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

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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 h at 4 °C prior to hot air drying at 60 °C for 4 h. 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.

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 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

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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., 2016). 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 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 × 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 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 was 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 °C. All samples were subsequently cured for 18 h at 4 °C. All cured beef jerky slices were dried using a hot air dryer (Gallendkamp Plus II, Weiss Technik, UK) at a temperature of 60 °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)

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 × 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 of each fatty acid was calculated using following equation (Eq. (1)).

$$FA\ content = \frac{Peak\ Area(FA)}{Peak\ Area(ISTD)} \times \frac{Weight\ (ISTD)}{Weight\ (Sample)} \times ISTDpurity \times 10 \times 0.96 \quad (1)$$

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.

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 Eqs. (2)–(4), respectively. Other nutritional quality indices namely ratio of *n*–6/*n*–3 PUFA and