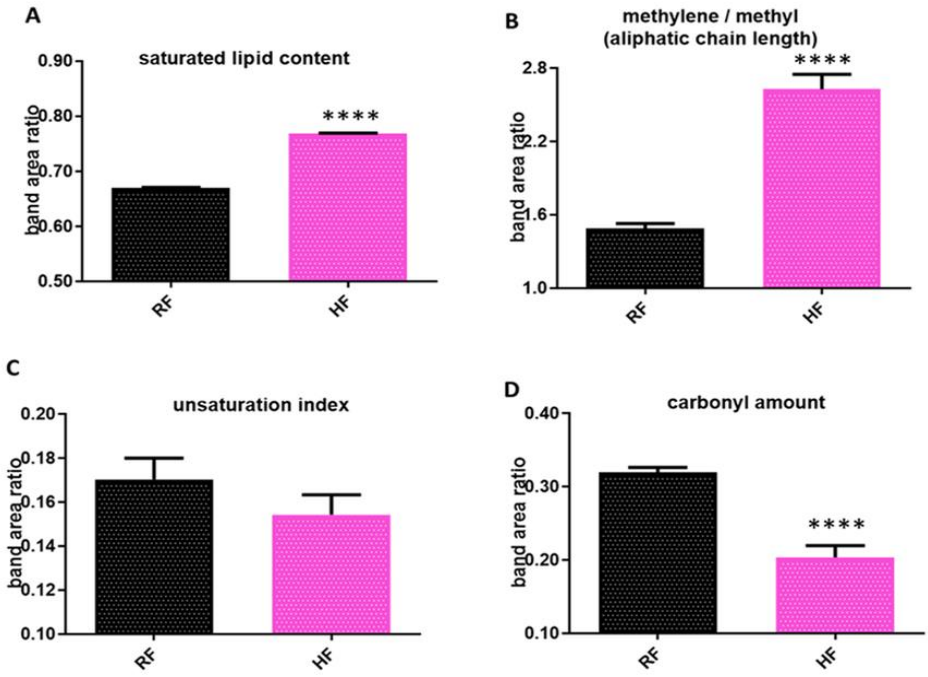

Figure 2. Bar graphs of some band area ratios related to relative amounts for RF and HF groups. A) saturated lipid content, B) aliphatic chain length, C) unsaturation index and D) carbonyl amount (The degree of significance for the comparison of the diseased groups with respect to the control group was denoted as * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001).

Fats are the primary energy source in infant formula, which provides 40-50% of the infant's energy needs. The fat composition of infant formula changes in accordance with the used fat sources and the potential biological effects are correlated with the diversity of saturated and unsaturated fatty acids. Cow and goat milk fats are not suitable as only fat sources in infant formulas due to large differences in fatty acid profiles, which compared to human milk. Human milk has a lower level of short-chain fatty acids and a higher fraction of unsaturated and long-chain polyunsaturated fatty acids than cow and goat milk. These differences in fatty acid profile are mostly due to differences in fatty acid synthesis mechanisms between them. Because of these differences, in infant formulas made from cow and goat milk, vegetable oils are used as the primary lipid source blended in a combination that provides a similar fatty acid profile to that of human milk (Delplanque et al., 2015; Byrne et al., 2021). The major differences between human milk and formulas are due to the various saturated and unsaturated fatty acids, cholesterol, and complex lipids. Fatty acid composition reflects nutritional quality. The lipid composition of infant formulas is essential, especially for babies with special needs, as dietary fats are the source of energy and may affect health. Long-chain polyunsaturated fatty acids (PUFAs) have functional and structural role in infant development. When there is a breakdown of the oral tolerance mechanisms, immune responses to ordinary food allergens can lead to food allergy and it has been hypothesized that certain nutrients, such as essential fatty acids, can positively influence this process. t*rans* fatty acids and metabolites of long-chain PUFAs can regulate immune function with their anti-inflammatory properties (Mazzocchi et al., 2018). Therefore, increased long-chain fatty acids and unsaturated fatty acids in hypoallergenic formulas are expected to be higher. Mendonça et al. (2017) aimed to investigate the lipid content and to identify the lipid profile of infant formulas. In that study, the percentages of saturated fatty acids in infant formula and human milk were given and a higher total saturated fatty acid was found in the

extensively hydrolyzed formula as compared to the regular ones (Mendonça et al., 2017). We found that the higher saturated lipid values obtained for the hypoallergenic infant formulas were in accordance with literature.

Proteins have major absorption bands such as the amide I (~1650 cm^{-1}) and amide II (~1540 cm^{-1}) bands in the mid-infrared region. The precise vibrational frequencies of the amide I and II bands depend on the attachment of nitrogen of the N-H and the carbon of the C=O bonds to the peptide bonds. While these bands are observed in regular formulas, they are not seen in hypoallergenic formulas. A carboxylic acid from the C-terminal and a primary amine from the N-terminal will be formed during the hydrolysis of the peptide bond. While the carbonyl group in the peptide will be subjected to electron donation from the nitrogen atom before hydrolysis, the C-terminal carbonyl group will not any more exposed to that donation after peptide bond cleavage and the vibrational frequency of C=O stretching will take away from the 1650 cm^{-1} to around 1700 cm^{-1} region (Poulsen et al., 2016). In this case, the absorbance increase at 1744 cm^{-1} which is carbonyl C=O stretching of triglyceride esters suggests that carbonyl groups in protein may also contribute to this increment. Moreover, the amide II band should reduce during hydrolysis since the C-N bond is cleaved. Due to the forming of a primary amine as hydrolysis occurs, N-H bending is found at approximately 1600 cm^{-1}. All these changes due to hydrolysis of proteins are predicted to give an alteration in IR spectrum (Fig 1).

The source of carbohydrates in breast milk is lactose. Generally, alternative glucose-derived polymers or sucrose-based carbohydrates are used instead of lactose in infant formulas (Strzalkowski et al., 2022). The absorption bands located in the 1200-800 cm^{-1} spectral region are considered as the fingerprint region of carbohydrates and dominated by deformational modes of the C-H/CH_2 and C-C-O groups. It is possible to distinguish bands characteristic for mono and polysaccharides by analyzing the functional groups in this region. The area ratios of some bands related to carbohydrates present in both formulas were evaluated and no significant changes were found. However, due to the complex structural properties of carbohydrates analyses were made statistically based on the spectral differences between the HF and RF samples.

Cluster Analysis and Principal Components Analysis

The HCA (Figs. 3 and 4) revealed the intragroup similarity within the studied sample groups and generated clusters in each group at the spectral regions of 1700-1500 and 1200-800 cm^{-1} regions. The successful differentiation in which all samples are clustered in their own group was obtained in the 1200-800 cm^{-1} spectral region (Fig. 3). A cluster with only one sample in the wrong group was observed in the region (1700-1500 cm^{-1}) which contains mainly the protein functional groups (Fig. 4). The sample observed in the wrong group is a fully hydrolyzed hypoallergenic formula containing rice protein.

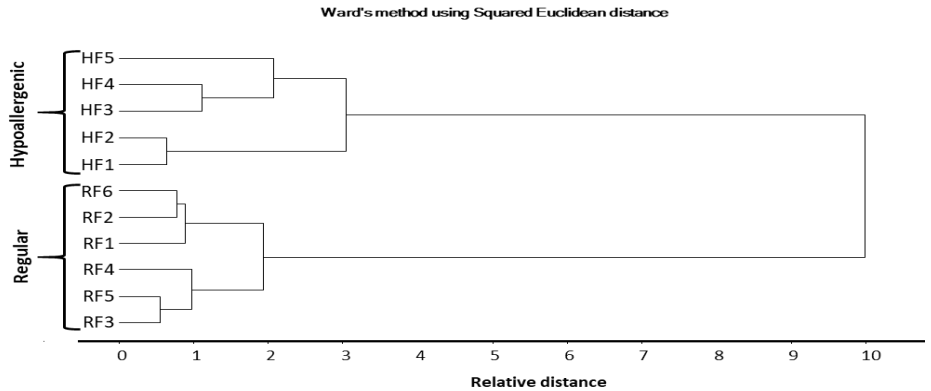

Figure 3. Hierarchical clustering of the studied samples in the 1200–800 cm^{-1} spectral region.

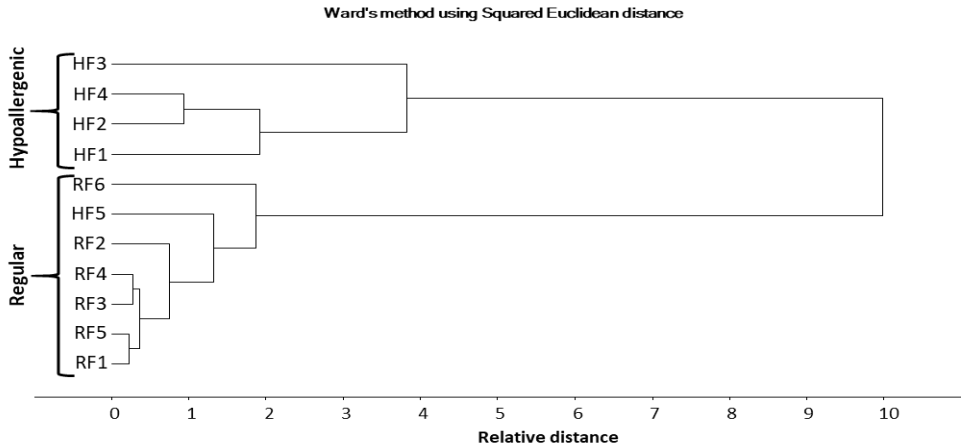

Figure 4. Hierarchical clustering of the studied samples in the 1700–1500 cm^{-1} spectral region.

Higher heterogeneity between the clusters demonstrates higher dissimilarity among analyzed groups. As seen from Figs. 3 and 4, the heterogeneity was about 10 in the differentiation of HF from RF that indicates higher heterogeneity between hypoallergenic and regular formulas.

In order to measure the efficiency of the discrimination, accuracy, specificity and sensitivity based on the obtained clusters were calculated as follows:

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN}$$

$$Sensitivity = \frac{TP}{TP + FN}$$

$$Specificity = \frac{TN}{TN + FP}$$

where;

-FP (false positive): number of RF samples classified as HF,

-FN (false negative): number of HF samples classified as RF,

-TP (true positive): number of HF samples classified as HF,

-TN (true negative): number of RF samples classified as RF.

The calculated accuracy, sensitivity and specificity values were all 100% for 1200-800 cm^{-1} spectral region, whereas 91, 80, 100% respectively for 1700-1500 cm^{-1} spectral region. The most excellent differentiation was achieved in the 1200–800 cm^{-1} region.

PCA was applied to FTIR spectra of both groups, producing a prominent discrimination (score plot) of the different regular (RF) and hypoallergenic infant formula (HF) samples. PCA score plots and the corresponding loading plots for these spectral ranges are presented in Figs. 5 and 6. The PCA scores plots shown in Fig. 5 demonstrated that the clusters of RF and HF samples were clearly separated from each other in the 1200-800 cm^{-1} spectral region. The main variation among the groups is along the first principal component (PC-1), which describes 83% of the variation (Fig. 5). The observed positive and negative peaks in the loading plots given on the bottom panel of figures indicate peaks which strongly affect the principal components and contribute to the discrimination of the groups under study. In Fig. 5, loadings plot shows the peaks at 1148, 995, 926 and 846 cm^{-1} can be assigned to C-O/ C-C stretching, C-O-C / C-C / C-O stretching, C-O/ C-C stretching of ring and C-O/ C-C stretching of carbohydrate, respectively (Wiercigroch et al., 2017). These are positively correlated with PC-1. Moreover, the absolute value of the peak at 1067 cm^{-1} is negative, indicating that PC-1 is negatively correlated to this variable. Even very small spectral differences of the carbohydrate functional groups contribute to the separation in this spectral region in PCA and HCA analyzes (Figs. 3 and 5).

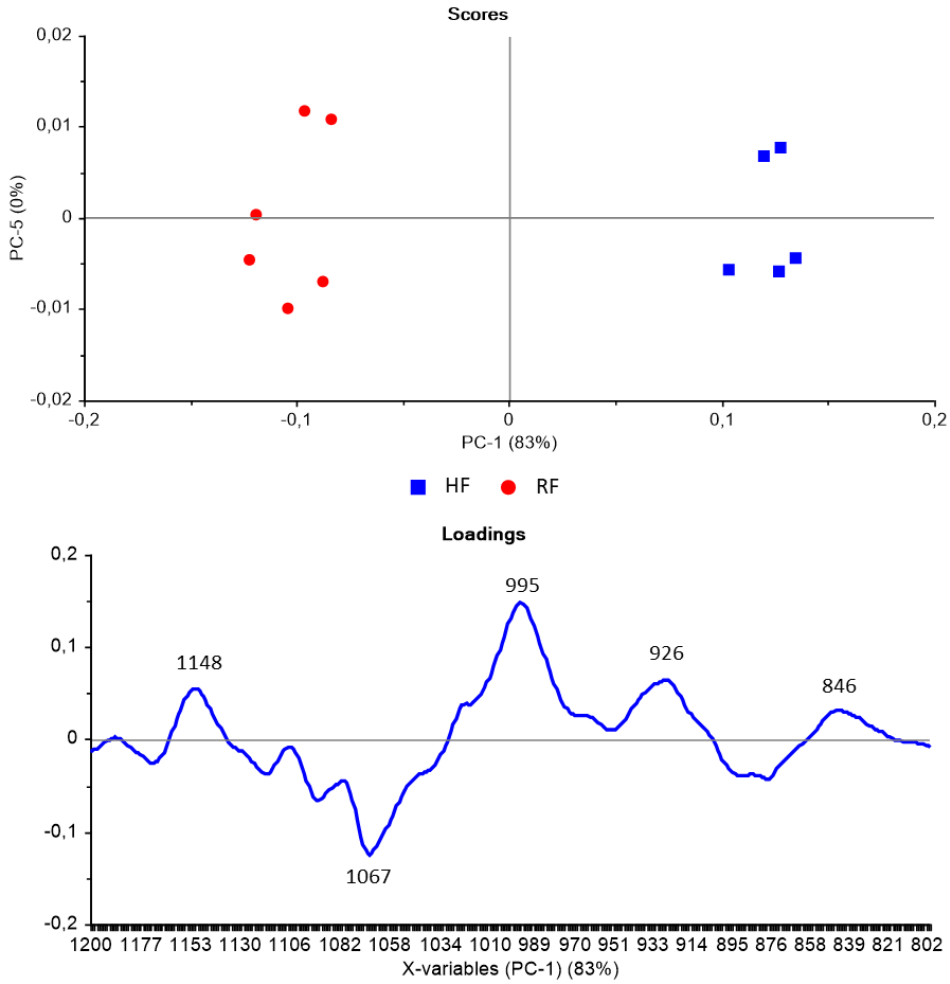

Figure 5. PCA scores and loadings plots for all of the samples over 1200–800 cm^{-1}

The successful differentiation was also obtained in the 1700-1500 cm^{-1} spectral region with only one sample in the wrong group (Fig. 6). The main variation among the groups is along PC-1, which describes 87% of the variation. The observed positive and negative peaks in the loading plots given on the bottom panel of figure indicate peaks which strongly affect the principal components and contribute to the discrimination of the groups under study.

In Fig. 6, loadings plot shows the peaks at 1608, 1583 and 1510 cm^{-1} which are positively correlated with PC-1 and at 1657 cm^{-1} which PC-1 is negatively correlated with that peak. These changes are thought due to the formation of primary amines from new N-terminals and formation of carboxylic acid from the C-terminals because of the peptide bond hydrolysis.

Although we could not show the contextual changes in the major protein bands amide I and II (not observed in HF samples), very clear clustering (high heterogeneity and 91% accuracy or high variation in PCA) was observed in the 1700-1500 cm^{-1} region from PCA and HCA results based on spectral changes in RF and HF. This is an indication that the changes related to the hydrolysis process of milk proteins in hypoallergenic formulas are directly reflected to their IR spectrum.

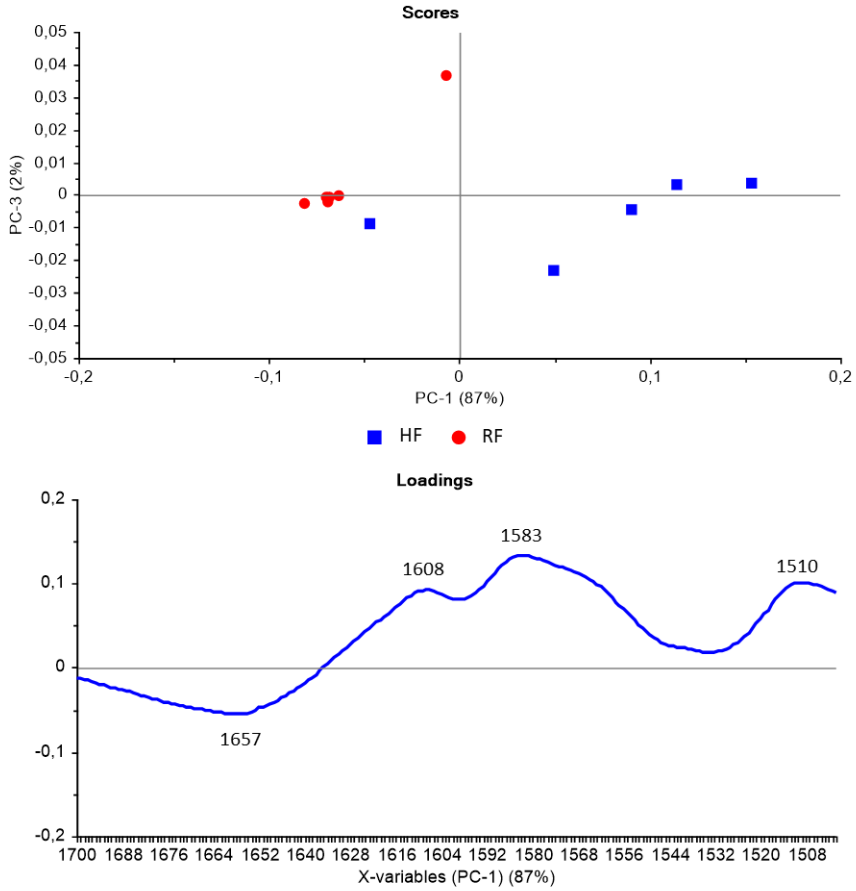

Figure 6. PCA scores and loadings plots for all of the samples over 1700–1500 cm^{-1}

CONCLUSION

IR spectroscopy was used to monitor the absorbance changes of infant formula powders. The results of the present study indicate that there are many reasonable variations between the spectral parameters of regular and hypoallergenic infant formula samples which come from the difference in ingredients and enzymatic hydrolysis of milk proteins.

A relatively high fat amount compared to protein was found in the infant formula powder. Higher saturated fatty acid content and qualitatively longer acyl chained fatty acids were observed in hypoallergenic formulas. ATR-FTIR spectroscopy combined with multivariate analysis allows the receiving of useful information by single rapid measurement for the classification of infant powder samples. Successful differentiation of regular and hypoallergenic formulas with 83% variation and 100% accuracy was obtained by HCA and PCA based on spectral differences.

This study reveals the potential power of FTIR spectroscopy for highly sensitive prediction of differences in molecular content of infant foods. FTIR spectroscopy can effectively distinguish protein hydrolysates and different enzymatic processes.

CONFLICT OF INTEREST

The authors declare no conflict of interest. The authors are only responsible for the content and writing of this paper.

AUTHORS CONTRIBUTIONS

DY. design and performed the experiments. DY, SHB. Analyses and interpretation, DY, SHB. contributed to the writing of the paper. All authors read and approved the manuscript.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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