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Application of chemometrics to assess the influence of ultrasound frequency, *Lactobacillus sakei* culture and drying on beef jerky manufacture: Impact on amino acid profile, organic acids, texture and colour

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Abbreviated running title: Ultrasound, *L. sakei* and drying effects on beef jerky production

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Abstract

The effects of ultrasound (US) frequency, addition of *Lactobacillus sakei* culture and drying time on key nutritional (protein, amino acids, and organic acids) and physicochemical properties (texture and colour) of cultured and uncultured beef jerky were evaluated. Cultured and uncultured jerky samples were subjected to US frequencies of 25 kHz, 33 kHz and 45 kHz for 30 min prior to marination and drying. Principal component analysis demonstrated a significant effect of beef jerky processing conditions on physicochemical properties. Taurine content of jerky samples was found to increase with an increase in ultrasonic frequencies for cultured samples. No significant changes in colour values were observed for ultrasound pre-treated and control samples. Interactive effects of culture treatment, drying and ultrasonic frequency were observed. This study demonstrates that the nutritional profile of beef jerky can be improved through the incorporation of *L. sakei*.

Keywords: Beef jerky production; ultrasound; *Lactobacillus sakei*; drying; amino acids; organic acids; physicochemical properties

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1. Introduction

Jerky, also known as charque/charqui is derived from the term *Ch'arki* which means dried salted meat. Jerky is one of the most popular ready to eat nutritious traditional meat based product which can be prepared from almost any lean meat including beef, pork, poultry, or game. According to U.S. Department of Agriculture (USDA), jerky is classified as a non-perishable heat treated, shelf stable ready-to-eat meat product. Nowadays, a range of jerky products available consists of formed meat compared to traditional sliced whole meat which may be cured/uncured, dried, smoked/unsmoked, and air or oven dried. Commercially available jerky samples have low water activity in a range of 0.70 – 0.85 and have a moisture to protein ratio of ≤0.75 (Nummer, Harrison, Harrison, Kendall, Sofos, & Andress, 2004).

Traditionally, jerky products are perceived as unhealthy. However, in recent years, consumption of jerky has increased significantly in Western meat-consuming countries. In a recent report of the Euromonitor International on sweet and savoury snacks in Ireland for 2015, it has been suggested that the air-dried protein product snack (beef jerky) was the "big breakout product". Additionally, IBIS World report highlighted that the beef jerky accounted for 79% jerky sales within USA in 2014. The growth of sweet and savoury snacks has been heavily influenced by the changing health attitudes. Moreover, jerky, as a protein source, is the main driver for its popularity. Typically chopped and formed beef jerky contains approximately 23.4% moisture, 33.2% proteins and 25.6% lipids depending on the formulation. Protein content as high as 81% has been reported for pork jerky (Ruiz-Ramírez, Arnau, Serra, & Gou, 2006).

Consumer demands for safe, minimally processed, nutritious, high quality with health benefits has led to a significant development for the production of jerky based products. The focus has been to improve nutritional quality of traditionally produced jerky samples. Numerous applications of novel ingredients including antioxidants (Kołożyn-Krajewska &

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Dolatowski, 2012; Udabage, Augustin, Versteeg, Puvanenthiran, Yoo, Allen, et al., 2010), stabilisers (Dobson, Sanozky-Dawes, & Klaenhammer, 2007), probiotics (Ruiz-Ramírez, Arnau, Serra, & Gou, 2006) and non-conventional technologies, such as irradiation (McLeod, Zagorec, Champomier-Vergès, Naterstad, & Axelsson, 2010), plasma (Hammes, Bantleon, & Min, 1990) have been reported to improve the physicochemical properties, nutritional and safety profiles of beef jerky.

Ultrasound (US) processing has been demonstrated to have the potential to improve food safety, extraction efficiency, emulsification/homogenization, crystallization, drying and fermentation processes (Chemat et al., 2017; Misra et al., 2017). This technique has shown an ability to save water, improve the reliability of processes, decrease emissions, improve product quality and enhance productivity compared to conventional processes (Li et al. 2012). US has shown promising applications in meat product manufacture (Purchas, Rutherfurd, Pearce, Vather, & Wilkinson, 2004; Ruiz-Ramírez, Arnau, Serra, & Gou, 2006; Troy, Ojha, Kerry, & Tiwari, 2016). For instance, US has been shown to improve the texture, salt diffusion rates, marination and water holding capacity of meat. For example, Smith (2011) reported a significant improvement in uptake of marination (91% water, 6% NaCl, 3% sodium tripolyphosphate) for 20 min US pre-treated chicken meat after 18 h of marination. Similar improvements in marination efficiency was reported for pork (Ozuna, Puig, García-Pérez, Mulet, & Cárcel, 2013) and chicken breast (Leal-Ramos, Alarcon-Rojo, Mason, Paniwnyk, & Alarjah, 2011).

Additionally, over the last years, the combination of US with traditional preservation techniques, such as fermentation has attracted much interest from both researchers and the food industry (Ojha, Mason, O'Donnell, Kerry, & Tiwari, 2017). In this line, *Lactobacillus* species have been established as important food-associated lactic acid bacteria, which are widely used as starter culture for industrial meat fermentation, and with great

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potential as a bio-preservative in meat products (Hammes, Bantleon, & Min, 1990; McLeod, Zagorec, Champomier-Vergès, Naterstad, & Axelsson, 2010)

Therefore, taking on the technological trend to use non-conventional processing techniques in the meat industry, the objective of this study was to investigate the effect of US frequency, the addition of *L. sakei* culture and drying time on key nutritional (protein, amino acids, and organic acids) and physicochemical properties (texture and colour) of cultured and uncultured beef jerky samples by employing a reliable multivariate statistical strategy.

2. Material and methods

2.1. Sample preparation

Eye of the round (*Semitendinosus*) obtained from a local supplier (Dublin Meat Company, Blanchardstown, Co. Dublin, Ireland) was used in this study. Muscles were stored at 4 °C and were then cut into slices of similar size with a meat slicer (10 × 4 × 0.2 cm, L × W × H). The beef slices were cured in two different curing solutions: (I) Cultured, containing 70% water, *L. sakei* DSM 15831 (10^4 cfu/mL), 1.5% salt, 1.0% sugar, 0.05% sodium nitrite and (II) Uncultured, containing 70% water, 1.5% salt, 1.0% sugar, 0.05% sodium nitrite (based on raw meat weight). The ingredients were thoroughly mixed, and samples from both cultured and uncultured groups were subjected to US pre-treatments at frequencies of 25 kHz (Elma Schmidbauer GmbH, Germany), 33 kHz (Jencons, (Jencons, Leighton Buzzard, UK) and 45 kHz (Elma Schmidbauer GmbH, Germany) for 30 min along with controls (no US treatment). US treatments were performed in US bath systems maintained at a temperature of 30 ºC. All samples were subsequently cured for 18 h at 4 ºC. All cured beef jerky slices were dried using a hot air dryer (Gallendkamp Plus II, Weiss Technik, UK) at a temperature of 60 ºC for 4 h. Samples were withdrawn at drying times of 0 (after marination), 1, 2, 3 and 4 h and freeze dried prior to subsequent analysis.

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2.2. Protein content and proteolysis index

Protein content of all the samples was determined using a LECO FP628 (LECO Corp., MI, USA) protein analyser based on the Dumas method according to the AOAC method 992.15 (1990). A sample extract of 0.25 g was used for protein estimation. Proteolysis index was determined as a percentage of the ratio between non protein nitrogen obtained by precipitation of proteins with trichloroacetic acid and total nitrogen obtained using the Dumas method (Ruiz-Ramírez, Arnau, Serra, & Gou, 2006).

2.3. Amino acid analysis

The amino acid analysis (free and total content) of beef jerky was carried out according to the procedures outlined by Gambuteanu and Alexe (2015) with the aid of JEOL JLC-500/V AminoTac™ amino acid analyser (JEOL Ltd., Herts, UK). Beef jerky samples were deproteinised to determine the free amino acids content using a trichloroacetic acid solution at 240 g/L for 10 min. Samples were centrifuged (14,400×*g* for 10 min) and the supernatant was diluted with a sodium citrate buffer (0.2 mol/L; pH 2.2) and the content was diluted (1:2 v/v) with an internal standard (Norleucine) prior to injection. A λ=440 nm was used to detect proline, while λ=570 nm was used to detect the other amino acids. Aiming to assess the total amino acids content, beef jerky samples were hydrolysed using a 6 mol/L HCl solution at 110 °C for 23 h and all analyses were performed in triplicate.

2.4. Organic acid analysis

Organic acid analysis was performed according to the method outlined by Gupta, Jaiswal, and Abu-Ghannam (2013). Briefly, 1 g of jerky sample was mixed with 25 mL of distilled water, vortexed and centrifuged at 8720×*g* for 10 min at 4 °C, whereas the fermented broth samples were directly centrifuged following the same conditions. The collected supernatant

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was filtered using 0.45 µm syringe filter and used for the determination of organic acids by HPLC. The HPLC analyses were carried out using the Waters Alliance HPLC (e2695 separation module) system equipped with W717 plus auto sampler, W486 UV detector and W410 differential refractometer detector connected in series. Chromatographic analysis was performed using an analytical Rezex ROA-Organic acid H^+ (8%) column (350 mm × 7.8 mm ID), fitted with a suitable guard cartridge (50 mm × 7.8 mm) (Phenomenex, UK). The analyses were carried out isocratically at a flow rate of 0.6 mL/min, employing 0.005 mol/L H_2SO_4 as mobile phase. A 20 µL aliquot was injected into a thermostatically controlled compartment set at 65 °C and the detection was carried out at 210 nm wavelength. The data acquisition and integration were performed using the Empower software package. The organic acids in the samples were identified by comparing the retention time and spectral data with that of standards, such as lactic acid and acetic acid (Sigma-Aldrich, Ireland).

2.5. Instrumental texture and colour

Textural properties of jerky samples from both cultured and uncultured group were measured using a Texture Analyzer (Model: TA-XT2i; Stable Microsystems, UK), with a 25 kg load celland HDP/M3PB rig. Firmness (g force) and toughness of (g.sec) was measured by placing samples on top of the two adjustable supports of the base plate with the upper blade at a speed of 5 mm/s. Eight samples from each treatment were measured and average values recorded.

Colour parameters were measured using a Hunter Lab colorimeter (Model: UltraScan XE, Reston, USA) dried beef jerky samples. The CIE *L** (lightness [0=black, 100=white]), *a** (–*a**=greenness, +*a**=redness) and *b** (–*b**=blueness, +*b**=yellowness) values were used to calculate the total colour difference (TCD) ($TCD=[(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$) , where Δ*L**,

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Δa^*, and Δb^* are differences between the untreated sample and the US-treated beef jerky. Five samples were measured for colour parameters and average was recorded.

2.6. Statistical analysis

Data were presented as means ± standard deviation of replicates (n=3). Differences between samples were assessed using three-way analysis of variance (ANOVA) and Tukey's test (Granato, de Araújo Calado, & Jarvis, 2014). In order to gain a broader view of the experimental data, principal component analysis (PCA) was applied to the whole dataset. The (I×J) data matrix was pre-treated using the unit variance standardization prior to PCA. Factor loadings analysis was also performed and graphs containing the experimental treatments were constructed using the first two principal components (PC1 vs PC2). Statistical analyses were performed using Statistica v.7 software (Statsoft, USA).

3. Results and discussion

3.1 Changes in protein, proteolysis index and amino acid profile

The true protein contents for uncultured samples ranged from 76.07±0.15 g/100 g of dry matter to 79.07±0.96 g/100 g of dry matter and 75.49±0.35 g/100 g to 76.03±0.12 g/100 g of dry matter for cultured beef jerky samples, respectively (Figure 1). From comparison, it can be seen that the cultured jerky has significantly less true protein than the uncultured jerky samples. No significant difference ($P>0.05$) was observed between true protein content of control cultured and uncultured samples whereas significant differences ($P<0.05$) were observed for US pre-treated samples. The highest true protein content was observed for samples processed at 25 kHz for uncultured samples (79.89±0.95 g/100 g of dry matter) and

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the lowest value was observed for beef jerky manufactured with 45 kHz (74.19±0.53 g/100 g of dry matter).

Beef jerky samples pre-treated at US frequencies of 25 kHz, 33 kHz and 45 kHz showed low level of proteolysis after 18 h of marination compared to dried beef jerky samples. The addition of *L. sakei* coupled with US would have hydrolysed beef proteins, thus yielding short chain polypeptides or free amino acids. Total amino acid content of control samples for cultured and uncultured samples was 64.25 g/100 g and 63.75 g/100 g, respectively with highest observed for cultured samples pre-treated with 25 kHz (67.96 g/100 g) and lowest for cultured samples pre-treated at 45 kHz.

In the case of free amino acids, no significant differences were observed for control cultured (27.89 mg/g) and uncultured (28.98 mg/g) samples. US pre-treated samples showed higher level of free amino acid at 25 kHz for uncultured (41.48 mg/g) samples and decreased with an increase in US frequencies. This indicates that US pre-treatment had significant effect during the marination and subsequent drying process on protein hydrolysis. It appears that the breakdown of beef proteins occurred due to ultrasound as well as the culture treatment. Effect of US on proteolysis has already been reported. For instance, Abadía-García, Castaño-Tostado, Ozimek, Romero-Gómez, Ozuna, and Amaya-Llano (2016) observed that the degree of whey protein hydrolysis was dependent on ultrasonic pre-treatment and enzymes. Similarly, Uluko, Li, Cui, Zhang, Liu, Chen, et al. (2013) observed US pre-treatment can enhance hydrolysis of milk protein concentrates to produce short chain peptides with various biological activities.

US pre-treatment may have caused loss of protein structure for various natural enzymes present in beef slices required for hydrolysis. Studies have shown that the protein structure can be altered after US processing due to partial cleavage of intermolecular hydrophobic

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interactions, rather than peptide or disulphide bonds (Jambrak, Mason, Lelas, Paniwnyk, & Herceg, 2014).

On the other hand, for cultured samples, it was not possible to establish a trend and the behaviour differed according the amino acid data and the US frequency. In fact, PCA was able to explain up to 71% of data variability using only two factors but no clear distinction between US frequencies was observed (Figure 2). The amounts of cystic acid, methionine sulfone, aspartic acid, threonine, serine, histidine, lysine, and arginine were higher in control (untreated) sample, while US frequency of 25 kHz gave the highest values for cysteine, 33 kHz for glycine and proline and 45 kHz for glutamic acid, alanine, valine, and phenylalanine. Using PCA, a separation of uncultured and cultured samples was basically based on the content of threonine, γ-aminobutyric acid (GABA), and aspartate.

Interestingly, taurine (2-aminoethanesulfonic acid) contents of control beef jerky samples for cultured and uncultured samples were 1.09±0.14 and 1.05±0.16 mg/g, respectively. However, taurine content was found to be higher for US pre-treated samples compared to control for both cultured and uncultured samples (Figure 3a). Taurine is a sulphur-containing β amino acid which is reported to be abundantly present in free form in foods of animal origin. Higher level of taurine in US pre-treated samples indicates the US pre-treatment would have released bound taurine in jerky samples (Figure 3a). Various biological and health benefits (eg. antioxidant, protection against ischemia-reperfusion injury, modulation of intracellular calcium concentration, antiatherogenic and blood pressure-lowering effects) of taurine have been reported in literature (Purchas, Rutherfurd, Pearce, Vather, & Wilkinson, 2004; Xu, Arneja, Tappia, & Dhalla, 2008; Zulli, 2011). Taurine is also reported to have a protective role in exercise induced muscle and is one of the key ingredients in sports nutrition (McPherson & Hardy, 2011).

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Changes in true protein content, proteolysis index and amino acid profile can be attributed to proteolysis induced when US pre-treatment is applied (Gambuteanu & Alexe, 2013; Jayasooriya, Torley, D'arcy, & Bhandari, 2007; Stadnik & Dolatowski, 2011). It is also reported that the US treatments can lead to a significant decrease in molecular weight and protein fractionation (Jambrak, Mason, Lelas, Paniwnyk, & Herceg, 2014). A higher taurine level in US pre-treated samples could be attributed to US-assisted extraction of taurine due to various sonochemical mechanisms. For instance, US have shown improved extraction yields of taurine from *Porphyra yezoensis* (Wang, Guo, Zhang, Wu, Wu, & Chen, 2015). Studies have shown that the US pre-treatment on hydrolysis of proteins using enzymes are mostly inconsistent. For example, US pre-treatment is shown to enhance the release of peptides from defatted wheat germ protein with no changes in degree of hydrolysis at various ultrasound power intensities and US frequencies range of 24–68 kHz (Stefanović, Jovanović, Grbavčić, Šekuljica, Manojlović, Bugarski, et al., 2014; Zhou, Ma, Yu, Liu, Yagoub, & Pan, 2013).

3.2. Influence of US frequency and drying time on organic acid composition of cultured and uncultured beef jerky samples

Changes in lactic acid and acetic acid content of jerky samples are shown in Figure 3b-c. ANOVA analysis revealed a significant influence ($P<0.05$) of US frequency, drying time and *L. sakei* on organic acid composition. As can be seen in Figure 3b-c, US frequency had a significant remarkable influence independent of *L. sakei* culture inclusion after 18 h of marination (0 h drying), observing a significant decrease when higher US frequencies of 33 and 45 kHz were applied. After 4 h drying, a decrease in lactic acid of US pre-treated cultured samples compared to control samples was observed. However, the trend was not clear for uncultured samples, obtaining a decrease of 25.6% after US application at 33 kHz while an increase of 9.2% was found at 45 kHz compared to control samples.

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An increase in acetic acid content was observed after 18 h of marination for cultured samples subjected to US frequencies of 25, 33 and 45 kHz whereas, a decrease in acetic acid content was observed with an increase in US frequencies for uncultured samples. After 4 h of drying a significant increase of 36.3 and 28.2% in acetic acid content was observed for cultured samples pre-treated at 33 kHz and 45 kHz respectively. However, for uncultured samples a significant decrease of 72.5% was observed for samples pre-treated at 45 kHz. PCA showed no clear differences in acidity for samples inoculated with or without *L. sakei*. This result can be observed as samples were mixed in all quadrants in the projection presented in Figure 4. However, a trend was observed, cultured samples submitted to US frequency (33 and 45 kHz) showed higher acetic acid content, while uncultured samples showed a higher lactic acid content for samples pre-treated at 25 and 45 kHz.

3.4 *Effect of ultrasound frequency on colour and texture of beef jerky*

Changes in firmness and toughness values of cultured and uncultured beef jerky samples are shown in Figure 5a-b. Overall, cultured samples showed higher firmness and toughness values compared to uncultured samples. However, in contrast to the results found in this study, Meng, Long-Hao, Hong-Sheng, Yan-Qing, & Xin (2013) found an increase in tenderness after beef jerky fermentation compared with uncultured samples.

Moreover, in the present study, significantly lower firmness and toughness values were observed for samples pre-treated at 33 kHz for both cultured and uncultured samples. Higher values of firmness values for cultured beef jerky samples pre-treated at 25 kHz can be attributed to decreased migration of marination solution into the beef jerky samples due to sonication, thus reducing hydration and swelling of myofibrils and increasing water holding capacity. This fact has a negative effect on tenderisation and juiciness of meat, thus resulting in firm jerky samples (Gault, 1985; Goli, Bohuon, Ricci, Trystram, & Collignan, 2011; Offer et al., 1989; Offer & Knight, 1988). Low power US frequency has shown enhanced diffusion

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of marination solution into the meat matrix. For example, Leal-Ramos, Alarcon-Rojo, Mason, Paniwnyk, and Alarjah (2011) reported improved marination rates for US-assisted marination of chicken breasts. Enhanced marination is reported to induce tenderisation due to several mechanisms including weakening of structures, due to swelling of meat, increased proteolysis by cathepsins (Berge, Ertbjerg, Larsen, Astruc, Vignon, & Møller, 2001). Higher firmness and toughness may be due to uptake of higher level of marination solution resulting in firm texture in dried jerky samples.

Hunter colour values of cultured and uncultured beef jerky samples subjected to US pre-treatments are shown in Table 1. As can be seen from the Table, there was not a clear trend in L*, a* and b* values after applying US treatments. Overall, non-significant changes in L*, *a** and b* values were found for cultured and uncultured samples, regardless of the US treatments. The results contrast with those obtained by Meng, Long-Hao, Hong-Sheng, Yan-Qing, & Xin (2013) who observed a significant increase in *a** and *b** values of fermented beef jerky samples compared to uncultured. However, it should be noted that fermentation conditions (i.e., fermentation time) were different from those reported in the current study. In addition, higher total colour differences were found for uncultured samples after applying US treatments compared to the US-treated cultured samples.

4. Conclusions

This study demonstrates that beef jerky can be obtained by incorporating *Lactobacillus sakei*. The result presented in this work showed that beef jerky samples with improved amino acid profile including taurine content can be obtained by employing US pre-treatment. Significant effects on true protein content, amino acid profile and organic acid contents were observed with no significant changes in colour values. Interactive effect of US frequencies and culture treatment was evident. However, the individual effects of *L. sakei* and

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US pre-treatment at various frequencies cannot be established based on this study. In addition, principal component analysis was shown to be a promising statistical approach to monitor the quality traits of beef jerky.

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Figure captions

Figure 1. Changes in protein content (g/100 g) for cultured (■) and uncultured (■) beef jerky samples. Ultrasound frequencies: 25, 33 and 45 kHz.

Figure 2. Principal component analysis (PCA) based on the amino acid profile of beef jerky subjected to ultrasound frequency (25, 33 and 45 kHz). NOC: Uncultured. 1 h, 2 h, and 3 h drying after 18 h marination.

Figure 3. a) Taurine (mg/g), b) lactic acid (µg/g) and (c) acetic acid (mg/g) contents for cultured (■) and uncultured (■) beef jerky samples.

Figure 4. Principal component analysis (PCA) based on the acetic and lactic acid profile of beef jerky subjected to ultrasound frequency (25, 33 and 45 kHz). NOC: Uncultured jerky. 0 h, 2 h, 3 h and 4 h drying after 18 h marination.

Figure 5. Changes in firmness (a) and toughness values for cultured (■) and uncultured (■) beef jerky samples.

Tables

Table 1. Changes in colour values for cultured and uncultured beef jerky samples after ultrasound (frequencies 25-45 kHz) treatments.

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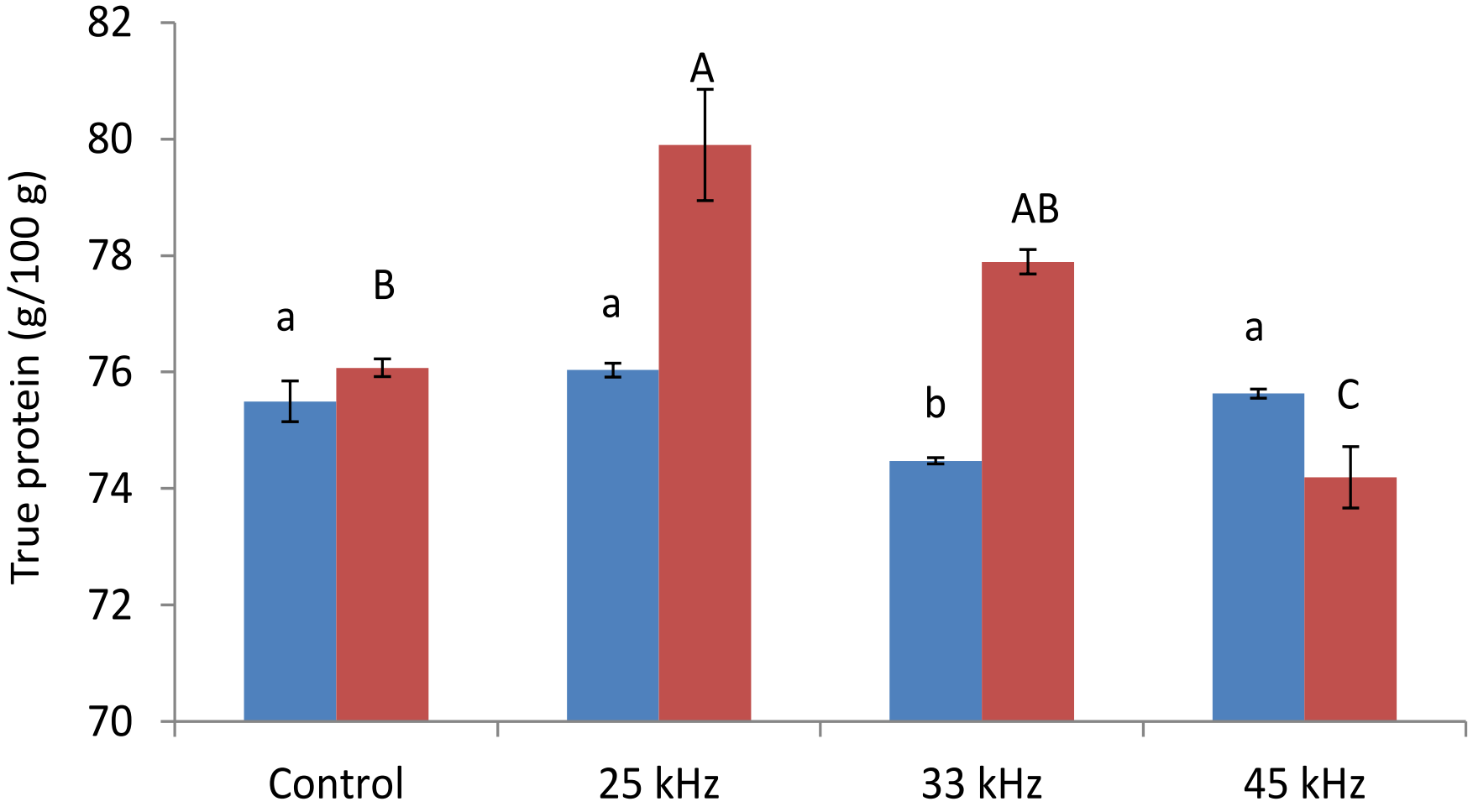
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Table 1. Changes in colour values for cultured and uncultured beef jerky samples after ultrasound (frequencies 25-45 kHz) treatments.

	Treatment	*L**	*a**	*b**	TCD
Cultured	Control	24.5±0.7^{ab}	5.9±1.0^{a}	3.5±0.5^{ab}	0.0±0.0^{c}
	25 kHz	24.4±1.7^{ab}	4.7±0.9^{a}	3.9±0.8^{ab}	2.2±1.2^{bc}
	33 kHz	25.7±1.7^{ab}	6.4±1.8^{a}	4.7±2.4^{ab}	2.4±2.4^{bc}
	45 kHz	23.1±0.8^{b}	4.4±0.9^{a}	2.7±0.8^{b}	2.9±1.2^{bc}
Uncultured	Control	26.5±3.1^{ab}	5.6±0.8^{a}	5.1±1.6^{ab}	0.0±0.0^{c}
	25 kHz	27.7±3.1^{a}	6.1±1.3^{a}	6.4±1.9^{a}	3.3±1.8^{abc}
	33 kHz	24.3±1.9^{ab}	4.0±0.8^{a}	2.9±1.6^{b}	6.7±2.1^{a}
	45 kHz	25.9±2.9^{ab}	5.0±1.8^{a}	4.5±2.1^{ab}	4.9±2.7^{ab}

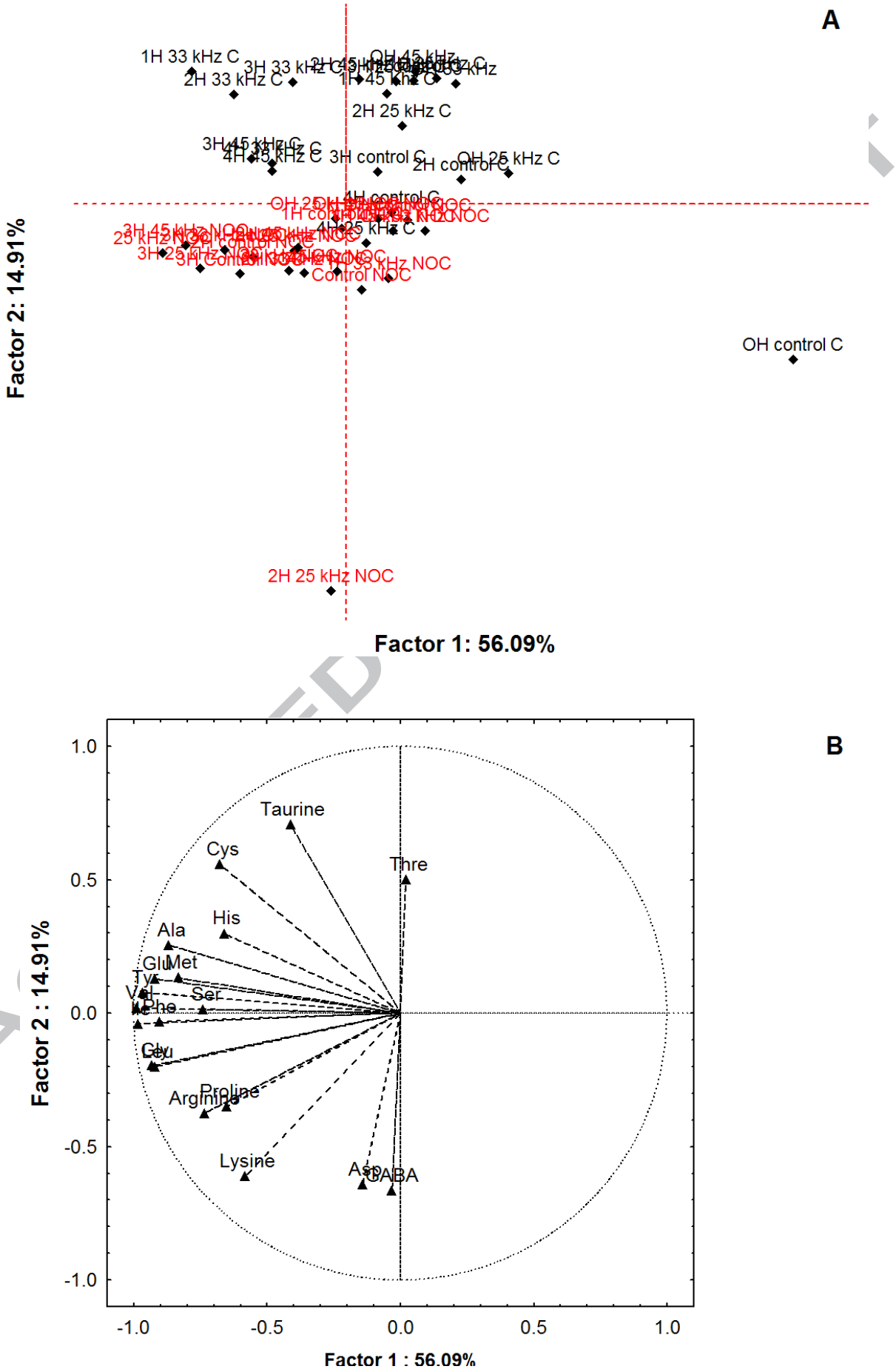
a-c Different letters in the same column indicate significant differences according to the applied treatment. *L**: Lightness. *a**: redness. *b**: blueness. TCD: Total colour difference

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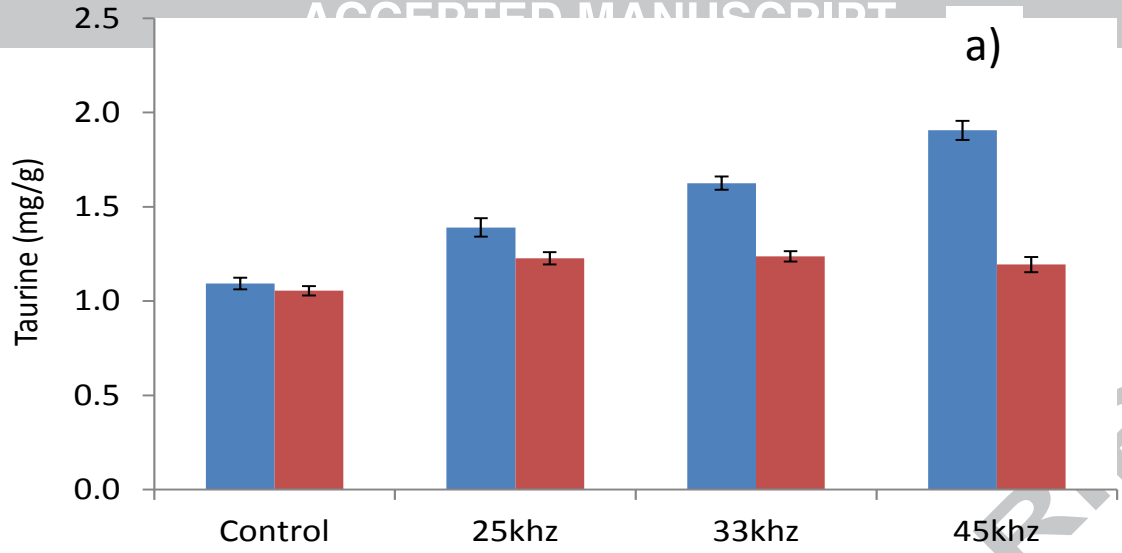

True protein (g/100 g)
82
80
78
76
74
72
70
a
B
a
A
b
AB
a
C
Control
25 kHz
33 kHz
45 kHz

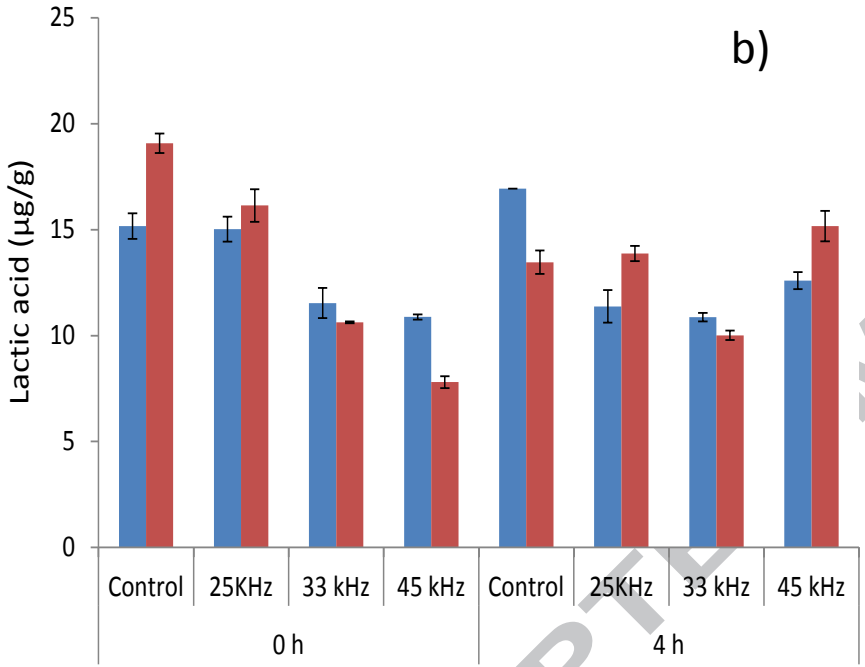
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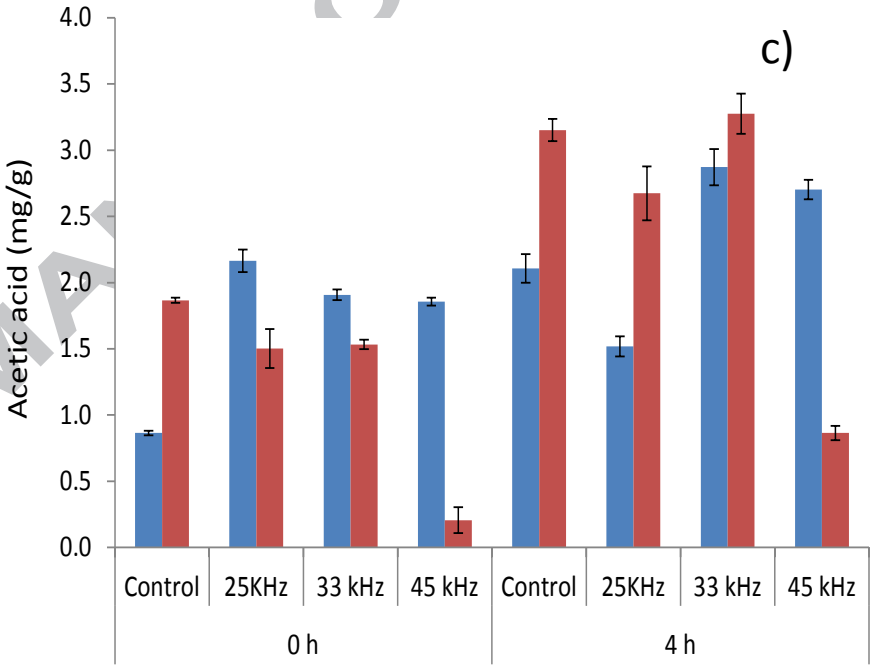
FIGURE 2



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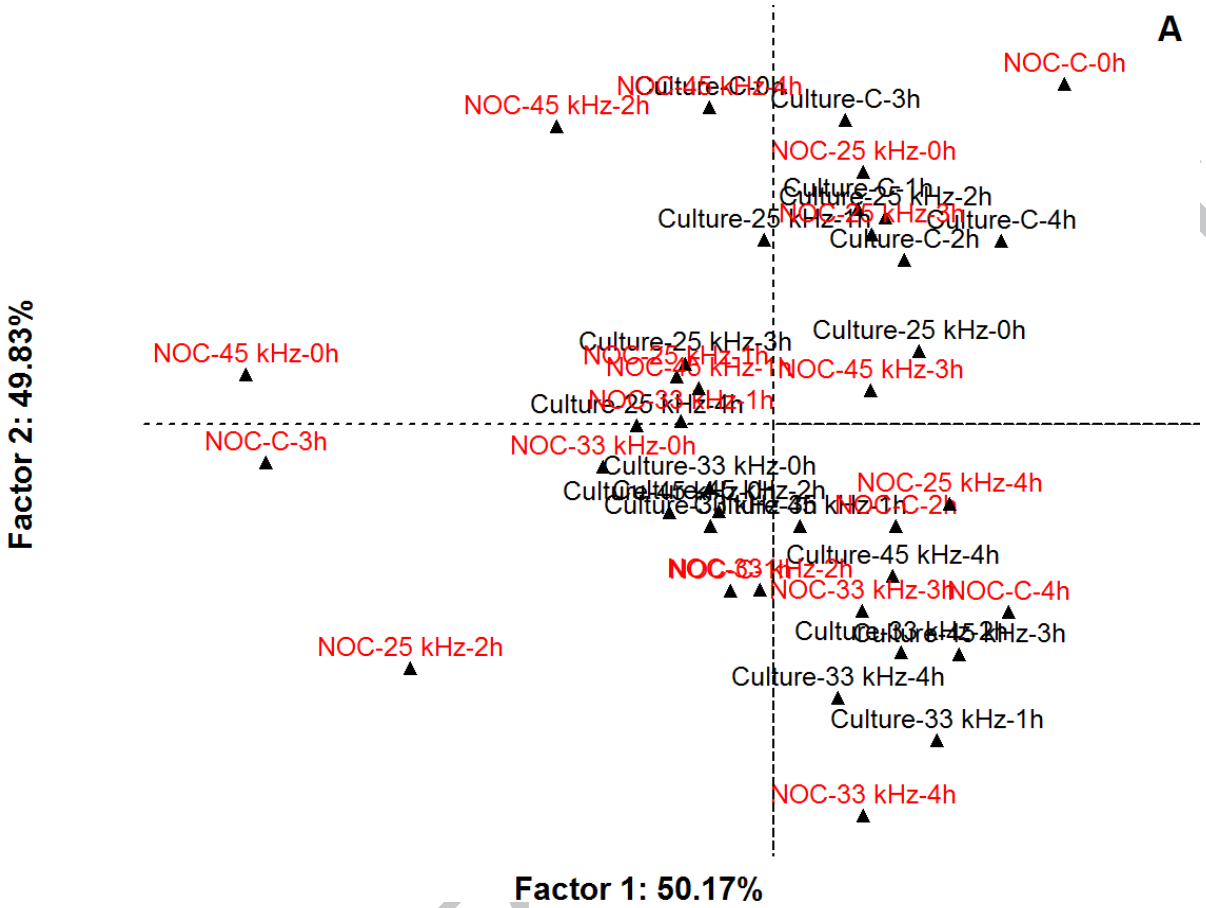


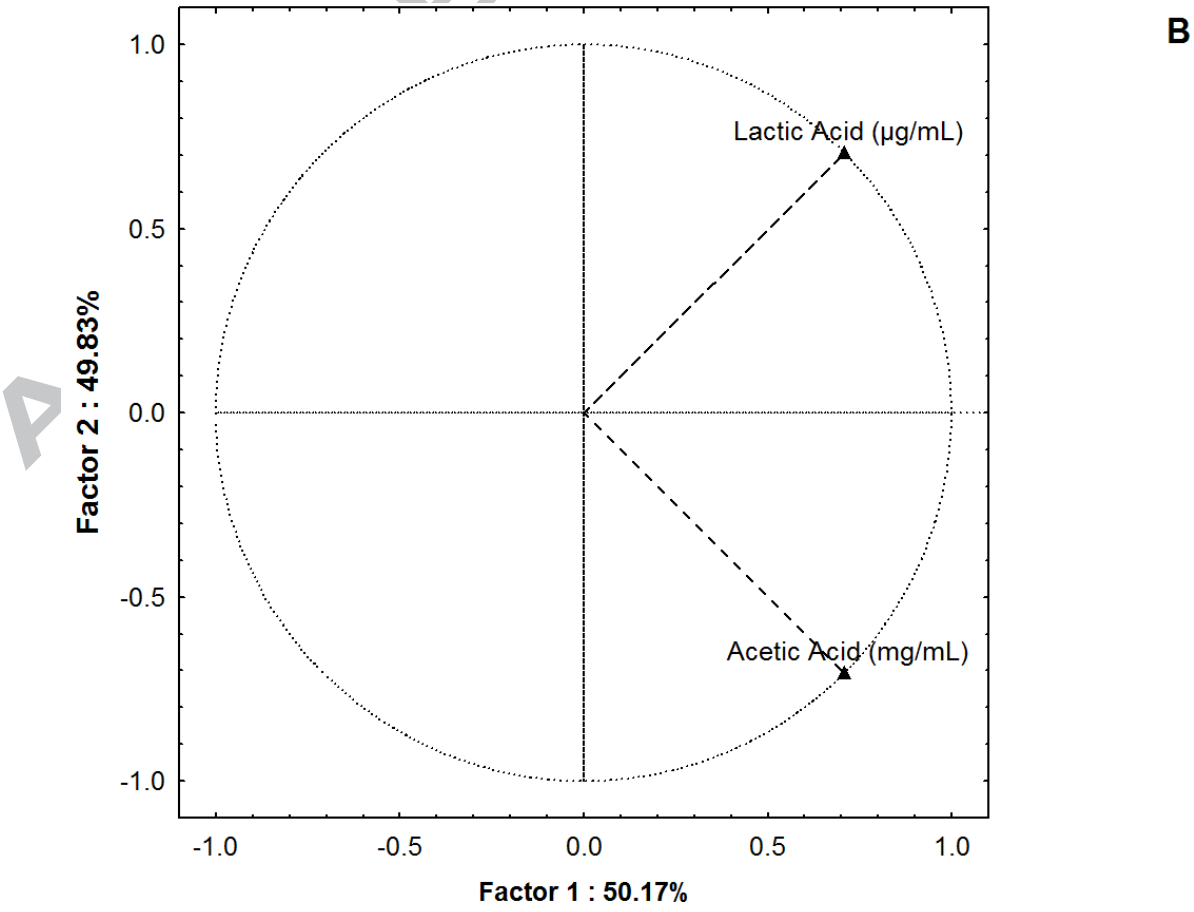




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FIGURE 4





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Highlights

- Significant effects of culture treatment, drying and ultrasound (US) on beef jerky
- Increased taurine of jerky with increased US frequencies for cultured samples
- Similar colour values for US pre-treated and control samples
- *L. sakei* and US can improve the nutritional profile of beef jerky.

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