Scientific Papers. Series D. Animal Science. Vol. LXVII, No. 1, 2024
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

FROM WASTE TO VALUABLE FOOD: DEVELOPMENT AND QUALITATIVE DIFFERENTIAL CHARACTERIZATION OF BONE BROTHS FROM JUVENILE AND ADULT CATTLE

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Abstract

Following the slaughtering and processing of animals for meat, only one-third of them is meat, while the remainder consists of byproducts and waste, which need to be processed and utilized appropriately. Industrial byproducts constitute costly losses for these industries and pose challenges in their eco-friendly disposal. These costs can be offset through innovation to generate value-added products that increase profitability. Efficient utilization of byproducts has a direct impact on the economy and the environment. This study explores the potential to create collagen-rich bone broths and to this end, four batches were developed, two made from bones sourced from adult cattle and two from bones sourced from juvenile cattle. These were analyzed physicochemically and microbiologically to characterize them qualitatively. The findings suggest that both types of bone broths offer significant nutritional value, with variations influenced by the age of the cattle and the vegetable additions, as evidenced by highly significant differences ($p < 0.001$) among batches obtained through statistical processing. These findings underscore the importance of efficiently exploiting resources and the potential to develop valuable food products from seemingly residual sources.

Key words*: animal by-products, bone broth, new valuable products, quality characterization.*

INTRODUCTION

Meat and meat products constitute an important segment of the human diet as they provide essential nutrients that cannot be easily obtained through vegetables and their derivatives (Byers et al., 2002). They offer a means to reduce malnutrition and increase food security in households (Chikwanha et al., 2018). Over the past 20 years, the demand for meat and meat products has increased in many parts of the world (including Africa, Asia, Europe, and the United States of America), leading to a rapid growth in animal production for sustainable food security (Sans & Combris, 2015).

The slaughter of animals not only provides meat but also valuable by-products. The yield of these by-products has been reported to represent approximately 10 to 15% of the value of the live animal in developed countries, but following the slaughter and processing of animals, only one-third is meat, while the rest are by-products and waste (two-thirds), which need to be processed and utilized appropriately. The global meat industry economy requires the utilization of animal by-products for the livestock industry to remain economically competitive against plant protein sources (Irshad & Sharma, 2015). Processing by-products can turn a low-value product or one requiring costly disposal into a product capable of covering all processing and disposal costs, with higher added value and reduced environmental impact (Toldrá et al., 2012). Furthermore, any industry that transforms residual products into valuable products should be commended (Irshad & Sharma, 2015).

Bone broth is defined as a liquid obtained by boiling bones, and optionally adding vegetables, herbs, and spices to the boiling process (Gimbar, 2017). Bone broth, a food with a centuries-old history, has experienced a significant increase in popularity in the food and health industry in recent years. This trend can be partially attributed to the rise of the Paleo diet, which emphasizes foods consumed in the pre-agricultural era, including meat from sustainable sources, fish, nuts, vegetables, and fruits while

avoiding legumes, dairy products, refined sugar, grains, and processed foods. Bone broth recipes are often found in popular cookbooks that follow the principles of the "Paleolithic" diet and are promoted as an effective means of alleviating various conditions, from arthritis to wound healing (Gimbar, 2017). The success of a product involves the combined efforts of various professional branches, especially nutritionists, epidemiologists, food technologists, chemists specializing in natural products, and others (Anchidin et al., 2023).

Bones are essentially connective tissue similar to cartilage, composed of cells located in lacunae and collagen fibers (Uddin et al., 2021). Bone consists of a cell present in each lacuna and is connected to others through a series of bones traversing a matrix. This matrix contains collagen fibers, albuminoid substances, and calcium salts (Mushtaq et al., 2022). Bone is a hard tissue in the body, composed of two types of tissue, namely compact tissue and spongy tissue, which contain almost the same amount of collagen (Alipal et al., 2021). The color of fresh bone is yellowish-white, and when boiled, it becomes completely white. Bone contains organic and inorganic materials, the majority of which are inorganic materials such as calcium phosphate and calcium carbonate. At the same time, the rest are ions such as magnesium, potassium, fluoride, and chlorine (Rigueto et al., 2022). The inorganic materials in bone function to provide hardness to the bone structure.

The bone tissue is composed of bone cells that remain alive throughout the animal's life (Aykın-Dinçer et al., 2021). Bone cells are responsible for the bone matrix containing salts of several minerals such as sodium, potassium, phosphorus, calcium, and magnesium, type I collagen, and other proteins (proteoglycans, glycoproteins, and sialoproteins), in addition to acid mucopolysaccharides (hyaluronic acid, chondroitin sulfate, and heparin) (Aljumaily, 2011; Hay & Dane, 2016; Haluk et al., 2018). Bovine bones represent a highly promising material as a new material, especially for applications in the food, medical, textile, and medical textile fields (El-Aassar et al., 2021). This is because the content of compounds in bovine bones is highly compatible to be accepted by the body, such as collagen and gelatin (Asadi et al., 2021). In general, inedible by-products, such as bones, are used in the manufacture of fertilizers, animal feed, and fuels, but there is also a growing market for their use in obtaining protein hydrolysates and collagen. The main sources for obtaining collagen are the by-products resulting from the slaughter of pigs and cattle (Jia et al., 2010; Silva & Penna, 2012). Obtaining these products, which have high added value, represents a better alternative for the use of these by-products, which would otherwise be disposed of.

The word "collagen" comes from ancient Greek, where "kola" means glue and "gen" means producer. Collagen is a fibrous structural protein present in the extracellular matrix and connective tissue of animals (Ramshaw et al., 2009). It is the most abundant protein in the animal kingdom. Collagen is not present in plants and unicellular organisms, where its role is taken over by polysaccharides and cellulose. In the case of invertebrates, it is present in the body walls and cuticles. Collagen accounts for 25-30% of the protein content of the entire body, especially in mammals. It is found in the cornea, bones, blood vessels, cartilage, etc. (Müller, 2003), and its abundance in the animal kingdom is attributed to its unique thermal and chemical stability characteristics, which are conferred by intermolecular and intramolecular forces (Shoulders & Raines, 2009).

According to research conducted by Grand View Research (2021), the easy availability of collagen sources will lead to an increase in collagen-based product production until 2027, at an estimated annual growth rate of 5.9%. The growing demand for collagen in food products, beverages, cosmetics, and medical applications is expected to drive the demand for collagen. A significant portion of the collagen market is dominated by Europe and North America, with an increasing application of collagen in food, nutraceuticals, and cosmetics. In the Middle East and Africa, bovine collagen applications are on the rise due to easy availability. Additionally, the demand for non-genetically modified products is increasing in these regions due to the demand for 100% natural products.

Many of the collagen-rich products originate from by-products of the beef industry due to its high production (Silva & Penna, 2012). Besides bovine collagen, various sources such as porcine, poultry, fish, and marine algae can be

utilized. In 2020, the global collagen market yielded 34.9% from bovine-derived products, which can be attributed to the large number of available bovine by-products (Grand View Research, 2023). Utilizing these by-products (tendons, skins, bones, hides) for collagen production represents an alternative for improving the circular economy of this sector.

Collagen is an economically renewable source (Yorgancioglu et al., 2020) because its extraction adds value to the by-products resulting from animal slaughter, and this reuse is considered of great importance in the quest for a clean, sustainable, and circular economy (Masilamani et al., 2016; Schmidt et al., 2016). In this context, skin processing by-products are valuable materials due to their composition, which contains substances ideal for producing gelatin and collagen peptides (Ali et al., 2020).

The necessity of optimizing the utilization of abattoir by-products represents a significant challenge for processing units globally. Bones, a by-product obtained from meat deboning, containing compounds of high value, can be processed into valuable products. This not only minimizes the environmental waste impact but also enhances the sustainability and profitability of the meat industry. The present study investigates the main characteristics of the physico-chemical and microbiological quality of a valuable product obtained from a by-product considered "waste" in the meat industry. This product is represented by bone broth, elaborated by utilizing bones from juvenile and adult cattle. The study aims to identify the qualitative differences between these two categories of raw materials and the influence brought by vegetable additives.

MATERIALS AND METHODS

The products subject to this study were obtained within the Meat Microproduction Workshop at the "Ion Ionescu de la Brad" University of Life Sciences in Iași.

In this study, cattle bones containing high amounts of marrow (femur and tibia), pelvic bones (coxal bone - consisting of ilium, ischium, and pubis), and the patella were used to obtain bone broth as raw material. These bones were purchased from a local abattoir, S.C. IASICARN S.R.L. (Tomești commune, Iași county). Root and bulb vegetables added to the broths included: parsley (root), carrot, and onion.

The process of making bone broth involved several steps, as follows: baking the bones at 120ºC for 60 minutes, slow boiling the baked bones for 12 hours at 110ºC, followed by two filtration stages to remove larger and smaller debris, portioning into jars and sterilizing them, thermostating, drying the jars, labeling, and storing at refrigeration temperatures (0-4ºC). In addition to these steps, vegetable-added bone broth involved several additional stages that occurred after filtering the bone broth and consisted of boiling the vegetables at 95-100ºC for 30 minutes and filtration, followed by the steps described above for bone broth without vegetable additives.

For the chemical determinations, samples of approximately 100-150 grams were collected from each batch. These samples were analyzed using the Food-Check apparatus (Bruins Instruments - a KPM Analytics brand, Puchheim, Germany) via an infrared light source (Boișteanu et al., 2023). This is a spectroscopic technique that utilizes the electromagnetic spectrum. The NIR (Near-Infrared) region is the spectrum defined by wavelengths ranging from 700 nm to 2500 nm.

The physical determinations of the bone broth batches consisted of pH value determinations, instrumental colorimetric determinations, and the calculation of process yield and losses. The pH values of the bone broth samples were determined using the Testo 206-pH2 pH meter for semi-solid food products (Testo Rom S.R.L., Cluj-Napoca, Romania). Instrumental color was determined in the CIELAB system using the luminosity scales (L*), red-green complementary color coordinates (a*), and yellow-blue complementary color coordinates (b*) using the Konica Minolta Chroma Meter CR-410 color analyzer, as in the study by Gucianu et al. (2023). The light source of the device was D65, and the observation angle was set at 10°C, in accordance with the work of Manoliu et al. (2023). Based on the results of the parameters concerning the CIE colorimetric coordinates a* and b*, Hue (H*) and Chroma (C*) were calculated using equations (1) and (2), according to Long et al. (2024). The C* value represents color saturation, indicating the distance covered by the gray achromatic central

axis of the color space, and the hue angle (H*) reflects the chromaticity or color tone, ranging from 0° (red) - 90° (yellow) - 180° (green) - 270° (blue) - 360° (red) (Long et al., 2024).

$$H(*) = tan^{-1} \times b^*/a^* \quad (1)$$

$$Chroma\ (C^*) = \sqrt{(a*)^2 + (b*)^2} \quad (2)$$

For the measurement of microorganism count (Plate Count Agar - PCA) and certain bacterial species - *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* - in the bone broth samples, the method of successive dilutions was used. The samples were collected in accordance with the specifications of ISO 6887-2:2017 standard. The procedure involved preparing dilutions (10^{-1}, 10^{-2}, and 10^{-3}) from the collected samples using peptone water. Then, for each dilution, 1 ml was taken and transferred onto individual Petri dishes. These plates were subsequently used for bacterial cultivation on three distinct culture media: Rapid *Staph.* Agar, Plate Count Agar, Rapid *E. coli*, and Rapid *Salmonella*. The selection of culture media was determined by the specific type of target microorganism identified in each dilution. Plates with specific culture media were incubated for 24-36 hours depending on the target microorganism. After completing this step, the plates were removed and visual examination was performed using a colony counter with a counting grid and digital counting pencil (BOECO Colony Counter CC-1, Germany).

For each batch, a total of five trials were conducted for each of the analyses described above.

The results obtained after conducting the physico-chemical analyses were subjected to a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test at a significance level of 5% ($p < 0.05$), to compare the mean values among the four batches of bone broth, as in the study by Ciobanu et al. (2023). IBM SPSS Statistics V21 software was used for statistical analysis.

RESULTS AND DISCUSSIONS

The results of the raw chemical composition of the bone broth samples studied are presented in Table 1. These consist of the mean values and standard deviation.

The moisture content of all bone broth samples ranged from 68.10% to 76.56%. As observed in Table 1, the highest moisture content values were recorded in the batches where the raw material was obtained from juvenile cattle (YBS1 and YBVS2). This may be due to a higher moisture content in the carcasses of young cattle, as observed by Coleman et al. (1993) for two different cattle breeds. Additionally, studies by Arthaud et al. (1977) show that the total moisture content of cattle carcasses decreases with age. The highest moisture content value was obtained in the sample of bone broth from juvenile cattle without vegetable additives (YBS1), which was 76.56 ± 0.089%, while the lowest was identified in the batch of broth obtained from bones of adult cattle with vegetable additives (ABVS4), which was 68.10 ± 0.600%. By consulting Table 1, it can be observed that the moisture content is higher in batches of bone broth without vegetable additives, regardless of the raw material used, even though they have high water content (Knez et al., 2022). These results may be due to the water absorption properties of root vegetables during heat treatment (Bradbury & Holloway, 1988). As these are eliminated at the end of the heat treatment, they led to a reduction in the total moisture content of the broths in which they were added.

The differences in moisture content between the four batches of bone broth were highly significant ($p < 0.001$). However, the mean values obtained were relatively similar within the batches where the same raw material was used, especially in the YBS1 and YBVS2 batches, where the difference was only 0.24%.

The lipid content varied considerably among the batches (Table 1), with highly significant differences ($p < 0.001$) observed upon statistical testing. The primary influence on this parameter was the origin of the raw material, with high values recorded in the batches where bones from adult animals were used, at 11.52 ± 0.760% (ABVS4) and 8.36 ± 1.677% (ABS3), compared to those where the bone tissue originated from young cattle, which had lower values of 1.26 ± 0.089% in the batch without vegetable additives

and 1.62 ± 0.084% in the batch with vegetable additives. By examining these data, a slight increase in this parameter value can be observed in both batches where bones from adult cattle and those from juvenile cattle with added root vegetables were used. The differences were much more significant in the batches where the raw material consisted of bones from adult cattle, with differences of 3.16% between the two batches. Even though the amounts of lipids contained in the added vegetable products are extremely low, it can be assumed that they solubilized during the heat treatment in the liquid where it took place (the future bone broth), as observed in the studies by Bradbury & Holloway (1988), where the fat content of root vegetables decreases after the heat treatment stage, resulting in values very close to or even equal to 0.00. However, the fat contribution provided by the vegetable addition is not sufficient to account for such a large difference (3.16%) between the batches where bones from adult cattle were used. For this reason, it can be assumed that the reason for the much higher fat content in batch ABVS4 compared to batch ABS3 may be due to a better extraction of this qualitative parameter. This superior extraction can be attributed to a more rigorous adherence to the heat treatment, exceeding the established values for it, or more frequent opening of the boiling kettle in the batch with a lower fat content (ABS3), which led to a decrease in temperature and slower return to the temperature set in the product technical sheet, resulting in a more deficient fat extraction. Additionally, another cause may be attributed to the non-uniformity in sample collection. The bone broth from adult animals exhibited a significant fat layer, constituting approximately 20 - 30% of the jar's volume, compared to the broths where the raw material originated from young animals, where the fat layer was <5% of the jar's volume. This latter cause is presumed to be correct, considering that the other parameters of the chemical quality studied were not affected.

Table 1. The proximate chemical composition of the bone broth batches

Parameters (%)	Batches				p-value
	YBS1	YBVS2	ABS3	ABVS4	
Moisture	76.56 ± 0.089^{c}	76.32 ± 0.045^{c}	70,70 ± 1,389^{b}	68.10 ± 0.600^{a}	0.000 (***)
Lipid	1.26 ± 0.089^{a}	1.62 ± 0.084^{a}	8.36 ± 1.677^{b}	11.52 ± 0.760^{c}	0.000 (***)
Protein	22.06 ± 0.054^{c}	21.96 ± 0.055^{c}	20.42 ± 0.396^{b}	19.68 ± 0.164^{a}	0.000 (***)
Collagen	20.44 ± 0.055^{c}	20.34 ± 0.054^{c}	18.64 ± 0.428^{a}	17.90 ± 0.200^{a}	0.000 (***)
pH	8.80 ± 0.060^{c}	6.44 ± 0.047^{a}	8.69 ± 0.087^{c}	7.49 ± 0.059^{b}	0.000 (***)

$^{a, b, c}$ – Superscripts on different means within the same row differ significantly, $p > 0.05$; *** $p < 0{,}001$.
YBS1 – juvenile bovine bone soup; YBVS2 – juvenile bovine bone soup with vegetable addition; ABS3 – adult bovine bone soup; ABVS4 – adult bovine bone soup with vegetable addition.

The protein content of the studied samples, as presented in Table 1, is higher for batches made from bones of juvenile cattle, where values of 22.06 ± 0.054% (YBS1) and 21.96 ± 0.055% (YBVS2) were obtained, compared to batches made from bones of adult cattle, which had values of 20.42 ± 0.396% (ABS3) and 19.68 ± 0.164% (ABVS4). These values are much higher than those obtained by Ozturk & Kerimoğlu (2022), where the highest protein content value in one of the bone broth batches studied was 16.58 ± 0.44%, under the condition that sheep meat was also added, and for the batch obtained exclusively from bones (tibia), the protein content was only 3.27 ± 0.18%.
Collagen also shows highly significant differences ($p < 0.001$) among the four studied batches (Table 1). Batches made with bones from adult animals (> 24 months) have a lower collagen content of 18.64 ± 0.428% (ABS3) and 17.90 ± 0.200% (ABVS4), compared to the values of the same parameter obtained in batches where bones from cattle still in the growth and development phase (< 24 months) were used as raw material. Although at first glance, these results seem to contradict those in the literature, which indicate an increase in collagen content in animal tissues directly proportional to age, Williamson et al. (2001) observed a slight decrease in collagen content in adult cattle compared to that recorded in calves, but higher than the collagen content in animals in the fetal stage. Their study concluded that this decrease from younger to adult cattle could be due to the normal evolution of the biochemical properties of bones and joint cartilage during

development. These results imply changes in the collagen component of articular cartilage as having important functional consequences during normal development and growth (Williamson et al., 2021). Furthermore, we can observe that batches of bone broth with vegetable additives have lower collagen content values, a fact motivated by the absence of collagen in plant-based products, which is specific only to animal tissues.

The average values obtained for the pH parameter are high (alkaline) in the case of three of the studied batches - YBS1 (8.80 ± 0.060%), ABS3 (8.69 ± 0.087%), and ABVS4 (7.49 ± 0.059%), as can be observed in Table 1. The results of the statistical test of variation of the samples (ANOVA) show that there are highly significant differences between all the studied samples in terms of the pH parameter, just like in the case of all the other parameters of the chemical quality of the bone broths. The relatively high average values of this parameter may be due to the relatively alkaline water in the municipality of Iași, as shown by the results obtained by Cohl et al. (2014). Another reason for the alkalinity of the studied batches could be the high microbiological load of the bones, which is not entirely destroyed during heat treatment, or the dissolution of minerals present in the bone tissue, especially calcium (Field et al., 1974), which can lead to an overall increase in the pH value of the analyzed products.

Table 2 presents the Pearson correlations between the studied chemical parameters. As can be seen in this table, the moisture content of the samples shows distinct significant correlations ($p < 0.01$) with all the other chemical parameters, with the exception of pH, where the interaction is nonsignificant ($p > 0.05$). Strong positive correlations of the moisture content of the samples are observed for the protein content and for the collagen content. In addition to these, there is also a negative correlation (-1.000**) between the moisture content of the samples and the lipid content, which is in line with the specialized literature (Cobos & Díaz, 2015).

Table 2. Pearson correlations between qualitative parameters of bone broth samples

Parameters	Moisture	Lipid	Protein	Collagen	pH
Moisture	1	**-1.000****	**.999****	**.999****	-.098
Lipid		1	**- .999****	**.999****	,093
Protein			1	**.998****	-.086
Collagen				1	-.096
pH					1

**Correlation is significant at the 0.01 level.

The fat content shows a significant negative correlation (- .999**) with the protein content (Table 2), as an increase in the latter implies a decrease in the former. Consequently, batches with higher lipid content (ABS3 and ABVS4) obtained lower scores for protein content (Table 1). The relatively significant differences in the average fat content between batches made with bones from juvenile cattle compared to those produced from bones from adult cattle are considered one of the causes of the highly significant differences ($p < 0.001$) in the average values for the protein parameter within the studied batches.

The proteins and collagen of the samples exhibit a significant correlation (.998**) between the studied samples, which represents a completely natural result, considering that collagen is a component of animal proteins. It is entirely normal for the collagen content to increase alongside the increase in crude protein content (Table 2).

The pH value does not significantly influence the other chemical parameters (Table 2), but its elevated values are most likely influenced by the presence of mineral elements (Field et al., 1974).

Color is considered the most important visual characteristic that can affect consumer preferences and satisfaction. Since it has been found that color parameters are influenced by recipe compositions in numerous meat systems (Ozturk & Kerimoğlu, 2022), we evaluated the variations in color parameters in the case of bone broths (Table 3).

The average values of the L* parameter can be observed to be higher in the case of broths made from bones from adult cattle (ABS3 and ABVS4, Table 3). The same trend can be

observed within the broths made with bones from the same age category, where the broths without vegetable additions recorded higher values for the colorimetric parameter L* (YBS1 and ABS3). The broth made from bones from adult cattle without vegetable additions (ABS3) obtained the highest value for the L* parameter (71.24 ± 0.903), followed by the broth made with the same raw material but with vegetable addition (ABVS4), which obtained a value of 67.65 ± 0.882. From this, we can deduce that the most significant influence on the brightness of the sample was the raw material used and, subsequently, the addition of root vegetables, the latter leading to a slight "darkening" of the samples. The higher values for the colorimetric parameter L* in the case of samples using bones from adult cattle can also be attributed to the much higher fat content in these batches (Table 1), which is opaque white in color. All values for L* are statistically significantly different ($p < 0.001$).

The parameter a* obtained higher and closer values within the YBVS2 and ABVS4 batches, which are 2.81 ± 0.387 and 2.91 ± 0.084, respectively (Table 3). By analyzing the values in Table 3, significant differences ($p < 0.05$) can be observed between the L* and a* parameters, which are negatively correlated (-.467). Therefore, batches YBS1 and ABS3, which have higher values of the L* parameter, obtained lower values for the a* parameter, which are 0.15 ± 0.048 and 0.47 ± 0.063, respectively (Table 3). The colorimetric coordinate b* recorded higher values in the batches where the value of the coordinate a* was higher and where the brightness (L*) was lower (Table 3). To reinforce this finding, we can analyze Table 4, where positive correlations (.995**) can be observed between the a* and b* parameters, which are significant at a level of 0.01. As with the colorimetric parameter a*, a negative correlation (-.493*) was recorded between the b* parameter and brightness, which is significant ($p < 0.05$). The correlations obtained for the CIE L*, CIE a*, and CIE b* parameters are in line with those obtained by Ozturk & Kerimoğlu (2022).

Table 3. Colour parameters (L* - lightness, a* - redness and b* - yellowness), chroma and hue values (mean ± st dev) of the batches

Parameters	Batches				p-value
	YBS1	YBVS2	ABS3	ABVS4	
L*	67.04 ± 1.417^{b}	62.13 ± 3.699^{a}	71.24 ± 0.903^{c}	67.65 ± 0.882^{bc}	0.000 (***)
a*	0.15 ± 0.048^{a}	2.81 ± 0.387^{b}	0.47 ± 0.063^{a}	2.91 ± 0.084^{b}	0.000 (***)
b*	14.66 ± 0.591^{a}	37.03 ± 2.367^{c}	18.06 ± 0.385^{b}	35.554 ± 0.374^{c}	0.000 (***)
Chroma	14.67 ± 0.591^{a}	37.14 ± 2.388^{c}	$18.07 \pm 0{,}376^{b}$	35.67 ± 0.376^{c}	0.000 (***)
Hue	1.56 ± 0.000^{c}	1.49 ± 0.009^{a}	1.55 ± 0.005^{b}	1.49 ± 0.000^{a}	0.000 (***)

a, b, c – Superscripts on different means within the same row differ significantly, $p > 0.05$; *** $p < 0{,}001$.
YBS1 – juvenile bovine bone soup; YBVS2 – juvenile bovine bone soup with vegetable addition; ABS3 – adult bovine bone soup; ABVS4 – adult bovine bone soup with vegetable addition.

Table 4. Correlations between color parameters (L* - lightness, a* - redness and b* - yellowness), chroma and hue

Parameters	L*	a*	b*	Chroma	Hue
L*	1	**-.467***	**-.493***	**-.493***	,420
a*		1	**.995****	**.995****	**-.992****
b*			1	**1.000****	**-.986****
Chroma				1	**-.986****
Hue					1

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

The degree of color saturation (Chroma) shows a direct proportional increase with the a* and b* coordinates of the CIELAB system, as seen in the table. Consulting Table 4, which presents the Pearson correlations of the studied color parameters, it can be observed that there are strongly positive and significant correlations ($p < 0.01$), of .995** (the correlation between Chroma and a*) and 1.000** (Chroma and the b* parameter), the latter being a perfect correlation. The highest values of Chroma, 37.14 ± 2.388 (YBVS2) and 35.67 ± 0.376 (ABVS4), are observed in the broths with

vegetable additions, indicating that this addition has the highest influence on this parameter.

The color tone (Hue) correlates positively only with the CIE L* coordinate, where we observe the value of .420 (Table 4), but this correlation is not significant. Negative and significant correlations ($p < 0.01$) are observed between hue and the other colorimetric parameters (a* -.992** and b* -.986**), as well as between hue and chroma (-.986**) (Table 4). Higher average values are observed in the broths without vegetable additions, which are 1.56 ±0.000 (YBS1) and 1.55 ± 0.005 (ABS3) (Table 3). Lots with higher values of color coordinates a* and b* have slightly lower values of the hue parameter (Table), which are 1.49 ± 0.009 and 1.49 ± 0.000 in the YBVS2 and ABVS4 lots, respectively.

Following the application of statistical tests, highly significant differences ($p < 0.001$) were obtained among all color parameters, chroma, and hue of the studied lots (Table 3).

Table 5 includes the microbial species for which microbiological analyses were conducted and the results obtained for them. All microbial species studied, except *Salmonella*, were identified in the samples examined.

Staphylococcus aureus exhibited the highest value (3.07 ± 0.045 log CFU/cm^2) in the ABVS4 batch, followed by the YBVS2 batch, with a value of 2.75 ± 0.010 log CFU/cm^2. Batches YBS1 and ABS3 obtained average values of 1.93 ± 0.025 log CFU/cm^2 and 2.34 ± 0.046 log CFU/cm^2, respectively (Table 5). From these results, it can be observed that batches without vegetable additives obtained lower values for this microorganism. Additionally, it can be noted that the values are slightly lower in batches where the raw material originated from juvenile cattle.

Escherichia coli, unlike *Staphylococcus aureus*, obtained lower values in batches where root vegetables were added (YBVS2 and ABVS4), at 1.83 ± 0.031 log CFU/cm^2 and 2.09 ± 0.036 log CFU/cm^2, respectively, compared to batches made exclusively from bones, where the recorded values were 2.14 ± 0.017 log CFU/cm^2 for YBS1 and 2.32 ± 0.021 log CFU/cm^2 for ABS3 (Table 5).

The PCA (Plate Count Agar) analysis was used to determine the number of aerobic bacteria in the samples studied. By analyzing the values in Table 5, it can be observed that the values resulting from this analysis are higher in batches where bones from young cattle were used for soup production and in batches where root vegetables were added. The highest value of the PCA analysis was 4.92 ± 0.020 log CFU/cm^2, obtained by batch YBVS2, which was made from both bones from young cattle and vegetable additions. The lowest value of this microbiological test was identified in batch ABS3, at 3.74 ± 0.032 log CFU/cm^2 (Table 5).

Salmonella was absent in all batches studied, consistent with the provisions of Regulation (EC) No. 2073/2005, as are the other results of the microbiological analyses conducted in this study.

All batches of bone broth (YBS1, YBVS2, ABS3, and ABVS4) showed significantly different results ($p < 0.001$) for the three microbiological analyses where colony-forming units were identified (*Staphylococcus aureus, Escherichia coli*, and Plate Count Agar) (Table 5).

Given that pH has a significant influence on the microbial contamination of meat and meat products (Sofos, 2014), we decided to perform Pearson correlations (Table 6) to observe if it might have had an influence on the microbiological quality of our products, considering that some of them recorded quite high pH values (Table 1) Moreover, its value also increases due to the high pressure during heat treatment, which promotes intensive and rapid multiplication of microorganisms that cause food spoilage (Hygreeva & Pandey, 2014). Certain protein-degrading bacteria can lead to the increase of pH in meat products, such as *Enterobacteriaceae* bacteria. The growth of microorganisms and their ability to cause spoilage in meat products depends on a variety of properties. These include intrinsic factors such as the type of organism causing spoilage and the initial load of bacteria present, pH, and water activity as well as the availability of substrates used for energy (Gribble, 2014).

Table 5. The average values of the microorganisms analyzed in the batches of bone broth studied

Batch	*Staphylococcus aureus* (log CFU/g)	*Escherichia coli* (log CFU/g)	Plate count agar (log CFU/g)	*Salmonella* (log CFU/25g)
YBS1	1.93 ± 0.025^{a}	2.14 ± 0.017^{c}	4.36 ± 0.026^{c}	Abs.
YBVS2	2.75 ± 0.010^{c}	1.83 ± 0.031^{a}	4.92 ± 0.020^{d}	Abs.
ABS3	2.34 ± 0.046^{b}	2.32 ± 0.021^{d}	3.74 ± 0.032^{a}	Abs.
ABVS4	3.07 ± 0.045^{d}	2.09 ± 0.036^{b}	4.11 ± 0.010^{b}	Abs.
p-value				
Sig.	0.000 (***)	0.000 (***)	0.000 (***)	-

a, b, c, d – Superscripts on different means within the same column differ significantly, $p > 0.05$; ns – non significant; *** $p < 0,001$.
YBS1 – juvenile bovine bone soup; YBVS2 – juvenile bovine bone soup with vegetable addition; ABS3 – adult bovine bone soup; ABVS4 – adult bovine bone soup with vegetable addition.

Table 6. Pearson correlations between the studied microorganism species and pH

Comparative indicators	*Staphylococcus aureus*	*Escherichia coli*	PCA	pH
Staphylococcus aureus	1	0.381	-0.180	0.266
Escherichia coli		1	**0.918****	**0.908****
PCA			1	**-0.735****
pH				1

** Correlation is significant at the 0.01 level.

The results obtained from the application of Pearson correlations were positively significant between *Escherichia coli* and PCA (0.918**) and between *Escherichia coli* and pH (0.908**). A highly significant negative correlation was identified between pH and PCA results (-.735**) (Table 6). The values for *Staphylococcus aureu*s did not show significant correlations either with the values of other microbiological analyses or with the pH value.
These results indicate that pH may have an influence on the presence of microorganisms in the samples studied, but not for all bacterial species.

CONCLUSIONS

The research conducted in this investigation highlights notable variations among bone broths based on the composition of the ingredients used, whether of animal or vegetable origin, in terms of physicochemical, colorimetric, and microbiological characteristics. Although derived from the same species, the choice of raw materials for bone broth production, whether it be from juvenile or adult bovine bones, has been associated with significant differences in the final product's quality. The influence of vegetable additives has also been crucial in determining the qualitative differences among batches of bone broth.
Specifically, broths prepared with bones from young bovines (under 24 months of age) exhibited higher protein and collagen contents compared to those obtained from adult bovine bones, and the addition of bones was associated with higher average lipid content in the samples studied. Additionally, vegetable additives had a significant impact on the pH of the broths, resulting in significant differences between those with and without vegetable additives.
Regarding the instrumental color of the samples studied, it was greatly influenced by the presence or absence of vegetable additives in the bone broth samples. The use of root vegetables in the production of two of the bone broth batches led to increased brightness (L*) and higher values of the colorimetric parameters a* (redness) and b* (yellowness) of the samples.
The results of the microbiological analyses conducted on the bone broth samples indicate significant variation between batches, except for *Salmonella*, which was absent in all samples. *Staphylococcus aureus*, PCA, and *Escherichia coli* showed different values depending on the composition and origin of the ingredients used. pH values were also variable, with some samples recording high values.
Ultimately, both bone broths derived from juvenile and adult cattle, with or without vegetable additives, exhibited a high level of quality, particularly in terms of protein and collagen content. However, the significant variations between these types of broth were evident and deserve special attention.

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