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CHARACTERIZATION OF INDUSTRIAL BONE BROTHS FORMULATED WITH VARIOUS MEAT AND NON-MEAT INGREDIENTS

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ABSTRACT
Bone broth has been utilized as food and as a favorite ingredient in many dishes for centuries; notwithstanding, due to the recent growing interest of the consumers, industrial production of bone broths has become widespread. This study evaluated physical and chemical quality features of industrial bone broths produced with beef bone, water, and seasonings (BC), including vegetable mixture (BV), beef trotter (BT), or beef trotter plus sheep head meat (BTH). BTH had the highest lipid and protein contents, and also lipid oxidation levels. The lowest free fatty acids were detected in BC, followed by BT, BV and BTH. SIMCA model provided distinct clusters with interclass distances of more than 3. Consequently, bone broths showed different quality characteristics when formulated with meat or non-meat ingredients. Specifically, FTIR combined with multivariate analysis might provide valuable information, but further studies are needed to quantify the amounts of ingredients added to the formulation.
Keywords: bone, marrow containing bone broth, animal by-products, Fourier-Transform infrared spectroscopy, lipid oxidation

ÇEŞİTLİ ET VE ET OLMAYAN BİLEŞENLERLE FORMÜLE EDİLEN ENDÜSTRİYEL KEMİK SULARININ KARAKTERİZASYONU

ÖZ
Son yıllarda, gıda ve gıda bileşeni olarak kullanılan kemik suyunun endüstriyel üretimi yaygınlaşmıştır. Çalışmada, dana kemik, su ve baharatlar (BC); dana kemik, su, baharatlar ve sebze karışımı (BV); dana kemik, su, baharatlar ve dana paça (BT), veya dana kemik, su, baharatlar, dana paça ve kuzu kelle eti ile (BTH) formüle edilmiş endüstriyel kemik sularının fiziksel ve kimyasal kalite özellikleri değerlendirilmiştir. En yüksek yağ ve protein miktarları ve aynı zamanda en yüksek lipit oksidasyonu BHT örneklerinde belirlenmiştir. En düşük serbest yağ asitliği BC örneklerinde bulunurken, bu grubu BT, BV ve BTH takip etmiştir. SIMCA modeli, örnek grupları için sınıflar arası mesafenin 3'ten büyük olduğunu göstermiştir. Sonuç olarak, kemik suyunun et veya et olmayan bileşenlerle formüle edilmesinin kalite karakteristiklerinde farklılıklar oluşturabileceği saptanmıştır. Spesifik olarak, çok değişkenli analizlerle kombine edilen FTIR analizinin, kemik suları hakkında önemli bilgiler sağlayabileceği belirlenmiştir. Ancak formülasyona eklenen bu bileşenlerin miktarlarını ölçmek için daha fazla çalışmaya ihtiyaç duyulmaktadır.
Anahtar kelimeler: Kemik, ilikli kemik suyu, hayvansal yan ürünler, Fourier dönüşümlü kızılötesi spektroskopisi, lipit oksidasyonu

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INTRODUCTION

Bone tissue is made up of bone cells that remain alive throughout the life of the animal (Aykın-Dinçer et al., 2021). The bone cells are responsible for the bone matrix that contains salts of several minerals like sodium, potassium, phosphorus, calcium and magnesium, type-I collagen, and other proteins (proteoglycans, glycoproteins and sialoproteins), besides acid mucopolysaccharides (hyaluronic acid, chondroitin sulfate and heparin) (Aljumaily, 2011; Hay and Dane, 2016; Ergezer et al., 2018).

Since most of the meat is deboned prior to packaging and retail, based on carcass weight, approximately 6-12% of bone is left (Song et al., 2016). Those bones are regarded as notable by-products to be further converted into high added-value products by the application of efficient strategies for ensuring sustainability (Toldrá et al., 2021). One of the effective strategies for the evaluation of bones is to produce bone broth which is an alternative food ingredient with excellent nutritional composition. Hay and Dane (2016) specified the four key reasons to consume bone broth as follows: Firstly, bone broth is a considerable source of bioavailable collagen that makes it a unique healing food. Secondly, bone broth contains several bioavailable nutrients such as amino acids, vitamins, minerals, and essential fatty acids. Third, as a by-product, bone broth could be regarded as a value-added ingredient that supports protecting the environment and presenting economical benefits. Lastly, bone broth is not only used as food itself but it could also be utilized as a healing flavor enhancer in many food formulations such as drinks, soups, and meals. Animal bone broths are associated with boosting the immune system activity, supporting the digestive system, showing anti-inflammatory properties, and building the blood cells due to high mineral concentrations (Chimeegee and Dashmaa, 2018; Mar-Solís et al., 2021). Specifically owing to its rich collagen, calcium, phosphorus, hyaluronic acid, and chondroitin sulfate contents, consumption of bone broth is highly recommended during the healing of wounds or the removal of toxins (Ergezer et al., 2018; Aykın-Dinçer et al., 2021). Considering all of these beneficial facts, attempts should be made to reveal the important characteristics of bone broths.

The major food markets around the world for bone broth appear to be in beef or chicken bone stocks that include various flavor enhancers, vegetables, or some preservatives (Chimeegee and Dashmaa, 2018). In Türkiye, there has been a rising trend in the production of bone broths due to the recent attention of the consumers that are willing to be well-nourished. According to the latest reports on red meat manufacturing statistics, total meat production has increased by 9.3% in 2021 when compared with the previous year (Turkish Statistical Institute, 2022). However, there is a lack of information on the certain production amounts of meat by-products. Nevertheless, it has been reported in the media that new entrepreneurial companies have initiated to produce various types of ready-to-eat bone broths (Anonymous, 2018; Anonymous, 2020; Anonymous, 2021). On the other side, the characterization studies on the evaluation of remarkable quality parameters of locally produced bone broths are quite limited. Accordingly, the aim of the present research was to evaluate the physical and chemical quality characteristics of different industrial bone broths formulated with meat and non-meat ingredients. In this context, visual, physical, compositional and oxidative quality parameters were assessed, and Fourier-Transform Infrared (FTIR) Spectroscopy characterization was performed with Soft Independent Modelling of Class Analogy (SIMCA) to generate classification models for different bone broths.

MATERIAL AND METHOD

Material

The bone broths used as the study material were recruited from an industrial producer located in İstanbul/Türkiye. Briefly, the production of bone broths covers the basic steps as bain-marie cooking of the marrow-containing beef tibia bones at 100 °C for 36 h with/without the addition of different vegetables, beef trotter, and/or skinless sheep head meat. After the cooking process, the mixtures were filtered using

cotton cheesecloth, filled in sterilized glass bottles, vacuum-sealed and refrigerated.

Four different bone broths were selected that were produced with different formulations as follows: Control (BC) sample was a single bone broth that contained water, tibia bone with marrow, and seasonings. BV sample included this single bone broth plus a mixture of vegetables containing carrot, mushroom, celery, and onion. BT sample was formulated with the single bone broth plus beef trotter, while BTH sample consisted of the single bone broth plus beef trotter and skinless sheep head meat. The formulation of the bone broth samples is presented in Table 1. Five portions (600 mL) from the same batch of each group were randomly sampled at the point of sales, controlling the batch number and expiry date as well as the presence of swelling or leakage. Non-damaged and appropriate cans were transported to the laboratory and stored at 4 °C prior to analyses.

Table 1. Formulations of the bone broth samples

Samples[1]	Ingredients (%)					
	Beef tibia bone	Water	Beef trotter	Skinless sheep head meat	Vegetable mix[2]	Seasoning mix[3]
BC	30.0	67.0	-	-	-	3.0
BV	30.0	57.0	-	-	10.0	3.0
BT	30.0	52.0	15.0	-	-	3.0
BTH	30.0	44.5	15.0	7.5	-	3.0

[1]The samples represent the following formulations: BC: Bone broth formulation containing beef bone, water, and seasonings (control), BV: Bone broth formulation containing beef bone, water, and seasonings plus vegetable mixture, BT: Bone broth formulation containing beef bone, water, and seasonings plus beef trotter, BTH: Bone broth formulation containing beef bone, water, and seasonings plus beef trotter and sheep head meat.
[2]Vegetable mixture consisted of fresh and chopped carrot (5.5%), mushroom (2.5%), onion (1.3%), and celery (0.7%).
[3]Seasoning mixture was a blend of %1.0 salt, 0.7% thyme, 0.4% garlic powder, 0.4% granule black pepper, 0.2% bay leaves, 0.2% ground ginger, and 0.1% granule clove.

All the bone broth samples were well-homogenized using a high-shear homogenizer (Ultra-Turrax, IKA, Germany) operated at 6500 rpm for 3 min before sampling. The analyses were carried out within 48 h after supplying the samples, while sampling for lipid oxidation analysis was conducted at the beginning of the storage and during two days of the refrigerated storage.

Analyses

Chemical Composition

Total moisture (oven drying method), protein (Kjeldahl method), and ash (dry combustion method) contents of the bone broths were determined according to AOAC (2012). Lipid content was analyzed by the chloroform-methanol extraction method (Flynn and Bramblett, 1975).

pH and Free Fatty Acids

pH values of the bone broths were measured at room temperature using a portable pH-meter (WTW 330i/SET, Germany) equipped with an immersion probe. Free fatty acid (FFA) content was analyzed according to Gökalp et al. (1995) by titration of the sample slurries with 0.1 N NaOH in ethanol and calculated as a percentage of oleic acid.

Refractive Index and Color

Total soluble matter (°Brix) of bone broths was measured with a refractometer at 20 °C (RFM 330; Bellingham + Stanley Limited, Tunbridge Wells, Kent, U.K.). The color of the samples was assessed by a desktop HunterLab colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA). White and black standard plates were used for calibration. The following color parameters

were measured and defined as follows: L* is an index associated with luminosity (0=black and 100=white); redness (a*) is an index that varies from green (−) to red (+) while yellowness (b*) is an index that varies from blue (−) to yellow (+).

Lipid oxidation

Lipid oxidation during 2 days of storage in bone broth samples was followed by Thiobarbituric Acid Reactive Substances (TBARS) analysis. In summary, 5 mL of the sample homogenate was mixed with 15 mL of distilled water. After that, 1 mL of this mixture was transferred to a test tube and mixed with 50 µL of 7.2% (w/v) butylated hydroxyanisole in ethanol and 2 mL of thiobarbituric acid (15 mM)-trichloroacetic acid (15%, w/v) solution. The tubes were incubated in a boiling water bath for 15 min, cooled, re-vortexed, and centrifuged at 2600 ×*g* for 15 min. The absorbance of the supernatant was recorded at 531 nm against the blank sample with 1 mL of water plus 2 mL of the solution. TBARS value was expressed as mg malonaldehyde/kg sample (Due and Ahn, 2002).

FTIR spectroscopy measurements

FTIR spectroscopy measurements of the bone broths were carried out using a Perkin Elmer Spectrum Two FT-IR Spectrometer (Perkin-Elmer Inc., Norwalk, CT, USA) equipped with a DTGS detector. The sampling station was equipped with an attenuated total reflection accessory (ATR). Bone broths were firstly lyophilized, and then placed on the ATR and infrared spectra were recorded between 4000 and 650 cm^{-1} wavenumbers with a resolution of 4 cm^{-1}. Totally 32 scans were collected for each sample spectrum. Single-beam spectra of the samples were obtained and corrected against the background of the sample holder to present the spectra in absorbance units. Six spectra were recorded for each sample.

Statistical Analysis

Statistical Package for Social Science (SPSS) version 28.0 (IBM, USA) was utilized to perform the statistical evaluation. One-Way Analysis of Variance (ANOVA) was used to detect the significant differences between product formulations, while two-way ANOVA was conducted to assess the significance depending on the formulations and storage time for lipid oxidation. Least-square differences (LSD) test was applied for comparison of the means and Duncan's Multiple Range Test was applied for posthoc comparisons at a 95% confidence interval.

Spectral data acquisition and data analysis were performed using a multivariate data analysis software Pirouette (3.11, Infometrix Inc., WA, USA). Soft Independent Modelling of Class Analogies (SIMCA) method was used to develop classification. SIMCA was evaluated based on three-dimension class projection and interclass distance (ICD).

RESULTS AND DISCUSSION

Chemical Composition, pH, and FFAs

The chemical compositions of bone broth samples are presented in Table 2. No significant differences were recorded for centesimal composition between BC and BV. On the other hand, the incorporation of different meat ingredients such as beef trotter and sheep head meat significantly affected the moisture, lipid, protein and ash contents of the bone broths (P <0.05). Total lipid content of the samples varied from 0.38% to 4.70%, and protein contents ranged between 3.27% and 16.58%. BC and BV samples had the lowest lipid and protein contents compared to those of BT and BTH (P <0.05). In particular, BTH samples had the highest lipid and protein contents (P <0.05), most probably due to the high protein and lipid contents of beef trotter and sheep head meat added to the formulation. The results showed that including both beef trotter and sheep head meat might be a good strategy to increase the nutritional value of bone broths, due to a significant increase in protein amount.

The pH value of bone broth is important for further processing and storage stability because of the capacity to change the hydrogen ion concentration. Average pH values of the bone broths, given in Table 2, were found between 6.56-8.22. A similar pH range was obtained in a

study conducted by Hsu et al. (2017) in which the properties of different animal bone broths were determined. It was found that pH of the bone broths was significantly influenced by the ingredients added to the formulation (P <0.05) (Table 2). BV had the lowest pH value while the highest value was found in BC. So the mixture of vegetables caused a significant decrease in pH levels of bone broths (P <0.05). On the other hand, beef trotter and sheep head meat had low pH values compared to control. Similarly, Choi et al. (2016) found variations in pH values of bone broths formulated with different meat mixtures. The authors stated that pH value of bone broth produced from only bones was higher than those of bone broth including meat.

Table 2. Centesimal composition, pH, and FFA contents of bone broth samples

Samples*	Moisture (%)	Lipid (%)	Protein (%)	Ash (%)	pH	FFA (%)
BC	$95.22^{a} \pm 0.05$	$0.38^{c} \pm 0.03$	$3.27^{c} \pm 0.18$	$0.26^{c} \pm 0.02$	$8.22^{a} \pm 0.01$	$0.38^{d} \pm 0.01$
BV	$95.00^{a} \pm 0.12$	$0.24^{c} \pm 0.02$	$3.78^{c} \pm 0.37$	$0.43^{c} \pm 0.05$	$6.56^{d} \pm 0.01$	$0.66^{b} \pm 0.01$
BT	$80.80^{b} \pm 1.67$	$3.44^{b} \pm 0.49$	$12.56^{b} \pm 0.32$	$2.13^{b} \pm 0.08$	$7.16^{b} \pm 0.02$	$0.48^{c} \pm 0.01$
BTH	$71.97^{c} \pm 1.41$	$4.70^{a} \pm 0.47$	$16.58^{a} \pm 0.44$	$2.93^{a} \pm 0.40$	$6.80^{c} \pm 0.01$	$0.84^{a} \pm 0.02$

*Bone broths formulated with BC: Bone, water, and seasonings (control), BV: Bone, water, and seasonings plus vegetable mixture, BT: Bone, water, and seasonings plus beef trotter, BTH: Bone, water, and seasonings plus beef trotter and sheep head meat.

Data were presented as the mean values ± standard deviation. a, b, c, ….: Means with a different letter in the same column are significantly different (P <0.05).

FFAs, the main product of enzymatic hydrolysis of triacylglycerides, are important for the formation of flavor and characteristics of many foods (Pegg and Shahidi, 2007). The FFA content should be examined to gain insight into the quality of the meat products. The values of FFA for BC, BV, BT and BTH were 0.38%, 0.66%, 0.48% and 0.84%, respectively (Table 2). An increase in FFA values was observed with the addition of different ingredients to the bone broth formulation. In particular, more than a two-fold increase in FFA was observed in bone broths formulated with both beef trotter and sheep head. Similarly, in the study conducted by Choi et al. (2016), significant changes were detected in the fatty acid composition of bone extracts due to the addition of meat mixtures. It was also reported in the study of Marco et al. (2006) that the majority of FFA were derived from the triglycerides. Therefore, the FFA released in bone broth samples comes mainly from triglycerides, so high amount of meat ingredients used in the broth formulation makes the triglyceride hydrolysis was more important. Although we observed significant changes with the addition of vegetables or meat ingredients to the formulation, FFA values were found in lower limits compared to the processed meat products studied by Kowale et al. (1996), Ercoşkun et al. (2010), and Demirok-Soncu et al. (2014).

Brix and Color

The Brix is expressed by the soluble solids of bone broths in the water. This quality parameter can affect the appearance, taste, viscosity, and other quality characteristics of the end products, and hence determines the consumer impression. The Brix values of bone broths, given in Table 3, were determined to be in the range of 6.10-10.28º. The lowest Brix value was found in BC whereas the highest value was obtained in BT samples (P <0.05). The addition of vegetable mixtures to the broth formulation resulted in a slight increase in Brix value, and further increases were observed by the incorporation of beef trotter and sheep head meat. This data demonstrated that higher dry matter content in the bone broths resulted in a higher Brix value. Different Brix values were obtained in the literature depending on the raw material and ingredients added to the formulation as well as depending on the process type and conditions (Aykın-Dinçer et al., 2021; Chiang et al., 2022).

Table 3. Refractive index (°Brix) and color of bone broth samples

Samples*	°Brix	L*	a*	b*
BC	$6.10^{d} \pm 0.08$	$26.30^{c} \pm 0.34$	$0.09^{b} \pm 0.04$	$6.94^{c} \pm 0.07$
BV	$6.50^{c} \pm 0.08$	$10.42^{d} \pm 0.07$	$1.94^{a} \pm 0.19$	$10.03^{b} \pm 0.29$
BT	$8.58^{b} \pm 0.22$	$30.28^{b} \pm 0.08$	$-1.33^{c} \pm 0.03$	$5.92^{d} \pm 0.13$
BTH	$10.28^{a} \pm 0.22$	$33.37^{a} \pm 0.20$	$0.08^{b} \pm 0.03$	$10.82^{a} \pm 0.22$

*Bone broths formulated with BC: Bone, water, and seasonings (control), BV: Bone, water, and seasonings plus vegetable mixture, BT: Bone, water, and seasonings plus beef trotter, BTH: Bone, water, and seasonings plus beef trotter and sheep head meat.

Data were presented as the mean values ± standard deviation. a, b, c, ….: Means with a different letter in the same column are significantly different (P <0.05).

Color is known as the most important visual attribute that can affect consumer preferences and satisfaction. Because color parameters were noted to be affected by formulation for many meat systems (Jiménez-Colmenero et al., 2010), the variations in the color parameters of bone broths were evaluated (Table 3). L* values of BV samples were found to be lower than all other samples (P <0.05). The highest L* value was recorded in BTH, which was followed by BT and BC (P <0.05). Thus the addition of vegetables to the mixture caused a serious decrease in the lightness of the bone broths, whereas the inclusion of meat ingredients led to an opposite impact in terms of lightness characteristics. Moreover, the lowest a* and b* values were detected in BT when compared with the other bone broth samples (P <0.05). In brief, our results showed that the usage of vegetable mixtures, beef trotter, and sheep head meat had significant effects on color attributes (P <0.05) which may also have an impact on the sensory quality characteristics and consumer acceptance. In a similar study on bone broths by Choi et al. (2016), the addition of mixed bone and brisket meat to the bone extracts significantly decreased the sensorial color scores. Kim (2006) also reported higher sensory evaluation scores for color in rib bone extract compared to shank bone extract.

Lipid oxidation

Lipid oxidation degree is one of the most important reasons for the sensory and nutritional deterioration of meat products (Nuñez De Gonzalez et al., 2008). In the present study, the oxidative stabilities of the bone broths were compared in terms of their TBARS values throughout 2 days of refrigerated storage (Figure 1). The ingredient type added to the formulation and storage time significantly affected the oxidation level (P <0.05). After the production and the first day of storage, no significant differences were found between BC and BV samples, which were found between 0.05 mg malonaldehyde/kg and 0.15 mg malonaldehyde/kg, respectively. During storage, the addition of beef trotter to the bone broth formulation caused a significant increase in the values, and further increment was observed in samples containing both beef trotter and sheep head meat (P <0.05). These results showed that including beef trotter with/without sheep head meat in the bone broth formulation triggered lipid oxidation, presumably due to the increase in the lipid content. Indeed the lipid oxidation results were higher in the samples with the highest lipid contents, pointing out the increase in TBARS values with the increase in lipid concentrations. It is well-known that the initiation and progress of lipid oxidation could be promoted by several factors such as raw material, fat and free fatty acid contents, metal ions concentration, moisture contents, as well as process and storage conditions. Similar to our findings, Choi et al. (2016) determined that TBARS values of shank bone extract increased as the mixed bone and brisket meat bone were added to the formulation. Aykın-Dinçer et al. (2021) reported that TBARS values increased with the increase in the temperature applied during the production of bone broth powder. Despite of the increase in oxidation rate of bone broths containing meat ingredients, addition of vegetables to the formulation did not increase the TBARS, and this may be attributed to the presence of antioxidant

compounds in vegetable mixtures that decelerated the oxidations.

In our study, during the 2-day storage time, TBARS values steadily increased in all the samples (P <0.05), which were measured between 0.10 mg malonaldehyde/kg and 0.58 mg malonaldehyde/kg. Such an increase in TBARS values was observed during the storage period for different bone broth powders produced by Ergezer et al. (2018). Nonetheless, in the present work, all of the bone broth samples had oxidation values within acceptable limits (<1 mg malonaldehyde/kg) during storage.

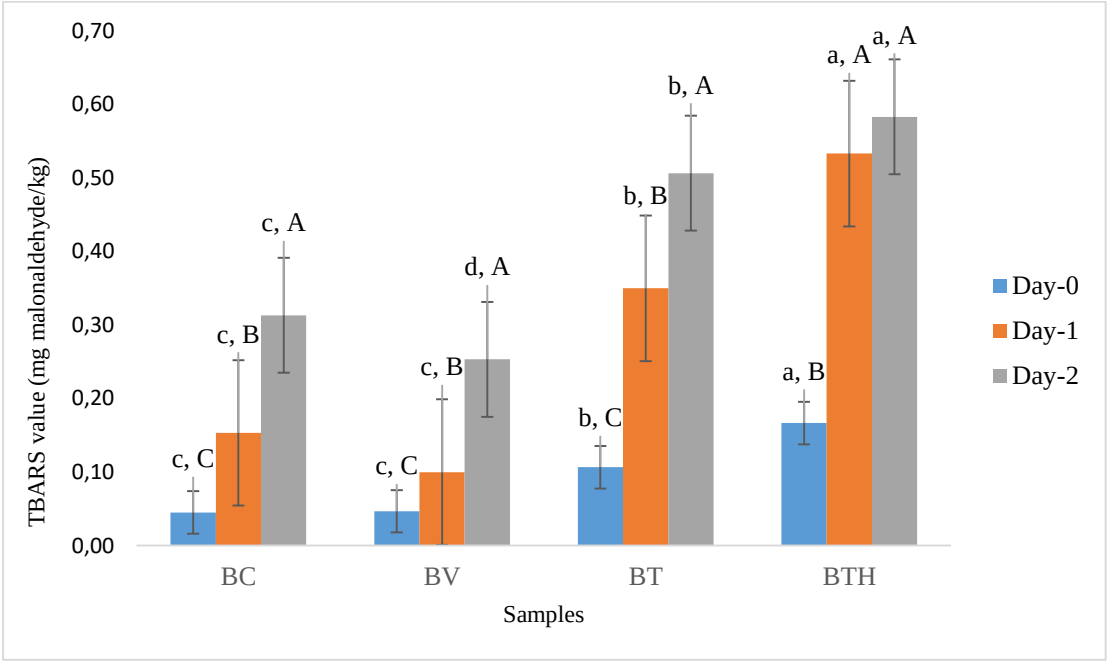

Figure 1. TBARS value of bone broth samples during short-term refrigerated storage

Samples represent the bone broths formulated with BC: Bone, water, and seasonings (control), BV: Bone, water, and seasonings plus vegetable mixture, BT: Bone, water, and seasonings plus beef trotter, BTH: Bone, water, and seasonings plus beef trotter and sheep head meat.
a, b, c, ...: The lower-case letters indicate significant differences among the samples within the same storage day (P <0.05).
A, B, C, ...: The upper-case letters indicate significant differences among the storage days within the same sample (P <0.05).

FTIR Spectra and SIMCA results

FTIR spectroscopy can be a unique biochemical fingerprinting method that enables rapid detection of compounds found even at a low concentration within complex food matrices (Ellis et al., 2015). Since the technique is very rapid, sensitive, and simple with minimal sample preparation and low operational costs, it can be used for routine analyses in laboratories. The importance of IR spectroscopy for qualitative analysis originates from the possibility to detect certain absorption bands related to specific functional groups (Bendini et al., 2007). In this study, the bone broths were analyzed using FTIR spectroscopy in the mid-infrared region (4000–650 cm^{-1}). The representative FTIR spectra of the bone broths are demonstrated in Figure 2, indicating some of the spectral bands arising from specific functional group vibrations. The spectrums of BC and BV samples were mainly shown very similar. Moreover, BT had vibrations similar to BTH. All spectra obtained from the bone broth samples displayed similar bands at around 2922, 2853, 1639 and 1464 cm^{-1}. These

bands were quite strong and well-separated in each spectrum. In literature, the bands determined at wavenumbers between 3000 and 2850 cm^{-1} region were reported to be mainly originated from C–H stretching vibrations in aliphatic hydrocarbon compounds such as lipids (Stuart, 2004, Lohumi et al., 2015). The intensities of these bands increased with the addition of beef trotter and sheep head meat to the bone broth. Previously, Deniz et al. (2018) reported that the region from 3000 to 2800 cm^{-1} could be defined as a characteristic unsaturated and saturated lipid region for beef mixtures. Therefore, the noticeable variations observed in this specific region of the spectrum might be due to the differences in the lipid profile of beef trotter and sheep head meat added to the formulation. On the other hand, it could be stated that the addition of vegetable mixtures to the bone broth formulation did not cause a significant variation in this region. Another signal obtained from all bone broths was located in the 1639 cm^{-1}, which was related to the Amide-I region of proteins. In literature, this band was reported to be generally originated from C=O stretching, N–H bending or C–N stretching proteins (Stuart, 2004; Deniz et al., 2018). This was the broadest band in the spectrum of BC and BV samples. In addition, a band at around 1464 cm^{-1}, which was related to the bending of the CH_2 and CH_3 aliphatic groups, was observed in all samples, but the area of this band was small in the spectrums of BC and BV samples. The spectrums of BT and BTH showed some other different vibrations at around 1743, 1378, 1239, 1161, 1117 and 1098 cm^{-1}. It was reported in the literature that the bands at the wavenumbers of 1743, 1378, 1239 and 1161 cm^{-1} could be related to aliphatic C=O stretching of esters, C–H bending bands of methyl groups, aliphatic P=O stretching of phosphorus compounds and stretching vibration of C-O and C–OH groups belonging to RNA or serine, threonine and tyrosine residues of cellular proteins, respectively (Stuart, 2004; Movasaghi et al., 2008; Deniz et al., 2018). The bands located at 1117 and 1098 cm^{-1} wavenumbers were reported to arise from vibrations due to C–H bending and CH deformation of fatty acids, respectively (Rohman et al., 2011). Moreover, Kurniawati et al. (2014) reported that this region (between 1117 and 1098 cm^{-1}) originated from the stretching vibrations of C-O in triacylglycerols could be varied due to the addition of lard to the meatball broth samples. These authors stated that the band located in this region could show variations due to the differences in the nature and composition of the meat products. Therefore, in our study, the differences in the indicated bands were related to the vibrations caused by the compositional differences of the bone broths, mainly due to the addition of beef trotter and sheep head meat.

Since some similar vibrations were observed in the spectrum of bone broths, the differences between samples were tried to be determined by using multivariate analysis. For SIMCA analysis, the spectra were 2^{nd} derivative and smooth transformed (with second order polynomial filter with a 35-point window) to improve the spectral characteristics. SIMCA three dimension class projection was illustrated in Figure 3. Using the spectral range of 1,800 to 1,200 cm^{-1}, bone broth samples from different ingredients were grouped in well-separated clusters by the SIMCA model. Infrared spectra analysis using SIMCA classification models permitted tight clustering and clear differentiation among bone broth samples. Therefore, it was found that the classification of bone broths with different compositions could be possible using their spectral information. The interclass distances (ICD) are Euclidean distances between centers of clusters and values above 3.0 are considered good for class discrimination. The ICD value was above 3, pointing out that the bone broths could not be considered similar (Table 4). The highest differences were obtained between BC and BTH due to the highest ICD value (18.8), while the lowest (4.1) was observed between BT and BTH. These results indicated that FTIR spectroscopy combined with SIMCA analysis could have the possibility to classify the bone broths.

Table 4. Interclass distances between bone broth samples based on the SIMCA class projections

Samples*	BC	BV	BT	BTH
BC	0.0	4.3	15.7	18.8
BV	4.3	0.0	9.8	10.2
BT	15.7	9.8	0.0	4.1
BTH	18.8	10.2	4.1	0.0

*Bone broths formulated with BC: Bone, water, and seasonings (control), BV: Bone, water, and seasonings plus vegetable mixture, BT: Bone, water, and seasonings plus beef trotter, BTH: Bone, water, and seasonings plus beef trotter and sheep head meat.

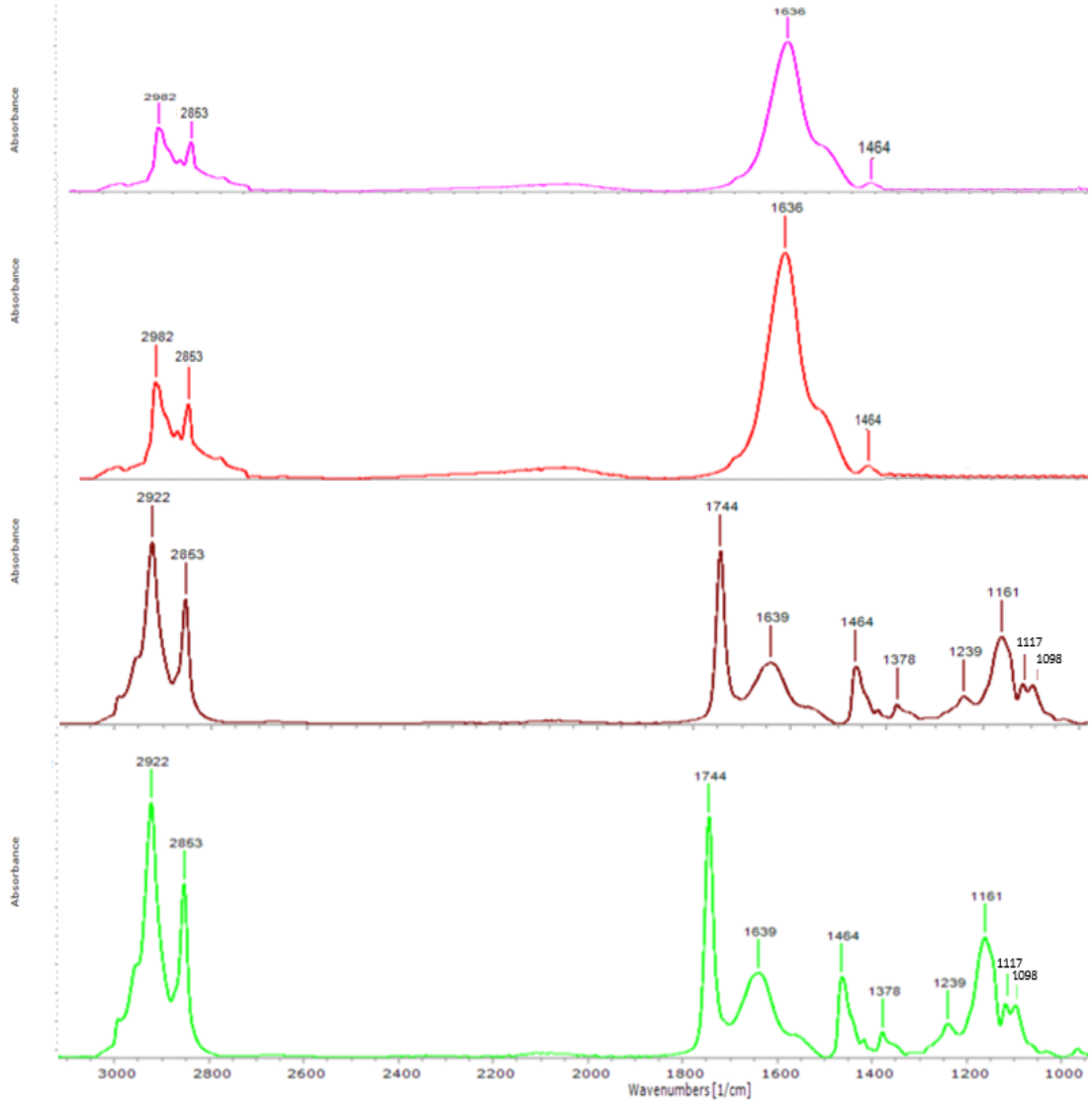

Figure 2. FTIR spectra of bone broth samples in the mid infrared region

Pink color: BC-Bone broths formulated with bone, water, and seasonings (control), Red color: BV-Bone broths formulated with bone, water, and seasonings plus vegetable mixture, Brown color: BT- bone broths formulated with bone, water, and seasonings plus beef trotter, Green color: BTH- bone broths formulated with bone, water, and seasonings plus beef trotter and sheep head meat.

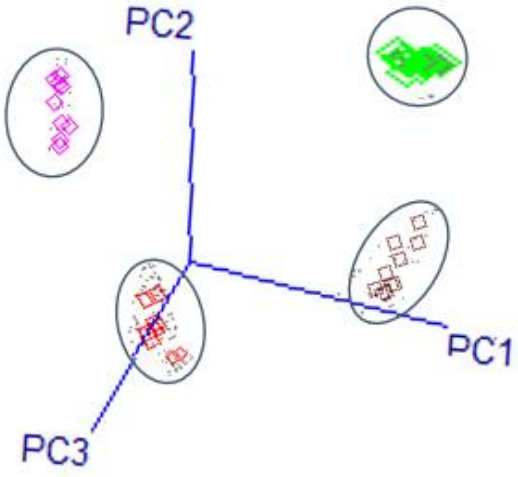

Figure 3. SIMCA class projections of transformed spectra of bone broths

Pink color: BC-Bone broths formulated with bone, water, and seasonings (control), Red color: BV-Bone broths formulated with bone, water, and seasonings plus vegetable mixture, Brown color: BT- bone broths formulated with bone, water, and seasonings plus beef trotter, Green color: BTH- bone broths formulated with bone, water, and seasonings plus beef trotter and sheep head meat.

CONCLUSION

The present research highlighted that when formulated with meat and non-meat ingredients, bone broths indicated pretty different physical and chemical quality characteristics. The incorporation of different meat-originated ingredients like beef trotter and sheep head meat into the formulation led to remarkable increments in protein and lipid contents, whereas it resulted in high FFA levels. Despite the addition of vegetable mixtures did not cause considerable alterations in composition, this addition caused an increase in FFA values. The use of vegetable mixtures, beef trotter, and sheep head meat had significant impacts on color attributes. Bone broths including beef trotter with/without sheep head meat triggered lipid oxidation during storage. However, no significant changes were observed in lipid oxidation of the bone broths including vegetable mix. Utilization of the FTIR spectrum with multivariate analysis could be useful for differentiating the bone broths. Since these results could be regarded as the first findings concerning bone broths, detailed investigations are underway in our laboratories for further characteristics and quantification of the ingredients added to the bone broth formulation.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHORS' CONTRIBUTIONS

Both authors significantly contributed to different processes in the article. The authors also declare that they read and approved the final version of the manuscript.

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