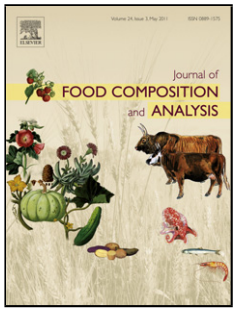
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**Original research article**

# Statistical approaches to access the effect of *Lactobacillus sakei* culture and ultrasound frequency on fatty acid profile of beef jerky

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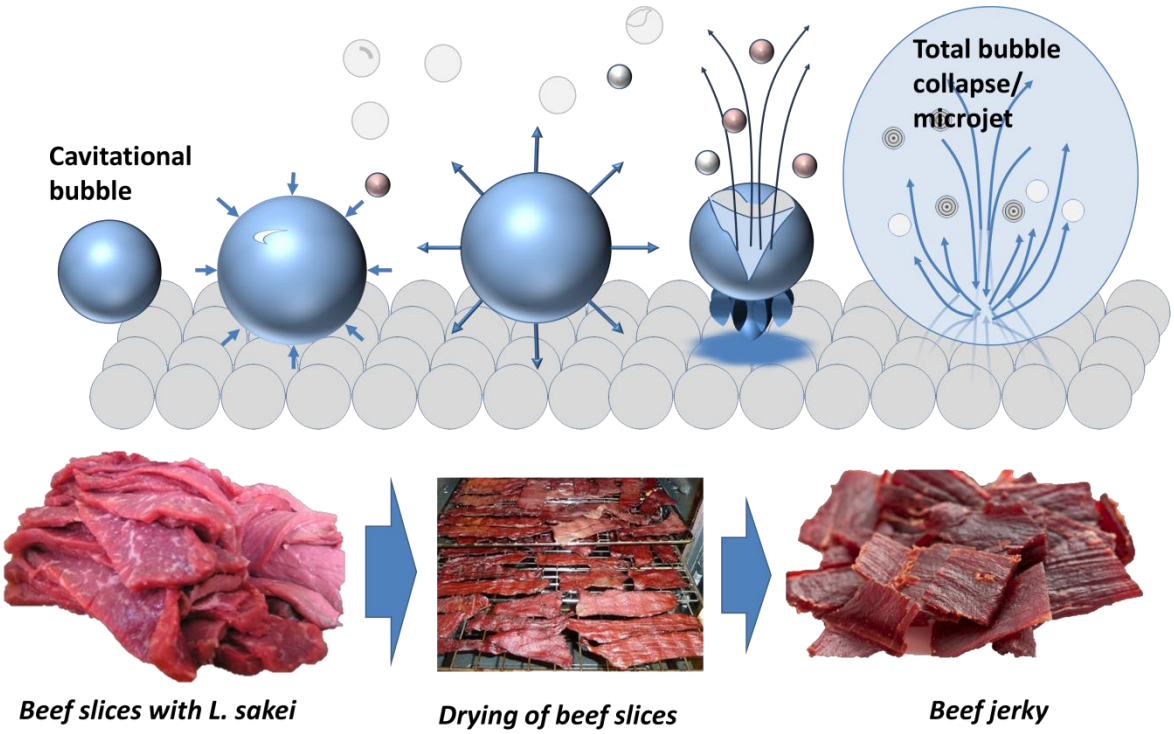
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**Graphical Abstract**




Research Highlights

1. Fatty acid profile of beef jerky

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2. L sakei can influence fatty acid profile of beef jerky samples
3. Ultrasonic effect is frequency dependent

**Abstract**

The objective of this study was to investigate the effect of ultrasonic frequencies and drying time on fatty acid profiles of beef jerky samples possessing different microbial compositions. Beef slices were cured using curing solutions formulated both with and without Lactobacillus *sakei*. Curing was carried out for 18 hr at 4$^{o}$C prior to hot air drying at 60$^{o}$C for 4 hr. Jerky samples from both treatment groups were then subjected to ultrasonic frequencies of 25 kHz, 33 kHz and 45 kHz for 30 min. Beef jerky samples were subsequently analysed for fatty acid profile using Gas Chromatography. In the present study, beef slices showed a high level of MUFAs, which accounted for 45.6 – 53.8%, followed by the SFAs (36.3 – 47.8%) and PUFAs (4.8 – 13.7%), respectively. Results demonstrated a significant effect of beef jerky processing on fatty acid profile. Various correlation analyses showed that changes in fatty acid profiles were significantly affected by individual and/or interactive effects of *L. sakei*, drying time and ultrasonic frequency.

**Keywords: Fatty acid composition, ultrasound frequency, drying, microwave-assisted extraction, Principal component analysis, probiotics, gas chromatography, atherogenicity Thrombogenicity, hypocholesterolemic.**

## 1. Introduction

Jerky is one of the oldest forms of cured and dried meat products. Jerky products possess a high protein content and a unique flavour and texture profile. Jerky is traditionally prepared from thinly sliced whole muscles marinated and subsequently dried to an $a_w$ value ranging from 0.70–0.85 (Yang et al., 2009). Commercially this intermediate moisture foodstuff is often preserved using a hurdle concept involving interventions such as reducing $a_w$ through the addition of preservatives such as organic acids, spices and nitrate/nitrite salts. The development of whole-muscle and/or restructured jerky from a range of meats by employing various curing ingredients, curing methods and drying conditions have been

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widely reported (Choi et al., 2008; Jang et al., 2015; Kucerova et al., 2015). With growing consumer demand for high quality foods with good flavour, texture, nutrition and safety profiles, various strategies are being investigated and applied to jerky production. Amongst these strategies the application of a starter culture (e.g. lactic acid bacteria) capable of preventing the growth of spoilage bacteria by producing bacteriocins has recently been reported (Biscola et al., 2013; O'Connor et al., 2015). For example Pinto et al. (2002) reported that the inoculation of starter cultures (e.g. *Staphylococcus carnosus* and *S. xylosus*) can substantially improve sensorial quality of beef jerky.

Whilst consumers are most interested in the organoleptic quality, safety and healthiness of food products, manufacturers must also consider the cost of manufacture and are increasingly examining the use of novel processing strategies capable of reducing energy requirements and accelerating processing times. In recent years, several studies have reported the effects of power ultrasound on fresh and processed meat to assist curing, brining, drying and tenderisation of meat. For example, ultrasound in combination with the application of a vacuum has been shown to increase drying rate of beef and chicken meat (Başlar et al., 2014). Application of ultrasound can also enhance mass transfer rates during brining/curing of meat mainly by disrupting the continuity of cellular membranes due to various physical and chemical effects of the technology (Ozuna et al., 2015). However, whilst the processing benefits of ultrasound are clear some authors have reported that it can cause undesirable compositional changes in some foods leading to a reduction in nutritional and eating quality (Pingret et al., 2013).

In particular, because of increasing concern and awareness of the health implications of the fatty acid composition of food products, manufacturers are under pressure to produce foods with optimal fatty acid profiles from a nutritional and health perspectives. Changes in the fatty acid profile can occur due to various processing and storage conditions depending on the properties of ingredients and processing parameters (e.g. temperature, pH and time). Starter cultures including Lactic acid bacteria (LAB) have been reported to change fatty acid profile of fermented dairy products (Florence et al., 2012; Manzo et al., 2015). However, effect of starter culture on fatty acid profile of beef jerky has not been reported to date. Additionally, ultrasound assisted extraction has shown to influence the fatty acid profile of oils depending on the food matrix and extraction conditions (Chemat et al., 2004a; Xu et al.). To date no study reports the effect of high power ultrasound on fatty acid profile of meat and/or fermented meat products. The objective of this study was to investigate the

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effect of ultrasonic frequency, starter culture (*Lactobacillus sakei*) and drying time on the fatty acid profile of beef jerky.

## 2. Material and methods

### 2.1. Chemicals and reagents

GC grade methanol, Supelco-37 FAME standard, tricosanoic acid methyl ester as well as potassium hydroxide, acetyl chloride and disodium sulphate were purchased from Sigma-Aldrich (Arklow, Co. Wicklow, Ireland). GC grade n-pentane as well as sodium chloride was purchased from Fisher Scientific (Dublin, Ireland). Type 2 water was obtained from a MilliQ water unit (Millipore, Massachusetts, USA).

### 2.2. Sample preparation

Eye of the round (*Semitendinosus*) obtained from a local supplier (Dublin Meat Company, Blanchardstown, Co. Dublin, Ireland) was used in this study. Muscle was stored at 4 °C and then cut into slices of similar size with a meat slicer (10 x 4 x 0.2 cm, L x W x H). The beef slices were cured in two different curing solutions: (I) Cultured, containing 70% water, *L. sakei* DSM 15831 culture ($10^4$ 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; v/w). The curing solution was calculated based on weight of beef slices in each sub-groups cultured (4 sub-groups: Control, 25 kHz, 33 kHz and 45 kHz) and uncultured (4 sub-groups: Control, 25 kHz, 33 kHz and 45 kHz). Curing solution along with beef slices from each sub-group were subjected to ultrasonic (US) treatments at frequencies of 25 kHz (Elma Schmidbauer GmbH, Germany), 33 kHz (Jencons, Leighton Buzzard, UK) and 45 kHz (Elma Schmidbauer GmbH, Germany) for 30 min along with controls (no US treatment). US treatments were carried out in ultrasonic bath systems maintained at a temperature of 30 $^{o}$C. All samples were subsequently cured for 18 h at 4 $^{o}$C. All cured beef jerky slices were dried using a hot air dryer (Gallendkamp Plus II, Weiss Technik, UK) at a temperature of 60 $^{o}$C for up to 4 h. Two beef slices were withdrawn at drying times of 0 (after 18 h curing), 1, 2, 3 and 4 h and freeze dried prior to fatty acid analysis.

### 2.3. Fatty acid profile

#### 2.3.1. Microwave- assisted preparation of fatty acid methyl esters (FAMEs)

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Microwave assisted FAME preparation was carried out using a MARS 6 Express 40 position Microwave Reaction System (CEM Corporation, Matthews, NC, USA) according to the method by Brunton et al. (2015). Briefly, 0.5 g of freeze dried beef jerky samples were added to the reaction vessel containing a 10 mm stir bar. To this 10 ml of potassium hydroxide (2.5%, w/v) in methanol was added along with 100 µL of internal standard (C23:0 methyl ester; final concentration following extraction is 0.1 mg/mL in pentane). The reaction vessel was heated in the MARS 6 Express system to 130°C over 4 min and held at this temperature for 4 min. The reaction vessels were then removed from the carousel and cooled on ice for 5 min or until they had reached room temperature before they were opened. The derivatisation was then carried out in Microwave Reaction System by adding 15 mL of 5% (v/v) acetyl chloride in MeOH solution and heating to 120°C over 4 min and holding at this temperature for 2 min. The reaction tubes were removed again and cooled on ice to room temperature. To the cooled tubes 10 mL of pentane was added and the reaction tubes were shaken to extract the FAMEs into the upper pentane layer. Following this, 15 mL of a saturated salt solution was added, and the solution was mixed again. Following separation of the layers, the top pentane layer was removed and aliquoted into amber GC vials (1.5 mL) containing sodium sulphate and analysed using gas chromatography.

### 2.3.2. Gas chromatography-flame ionisation detector (GC-FID) analysis

Gas chromatography was carried out using a Clarus 580 Gas Chromatograph fitted with a flame ionisation detector. A CP-Sil 88 capillary column (Agilent, Santa Clara, California, USA) with a length of 100 m x 0.25 mm ID and 0.2 µm film was used for the separation. The injection volume was 0.5 µL, and the injection port was set to 250 °C. The oven was set to 80 °C with an initial temperature ramp of 6.2 °C/min to 220 °C which was held for 3.2 min. A second temperature ramp of 6.3 °C to 240°C followed and was held for 6.5 min (runtime 35 min). The carrier gas was hydrogen at a constant flow of 1.25 mL/min, and the split ratio was set at 10:1. The FID was set at 270°C. Compounds were identified by comparing their retention times with those of authentic FAMEs from the Supelco 37 FAME mix. The content off each fatty acid was calculated using following equation (Eq. 1).

$$FA\ content = \frac{\square\square\square\square\ \square\square\square\square\ (\square\square\square\square)}{\square\square\square\square\ \square\square\square\square\ (\square\square\square\square)} \times \frac{\square\square\square\square\ \square\square\square\square h\square}{\square\square\square\square\square\square\ \square\square\square\square h\square} \times ISTD\ purity \times 10 \times 0.96 \quad \text{(Eq. 1)}$$

Where, FA content is the amount of a given fatty acid in the sample (mg/g), 10 is dilution factor and 0.96 is the conversion factor for the internal standard which is already a FAME.

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2.3.3. Nutritional quality indexes

Nutritional quality indices of beef jerky samples were analyzed from fatty acids composition data. The indexes of atherogenicity (AI) and thrombogenicity (TI) were calculated as proposed by Ulbricht and Southgate (Ulbricht and Southgate, 1991) and hypocholesterolemic/hypercholesterolemic (HH) index was calculated according to Santos-Silva et al. (Santos-Silva et al., 2002). AI, TI and HH indices were calculated using Equation 2 – 4, respectively. Other nutritional quality indices namely ratio of *n*–6/ *n*–3 PUFA and PUFA/SFA were also determined.

$$\square\square = \frac{[\square 12{:}0 + 4\times(\square 14{:}0) + \square 16{:}0]}{[\sum \square\square\square\square + \sum \square\square\square\square]} \quad \text{[Eq 2]}$$

$$\square\square = \frac{[\square 14{:}0 + \square 16{:}0 + \square 18{:}0]}{[0.5\times(\sum \square\square\square\square + \sum \square 6) + 3\times\sum \square 3 + \frac{\sum \square 3}{\sum \square 6}]} \quad \text{[Eq 3]}$$

$$\square\square = \frac{[\square 18{:}1\square\square\square 9 + \square 18{:}2\square 6 + \square 20{:}4\square 6 + \square 18{:}3\square 3 + \square 20{:}5\square 3 + \square 22{:}5\square 3 + \square 22{:}6\square 3]}{[\square 14{:}0 + \square 16{:}0]} \quad \text{[Eq 4]}$$

## 2.4. Statistical Analysis

Analysis of variance (ANOVA) of dependent variables was performed using the SAS procedure (SAS Version 9.1.3, statistical Analysis Systems). Tukey's multiple comparison was used to compare treatment means. PROC GLM was performed on major fatty acids to investigate individual and interaction effects of independent variables of culture treatment (C), ultrasonic frequency (UF, kHz) and drying time (DT, h). PROC CORR procedure of SAS was employed to determine Pearson's correlation coefficients (*r*) which were analysed to determine a relationship between various parameters.

Multivariate analysis of fatty acid profiles for all treatments except for various nutritional indices was carried out using Factor analysis method. Factor analysis method is more elaborate compared to principal component analysis which can be employed to describe variability among observed, correlated variables in terms of unobserved variables called factors. The observed variables can be projected as linear functions of the "factors." Factor analysis with VARIMAX rotation was performed on the mean data matrix to classify samples without any presumption. The VARIMAX rotation is carried out to obtain a clear pattern of factor loading and is aimed at maximizing the variances of the squared normalized factor

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loadings across variables for each factor. Factor analysis was carried out using MINITAB (v17.0) software package.

## 3. Result and Discussions

### *3.1. Fatty acid profiling*

Thirty-one fatty acids were identified and quantified in beef jerky samples. A representative chromatogram of beef jerky sample is shown in Figure S1 (see Supplementary Material online). Medium and long chain fatty acids were identified and quantified at various drying times (0, 1, 2, 3 and 4 h) for cultured and uncultured beef jerky samples with or without ultrasound pre-treatment are presented in Table S1 (See Supplementary Material online). In general, fatty acids in beef vary depending on the genotype, muscle type and feeding regime (Mapiye et al., 2013; Scollan et al., 2014). In the present study, beef slices had a high level of MUFAs, accounting for 45.6 – 53.8%, followed by the SFAs (36.3 – 47.8%) and PUFAs (4.8 – 13.7%). Similar fatty acid profiles of beef jerky samples were reported by Yang et al. (2009); these contained 42.9% SFAs, 53.2% MUFAs and 3.8% PUFAs. Among PUFAs, linoleic acid (C18:2*n*–6), α-linolenic acid (C18:3*n*–3), Arachidonic acid (C20:4*n*–6), Eicosapentaenoic acid (C20:5*n*–3) and Docosapentaenoic acid (C22:5*n*–3) were the main fatty acids identified and quantified in beef jerky samples. Presence of these long chain *n*–3 and *n*–6 PUFAs has been reported to confer various health benefits including improvement of maternal and offspring health, growth and development, cognitive function and psychological status (Mapiye et al., 2015; Pelliccia et al., 2013).

### 3.2. Effect of culture of fatty acid profile

In this section, controls from both groups (cultured and uncultured) are compared to investigate the effect of culture on fatty acid profile of marinated beef slices. The proportion ∑SFA and ∑MUFA were significantly higher for cultured samples (42.98 g/100g and 50.41 g/100g of FA) compared to uncultured samples (40.81 g/100g and 46.85 g/ 100 g of FA) respectively. Whereas, ∑PUFA was higher for uncultured (12.23 g/100 g FA) compared to cultured samples (6.57 g/100g FA). Among the 31 fatty acids identified and quantified Oleic acid (C18:1), Palmitic acid (C16:0), Stearic acid (C18:0), Palmitoleic acid (C16:1) were the most abundant for both cultured and uncultured beef jerky slices. Other authors have also reported that palmitic acid, stearic acid, oleic acid, are the predominant fatty acids in beef

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jerky samples (Kim et al., 2014). Culturing of the samples would have caused a higher extent of oxidation resulting in higher losses of PUFA's as these are more susceptible to oxidation. Studies show that the bacteria accelerate the oxidation of UFA's (Ansorena and Astiasarán, 2004; Chizzolini et al., 1998). Higher degradation of ∑PUFA for cultured samples can be due to lipolysis of long chain fatty acids owing to the inclusion of *Lactobacillus* culture along with the action of endogenous enzymes. For example, Hierro et al (1997) investigated the role of the starter culture (*Lactobacillus*, *Staphylococcus* and *Micrococcus*) on lipolysis of dry fermented sausages and concluded that the lipolysis is not only due to microbial lipases and also due to endogenous lipases present in meat. Contrary to this, Hu et al. (2007) observed higher PUFA content in sausages with mixed cultures of *Staphylococcus xylosus*, *Lactobacillus plantarum*, *Pediococcus pentosaceus* and *Lactobacillus casei* compare to the control silver carp (*Hypophthalmichthys molitrix*) sausages. The effect of starter culture on fatty acid composition in a range of fermented meat products, including sausages, during fermentation, ripening and storage has been reported (Karsloğlu et al., 2014; Qiu et al., 2013; Visessanguan et al., 2006). Studies show that there is a tendency to hydrolyse certain fatty acids (e.g. linoleic, oleic, and stearic acid) probably because of the action of microbial lipases, which is dependent on the position and structural conformation of the fatty acids in the glycerides (Alford et al., 1971; Gambacorta et al., 2009).In the present study among the main fatty acid identified in samples, culture treatment had a significant effect (*P<0.0001*) on C14:0, C16:0, C16:1, C17:0, C18:0, C18:1, C18:2*n*–6, & C18:3*n*–3 levels. Correlation analysis (Table 1) showed a negative relationship between culture inoculation and ∑SFA (*r=-0.465*, *P<0.01*), ∑MUFA (*r= - 0.430, P<0.01*) whereas, a positive correlations were observed for PUFA (*r=0.379, P<0.05*) and *n*–6 PUFA (*r=0.497, P<0.001*) indicating a positive effect of culture treatment on PUFA.

### 3.3. Effect of ultrasound frequency and drying on fatty acid profile

Ultrasonic frequency (kHz) and drying time (h) had a significant impact on fatty acid profiles. In the case of cultured beef jerky samples after 18 h of marination, a significant increase in SFA (%) was observed for samples sonicated at 33 and 45 kHz compared to control whereas, uncultured samples sonicated at 33 kHz had a higher proportion of SFA compared to control (Figure 1). The proportion of MUFA was found to decrease significantly ($p<0.05$) from 52.0% (control) to 48.2% (45 kHz) with increase in ultrasonic frequency for cultured samples while the reverse was observed in the case of uncultured dried beef jerky samples. In the case of

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cultured beef jerky samples, a significant increase in PUFAs was observed with an increase in ultrasonic frequency with highest being observed for dried beef jerky samples pre-treated at 45 kHz (9.4%) compared to control (4.8%); a reverse trend was observed for uncultured dried beef jerky samples. ANOVA revealed that drying time was found to have a significant effect on C18:0 ($P<0.01$), C18:2*n*–6 ($P<0.0001$) and C18:3*n*–3 ($P<0.0001$) while drying time did not significantly affect other major fatty acids (Table S2, see Supplementary Material online). A significant Interaction between drying time and culture was observed for C18:0, C18:2*n*–6 and C18:3*n*–3 only. Drying time and ultrasonic frequency interacted significantly for all major fatty acids with the exception of C16:1. Ultrasonic frequency did not affect most of the major fatty acids with the exception of C16:1 ($P<0.05$) and C18:2*n*–6 ($P<0.01$) whereas, interaction effects of ultrasonic frequency and culture was found to be significant for all major fatty acids. Correlation analysis showed a negative relationship between drying time and ∑PUFA ($r = –0.667, P<0.001$) and no significant correlations were observed between drying time and ∑SFA ($r=–0.222$) or ∑MUFA ($r=–0.155$) (Table 1). A significant decrease in ∑PUFA during drying of beef jerky samples could be due to oxidation of PUFAs to secondary oxidation products such as aldehydes, alcohols, ketones and furans. For example, formation of various compounds including n-alkanals, 2-alkenals, 1-alkanols, and alkylfurans are reported due to oxidation of abundant MUFA or PUFA present in beef during cooking (Elmore et al., 1999). Ultrasound has been reported to have minimal or no effect on fatty acid profiles of various foods when ultrasound is employed for extraction of oils from food matrices (Zhang et al., 2008). However, some studies highlight the degradation of some fatty acids due to ultrasound treatment to form volatile fatty acids leading to changes in flavour (Chemat et al., 2004b). Moreover, cavitation induced by ultrasound will cause damage to cell membranes, which are rich in unsaturated fatty acids and therefore can contribute to fatty acid oxidation.

### 3.4. Effect of ultrasound frequency, culture and drying time on nutritional indices

The nutritional indices (AI, TI, HH, *n*–6/*n*–3 and PUFA/SFA) for dried beef jerky samples are shown in Table 2. Certain fatty acids have proatherogenic and antiatherogenic effects on cholesterol. Saturated fatty acids mainly C12:0, C14:0 and C16:0 are proatherogenic whereas, MUFAs and PUFAs are antiatherogenic fatty acids related by the index of atherogenicity (AI). AI relates to the risk of atherosclerosis and is considered as an indicator of the impact of fat on the cholesterol concentration. AI is based on those fatty acids which can increase (C12:0, C14:0 and C16:0) or decrease (∑MUFA, ∑PUFA) the level of cholesterol

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(Stajić et al., 2011). The AI was found to vary from 0.63 to 0.68 in the case of cultured samples and from 0.52 to 0.60 in the case of uncultured samples. López-López et al. (2011) reported similar AI values of 0.71 and 0.68 for raw and cooked formulated beef patties, respectively. In general, a lower AI value corroborates to the lower atherogenic potential. TI is an index of thrombogenicity (TI) i.e. formation of clots within blood vessels. It is a ratio of prothrombogenic (C14:0, C16:0 and C18:0) and antithrombogenic fatty acids (MUFAs and PUFAs). Interestingly, C18:0 is believed to be thrombogenic but not atherogenic (Laudadio and Tufarelli, 2011). TI ranged from 1.04 to 1.30 and 0.89 to 1.12 for cultured and uncultured samples, respectively. Low values of AI and TI are preferred and indicate positive health benefits derived from the product. HH index, a ratio of hypocholesterolemic fatty acids and hypercholesterolemic fatty acids is related to the metabolism of cholesterol. Most PUFAs cause the strong hypocholesterolemic effects whereas, C12:0 and C14:0 have hypercholesterolemic effects. Higher value of HH index demonstrates low presence of cholesterol. HH index was found to vary from 1.546 to 1.615 and 1.654 to 1.808 for cultured and uncultured dried beef jerky samples, respectively. The PUFA:SFA ratio in beef jerky samples varied from 0.112 to 0.222 and 0.148 to 0.314 for cultured and uncultured samples, respectively. It has been reported that the ratio of PUFA:SFA in human diet should be >0.45(Sobczuk-Szul et al., 2013). The ratio of *n*–6/ *n*–3 for cultured samples was generally lower (1.398 to 1. 614) compared to uncultured samples (1.681 to 2.255). These values are less than <5.0 as recommended by WHO/FAO (Migdał et al., 2009).Diets containing higher amount of *n*–6 PUFA or high *n*–6/ *n*–3 ratio have been reported to promote the pathogenesis of cancer, inflammatory and cardiovascular diseases (Simopoulos, 2002, 2008). Both PUFA:SFA and *n*–6/ *n*–3 ratio are considered as good indicators of nutritional value of dietary fat.

### 3.5. Multivariate analysis

In order to classify the samples according to their fatty acid profile, multivariate analysis was performed on levels of individual fatty acids in beef jerky samples subjected to the various treatments under investigation (see supplementary file). Three factors were retained based on the amount of variance explained and eigenvalues. According to factor analysis, 84.5% of total variance was explained by three factors as shown in Table 3. Factor 1 explained 53.9% variation and was marked by high positive loadings for C12:0 –C21:0, C16:1, C17:1, C18:1,

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C20:1, C22:2. Factor 2 which explained 21.5% variation was marked by high negative loadings for C15:1, C18:2*n*–6, C20:3*n*–6, C20:4*n*–6, C20:5*n*–3, C22:5*n*–3 and C22:6*n*–3. Finally, factor 3 explained 8.0% variation, was marked with high positive loadings for C20:2 and C22:5*n*–3 and high negative loadings for C21:0 and C22:0 respectively. Factor 1 can be interpreted as the presence of higher level of saturated fats in beef jerky samples. The score plot of Factor 1 and 2 explaining total variability of 76.3% is shown in Figure 2. Figure 2 showed the position of cultured and uncultured beef jerky samples. The clear separation between samples is visible with cultured samples appearing in the upper part of the graph while samples without culture appearing in the bottom quadrant with negative factor values.

**Conclusions**

Results presented in this study demonstrate the fatty acid profile of beef jerky samples is influenced by various processing parameters. Interaction effect of ultrasound frequency with culture and drying time were more pronounced compared to individual effects.

Supplementary material. Figure S1, Tables S1-S2 [to be uploaded online by typesetters]

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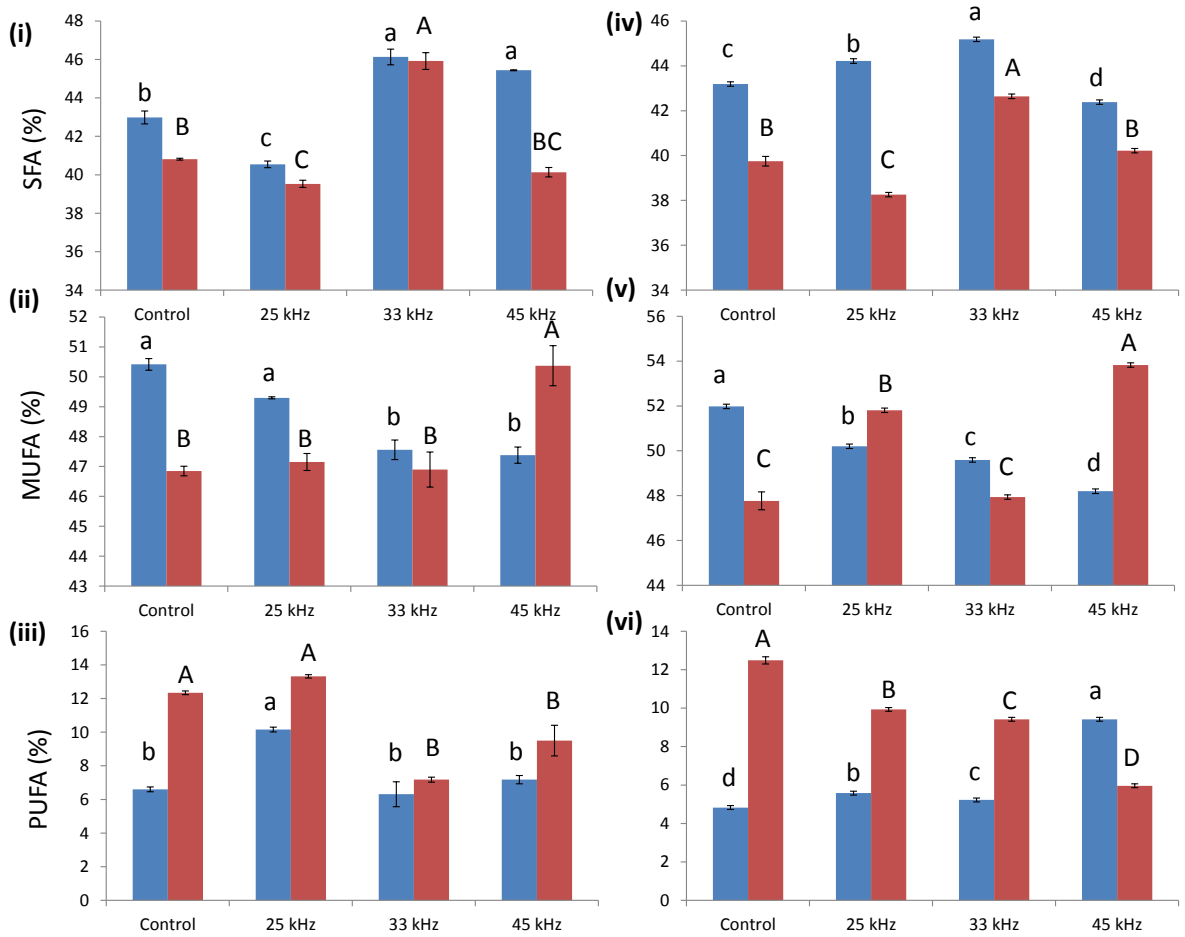
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**Figure captions**

**Figure 1.** Proportion of SFA (%FA), MUFA (%FA) and PUFA (%FA) after marination (i – iii) and 4 h drying period (iv – vi) for cultured (■) and uncultured (■) beef jerky samples. [a-d, A-D; columns followed by same letters are not significantly different at P<0.05].

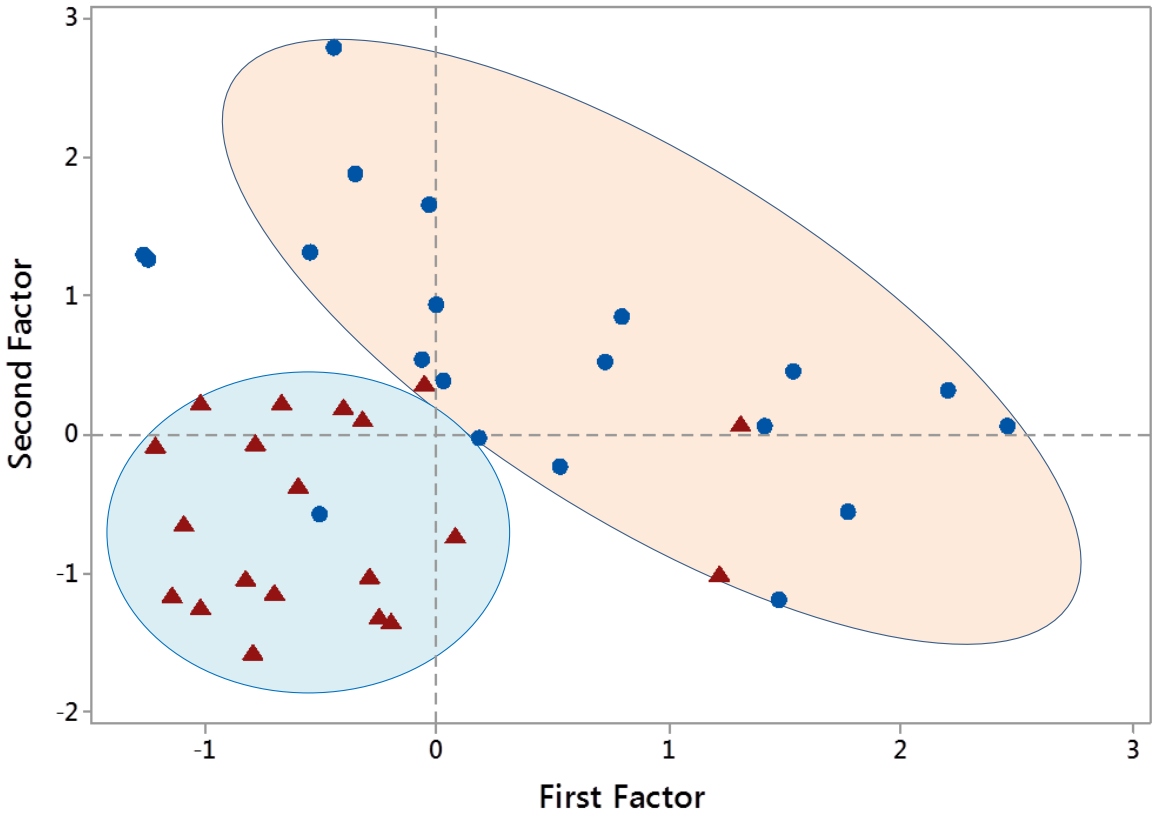
**Figure 2.** Score plot for Factor 1 and 2 for beef jerky cultured samples (●) and uncultured (▲) samples.

**Figure 1.**



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Figure 2.



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**Table 1. Correlation matrix for fatty acid derived nutrition indices of control and ultrasound pre-treated (25 kHz, 33 kHz and 45 kHz for 30 min) beef jerky samples from uncultured and cultured groups followed by drying for 4 hours at 60°C**

| | Culture | Drying time | US treatment | ∑SFA | ∑MUFA | ∑PUFA | ∑PUFA(*n*–3) | ∑PUFA (*n*–6) | *n*–6/ *n*–3 | ∑MUFA/ ∑SFA | ∑PUFA/ ∑SFA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Culture** | 1.000 | 0.000 | 0.000 | -0.465$^{**}$ | -0.430$^{**}$ | 0.379$^{*}$ | 0.102$^{ns}$ | 0.497$^{***}$ | 0.549$^{***}$ | 0.389$^{**}$ | 0.710$^{****}$ |
| **Drying time** | | 1.000 | 0.000 | -0.212$^{ns}$ | -0.155$^{ns}$ | -0.664$^{****}$ | -0.620$^{****}$ | -0.634$^{***}$ | -0.161$^{ns}$ | 0.260$^{ns}$ | -0.124$^{ns}$ |
| **US treatment** | | | 1.000 | 0.038$^{ns}$ | -0.011$^{ns}$ | -0.140$^{ns}$ | -0.130$^{ns}$ | -0.133$^{ns}$ | -0.062$^{ns}$ | -0.189$^{ns}$ | -0.183$^{ns}$ |
| **∑SFA** | | | | 1.000 | 0.974$^{****}$ | 0.407$^{**}$ | 0.548$^{***}$ | 0.299$^{ns}$ | -0.232$^{ns}$ | -0.776$^{****}$ | -0.776$^{****}$ |
| **∑MUFA** | | | | | 1.000 | 0.380$^{*}$ | 0.536$^{***}$ | 0.266$^{ns}$ | -0.260$^{ns}$ | -0.762$^{****}$ | -0.762$^{****}$ |
| **∑PUFA** | | | | | | 1.000 | 0.898$^{****}$ | 0.972$^{****}$ | 0.270$^{ns}$ | 0.180$^{ns}$ | 0.180$^{ns}$ |
| **∑PUFA(*n*–3)** | | | | | | | 1.000 | 0.771$^{****}$ | -0.169$^{ns}$ | 0.027$^{ns}$ | 0.025$^{ns}$ |
| **∑PUFA(*n*–6)** | | | | | | | | 1.000 | 0.481$^{**}$ | 0.247$^{ns}$ | 0.248$^{ns}$ |
| ***n*–6/*n*–3** | | | | | | | | | 1.000 | 0.283$^{ns}$ | 0.284$^{ns}$ |
| **∑MUFA/∑SFA** | | | | | | | | | | 1.000 | -0.463$^{***}$ |
| **∑PUFA/∑SFA** | | | | | | | | | | | 1.000 |

$^{*}$Significant at p≤0.05; $^{**}$Significant at p≤0.01; $^{***}$Significant at p≤0.001; $^{****}$Significant at p≤0.0001; $^{ns}$Not significant

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**Table 2. ANOVA of independent factors on major fatty acids of beef jerky samples**

| | Cultured | | | | | Uncultured | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Frequency** | AI | TI | HH | *n*–6/ *n*–3 | PUFA/SFA | AI | TI | HH | *n*–6/ *n*–3 | PUFA/SFA |
| **Control (no ultrasound)** | 0.642$^{b}$ | 1.187$^{c}$ | 1.615$^{b}$ | 1.398$^{d}$ | 0.112$^{c}$ | 0.536$^{c}$ | 0.913$^{c}$ | 1.808$^{a}$ | 1.882$^{b}$ | 0.314$^{a}$ |
| **25 kHz** | 0.641$^{b}$ | 1.231$^{b}$ | 1.631$^{q}$ | 1.534$^{b}$ | 0.125$^{b}$ | 0.523$^{c}$ | 0.891$^{d}$ | 1.787$^{a}$ | 1.681$^{c}$ | 0.260$^{b}$ |
| **33 kHz** | 0.681$^{a}$ | 1.296$^{a}$ | 1.546$^{c}$ | 1.614$^{a}$ | 0.116$^{c}$ | 0.604$^{a}$ | 1.121$^{a}$ | 1.654$^{b}$ | 2.255$^{a}$ | 0.221$^{c}$ |
| **45 kHz** | 0.632$^{c}$ | 1.036$^{d}$ | 1.608$^{b}$ | 1.450$^{c}$ | 0.222$^{a}$ | 0.564$^{b}$ | 1.043$^{b}$ | 1.766$^{a}$ | 1.684$^{c}$ | 0.148$^{d}$ |

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Table 3. Eigenvalues and varimax rotated factor loadings (Eigenvectors) for the fatty acid profile of beef jerky samples

| | **Factor 1** | **Factor 2** | **Factor 3** |
|---|---|---|---|
| **Eigenvalue** | 13.873 | 5.583 | 2.085 |
| **Variance explained (%)** | 53.4 | 21.5 | 8.0 |
| **Eigenvectors (↓)** | | | |
| **Lauric Acid (C12:0)** | 0.812 | 0.094 | -0.389 |
| **Myristic acid (C14:0)** | 0.985 | 0.106 | -0.026 |
| **Myristoleic Acid (C14:1)** | 0.855 | 0.198 | 0.242 |
| **Pentadecanoic acid (C15:0)** | 0.984 | 0.075 | -0.058 |
| **cis-10-Pentadecenoic acid (C15:1)** | -0.093 | -0.725 | 0.475 |
| **Palmitic acid (C16:0)** | 0.995 | 0.025 | 0.011 |
| **Palmitoleic acid (C16:1)** | 0.869 | 0.034 | 0.384 |
| **Heptadecanoic Acid (C17:0)** | 0.972 | -0.038 | -0.07 |
| **cis-10-Heptadecenoic acid (C17:1)** | 0.941 | -0.103 | 0.205 |
| **Stearic acid (C18:0)** | 0.953 | -0.066 | -0.153 |
| **Elaidic acid (C18:1*n*–9t)** | 0.831 | -0.098 | -0.031 |
| **Oleic acid (C18:1 *n*–9c)** | 0.991 | -0.004 | 0.095 |
| **Vaccenic acid (C18:1*n*–7t)** | 0.820 | -0.108 | 0.33 |
| **Linoleic acid (C18:2 *n*–6c)** | 0.330 | -0.787 | -0.082 |
| **Arachidic Acid (C20:0)** | 0.919 | -0.069 | -0.193 |
| **cis-11-Eicosenoic acid (C20:1 *n*–9)** | 0.960 | -0.03 | 0.136 |
| **alpha-linolenic acid (C18:3 *n*–3)** | 0.912 | -0.154 | -0.222 |
| **Henicosanoic acid (C21:0)** | 0.744 | -0.087 | 0.548 |
| **cis-11,14-Eicosadienoic acid (C20:2)** | -0.001 | -0.367 | -0.56 |
| **Behenic acid (C22:0)** | 0.093 | -0.356 | 0.427 |
| **cis8,11,14-Eicosatrienoic acid (C20:3 *n*–6)** | 0.033 | -0.952 | -0.185 |
| **Arachidonic acid (C20:4 *n*–6)** | -0.034 | -0.960 | -0.026 |
| **cis-13,16,Docosadienoic acid (C22:2)** | 0.637 | -0.355 | -0.514 |
| **Eicosapentaenoic acid (C20:5 *n*–3)** | -0.018 | -0.905 | -0.268 |
| **Docosapentaenoic acid (C22:5 *n*–3)** | 0.035 | -0.653 | 0.172 |
| **Docosahexaenoic acid (C22:6 *n*–3)** | -0.163 | -0.915 | -0.012 |