LWT - Food Science and Technology 161 (2022) 113315

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Effect of drying temperature on nutritional, functional and pasting properties and storage stability of beef lung powder, a prospective protein ingredient for food supplements

S. Reshan Jayawardena [a], James D. Morton [a,*], Alaa El-Din A. Bekhit [b], Zuhaib F. Bhat [a,c], Charles S. Brennan [a,d]

[a] Department of Wine Food and Molecular Biosciences, Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln, 7647, Christchurch, New Zealand
[b] Department of Food Sciences, University of Otago, P.O. Box 56, Dunedin, 9054, New Zealand
[c] Division of Livestock Products Technology, SKUAST, Jammu, India
[d] Department of Food Sciences and Nutrition, School of Science, RMIT, Australia

ARTICLE INFO

Keywords:
Beef lungs
Air drying
Protein powder
Amino acid analysis
Mineral analysis
Physicochemical properties
Storage quality
Rheological properties

ABSTRACT

The present study utilized beef-lungs to develop a protein ingredient for affordable human food supplements. Beef-lungs were dried in an air drier at 50, 70 and 100 °C and ground to develop a powder-based intermediate product. All beef-lung powder (BLP) samples dried at different temperatures showed a 300% water-binding capacity. Drying temperature significantly decreased the haem-iron content and protein quality with highest haem-iron content observed for the samples dried at 50 °C (41% of total-iron content). A significant increase in glycine content was observed with increasing drying temperature. SDS-PAGE results showed lowest protein degradation for BLP-samples dried at 50 °C. While microbiologically safe products were produced at all drying temperatures, BLP-samples dried at 50 °C showed highest protein- and lipid-oxidative stability. The mean values of all the microbial counts were within acceptable limits beyond six-months of storage. Addition of 10% of BLP dried at 50 °C to semolina dough showed no effect on apparent viscosity, however, addition of 10% BLP dried at 70 and 100 °C decreased ($P < 0.05$) the viscosity. Drying of beef-lungs at 50 °C resulted in a superior quality product. The results demonstrated the potential of BLP as a prospective and cost-effective ingredient for protein-supplement industry.

1. Introduction

Protein supplements play a crucial role in improving the nutritional status of various groups of people in society such as the malnourished, the elderly, pregnant women and those recovering from surgery. Expensive protein sources and high demand for protein supplements have caused a global increase in the price of protein supplements. Both scientists and the food industry have started evaluating underutilised protein sources for the production of affordable high-quality protein products. As carcase constitutes only 45–60% of an animal's live weight (Muir, Thomson, & Askin, 2008) and much of the rest is protein-rich co-products (lungs, kidney, heart and tongue), there is an ample opportunity to utilise these co-products as a source of protein products (such as protein concentrates and protein hydrolysates). Converting underutilised co-products into nutritious and safe protein supplements is a good strategy since co-products have a long history of safe consumption by many people around the world. This use of co-products will help to reduce environmental impact caused by disposing the material and will improve the profit margins for the industry. Since beef industry has a high cost of production, a focus on utilising co-products, such as beef lungs, will help increase the production efficiency. Beef lungs have a high protein content, 87% on a dry weight basis (Jayawardena, Morton, Brennan, & Bekhit, 2019), and have been used as food in many cultures. However, it has low aesthetic appeal and often faces regulatory hurdles as a human food (Jayawardena et al., 2019). Therefore, beef lungs are mostly rendered and end-up as a low value material or are rarely used for the production of pet food.

Co-products in general and lungs in particular are highly prone to

* Corresponding author.
E-mail addresses: James.Morton@lincoln.ac.nz (J.D. Morton), aladin.bekhit@otago.ac.nz (A.E.-D.A. Bekhit), zuhaibbhatvet@gmail.com (Z.F. Bhat), charles.brennan@rmit.edu.au (C.S. Brennan).

https://doi.org/10.1016/j.lwt.2022.113315
Received 9 August 2021; Received in revised form 22 February 2022; Accepted 28 February 2022
Available online 26 March 2022

contamination and microbial spoilage. Drying is one of the simplest ways to improve storage stability of co-products and dried powders have been successfully developed from different protein sources (Bishnoi et al., 2015; Ohkuma et al., 2008). In our previous study, we demonstrated the superior nutritional properties of beef lung protein powder (BLP) that showed its potential as an ingredient for development of cost effective protein based food supplements (Jayawardena et al., 2019). Such a product can play a crucial role in fulfilling the protein requirements of the people, especially children and seniors, living in developing and low-income countries. The present study used hot air-drying method for production of BLP powder. While several other methods of drying are available such as freeze drying, infrared drying, fluidized bed drying, dielectric drying, refractance window, and contact drying which can be used alone or in combination for drying of beef lungs. However, hot air-drying method requires lower initial capital, has low operational cost and is widely available in low-income countries, making it a highly suitable method for commercial production of cost-effective BLP powder. Being a good source of animal proteins and highly bioavailable iron, there is a need for optimization of drying and other processing conditions to preserve these valuable nutrients in the final product. Further, comprehensive characterisation of BLP powder and evaluation of its storage stability is essential before proposing it as an ingredient for development of protein supplements. The objective of the present study was to evaluate the effect of different drying temperatures on the quality characteristics of BLP powder which included physicochemical, nutritional, microbiological, pasting properties, and protein- and lipid oxidative stability.

2. Materials and methods

2.1. Processing of beef lung powder

Hygienically derived frozen beef lungs without trachea (10 kg) were processed into BLP powder using the facilities in the food-processing laboratory of Lincoln University (Lincoln, New Zealand). Beef lungs were thawed overnight at 4 °C and discoloured areas were trimmed to obtain clean lung tissues. The cleaned lungs were randomly divided into nine equal parts (970 ± 122 g) and minced separately using a mincer with a 3 mm plate. The minced samples were evenly laid over the drying trays in layers of 1 cm thickness and air dried (E32M, Bakbar, Christchurch, NZ) till a constant weight was reached. The study used three different temperatures viz. 50, 70 and 100 °C for drying the samples. The drying time and temperatures were the key parameters of the study and preliminary trials were conducted to optimise minimum and maximum temperatures on the basis of the product quality. While temperatures below 50 °C compromised microbiological safety, temperatures higher than 100 °C affected the nutritional and sensory quality of the powder. The detailed nutritional and sensory information of BLP powder and its potential as an ingredient for development of cereal-based food products has been published in our earlier paper (Jayawardena et al., 2019). The drying process took about 1400 min at 50 °C, 655 min at 70 °C and 360 min at 100 °C. The weight of the dried samples was measured frequently and the surface crust was broken with a knife at 30 min intervals and mixed uniformly for steady moisture evaporation. The whole trays were weighted before and after drying to determine the moisture loss. The dried beef lungs were ground to powder using an FP920 grinder (Kenwood, China) for 15 min, vacuum-packed in LDPE packaging and stored at room temperature (25 °C) for further analysis. The BLP samples were assessed for storage stability by determining protein oxidation, lipid oxidation and microbiological counts for six months.

2.2. Particle size distribution, bulk density, absolute density, porosity & flowability

Dried BLP samples were passed through a series of standard sieves (0.5, 1.18 and 2.26 mm) to determine the particle size distribution of each sample. Other physical properties were measured after sieving the samples through 0.5 mm sieve.

Bulk density (þbulk) was measured according to the method of Mahdavi, Jafari, Assadpoor, and Dehnad (2016) using 1 g of beef lung powder in a 10 ml plastic measuring cylinder. The measuring cylinder was tapped and a constant volume was reached. The bulk density of the sample was calculated as the ratio between the weight of powder in the cylinder and the filled volume. The absolute density (þabs) was measured with a pycnometer using RO water as a standard.

The porosity (ε) was calculated using the bulk density to absolute density ratio (Rahman, Perera, Chen, Driscoll, & Potluri, 1996):

$$\varepsilon = 1e(bbulk / babs)$$

Flowability of BLP was determined according to Hausner (1967). BLP (10 g) was placed in a 25 ml measuring cylinder and the initial volume (Vb) was recorded. The filled cylinder was tapped 10 times and the final volume (Vf) was recorded.

$$\text{Hausner ratio (HR)} = \text{Vb/Vf}$$

2.3. Hygroscopicity of beef lung powder

Hygroscopicity of beef lung powder was measured according to the method of Cai and Corke (2000) with minor modifications. Triplicate samples (2 g) in pre-weighed Petri dishes were placed in an airtight plastic container with saturated sodium chloride solution. The container was placed in an incubator at 30 °C for 7 days to obtain constant relative humidity (75.09% RH). The hygroscopic moisture was measured by the difference of initial and final weights and was expressed as absorbed moisture per 100 g of dry matter.

2.4. Water solubility index (WSI) and water binding capacity (WBC)

The water solubility index was measured according to the method described by Mahdavi et al. (2016). BLP power (12.5 g) was vortexed thoroughly with 30 ml water (filtered through reverse osmosis system) in a 50 ml centrifuge tube for 2 min, incubated in a 37 °C water bath for 30 min and centrifuged at 17,640×*g* for 20 min at 4 °C. The supernatant was collected into a pre-weighed beaker and dried at 105 °C overnight. The weight difference was determined after drying and WSI was calculated. The remaining pellet in the centrifuge tube was weighed to determine the water-binding capacity (WBC).

$$\text{WSI} = \frac{(\text{weight difference of the supernatant after drying})}{\text{Initial sample weight}} \times 100$$

$$\text{WBC} = \frac{(\text{weight of pellet} - \text{Initial sample weight})}{\text{Initial sample weight}} \times 100$$

2.5. Colour measurements

Colour values 'L*', 'a*' and 'b*' parameters were determined using a Chroma CR 400 colourimeter (Konica Minolta INC., Tokyo, Japan) with the illuminant C (CIE, standard, 6774 K). The colorimeter was calibrated using a standard white tile (L = 98.03, a = −0.23 and b = 0.25). The colour values were measured at six random places on beef lung powder placed on black coloured paper (Bhat, Morton, Zhang, Mason, & Bekhit, 2020). All values were means of six replicates.

2.6. Protein and moisture content

Nitrogen content of BLP was determined using Dumas method as described by Bhat, Morton, Mason, and Bekhit (2018a). Protein content was calculated by multiplying the nitrogen content with 6.25 (Bhat & Pathak, 2009). Method described by Bhat et al. (2019a) was used to

determine the moisture content of the samples.

2.7. SDS-PAGE analysis

Protein from BLP and raw lung samples was extracted and analysed following the procedure of Bhat, Morton, Mason, and Bekhit (2018b). Fresh lung (1 g) or BLP samples (0.5 g) were homogenised in 10 ml extraction buffer (0.1 M KCl, 1 mM EDTA, 1 mM sodium azide, 25 mM potassium phosphate buffer at pH 7.0) for 60 s with a Polytron homogeniser at 10,000 rpm. The homogenate was centrifuged at 1000 g for 15 min at 4 °C, the pellet was resuspended in 10 ml extraction buffer and the procedure was repeated twice for maximum extraction. The supernatants were combined, and protein concentration was determined using the Bicinchoninic acid (BCA) method (Pierce Chemical Company, Illinois, USA). Samples (20 μg protein) were loaded on a 12% SDS-PAGE mini-gel (Biorad), separated at 120 V for 70 min, fixed with 50% methanol: 7% acetic acid for 15 min and stained with GelCode® Blue Stain Reagent (Pierce).

2.8. Amino acid and mineral profile

Amino acid and mineral profiles were determined according to Jayawardena et al. (2019) and Bhat, Morton, Mason, and Bekhit (2019), respectively. All amino acids were analysed by HPLC 1100 series (Agilent Technologies, Waldrbom, Germany) provided with an autosampler and fluorescence detector. HPLC column was ACE, 3 μm C-18 (150 mm × 4.6 mm) with 40 °C column temperature. Beef lung powder samples were added into culture tubes. The samples were resuspended in 5.0 mL of 6 M HCl and 10.0 μL 0.5 M amino-butyric acid was added as a standard. Samples were vortexed and sonicated for 5.0 min. Each tube was purged with nitrogen and immediately made airtight by closing the top. Samples were digested at 110 °C for 20 h. After cooling to room temperature, the hydrolysate was obtained and the tube was washed with 5 ml water two times and added to the hydrolysate. Then, the hydrolysate was dried in a rotary vacuum evaporator at 45 °C. The hydrolysate residue was resuspended in nanopure water, transferred to a volumetric flask and the volume was made to 50 ml. The samples were filtered through a 0.45- μL syringe into a 2 ml HPLC vial for the HPLC analysis.

The mineral profile was measured using an Inductively Coupled Plasma Optical Emission Spectrophotometer (Varian 720 ICP-OES, Melbourne, Australia). Dried BLP (0.2 g) sample was mixed with 2.0 ml of nitric acid (69%) and 2.0 ml of hydrogen peroxide (30%) and were digested in a microwave (CEM MARS Xpress, Matthews, NC, USA) using a temperature programme of a linear increase from ambient to 90 °C over 15 min and thereafter held at 90 °C for 5 min. Settings were Plasma gas flow-15.0 L min-1, Aux-1.5 L min-1, Nebuliser 0.9 L min-1 with SeaSpray nebuliser and cyclonic spray chamber. Calibration standards and internal standards were serially diluted using ICP standard solutions using MilliQ water. Calibration curves were generated using four standards and standard blank.

2.9. Haem iron analysis

Haem iron from 0.5 g of BLP was extracted in 14 ml acidified acetone and its absorbance measured at 640 nm as described by Hornsey (1956). For raw beef lung, a 2 g sample was used for the experiment and the moisture content of the sample was determined. The haem coefficient was confirmed using a standard haematin sample (product No: H3281, Sigma Aldrich, MO, USA) and the iron content calculated by multiplying by a factor of 0.0882 μg iron/μg haematin.

2.10. Protein and lipid oxidation

The extent of protein oxidation was determined by evaluating the total carbonyl content using dinitrophenylhydrazine (DNPH) as described by Bhat, Morton, Mason, and Bekhit (2020). All samples were analysed in triplicate. Dried beef lung powder sample (1 g) was weighed in 50 ml Falcon tube and was homogenised using 1:10 (w/v) 20 mM of sodium phosphate buffer (10 ml) containing 0.6 M NaCl (pH 6.5) for 30 s at 10000 rpm. Homogenate (0.2 ml) was transferred into separate Eppendorf tubes for protein and carbonyl measurements. Then, cold TCA (10%) was added and centrifuged at 5000 rpm for 5 min to precipitate the protein. After discarding the supernatant, 1 ml of 2 M HCl was added to one pellet for protein measurement and 1 ml of 0.2% (w/v) DNPH in 2 M HCl was added to other pellet for carbonyl measurement. Samples were incubated at room temperature for 1 h and vortexed every 15 min. TCA (10%) was added and the samples were centrifuged (Centra GP6R, Thermo IEC, Needham Heights, MA, USA) at 5000 rpm for 5 min to separate the pellet. All pellets were washed 3 times with ethanol: ethyl acetate (1:1, v/v) to remove excess DNPH. Then the pellet was dissolved in 1.5 ml of 20 mM sodium phosphate buffer containing 6 M guanidine HCl (pH 6.5). Samples were stirred and centrifuged (Centra GP6R, Thermo IEC, Needham Heights, MA, USA) at 10000 rpm for 5 min to take the supernatant. Protein concentration of the supernatant was measured by using BCA protein assay kit at 562 nm. The absorbance of the samples was measured at 370 nm using 1 cm path length cuvettes. The carbonyl content was measured using OD and coefficient (21 $nM^{-1}cm^{-1}$) for protein hydrazones. The carbonyl content expressed as nmol per milligram of protein using below equation

$$\text{Carbonyl concentration} = [\text{Absorbance (370 nm)}/21\ nM^{-1}cm^{-1}]/\text{protein concentration (mg/ml)}$$

Lipid oxidation was assessed by measuring the TBARS in the samples using the method described by Singh, Kumar, Bhat, Kumar, and Kumar (2015). A 5 g of minced raw sample or beef lung powder was homogenised in 25 ml distilled water for 1 min using a Polytron homogeniser at 9000 rpm. A 3 ml of sample homogenate solution was added to 3 ml TBARS/TCA (0.032 M 2-thiobarbituric acid, 1.14 M trichloroacetic acid in 0.32 M HCl) solution. Samples were incubated at 94 °C for 15 min, centrifuged (2500 g for 15 min) and the absorbance of the supernatant measured at 535 nm. A standard calibration curve was prepared using 1, 1,3,3-tetraethoxypropane.

2.11. Microbial analysis

Ten grams of the samples were taken under complete aseptic conditions inside a laminar flow and were homogenised with 90 ml of sterile peptone water (Marks, Darmstadt, Germany) in a sterile bag for 2 min using stomacher. Tenfold serial dilutions were prepared from the original homogenates inside the laminar flow and used for inoculation using spread plate technique. Total viable counts were enumerated by spreading 100 μl from each dilution on the surface of plate count agar and incubating at 35 °C for 48 h (Morton, 2001). Dilutions showing 30 to 300 colonies were selected for enumeration and expressed as colony forming units per gram (cfu/g) of sample. Coliform and other gram-negative pathogens were enumerated on MacConkey agar. The plates were incubated at 35 ± 2 °C for 48 h and counts were determined as cfu/g of sample. Yeast and moulds were enumerated on Sabaroud dextrose agar plates incubated at 25 ± 2 °C for 5 days (Bhat & Pathak, 2012) and the counts were determined as cfu/g.

2.12. Pasting properties of BLP added semolina dough

Dough was prepared using semolina flour incorporating 10, 15 or 20% BLP. Semolina without BLP was used as a control. The apparent viscosity of the samples was measured by Rapid Visco Analyzer RVA S4 (Perten Instruments Pty. Ltd., Warriewood, Australia). Sample (3 g) was transferred into a canister and 25 ml of distilled water was added to it before operating the analyser. Standard general pasting method No. 1 heating and cooling cycle program was used where the samples were held at 50 °C and heated to 95 °C at 12 °C/min, held at 95 °C for 3.5 min,

then cooled from 95 to 50 °C at 10 °C/min, followed by a holding phase at 50 °C for 2 min. Measurements of peak viscosity, trough viscosity, breakdown, final viscosity, setback and pasting temperature were directly recorded. The derived parameters of stability ratio (trough viscosity/peak viscosity), (setback ratio final viscosity/trough viscosity) and relative breakdown (breakdown viscosity/setback viscosity) were determined from direct measurements as described by Kumar, Brennan, Zheng, and Brennan (2018).

2.13. Statistical analysis

The data generated by repeating the experiments for different parameters were compiled and analysed using SPSS (IBM SPSS Statistics V22.0, Armonk, NY, USA). All experiments were performed in triplicate unless otherwise stated. All data were reported as means ± standard deviation. Differences between the treatments were analysed by one-way analysis of variance. When the ANOVA was significant ($P < 0.05$), means were separated by a pairwise comparison using Tukey's comparison test.

3. Results and discussion

3.1. Effect of drying temperature on physical properties

Drying temperature significantly ($P < 0.05$) reduced the particle size of BLP samples (Table 1). Increasing the drying temperature from 50 to 100 °C increased the percentage of particles of diameter less than 0.5 mm from 45% to 70%. The moisture content of the BLP samples reduced significantly ($P < 0.05$) as the drying temperature increased from 50 °C to 100 °C. Drying at 50 °C caused a caking effect due to a high moisture content. Tonon, Brabet, and Hubinger (2010) observed that low moisture powder has a higher capacity to absorb moisture as a result of the water concentration gradient between the product and its surrounding. While the water binding capacity of BLP samples was not affected by drying temperature, the water-soluble index (WSI) and hygroscopicity showed significant ($P < 0.05$) decrease at 70 °C and 100 °C. This might be attributed to denaturation of proteins at higher temperatures and subsequent increase of surface hydrophobicity, generating a repulsive force towards water molecules.

Increased drying temperature resulted in increased absolute density (Table 1). The bulk density showed a tendency to increase with higher temperature, but this was not significant. Porosity decreased significantly ($P < 0.05$) with increasing drying temperature and similar results were reported by Rostami, Dehnad, Jafari, and Tavakoli (2018) for meat powder dried by refractance window method. Changes in porosity can potentially alter access to enzymes and biochemical processes such as digestion (Sujka & Jamroz, 2007). The Hausner ratio for all the BLP samples was in the range where flow would be difficult (Hausner (1967). All the BLP samples showed higher water binding capacity (WBC) around the values of 315 which indicates the usefulness of these powders in the food processing industry (Southward, 2003). The results of these physical parameters suggest that BLP samples should be packed in moisture impermeable packages soon after processing and stored in a dry and cold place.

Higher drying temperatures led to a decrease in all colour parameters (L*, a*, and b*) of BLP samples. BLP samples dried at 50 °C were lighter (L* value) and yellowish (b* value) in colour, that makes them suitable for incorporating into light coloured food products. Drying at 100 °C significantly ($P < 0.05$) reduced the redness (a* value) of BLP samples. This was probably due to oxidation of myoglobin to metmyoglobin. Similar results have been reported for dried beef jerky (Kučerová, Marek, & Banout, 2018).

Table 1
Effect of drying temperature on physicochemical characteristics, colour values and protein content of beef lung powder.

Powder characteristics	Drying Temperature		
	50 °C	70 °C	100 °C
Moisture content % (w. b.)	4.79 ± 0.63[a]	1.01 ± 0.82[b]	0.40 ± 0.49[c]
Absolute density (g/ml)	1.18 ± 0.02[c]	1.27 ± 0.03[b]	1.48 ± 0.03[a]
Bulk density (g/ml)	0.56 ± 0.03	0.59 ± 0.04	0.65 ± 0.02
Porosity	0.62 ± 0.02[a]	0.54 ± 0.03[b]	0.45 ± 0.02[c]
Hausner ratio (HR)	1.29 ± 0.04	1.28 ± 0.03	1.32 ± 0.01
WBC (Water binding capacity) %	314 ± 4.3	316 ± 1.8	317 ± 1.8
WSI (Water solubility index) %	16.69 ± 0.91[a]	11.76 ± 0.19[b]	10.36 ± 0.15[c]
Hygroscopicity %	15.97 ± 1.03[a]	13.22 ± 0.55[b]	13.48 ± 0.81[b]
L* (Lightness)	62.72 ± 1.60[a]	55.20 ± 1.59[b]	53.61 ± 1.41[b]
a* (Redness)	4.81 ± 0.90[a]	5.12 ± 0.15[a]	3.89 ± 0.17[b]
b* (Yellowness)	18.26 ± 0.60[a]	13.07 ± 0.96[b]	12.35 ± 0.50[b]
Protein (% dry matter)	85.44 ± 1.63	84.88 ± 0.44	84.63 ± 0.38

Mean ± Standard deviation in a row with different superscripts differ significantly ($p < 0.05$).

3.2. Microbial safety

Raw minced beef lung samples showed a mean total viable count of 1.7×10^4 CFU (Table 2) which was within the reference limit of 5×10^5 CFU suggested for manufactured meats (MPI, 1995). Drying reduced the microbial load by at least two orders of magnitude. The aerobic microbial limit suggested for ready to eat meat products is 1×10^3 (MPI, 1995) and the microbial counts of all the BLP samples were within this limit even after six months of storage. BLP samples dried at 100 °C showed microbial results similar to commercial sterility as no trace of bacterial growth, coliforms or yeast/mould were detected. Previous studies have reported non-detection of coliforms for meat products heated to such a higher temperature (Kaur, Kumar, Bhat, & Kumar, 2015; Singh, Kumar, Kumar, & Bhat, 2014). Yeast and moulds were

Table 2
The effect of drying on amino acid composition of beef lung powder on a molar basis.

Amino acids (molar basis)	50 °C BLP mol %	70 °C BLP mol %	100 °C BLP mol %
Essential amino acids (EAA)			
Arginine	4.4 ± 0.1	4.2 ± 0.1	4.4 ± 0.3
Histidine	1.6 ± 0.1	1.8 ± 0.1	1.7 ± 0.1
Isoleucine	3.5 ± 0.1	3.4 ± 0.1	3.3 ± 0.1
Leucine	8.6 ± 0.3	8.7 ± 0.3	8.1 ± 0.2
Lysine	5.3 ± 0.1	5.4 ± 0.1	5.0 ± 0.2
Methionine	1.7 ± 0.0	1.7 ± 0.07	1.6 ± 0.1
Phenylalanine	3.5 ± 0.1	3.5 ± 0.1	3.3 ± 0.1
Threonine	4.5 ± 0.2	4.7 ± 0.1	4.4 ± 0.15
Tryptophan	1.8 ± 0.6	1.8 ± 0.04	1.7 ± 0.1
Valine	6.6 ± 0.3	6.4 ± 0.2	6.1 ± 0.1
Total EAA %	41.4 ± 2.0	41.6 ± 1.2	39.6 ± 1.4
Non-essential amino acids (NEAA)			
Amino acid	50 BLP mol%	70 BLP mol %	100 BLP mol %
Alanine	10.9 ± 0.2	10.5 ± 0.1	10.8 ± 0.1
Asparagine	0.6 ± 0.03	0.6 ± 0.02	0.7 ± 0.02
Aspartic	6.3 ± 0.1	6.4 ± 0.2	5.9 ± 0.2
cysteine	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
Glutamic acid	9.3 ± 0.4	9.4 ± 0.1	9.0 ± 0.2
Glutamine	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.001
Glycine	16.5 ± 1.0[b]	16.0 ± 1.0[b]	18.6 ± 0.8[a]
Proline	6.7 ± 0.3	6.7 ± 0.4	7.1 ± 0.3
Serine	4.5 ± 0.5	5.0 ± 0.1	4.7 ± 0.1
Tyrosine	2.3 ± 0.1	2.3 ± 0.1	2.2 ± 0.05
Total NEAA %	58.5 ± 2.8	58.4 ± 2.0	60.4 ± 1.9

Mean ± Standard deviation in a row with different superscripts differ significantly ($p < 0.05$).

found only in 50 °C BLP samples and did not increase during storage.

3.3. Effect of drying on proteins and lipids

The protein content of BLP samples was around 85% (dry weight basis) and was not affected by drying temperature. This was similar to the protein percent values (87% and 79%, respectively) reported by Jayawardena et al. (2019) and USDA food data central (USDA, 2020). The protein profile of BLP samples was compared with the fresh beef lung protein extract using SDS-PAGE (Fig. 1). The highest number and most intense bands were present for control samples followed by the BLP samples dried at 50 °C whereas least number and least intense bands were present for BLP samples dried at 100 °C. Two thick bands were present in fresh lung at 67 and 10 kDa. The band at 67 kDa decreased in intensity and thickness and ultimately disappeared at 100 °C. Darine, Christophe, and Gholamreza (2010) also reported the presence of a 67 kDa band from protein samples extracted from beef lungs. A thick 44 kDa band was also present in the beef lung concentrate of Darine et al. (2010). The heat labile band at 27 kDa had completely disappeared at 70 °C. The 17 kDa band was not present in the raw samples and increased in intensity with temperature and might have been produced during heat induced degradation of larger protein molecules in the dried powder. The thickest band present in the raw samples was 10 kDa and was most intense in raw samples and faded in the BLP samples dried at 50 °C and was absent at 70 and 100 °C.

Table 3 presents the amino acid profile of BLP samples. Glycine was the most abundant amino acid in BLP samples and significantly ($P < 0.05$) increased with drying temperature. This was probably due to loss of side chains from other amino acids. Glycine is the primary amino acid in collagen and the results reflected the fact that beef lung is a collagen-rich tissue (Francis & Thomas, 1975). The temperature did not have a significant effect on other individual amino acids, but the total percentage of essential amino acids was significantly ($P < 0.05$) decreased after drying at 100 °C. Among the essential amino acids, leucine, valine, lysine, threonine, and arginine were highly available on molar basis and leucine, lysine, arginine and valine on weight basis (Table 3). These

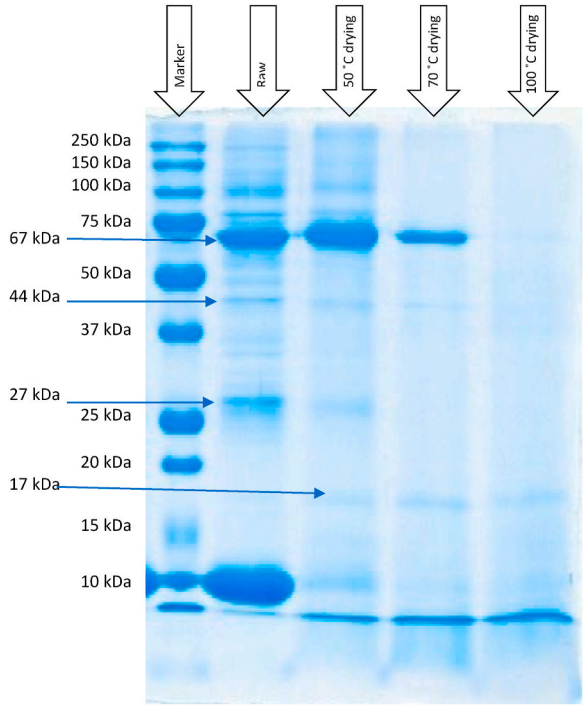

Fig. 1. SDS-PAGE protein profile of raw beef lung and BLP prepared at different drying temperatures (20 μg samples were loaded in each lane).

results were in agreement with the findings of Cardoso-Santiago and Arêas (2001) for beef lungs.

Drying can oxidise biological molecules and affect product appearance, taste and bioavailability. The total carbonyl content as measured by DNPH method can be used as an indicator of protein oxidation. Protein oxidation of BLP samples increased significantly ($P < 0.05$) with drying temperature (Table 3). Mean carbonyl values of the BLP samples also showed a non-significant increasing trend with storage time. Thermal processing at higher temperatures has been reported to increase the carbonyl content of meat and meat products. Hu et al. (2017) reported that temperature and cooking methods are directly responsible for the carbonyl production during protein oxidation. Carbonyl groups can react with non-oxidised free amino acids to form amide bonds (Liu & Xiong, 2000). This may lead to protein aggregation and may cause a reduction of digestibility and nutritional value. The BLP samples dried at 50 °C showed least amount of protein oxidation.

Thiobarbituric acid reactive substances (TBARS) was used to determine the lipid oxidation in the samples during storage. TBARS is a popular method of quantifying the lipid oxidation by determining the amount of malondialdehyde/kg of a sample (Kalem, Bhat, Kumar, Noor, & Desai, 2018, b). The TBARS values of BLP samples increased significantly ($P < 0.05$) from 0.25 to 0.55 mg/kg with 50–100 °C drying temperature. This was probably due to the rapid production of free radicals with high temperature which can cause lipid oxidation. TBARS values also showed a significant ($P < 0.05$) increase over storage time (Table 3). The mean TBARS values were less than those reported for beef jerky dried at 70 °C (Lim et al., 2014). The higher TBARS values of dried jerky products were due to higher levels of added salt which is known to catalyse the lipid oxidation (Jamwal, Kumar, Bhat, Kumar, & Kaur, 2015). Lipid oxidation is one of the primary causes of quality deterioration and is known to affect the sensory attributes such as flavour, colour, texture and appearance of meat and meat products (Bhat, Morton, Mason, Bekhit, & Bhat, 2019; Dua, Bhat, & Kumar, 2015). The relationship between the off flavour development and TBARS value was established by Greene and Cumuze (1982) during a sensory study and reported a value of 0.6–2.0 mg MDA/kg for a perceptive off-flavour development in beef. The values in the range of 0.51–0.54 mg MDA/kg were observed for the BLP samples dried at 50 and 70 °C after six months of storage and were fit for consumption. However, the TBARS values of BLP samples dried at 100 °C exceeded the threshold limit of 0.6 mg MDA/kg and were not fit for consumption after six months of storage.

3.4. Mineral analysis and haem iron

Mineral analysis of BLP samples revealed that the heavy metals Cd and Pb were below detection levels (Table 4). Drying temperatures did not have any significant effect on mineral content (data not shown). Iron content present in the foods is a vital micronutrient to prevent iron deficiency anaemia. Iron deficiency is the primary mineral deficiency worldwide and has been identified as one of the six priorities of World Health Organisation (WHO). The total iron content observed in BLP samples was 0.6 mg/g on dry weight basis (Table 4). This is lower than the value (1 mg/g) reported in our previous study (Jayawardena et al., 2019) and was attributed to leaching out of blood from lungs during mincing process. This is still higher than the value of 0.38 mg/g reported by the USDA for lungs. Further, haem iron content showed a decline with increase in drying temperature. BLP samples dried at 50 °C contained haem iron equal to 40% of the total iron which decreased to a level of 29% in BLP samples dried at 100 °C. High-temperature drying can induce oxidative cleavage of porphyrin structure of haem iron and change it to non-haem (Schricker & Miller, 1983). Contributed by the blood, 15–25% of haem iron is absorbed in human gut whereas only 5–12% of non-haem form present in animal tissues is absorbed (Hurrell & Egli, 2010).

Copper is an essential micronutrient for metabolically active tissues

Table 3
Effect of drying temperature on the microbial characteristics, lipid oxidation and protein oxidation of beef lung poowder

Parameters	Day 0				After 6 months of storage		
	Raw beef lungs	50 °C BLP	70 °C BLP	100 °C BLP	50 °C BLP	70 °C BLP	100 °C BLP
Total viable count	17×10^3	2×10^2	1×10^2	<100	3×10^2	1×10^2	<100
Coliform	<100	<100	<100	<100	<100	<100	<100
Yeast and Mould	8×10^2	1×10^2	<100	<100	1×10^2	<100	<100
TBARS (MDA mg/kg sample)	0.058 ± 0.006^{e}	0.25 ± 0.01^{d}	0.38 ± 0.02^{c}	0.55 ± 0.01^{b}	0.51 ± 0.03^{b}	0.54 ± 0.01^{b}	0.69 ± 0.07^{a}
Carbonyl content (nmol/mg protein)	1.6 ± 0.38^{d}	7.1 ± 0.4^{c}	8.1 ± 0.42^{bc}	11.3 ± 0.97^{a}	8.6 ± 0.92^{bc}	10 ± 0.4^{ab}	12 ± 1.58^{a}

Mean ± Standard deviation in a row with different superscripts differ significantly ($p < 0.05$).
TBARS = Thiobarbituric acid reacting substances.
BLP = Beef lung powder.

Table 4
Effect of drying temperature on specific minerals and haem iron of beef lung powder (dry matter basis).

Minerals	BLP 50 °C (μg/g)	BLP 70 °C (μg/g)	BLP 100 °C (μg/g)
Cadmium	ND	ND	ND
Copper	6.17 ± 0.24	6.00 ± 0.16	5.93 ± 0.06
Total iron	617 ± 17	605 ± 13	593 ± 19
Haem iron	247 ± 10^{a}	189 ± 13^{b}	171 ± 9^{b}
Percent of total iron	40%	31%	29%
Magnesium	509 ± 5	496 ± 3	498 ± 3
Lead	ND	ND	ND

Mean ± Standard deviation in a row with different superscripts differ significantly ($p < 0.05$).
BLP = Beef lung powder.
Haem iron percentage was calculated using total iron content.

like brain, heart and liver (Trumbo, Yates, Schlicker, & Poos, 2001). Recommended dietary allowance (RDA) of copper for adult men and women is 1.2–1.7 (mg/day) and recommended upper level is 10 mg/ml (Baghurst, 2006). Results presented in Table 4 revealed that the BLP samples can supply a considerable percentage of RDA value and are unlikely to reach toxic levels.

Present as a cofactor for more than 300 enzymes, magnesium plays an important role in the human body and is involved in both aerobic and anaerobic metabolism (Jahnen-Dechent & Ketteler, 2012). The results showed that BLP samples were an excellent source and contained around 500 μg/g of magnesium. Given that the upper intake limit for magnesium is 400 mg/day, BLP samples are unlikely to exceed this level.

3.5. Viscosity parameters

The viscosity parameters of semolina dough (peak viscosity, through viscosity, final viscosity and break down and setback values) decreased after adding beef lung powder to the semolina flour (Table 5). Similar results have been reported after addition of protein rich ingredients to taro flour (Onwulata & Konstance, 2002) and rice flour (Shin, Gang, & Song, 2010). Drying temperature can affect the viscosity of the flour mixture and this was confirmed by different temperature curves at 10% substitution (Fig. 2). While control semolina samples showed highest viscosity, viscosity of the mixture flours (semolina and 10% BLP) showed a decreasing trend with increasing drying temperature and lowest values were observed for mixtures containing BLP samples dried at 100 °C.

High peak viscosity curves generally lead to high breakdown viscosity (Tsakama, Mwangwela, Manani, & Mahungu, 2010) and high breakdown viscosity leads to unstable gel structures. Kumar et al. (2018) observed that lower breakdown and higher stability ratio indicated low hydration, low swelling power, and high shear resistance, which led to

Table 5
RVA viscosity parameters of pasta made from semolina flour and beef lung powder.

Samples	Peak viscosity (cP)	Trough viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)	Peak Time (min)	Pasting Temp (°C)	Stability ratio	Setback ratio	Relative breakdown
Control semolina	2570 ± 233^{a}	1940 ± 105^{a}	631 ± 127^{a}	3587 ± 202^{a}	1641 ± 101^{a}	5.51 ± 0.03^{b}	87 ± 0.51^{f}	0.76 ± 0.029^{c}	1.84 ± 0.02^{e}	0.38 ± 0.06^{a}
50 °C -10% BLP	2034 ± 81^{b}	1610 ± 92^{b}	451 ± 5^{b}	3153 ± 134^{b}	1574 ± 47^{ab}	5.47 ± 0.00^{bc}	89 ± 0.03^{bcd}	0.79 ± 0.024^{c}	1.96 ± 0.06^{cd}	0.29 ± 0.01^{b}
50 °C -15% BLP	1496 ± 87^{de}	1270 ± 49^{de}	227 ± 38^{cd}	2664 ± 101^{cd}	1394 ± 53^{cd}	5.42 ± 0.04^{bcd}	90 ± 0.46^{ab}	0.85 ± 0.017^{b}	2.10 ± 0.005^{ab}	0.16 ± 0.02^{cd}
50 °C -20% BLP	1155 ± 81^{f}	1062 ± 66^{f}	93 ± 14^{de}	2332 ± 129^{e}	1270 ± 62^{de}	5.40 ± 0.07^{cd}	91 ± 0.46^{a}	0.92 ± 0.007^{a}	2.20 ± 0.02^{a}	0.07 ± 0.01^{e}
70 °C -10% BLP	1781 ± 71^{bc}	1536 ± 23^{b}	245 ± 52^{c}	2970 ± 9^{bc}	1434 ± 16^{bc}	5.35 ± 0.04^{d}	89 ± 0.40^{cde}	0.86 ± 0.024^{b}	1.93 ± 0.02^{cde}	0.17 ± 0.04^{c}
70 °C -15% BLP	1409 ± 24^{def}	1332 ± 37^{cde}	76 ± 15^{e}	2686 ± 53^{cd}	1354 ± 67^{cde}	7.00 ± 0.00^{a}	89 ± 0.05^{bcd}	0.95 ± 0.012^{a}	2.02 ± 0.07^{bc}	0.06 ± 0.01^{e}
70 °C -20% BLP	1235 ± 89^{ef}	1158 ± 82^{ef}	77 ± 8^{e}	2418 ± 135^{de}	1260 ± 53^{de}	7.00 ± 0.00^{a}	89 ± 0.45^{bc}	0.94 ± 0.003^{a}	2.09 ± 0.03^{b}	0.06 ± 0.005^{e}
100 °C -10%-BLP	1650 ± 22^{cd}	1515 ± 45^{bc}	135 ± 32^{cde}	2904 ± 76^{bc}	1389 ± 31^{cd}	5.22 ± 0.04^{e}	87 ± 0.49^{ef}	0.92 ± 0.020^{a}	1.92 ± 0.01^{cde}	0.10 ± 0.03^{de}
100 °C -15% BLP	1499 ± 44^{cde}	1438 ± 37^{bcd}	61 ± 8^{e}	2714 ± 58^{cd}	1276 ± 23^{de}	7.00 ± 0.00^{a}	88 ± 0.83^{de}	0.96 ± 0.005^{a}	1.89 ± 0.01^{de}	0.05 ± 0.01^{e}
100 °C -20% BLP	1298 ± 70^{ef}	1208 ± 76^{ef}	90 ± 6^{e}	2411 ± 103^{de}	1203 ± 29^{e}	7.00 ± 0.00^{a}	86 ± 0.06^{f}	0.93 ± 0.008^{a}	2.00 ± 0.04^{bc}	0.07 ± 0.01^{e}

Mean ± Standard deviation in a row with different superscripts differ significantly ($p < 0.05$).
BLP = Beef lung powder.
-10% BLP = Samples containing 90% semolina flour and 10% BLP.
-15% BLP = Samples containing 85% semolina flour and 15% BLP.
-20% BLP = Samples containing 80% semolina flour and 20% BLP.

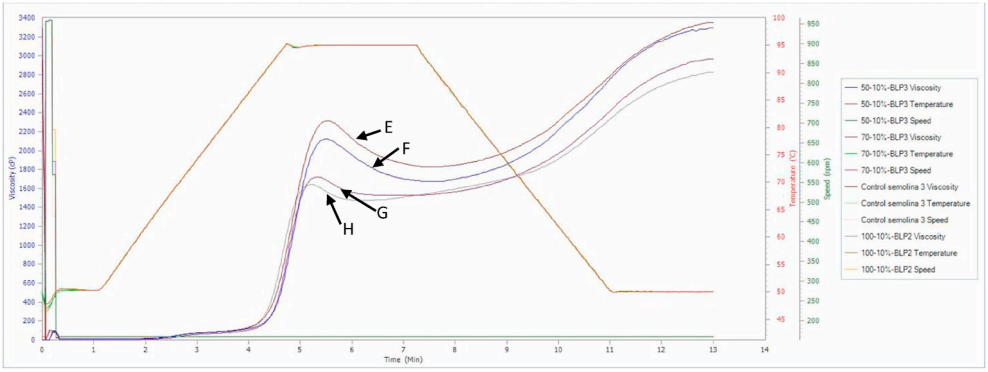

Fig. 2. Effect of drying temperature on viscosity of BLP-semolina flour mixture.

more stable gels. In agreement with these findings, the results of 50 °C BLP substitutions showed an inverse relationship between breakdown viscosity and stability ratio (Table 5). Higher substitution with 50 °C BLP significantly increased the stability ratio which means that the addition of BLP leads to stable gel structure. The stable gels eventually could lead to increased firmness of the products. Brabet et al. (2013) showed a correlation between the stability ratio and firmness of the noodles.

Setback ratio is defined as the ratio of viscosity after completion of cooling (final viscosity) to the viscosity at the onset of cooling (trough viscosity) (Kim, Wiesenborn, Lorenzen, & Berglund, 1996). It is a predictive parameter of retrogradation which directly affects the storage stability (Kim et al., 1996). Increasing the concentration of BLP or the drying temperature, both decreased the setback ratio (Table 5). Further, increasing protein percentage reduced the retrogradation due to reduction of starch concentration (Chen, Schols, & Voragen, 2003). High level of BLP incorporation and high drying temperature appears to favour the BLP supplemented product from retrogradation point of view.

In agreement with the findings of Kumar et al. (2018), relative break down of semolina dough decreased with increased BLP addition. At the breakdown, swollen starch granules break further, and amylose leaches to the solution (Zaidul, Norulaini, Omar, Yamauchi, & Noda, 2007). However, no clear pattern could be identified in relation to drying temperature. The pasting temperature increased by 4° after addition of 10% BLP dried at 50 °C similar to the results observed during addition of whey protein and caseinate (Shin et al., 2010), however, no change was recorded in the temperature after addition of BLP dried at 70 °C and 100 °C. Based on the results of the viscosity parameters, mixtures incorporated with 10% BLP samples dried at 50 °C maintained the viscosity characteristics of semolina dough.

4. Conclusions

The present study demonstrated the feasibility of production of high-quality beef lung powder using air drier at three different temperatures. While all the dried BLP samples were able to bind to three times their weight of water, Hausner ratio for all the BLPs was in the difficult flow range. While 41% of the total iron content in the BLP samples dried at 50 °C was haem iron, it significantly reduced to 29% in BLP samples dried at 100 °C. Specially readily absorbable haem iron was significantly preserved (247 μg/g) at 50 °C temperature. All BLP samples dried at different temperatures produced microbiologically safe products and the microbial counts (total viable, yeast/mould and coliform) remained within the permissible limits even after six months of storage. BLP samples dried at 50 °C showed comparatively better functional properties and protein- and lipid-oxidative stability during storage. The study has expanded our understanding about the effect of drying temperatures on quality and storage stability of protein-based matrices. Results of the study indicated the potential of BLP dried at 50 °C as a protein ingredient for development of cost-effective human food supplements. Future studies should evaluate the effect of other drying methods such as freeze drying and refractance window method on quality and functional properties of BLP powder and compare them with hot air drying. The results of the present study can help future workers in optimizing the processing conditions for further investigations involving other drying methods.

CRediT authorship contribution statement

S. Reshan Jayawardena: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **James D. Morton:** Funding acquisition, Project administration, Conceptualization, Supervision, Writing – review & editing. **Alaa El-Din A. Bekhit:** Conceptualization, Supervision, Writing – review & editing. **Zuhaib F. Bhat:** Investigation, Methodology, Writing – review & editing. **Charles S. Brennan:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that there is no conflict of interest.

Acknowledgement

This manuscript was part of a doctoral thesis submitted to the Department of Wine Food and Molecular Biosciences, Lincoln University, New Zealand.

References

Baghurst, K. (2006). *Nutrient reference values for Australia and New Zealand: Including recommended dietary intakes*. Canberra: National Health and Medical Research Council. https://www.nhmrc.gov.au/about-us/publications/nutrient-reference-values-australia-and-new-zealand-including-recommended-dietary-intakes.

Bhat, Z. F., Morton, J. D., Mason, S. L., & Bekhit, A. E. D. A. (2018a). Calpain activity, myofibrillar protein profile and physicochemical properties of beef *Semimembranosus* and *Biceps femoris* from culled dairy cows during ageing. *Journal of Food Processing and Preservation, 42*. e-13835.

Bhat, Z. F., Morton, J. D., Mason, S. L., & Bekhit, A. E. D. A. (2018b). Pulsed electric field: Role in protein digestion of beef Biceps femoris. *Innovative Food Science & Emerging Technologies, 50*, 132–138.

Bhat, Z. F., Morton, J. D., Mason, S. L., & Bekhit, A. E. D. A. (2019b). Pulsed electric field operates enzymatically by causing early activation of calpains in beef during ageing. *Meat Science, 153*, 144–151.

Bhat, Z. F., Morton, J. D., Mason, S. L., & Bekhit, A. E. D. A. (2020b). The application of pulsed electric field as a sodium reducing strategy for meat products. *Food Chemistry, 306*. https://doi.org/10.1016/j.foodchem.2019.125622

Bhat, Z. F., Morton, J. D., Mason, S., Bekhit, A. E. B., & Bhat, H. F. (2019). Obesity and neurological disorders: Dietary perspective of a global menace. *Critical Reviews in Food Science and Nutrition, 59*, 1294–1310.

Bhat, Z. F., Morton, J. D., Mason, S. L., Jayawardena, R., & Bekhit, A. E. D. A. (2019). Pulsed electric field: A new way to improve digestibility of cooked meat. *Meat Science, 155*, 79–84.

Bhat, Z. F., Morton, J. D., Zhang, X., Mason, S. L., & Bekhit, A. E. D. A. (2020). Sous-vide cooking improves the quality and *in-vitro* digestibility of *Semitendinosus* from culled dairy cows. *Food Research International, 127*, 108708.

Bhat, Z. F., & Pathak, V. (2009). Effect of mung bean (*Vigna radiata*) on quality characteristics of oven roasted chicken *seekh kababs*. *Fleischwirtschaft International, 6*, 58–60.
Bhat, Z. F., & Pathak, V. (2012). Quality evaluation of mutton *harrisa* during one week refrigerated storage. *Journal of Food Science & Technology, 49*, 620–625.
Bishnoi, S., Khanna, N., Bishnoi, N., Tewari, A., Ghadwal, S., & Ahlawat, S. S. (2015). Development of chicken meat powder incorporated instant idli mixes. *Journal of Animal Research, 5*(3), 527.
Brabet, C., Reynoso, D., Dufour, D., Mestres, C., Arredondo, J., & Scott, G. (2013). Starch content and properties of 106 sweetpotato clones from the world germplasm collection held at CIP, Peru, Korea. In *CIP Program report 1997-98* (pp. 279–286). CIP. CIP, Lima.
Cai, Y., & Corke, H. (2000). Production and properties of spray-dried amaranthus betacyanin pigments. *Journal of Food Science, 65*(7), 1248–1252.
Cardoso-Santiago, R. A., & Arêas, J. A. G. (2001). Nutritional evaluation of snacks obtained from chickpea and bovine lung blends. *Food Chemistry, 74*(1), 35–40.
Chen, Z., Schols, H., & Voragen, A. (2003). Physicochemical properties of starches obtained from three varieties of Chinese sweet potatoes. *Journal of Food Science, 68*(2), 431–437.
Darine, S., Christophe, V., & Gholamreza, D. (2010). Production and functional properties of beef lung protein concentrates. *Meat Science, 84*(3), 315–322.
Dua, S., Bhat, Z. F., & Kumar, S. (2015). Effect of oleuropein on the oxidative stability and storage quality of *Tabaq-Maz*, fried mutton ribs. *Food Bioscience, 12*, 84–92.
Francis, G., & Thomas, J. (1975). Isolation and chemical characterization of collagen in bovine pulmonary tissues. *Biochemical Journal, 145*(2), 287–297.
Greene, B., & Cumuze, T. (1982). Relationship between TBA numbers and inexperienced panelists' assessments of oxidized flavor in cooked beef. *Journal of Food Science, 47*(1), 52–54.
Hausner, H. H. (1967). *Friction conditions in a mass of metal powder*. Los Angeles: Polytechnic Inst. of Brooklyn. Univ. of California.
Hornsey, H. (1956). The colour of cooked cured pork. I.-estimation of the nitric oxide-haem pigments. *Journal of the Science of Food and Agriculture, 7*(8), 534–540.
Hu, L., Ren, S., Shen, Q., Chen, J., Ye, X., & Ling, J. (2017). Proteomic study of the effect of different cooking methods on protein oxidation in fish fillets. *RSC Advances, 7*(44), 27496–27505.
Hurrell, R., & Egli, I. (2010). Iron bioavailability and dietary reference values. *The American Journal of Clinical Nutrition, 91*(5), 1461S–1467S.
Jahnen-Dechent, W., & Ketteler, M. (2012). Magnesium basics. *Clinical Kidney Journal, 5*(Suppl 1), i3–i14. https://doi.org/10.1093/ndtplus/sfr163
Jamwal, A., Kumar, S., Bhat, Z. F., Kumar, A., & Kaur, S. (2015). The quality and storage stability of chicken patties prepared with different additives. *Nutrition & Food Science, 45*, 728–739.
Jayawardena, S. R., Morton, J. D., Brennan, C. S., & Bekhit, A. E. A. (2019). Utilisation of beef lung protein powder as a functional ingredient to enhance protein and iron content of fresh pasta. *International Journal of Food Science and Technology, 54*(3), 610–618. https://doi.org/10.1111/ijfs.13927
Kalem, I. K., Bhat, Z. F., Kumar, S., Noor, S., & Desai, A. (2018). The effects of bioactive edible film containing *Terminalia arjuna* on the stability of some quality attributes of chevon sausage. *Meat Science, 140*, 38–43.
Kalem, I. K., Bhat, Z. F., Kumar, S., Wang, L., & Jayawardena, S. R. (2018b). *Tinospora cordifolia*: A novel bioactive ingredient for edible films for improved lipid oxidative and microbial stability of meat products. *Journal of Food Processing and Preservation*, 2018e13774.
Kaur, S., Kumar, S., Bhat, Z. F., & Kumar, A. (2015). Effect of pomegranate seed powder, grape seed extract and tomato powder on the quality characteristics of chicken nuggets. *Nutrition & Food Science, 45*, 583–594.
Kim, Y. S., Wiesenborn, D. P., Lorenzen, J. H., & Berglund, P. (1996). Suitability of edible bean and potato starches for starch noodles. *Cereal Chemistry, 73*(3), 302–308.
Kučerová, I., Marek, Š., & Banout, J. (2018). Solar drying and sensory attributes of eland (*Taurotragus oryx*) jerky. *Journal of Food Quality*. https://doi.org/10.1155/2018/1067672. Article ID 1067672.
Kumar, L., Brennan, M., Zheng, H., & Brennan, C. (2018). The effects of dairy ingredients on the pasting, textural, rheological, freeze-thaw properties and swelling behaviour of oat starch. *Food Chemistry, 245*, 518–524.
Lim, H., Kim, G., Jung, E., Seo, H., Joo, S., Jin, S., et al. (2014). Effect of curing time on the physicochemical and sensory properties of beef jerky replaced salt with soy sauce, red pepper paste and soybean paste. *Asian-Australasian Journal of Animal Sciences, 27*(8), 1174.
Liu, G., & Xiong, Y. L. (2000). Oxidatively induced chemical changes and interactions of mixed myosin, β-lactoglobulin and soy 7S globulin. *Journal of the Science of Food and Agriculture, 80*(11), 1601–1607.
Mahdavi, S. A., Jafari, S. M., Assadpoor, E., & Dehnad, D. (2016). Microencapsulation optimization of natural anthocyanins with maltodextrin, gum Arabic and gelatin. *International Journal of Biological Macromolecules, 85*, 379–385.
Morton, R. D. (2001). In *Aerobic plate count. Compendium for methods of the microbiological examination of foods* (4th ed., pp. 63–67). Washington: American Public Health Association.
MPI. (1995). *Microbiological reference criteria for food. New Zealand*. Retrieved from https://www.mpi.govt.nz/dmsdocument/21185-microbiological-reference-criteria-for-food.
Muir, P., Thomson, B., & Askin, D. (2008). *A review of dressing out percentage in New Zealand livestock*. Wellington: Ministry of Agriculture and Fisheries.
Ohkuma, C., Kawai, K., Viriyarattanasak, C., Mahawanich, T., Tantratian, S., Takai, R., et al. (2008). Glass transition properties of frozen and freeze-dried surimi products: Effects of sugar and moisture on the glass transition temperature. *Food Hydrocolloids, 22*(2), 255–262.
Onwulata, C., & Konstance, R. (2002). Viscous properties of taro flour extruded with whey proteins to simulate weaning foods. *Journal of Food Processing and Preservation, 26*(3), 179–194.
Rahman, M. S., Perera, C. O., Chen, X. D., Driscoll, R. H., & Potluri, P. L. (1996). Density, shrinkage and porosity of calamari mantle meat during air drying in a cabinet dryer as a function of water content. *Journal of Food Engineering, 30*(1), 135–145.
Rostami, H., Dehnad, D., Jafari, S. M., & Tavakoli, H. R. (2018). Evaluation of physical, rheological, microbial, and organoleptic properties of meat powder produced by Refractance Window drying. *Drying Technology, 36*(9), 1076–1085.
Schricker, B., & Miller, D. (1983). Effects of cooking and chemical treatment on heme and nonheme iron in meat. *Journal of Food Science, 48*(4), 1340–1343.
Shin, M., Gang, D.-O., & Song, J.-Y. (2010). Effects of protein and transglutaminase on the preparation of gluten-free rice bread. *Food Science and Biotechnology, 19*(4), 951–956.
Singh, P. K., Kumar, S., Bhat, Z. F., Kumar, P., & Kumar, A. (2015). Effect of processed oats and clove oil on the characteristics and storage quality of aerobically packaged chevon cutlets. *Indian Journal of Small Ruminants, 21*, 76–84.
Singh, P. K., Kumar, S., Kumar, P., & Bhat, Z. F. (2014). Effect of mincing on the quality characteristics of chevon cutlets. *Journal of Animal Research, 4*(2), 193–200.
Southward, C. R. (2003). Casein and caseinates: Uses in the food industry. In B. Caballero (Ed.), *Encyclopedia of food sciences and nutrition* (2nd ed., pp. 948–958). Oxford: Academic Press.
Sujka, M., & Jamroz, J. (2007). Starch granule porosity and its changes by means of amylolysis. *International Agrophysics, 21*(1), 107.
Tonon, R. V., Brabet, C., & Hubinger, M. D. (2010). Anthocyanin stability and antioxidant activity of spray-dried açai (*Euterpe oleracea* Mart.) juice produced with different carrier agents. *Food Research International, 43*(3), 907–914.
Trumbo, P., Yates, A. A., Schlicker, S., & Poos, M. (2001). Dietary reference intakes: Vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. *Journal of the Academy of Nutrition and Dietetics, 101*(3), 294.
Tsakama, M., Mwangwela, A., Manani, T., & Mahungu, N. (2010). Physicochemical and pasting properties of starch extracted from eleven sweet potato varieties. *African Journal of Food Science and Technology, 1*(4), 90–98.
Available from USDA. (2020). *USDA food data central*. USDA Agricultural Research Service. Retrieved 2020/10/01 https://fdc.nal.usda.gov/.
Zaidul, I., Norulaini, N. N., Omar, A. M., Yamauchi, H., & Noda, T. (2007). RVA analysis of mixtures of wheat flour and potato, sweet potato, yam, and cassava starches. *Carbohydrate Polymers, 69*(4), 784–791.