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The Effect of Buffalo Meat on Composition, Instrumental and Sensory Characteristics of Traditional Greek Sausages

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Abstract

Five (5) mixtures of buffalo/pork meat (70/0, 52.5/17.5, 35/35, 17.5/52.5 and 0/70), maintaining stable the amount of pork backfat, were prepared and analyzed for their chemical composition, fatty acids profile, instrumental parameters and sensory attributes. The results of the study showed that the addition of buffalo meat produced sausages with higher protein and less fat content. A slight decrease in ω6/ω3 ratio was observed and an increase in CLA fatty acids. Principal Component Analysis revealed that the lower fat content in the sausages the higher the levels of CLA18:10trans 12cis and CLA18:9cis 11trans, whereas, SFA is abundant at the highest fat levels. Redness and hardness instrumentally increased in the sausages with the addition of buffalo meat, while juiciness decreased, probably due to the decreased lipid content. The mixture with the ratio of 52.5/17.5 buffalo/pork positively maximized sensorial consistency, elasticity and cohesiveness of the traditional sausages, while the addition of 70.0/0.0 mixture, hardness and redness.

Keywords: Greek buffalo meat, traditional Greek sausages, FAME, instrumental, sensory

1. Introduction

Essential fatty acids, fat soluble vitamins and energy are the three basic physiological functions contributed to the fat content of any food (Muguerza, Fista, Ansorena, Astiasaran, & Bloukas, 2002). In meat products fat is a crucial parameter since it contributes to flavor, texture, mouth feel, juiciness and overall sensation of lubricity of the product. Therefore, any fat reduction in meat products can affect their acceptability by the consumers (Giese, 1996). However, it has been worldwide scientifically announced that lowering the amount of fat, particularly saturated fatty acids as well as linoleic acid and increased ratio of ω–6/ω–3 fatty acids is a major risk factor for western type cancers, thrombotic diseases, apoplexy, allergic hyperreactivity and diseases for which anti-inflammatory drugs are effective (AHA, 1986; Zotos & Vouzanidou, 2012).

Greeks are quite keen on traditional sausages which are very popular all over the country. According to Greek Food Legislation (1987) they are characterized as fresh (non-cooked) sausages which should not contain more than 35% fat although Papadima, Arvanitoyannis, Bloukas, and Fournitzis (1999) have found that the fat content ranges from 15.4 to 56.8%. Neverthereless, fat acts as a reservoir for flavor compounds and contributes to product texture and juiciness (Papadima & Bloukas, 1999). The granulated fat in non-cooked sausages helps the sausage mixture to be loosened and consequently it attributes to the release of moisture from the inner layers of the product (Wirth, 1988). Therefore, reducing the fat content in sausages may alter product quality.

Most Greek agricultural families produce traditional sausages. They are mainly consisted by pork meat and fat which are chopped and thoroughly mixed with salt, leek and seasonings. The sausage mixtures were stuffed in natural casings obtaining from the cleaned small intestine of pigs. The sausages were pierced on all sides to allow ensnared air to get away and stored for some days in a warm room in order the expected dryness to be achieved. Sausages which are going to be consumed within a few weeks stored at low temperature rooms (Papadima & Bloukas, 1999).

The Food and Agriculture Organization of the United Nations refers to the Greek buffalo (*Bubalus bubalis*) population as a separate breed named "Ellinikos vouvalos (Greek buffalo)", and characterizes this population as endangered-maintained (FAO, 2007) species. The Greek buffalo is part of biodiversity in many Greek wetlands enriching ecosystems with its aesthetic value. Moreover, the Greek buffalo, as a food producing animal, provides valuable products (milk, meat; Zotos & Bampidis, 2014), which constitute exquisite dishes, contributing to economic growth in the surrounding areas of farming thus there is an increasing interest nowadays for its breeding in Greece, with its population being approximately 4,000 (GBBC, 2014). While Greek buffalo meat gaining popularity in Greece, there are opportunities for the development of the buffalo meat industry to cater for the needs of the Greek market. The advantages of buffalo meat are the high protein, low fat and low cholesterol contents as well as the attribution of lower calories than beef meat (Vasanthi, Venkataramanujam, & Dushyanthan, 2007). Thus, the aim of this work was to study the effect of Greek buffalo (*Bubalus bubalis*) meat on the proximate composition, fatty acids profile, instrumental and objective sensory characteristics of traditional Greek sausages, maintaining at the same time a consistent addition of pork backfat.

2. Materials and Methods

2.1 Preparation of Samples

The raw materials used for the preparation of traditional sausages such Greek buffalo (thigh) and pork (thigh) meat, pork backfat and natural cases of porcine small intestine were provided by Kerkini market, North Greece (41°14' N, 23°06' E) and stored at 4 °C. The used additives such as oregano, pepper, salt and cumin were purchased from the local market. The quantities were buffalo meat 10.5 kg, pork meat 10.5 kg and pork backfat 9 kg. The mixtures of the above raw materials are shown in Table 1.

Raw materials were sliced into small pieces and mixed thoroughly with the above mentioned additives. They were then minced using a Seydelmann mince machine with internal mesh of 8 mm and external 5 mm.

The batter was encased in natural cases of porcine small intestine 30-32 mm using a TalsaH26PA filler machine. The cases were densely pierced on all sides to allow entrapped air to escape. Then sausages were mounted on trolleys and placed in the chamber at 4 °C for 72 hours in order a natural drying process to be achieved. The drying process followed by a grilling process for approximately 20 min and the samples were presented to panelists after being cooled at approximately 30 °C. Samples for further analysis were refrigerated at 2±1 °C.

Table 1. The five mixtures of the raw materials used to produce traditional sausages

Buffalo meat %	Pork meat %	Buffalo meat (Kg)	Pork meat (Kg)	30% Pork backfat (Kg)	2% Salt (Kg)	0.2% Pepper (Kg)	0.3% Cumin (Kg)	0.3% Oregano (Kg)	Sausages (Kg)
52.5	17.5	3.15	1.05	1.8	0.12	0.012	0.018	0.018	6.168
17.5	52.5	1.05	3.15	1.8	0.12	0.012	0.018	0.018	6.168
70	0	4.20	0	1.8	0.12	0.012	0.018	0.018	6.168
0	70	0	4.20	1.8	0.12	0.012	0.018	0.018	6.168
35	35	2.10	2.10	1.8	0.12	0.012	0.018	0.018	6.168
Sum (Kg)		10.50	10.50	9.0	0.60	0.060	0.090	0.090	30.840

2.2 Proximate Analysis

The recommended method by the CEC (Commission of European Communities) ISO 1442-1973 was used to determine moisture content (CEC, 1979). The method of Hanson and Olley (1963) was applied to determine lipid content as well as to extract lipids. Total protein (crude protein, N × 6.25) content of the samples was determined using the Kjeldahl method according to standard AOAC method (AOAC, 2002a). The ash content was determined by mineralization at 550°C according to standard AOAC method (AOAC, 2002b).

2.3 Fatty Acid Analysis

The extracted lipids were further prepared for fatty acid analysis according to the procedure of Zotos, Hole and Smith (1995). Fatty acids methyl esters were analyzed using a Focus GC (Thermo Finnigan, USA) with an FID detector, equipped with auto sampler and a capillary column AT AquaWax 60 m × 0.25 mm ID, thickness 0.25

μm (Alltech, USA). The oven temperature set at 150 °C for 1 min and programmed to 220 °C at 3 °C min^{-1} and held there for 30 min, the total analysis time was 54 min. The volume of the sample was 1 μL, the injection temperature 220 °C at splitless mode with 0.80 s splitless time and the detection temperature 250 °C. The carrier gas was high purity helium with a linear flow rate 1.2 mL min^{-1}. The various fatty acids were identified by comparison with standard fatty acids methyl esters (C14:0, C16:0, C16:1ω-7, C18:0, C18:1ω-9, C18:1ω-7, C18:2ω-6, CLA (C18 9cis 11trans, C18 9cis 11cis, C18 9trans 11trans, C18 10trans 12cis), C18:3ω-3, C20:1ω-9, C20:4ω-6, C20:5ω-3 & C22:6ω-3, Larodan Fine Chemicals, Sweden).

2.4 Instrumental Analysis (TPA)

Sausages were instrumentally analyzed by the Texture Profile Analysis (Friedman, Whitney & Szczesniak 1963; Bourne, 1978), a well known method for texture characterization (Chen, 2009) which gives good correlation relationships between mechanical and sensory attributes for meat products such as rib steaks (Caine, Aalhus, Best, Dugan, & Jeremiah, 2003) and frankfurter-type sausages (Yang, Keeton, Beilken & Trout, 2001; Ritzoulis, Petridis, Derlikis, Fytianos, & Asteriou, 2010). The principle of the method is the application of two successive compressions to a test sample using a instrumental testing machine in imitation of a chewing process. The obtained force–displacement/time curves can be used for an approximate quantification of a number of kinesthetic parameters such as hardness, cohesiveness, viscosity, elasticity, adhesiveness, springiness, brittleness, chewiness, and gumminess. Hardness, cohesiveness, chewiness, springiness and gumminess were measured in this work. Experiments were performed using a TA-XT texture analyzer (TA instruments, New Castle, DE), as described before by Ritzoulis et al. (2010). The analyzer was equipped with a 50-mm-diameter aluminum cylinder, operating with a compression rate of 5 mm/sec. Samples, 20 mm in length, were cut using a dedicated template ring, and axially compressed to 40% of their original height. The capacity of the load cell used was 30 g. All tests were performed at least six times. The OriginPro 8.0 (OriginLab Corporation, Northampton, MA) computer program was chosen to obtain graphic displays of the texture analyzer data and perform the necessary calculations.

2.5 Sensory Analysis

Sensory evaluation was performed using a balanced incomplete block design with the following features (plain 11.1a, Cochran & Cox, 1957): t=5 treatments (samples), k=3 treatments assessed by each panelist, b=10 panelists, r=6 replicates per treatment και λ=3 similar pairs of treatments in the design. Each slice was cut into 3-cm cylinders. Samples were assessed at room temperature and were presented to panelists hosted in special booths in white plastic dishes. The order of assessment was randomized within each session. The samples were taken out from refrigeration (4 °C) 30 min prior to their testing and left for equilibration at room temperature and were immersed in boiling water for 3 min immediately prior to testing. Panelists were asked to evaluate five sensory attributes in terms of perception intensity:

- Hardness as the force required to penetrate sample with molar teeth.
- Consistency as the evaluation of the amount of deformation before rupture.
- Elasticity as the degree of bouncing between two consecutive bites.
- Juiciness as juice feeling in the mouth and gum.
- Red color intensity as the result of comparison of the degree of redness between slices of the various samples under white light.

A panel of 8 members of the School's staff plus two research students were chosen to participate in the sensory evaluation. They were previously allowed to gain experience by frequently consuming meat products available in the lab and were particularly trained on how to test various sausage formulations deliberately differing in sensory characteristics such as hardness, cohesiveness etc. Ten booths installed in the laboratory of sensory studies of the department were used to host the panelists who were asked to draw a vertical line on a 15 cm long unstructured scale (Muñoz & Civille, 1998) corresponding to a particular perceptive intensity of an attribute. The left end (0 cm) of the line was marked as not at all hard to bite, elastic, fatty, texturally consistent, and reddish. The right end (15 cm) was marked for the texture as very hard to bite and so forth. The experiment was conducted three times and adjusted means of attributes from each run were deduced forming eventually three replicates per sample.

2.6 Statistical Analysis

Descriptive statistics were employed for the chemical composition of data. Instrumental and sensory attributes were tested statistically using one way analysis of variance (fixed effect-sausage samples with five treatments) and those found significant at 0.05 probability level were checked for differences between buffalo treatments by plotting the 95% confidence intervals of means. Statistically significant pair-wise differences of means assume non-overlap intervals. Principal Component Analysis (PCA) was furthermore conducted to detect potential relationships between fatty acids and fat level of samples and also between the texture and sensory profile of sausages and the buffalo addition in samples. Three replicates per treatment were taken for chemical analysis, six to eight for instrumental analysis and three for sensory evaluation.

3. Results and Discussion

3.1 Proximate Composition of Raw Material, Unprocessed and Grilled Sausages

The proximate composition of the raw materials used in this work as well as of the unprocessed and grilled sausages are shown in Table 2. As can be observed the buffalo meat has higher protein content than pork meat and less fat content. These differences affected the proximate composition of either unprocessed or grilled sausages. Thus, in the mixture 70.0/0.0 (buffalo/pork) the higher protein content was detected (approximately 42% in the unprocessed and 44% in the grilled sausages) on the contrary in mixture 0.0/70.0 (buffalo/pork) protein content shown the lowest value (approximately 36.5% in the unprocessed and 39% in the grilled sausages). Similar results were also obtained for fat content (Table 2). Proportional variations between moisture, protein and fat in buffalo sausages according to fat content were also reported by Krishnan & Sharma (1990). Thus, they were reported that moisture was 66.19, 63.07 and 60.59%, protein 17.01, 16.39 and 15.44% and fat 13.85, 17.45 and 21.06% in sausages with 85/15, 80/20 and 75/25 meat and pork fat respectively.

Table 2. Proximate composition of raw meat materials on wet weight basis (mean ± StDev) and of unprocessed and grilled sausages on dry weight basis

Raw material	Moisture %	Ash %	Fat %	Protein %
Pork meat	73.11±6.6	1.07±0.18	5.18±0.40	19.74±1.37
Buffalo meat	74.88±0.22	1.11±0.05	2.14±0.05	23.74±2.06
Pork backfat	10.99±0.57	0.20±0.02	87.49±0.48	2.93±0.01

Unprocessed sausages			
Buffalo/Pork meat	Ash %	Fat %	Protein %
52.5 / 17.5	6.15±0,04	56.10±0.73	38.74±0.20
17.5 / 52.5	5.68±0.01	58.33±0.69	38.58±1.87
70.0 / 0.0	6.68±0.20	52.36±0.16	41.95±4.40
0.0 / 70.0	5.78±0.01	56.10±1.36	36.64±0.01
35.0 / 35.0	6.71±0.10	52.54±0.31	40.88±1.03

Grilled sausages			
Buffalo/Pork meat	Ash %	Fat %	Protein %
52.5 / 17.5	5.16±0.04	53.62±0.62	42.38±0.51
17.5 / 52.5	5.52±0.07	53.88±0.12	41.06±1.78
70.0 / 0.0	5.73±0.21	50.68±0.44	44.04±3.23
0.0 / 70.0	5.28±0.12	56.25±0.43	39.18±0.64
35.0 / 35.0	5.71±0.02	52.88±0.36	41.65±1.41

Data are means of triplicate determinations ± standard deviation.

3.2 Fatty Acids Profile and Relationships

The fatty acid profiles of the five mixtures (buffalo/pork meat) are shown in Table 3. As it can be observed the main fatty acids are the oleic (C18:1ω-9), palmitic (C16:0), stearic (C18:0) and linoleic (C18:2ω-6). It can also be observed that the contribution of the buffalo meat is not significant since the fatty acid profiles remained almost similar. However, a slight decrease of ω6 fatty acids was detected due to addition of buffalo meat having an effect on the proportional slight decrease of the ω6/ω3 ratio. The CLA fatty acids were also presented in higher concentrations in the sausages where buffalo meat was added (Table 3). The aforementioned fatty acids with the same order were also reported as the main fatty acids in dry fermented sausages (Muguerza, Gimenoa, Ansorena, Bloukas, & Astiasarán, 2001).

Table 3. Fatty acid profiles of the 5 above mentioned sausage mixture (buffalo/pork)

Fatty acids %	52.5/17.5	17.5/52.5	70.0/00.0	0.0/70.0	35.0/35.0
C14:0	1.40±0.01	1.46±0.01	1.37±0.01	1.53±0.13	1.43±0.16
C15:0	0.69±0.01	0.30±0.01	0.24±0.01	0.07±0.01	0.42±0.08
C16:0	26.75±0.06	26.46±0.52	26.07±0.22	26.88±0.80	26.09±1.29
C16:1ω7	2.65±0.01	2.45±0.01	2.49±0.01	2.90±0.11	2.72±0.33
C17:0	0.91±0.01	0.44±0.01	0.53±0.04	0.35±0.01	0.46±0.05
C18:0	12.55±0.10	12.94±0.10	12.86±0.06	12.57±0.28	12.53±0.16
C18:1ω-9	37.00±0.29	38.28±0.28	41.64±0.01	40.99±0.99	38.79±0.30
C18:2ω-6	11.54±0.07	11.24±0.08	10.36±0.03	11.25±0.06	11.11±0.05
CLA C18:9cis 11trans	0.92±0.04	0.77±0.01	1.09±0.04	0.63±0.01	0.87±0.02
CLA C18:10trans 12cis	0.22±0.01	0.18±0.01	0.27±0.01	0.19±0.01	0.20±0.01
C18:3ω-3	0.89±0.01	0.84±0.01	0.89±0.01	0.77±0.02	0.90±0.01
C18:3ω-6	0.23±0.03	0.37±0.04	0.18±0.02	0.38±0.01	0.23±0.01
C20:1ω-9	0.69±0.02	0.67±0.04	0.55±0.01	0.39±0.01	0.50±0.03
C20:2ω-6	0.11±0.01	0.09±0.01	0.14±0.05	0.07±0.01	0.15±0.03
C20:4ω-6	0.20±0.05	0.20±0.01	0.20±0.01	0.28±0.01	0.21±0.01
Saturated (SFA)	42.30±0.19	41.60±0.60	41.06±0.11	41.38±0.66	40.91±1.16
Monounsaturated (MUFA)	40.33±0.26	41.40±0.23	44.68±0.01	44.27±0.89	42.00±0.06
Polyunsaturated (PUFA)	14.10±0.08	13.67±0.08	13.11±0.13	13.56±0.02	13.65±0.06
Σ ω3	0.89±0.01	0.84±0.01	0.89±0.01	0.77±0.02	0.90±0.01
Σ ω6	12.29±0.03	12.06±0.09	11.14±0.09	12.16±0.04	11.89±0.04
ω6/ω3	13.81±0.19	14.35±0.11	12.58±0.20	15.79±0.42	13.21±0.04

Data are means of triplicate determinations ± standard deviation.

The effect of fat content on fatty acid content is shown in Figure 1 and the correlation coefficients between fatty acids and the two major components of PCA are shown in Table 4. Longer arrows in Figure 1 indicate attributes with higher effects, oblique and obtuse angles positive and negative correlations. The lower (oblique) or higher (obtuse) aperture between two lines the higher the correlation coefficient. Vertical lines indicate angle with zero correlation. Samples located near to an arrow exhibit strong effect on that attribute. PCA reveals an interesting relationship between fatty acids and fat content, bearing in mind that the initial preparation of sausages included steadily pork backfat concentration 30%. Fatty acids such as C20:4ω-6, MUFA, PUFA, C18:3ω-3 and C18:2ω-6 are the most responsible for the formation of major axis 1 (Table 4) explaining 55% of the total variation and SFA and CLA18:10trans 12cis for the formation of axis 2 explaining another 25.6% summing up to 80.64% variation. On the other hand, CLA18:9cis 11trans shares nearly equal correlations with both axes acting therefore equivalently. The fat content of the sausages correlates strongly and negatively only with axis 2 ($r=-0.913$) and that is clearly exemplified in Figure 1. The lowest fat levels appear in the top of axis 2 (25.76%) coinciding with high % levels of CLA18:10trans 12cis and CLA18:9cis 11trans. On the contrary, SFA is abundant at the highest fat levels (29.15 and 29.73%) at the bottom of axis 2, whereas PUFA, C18:3ω-6 and C20:4ω-6, are distributed, the latter in an inverse direction, at medium fat levels (27.90%) close to axis 1. MUFA correlates inversely (negatively) and strongly with PUFA, C18:2ω-6 and SFA.

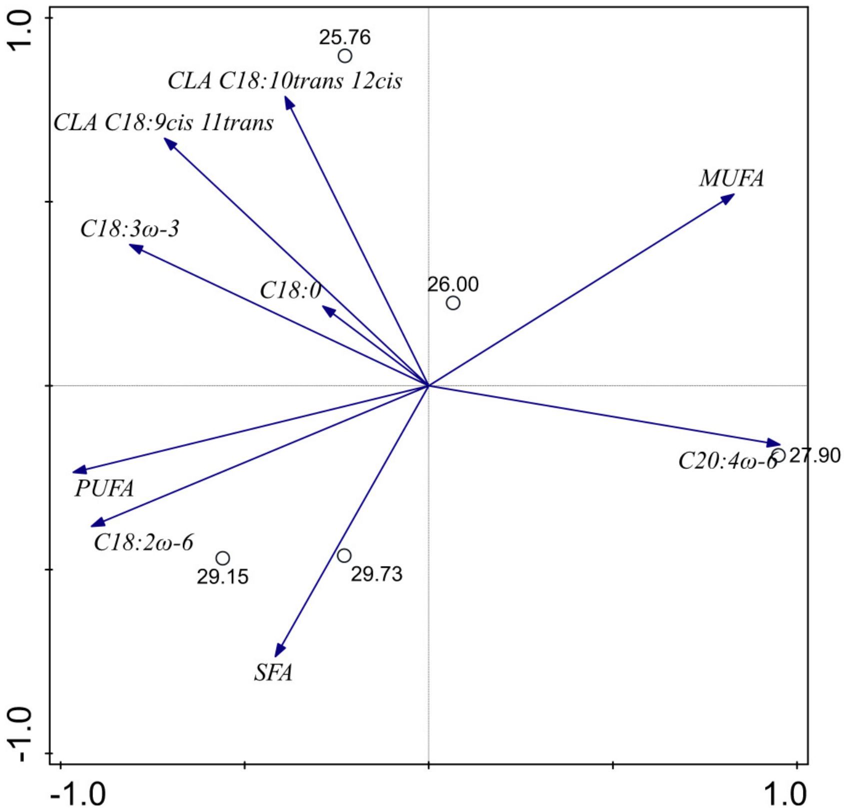

Figure 1. PCA biplot showing the correlations among fatty acids and the % distribution of fat in sausage mixtures. Lipid content on wet weight basis

Table 4. Correlation coefficients between fatty acids and the two major components. Values greater than |0.700| are shown in boldface. Asterisk denotes the correlation of fat concentration in the sausages, entered as a supplementary variable in the PCA, with the two axes

Fatty acids	Factor 1	Factor 2
C20:4ω-6	**-0.954**	-0.161
MUFA	**-0.829**	0.521
C18:0	0.288	0.216
CLA C18:10trans 12cis	0.390	**0.786**
SFA	0.417	**-0.737**
CLA C18:9cis 11trans	0.717	0.673
C18:3ω-3	**0.812**	0.383
C18:2ω-6	**0.916**	-0.382
PUFA	**0.966**	-0.236
*Fat %	0.183	**-0.913**

3.3 Instrumental and Sensory Properties of the Traditional Sausages

Three instrumental attributes, hardness, chewiness and gumminess, were found statistically significant and all appeared to increase proportionally to the buffalo content increase in the samples, more specifically at levels greater than 35% concentration (Figure 2). This should be expected since these sausages have higher protein and less lipid content. It is worth noted that any fat reduction can affect the acceptability of the products (Muguerza et al., 2002). Three also sensory attributes differentiate their intensity among buffalo levels (Figure 2). Redness elevated significantly to the buffalo content increase and distinctly between levels starting from as low as 5cm (low redness) and ending up to 11.5 cm (fair redness) proving to become an excellent indicator of buffalo amount in the sausages. An increase of sensory hardness is obvious with buffalo supplementation, actually distinct between 17.5 and 70% (confidence intervals do not overlap). Finally, juiciness declines with buffalo enrichment down to 7 cm (moderate intensity), presumably due to gradual pork content increase in the samples.

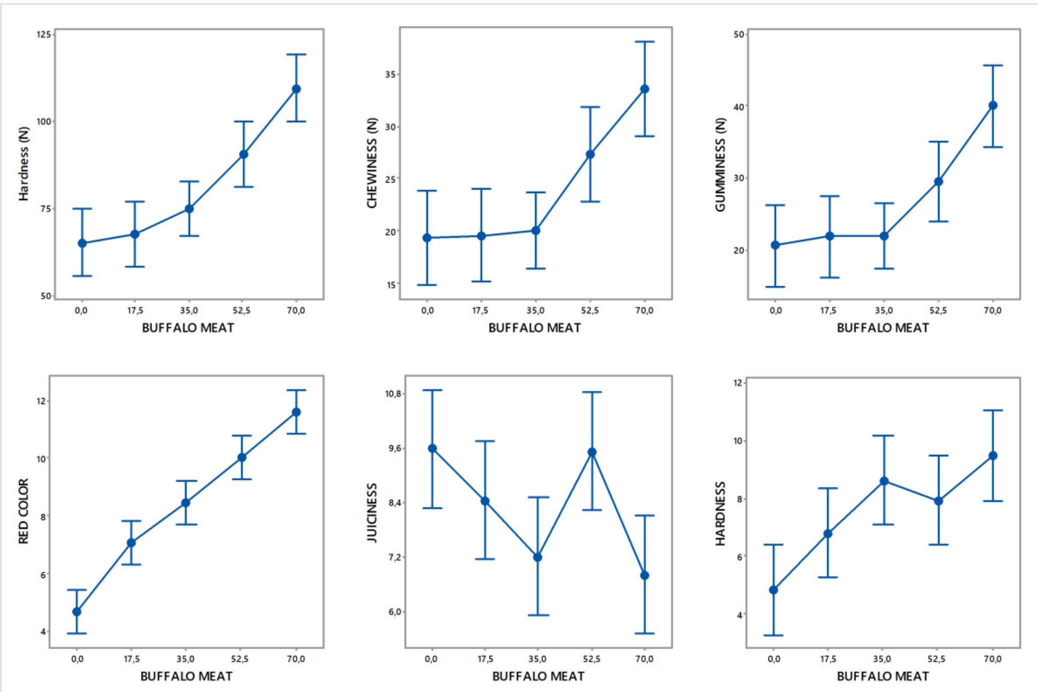

Figure 2. Mean change of instrumental and sensory attributes along the buffalo percentage increase. Vertical bars represent the 95% confidence intervals of means based on the mean square of ANOVA

The composition, instrumental and sensory profile of traditional sausages is explicitly described by the two major components in Table 5 and Figure 3. Sensory redness, juiciness and hardness, and instrumental hardness, gumminess, chewiness and springiness in joint with protein content best explain axis 1 (60.1% of the total variation) and ash and fat content axis 2 (21.3% totaling 81.5%). The relationships are further transitioned in Figure 3, in which three bundles of common attributes prevail. Between 0% and 17.5% buffalo amount in the samples, three variables of different origin correlate strongly: springiness, juiciness and fat content. Around 52.5% buffalo content, consistency, elasticity and cohesiveness maximize positively their performance. When buffalo occurs exclusively in the sausages (70% and pork 0%), then the protein content reaches high levels accompanied by high intensity of sensory hardness and redness, and all of them are adequately justified by high effects of instrumental hardness, gumminess and chewiness. Similar results regarding hardness were also reported by Rey, Martinez and Urrea (2011). They found an average hardness of 28.89 N and 19.63 N for sausages from buffalo and beef meat respectively. It was further reported that reducing the fat content of the sausages significantly increased ($p<0.05$) instrumental hardness and firmness (Muguerza et al., 2002).

Table 5. Correlation coefficients between each attribute and the two major components. Values greater than |0.700| are boldfaced. Asterisk denotes the correlation of buffalo concentration in sausages, entered as a supplementary variable in the PCA, with the two axes. Upper cases denote sensory and chemical variables and lower cases mechanical

Attribute	Factor 1	Factor 2
COLOR	**-0.959**	-0.167
Hardness	**-0.953**	-0.125
HARDNESS	**-0.924**	0.076
Gumminess	**-0.921**	-0.187
Chewiness	**-0.902**	-0.222
PROTEIN	**-0.875**	-0.101
CONSISTENCY	-0.668	-0.479
ASH	-0.595	**0.803**
ELASTICITY	-0.534	-0.574
Cohesiveness	-0.356	-0.685
FAT	0.578	**-0.802**
JUICINESS	**0.713**	-0.429
Springiness	**0.808**	-0.363
*Buffalo meat	**-0.966**	-0.129

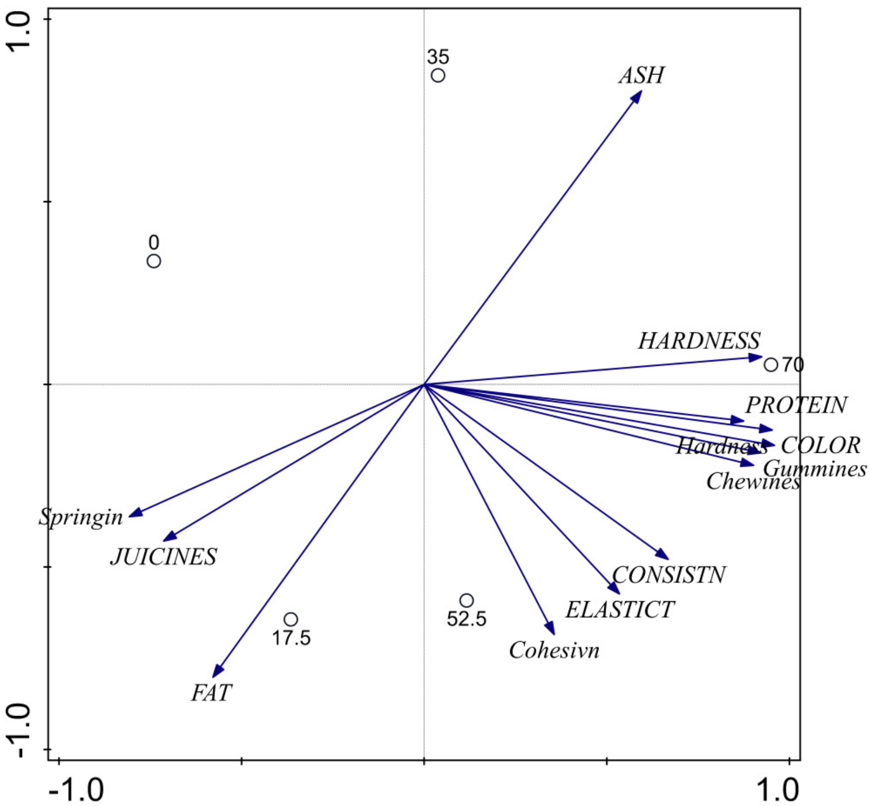

Figure 3. PCA biplot showing the correlations among attributes and the % distribution of buffalo meat samples. Upper cases denote sensory and chemical variables and lower cases instrumental

4. Conclusions

The addition of buffalo meat in traditional Greek sausages slightly increased protein and decreased fat content. A slight decrease of ω6/ω3 ratio and an increase of C18:10trans 12cis and C18:9cis 11trans fatty acids were also observed. Both C18:10trans 12cis and C18:9cis 11trans fatty acids are related to the fat content, thus the less fat content the higher the levels of the CLA fatty acids, while on the contrary, SFA is abundant at the highest fat levels. Instrumental redness and hardness increased with the addition of buffalo meat while juiciness decreased. The addition of 52.5/17.5 buffalo/pork positively maximized sensorial consistency, elasticity and cohesiveness while the addition of 70.0/0.0 mixture hardness and redness.

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