Meat-pulse bars: A novel dried meat snack with added pulse flour and its physicochemical, storage, and sensory properties

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Abstract

Novel beef-pulse bars with addition of pulse flours were produced in this study. Effects of pulse addition on the physicochemical and sensory properties of meat-pulse bars were evaluated, and functional behaviours of pulse flours were revealed and guidance for consumer preferences toward the products were provided.

In Study 1, 12% (w / w) pulse flours were added to beef bars and various treatments were prepared with different pulse flours (black bean, lentil, both infrared (IR) heated), product acidity (pH < 5.0, 5.3) and water activity ($A_w < 0.85, 0.90$) (regulation standards of being shelf-stable). Processing parameters, physicochemical properties, storage behavior, and sensory properties of the products were evaluated. No significant differences were found in processing parameters between beef-black bean and beef-lentil bars, although beef-black bean bars had a darker color and lower Warner-Bratzler (WB) shear values compared to beef-lentil ones. Higher target water activity (0.90) reduced product drying time and cook loss, but did not affect pH and color. Product acidity increased upon the usage of glucono delta-lactone and encapsulated citric acid (to pH below 5.3), but had no impact on other parameters. Storage at high temperature (HT, 40 ºC) introduced greater decrease in product water activity, moisture content, color parameters (L^*, a^*, b^* values), and increase in WB shear values than at room temperature (RT, 20 °C), but relatively stable properties were observed during RT storage. No difference in storage behaviour was observed between beef-black bean and beef-lentil samples, but beef-lentil bars at higher target water activity and hence higher moisture exhibited less changes and more stable properties over storage. Consumer sensory evaluation of the products was performed by young consumers (age 9 - 17) as an in-class activity. They were also asked to complete a survey on their snacking behaviours and attitude toward the products. Hedonic scores of products of all treatments were relatively high (5 out of 6), indicating high consumer acceptance. Consumers from lower age group (9 - 14, n = 72) showed a preference toward less firm texture, and consumers from both groups (9 - 19, n = 95) indicated preference toward moister texture and enhanced flavor. Young consumers agreed that this novel product appeared to be a healthier snack option than those they usually consume, which might help improve the nutrition quality of their diet.

Study 2 was a more comprehensive analysis of the effect of different addition levels of lentil flour (0, 6, 12, 18%, w / w). Lentil flour was also subjected to tempering the seeds for 24 h and subsequent IR heating to achieve higher starch gelatinization level. Beef-lentil bars were added with 6%, 12%, and 18% non-tempered (NT), IR heated flour, and 6% and 12% higher-gelatinized (HG), IR heated flour, and a meat-only control. Neither the addition level nor the seeds tempering, and hence starch gelatinization, of lentil flour showed influence on product cook loss and drying period. Product pH was not changed with addition levels and starch gelatinization of the lentil flour. As the addition level increased, carbohydrate content of meat-lentil bars increased, fat content decreased, but protein content remained constant. During storage, all samples experienced reduced water activity and moisture content, darkening color, increased textural strength, as determined by Warner Bratzler shear and three-point bending tests, and promoted lipid oxidation. Samples with higher addition levels and starch gelatinization of lentil flour demonstrated a firmer and more cohesive texture, potentially contributing to the lesser texture change over storage. Samples with higher addition levels and starch gelatinization of lentil flour displayed a brighter and more yellow color, also associated with lesser change and more stable color over storage. Lentil flour treatment at higher levels (12% and above) was associated with slower lipid oxidation during storage, while differences in starch gelatinization level did not influence lipid oxidation. Take-home packages of samples and accompanying scoresheets were provided to consumers (age 19 - 69, n = 47) for tasting at their convenience. Intensity and hedonic scores of all samples were generally acceptable (6 out of 8), with no difference detected for aroma, flavor intensities and overall acceptability among treatments. However, meat-lentil bars at high lentil flour addition (18% NT) showed color (most yellow) and texture (firmer, driest) differences than control, resulting in lower color and texture acceptances.

In conclusion, the meat-pulse bars developed in this work received high consumer acceptance by both young and adult consumers. With both young and adult consumers viewed the meat-pulse bars as a tasty and healthy snacking option, this positive perception could be leveraged in marketing campaigns to promote the consumption of these products.

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1. Introduction

The worldwide market for meat snacks is projected to grow at a compound annual growth rate of approximately 7% during the forecast period from 2019 to 2029 (Transparency Market Research, 2021). Common meat snacks such as jerky and sausage sticks are dried meat snacks that can be stored in small packets for a long period, making them highly convenient. Also, meat snacks are tasty and savory, high in protein content, and low in calories content, especially for jerky-based products (Schafer, 2014). This allow meat snacks to function as appetite suppressors and energy boosters that are attractive to many other market sectors, especially to the millennial generation (Transparency Market Research, 2021). The meat snack market is an attractive market with a large untapped sector and excellent future growth. Recently, innovative flavors, novel ingredients, and easily accessible packages, have been introduced to the markets and brining new consumers in (Schafer, 2014; Transparency Market Research, 2021). One of the emerging new products, is meat snack bars.

The term “meat bar” was first introduced to the meat snack market by Epic Bar in 2013 (Solomon, 2015). Snack bars made from non-meat ingredients such as granola and whey protein have become highly popular, but many of them are high in sugar, calories, and contain synthetic additives and preservatives. As consumers preference have shifted away from carbohydrates and sugars toward protein and natural products, meat snacks show advantages on low in fat and high in protein contents, limited use of additives and preservatives, and generally perceived as natural and healthy products (Solomon, 2015; Transparency Market Research, 2021). The meat bar was then developed as a jerky- or sausage- like cooked meat product, but in a snack bar form (Solomon, 2015). This allows the product to, while maintaining meat snacks properties, be able to be incorporated with various innovative ingredients, such as fruits and new proteins, and a novel packaging showing better accessibility. The product has soon become consumer-attractive, as the sales of Epic bar tripled on the next year of its launch (Solomon, 2015).

On the other hand, the global demand for animal protein is expected to double by 2050 due to population increase (Alves & Tavares, 2019). This demands the increased production and consumption of plant-based proteins, which show reduced greenhouse gas emissions and lower land demand than animal proteins production, and hence benefits on sustainability and food security (Alves & Tavares, 2019). However, many consumers are not ready to remove animal proteins entirely from their diet. The approach as mixed foods or hybrid proteins, namely, blending consumption of both animal and plant-based proteins, have been suggested as healthier and more sustainable option, and have gained popularity among consumers (Alves & Tavares, 2019; Joseph et al., 2020). Given the inherent adaptability of meat bars to integrate different kinds of ingredients, it emerges as an ideal candidate for this hybrid prototype.

Specifically, this study aimed to develop a snack meat-pulse bar that would be attractive to young consumers as elementary and high school students. Pulses, are dry, edible seeds of plants in the legume family *Leguminosae*, generally containing high protein content and as good sources of plant-based proteins (Hall et al., 2017; Pulse Canada, n.d.). Pulse consumption has been associated with various health benefits, one of which is reduced energy intake and weight management (McCrory et al., 2010). The reason of selecting students as target consumers is that the increased incidences of obesity among children and teenagers have been associated with their increased consumption of energy-dense snacks (Oellingrath et al., 2011). It is expected that the hybrid meat-pulse bars developed in this study will offer a more nutritious, healthier option to consumers than the snacks they usually consume. Early exposure of pulse-containing foods could also reduce food selectivity, decrease food neophobia, enhance taste experience, and change attitude (Galpin et al., 2018; Nystrand & Fjørtoft, 2015). This then help to develop a healthier eating behaviour and improved diet quality.

Furthermore, pulses contain ingredients such as fiber, proteins, and antioxidants that have shown functionalities in food industry. Some studies have utilized pulses and pulse fractions to produce food products, such as cake and pasta, with improved acceptance and nutritional profile (Abu-Ghannam & Gowen, 2021). Pulses and pulse fractions have also been incorporated with meat products, but were mostly focused on products with high moisture and fat contents, such as meatballs and burger patties, but limited on the convenient, shelf-stable meat snacks (Kiosseoglou et al., 2021). The purpose of this study is to develop and produce the novel hybrid snack, meat-pulse bar, and to examine the effect of pulse addition to the physicochemical and sensorial properties of the hybrid bars.

1.1 Study objectives and hypothesis

Objectives, Study 1:

I. To develop and produce novel meat-pulse bars with different pulse flours, and pH and water activity (regulatory factors)

II. To evaluate the physicochemical properties of meat-pulse bars upon treatments on different pulse flours, and pH and water activity (regulatory factors), also to monitor properties change over storage

III. To evaluate the sensory acceptance and consumer preference of the novel meat-pulse bars, specifically for young consumers, and to understand their attitudes toward the novel meat-pulse bars as being a healthier snack option

Hypothesis, Study 1:

I. Novel meat-pulse bars are acceptable hybrid products that fulfill regulatory requirements, and considered as a healthier snack option by young consumers

II. Lower target pH is associated with less firm product texture, brighter color, and more vulnerable to changes during storage

III. Lower target water activity decreases product moisture, and results in firmer texture, darker color, and more vulnerable to changes during storage

IV. Young consumers prefer products with higher pH (lower acidity, brighter color) and water activity (higher moisture, less firm texture)

Objectives, Study 2:

I. To evaluate the physicochemical properties of meat-pulse (lentil) upon treatments on addition level of pulse (lentil) flour, also the storage behaviour upon treatments

II. To produce pulse (lentil) flour with different starch gelatinization levels, and to evaluate the physicochemical properties of meat-pulse (lentil) upon treatments on starch gelatinization level of pulse (lentil) flour, also the storage behaviour upon treatments

III. To evaluate the sensory properties of meat-pulse (lentil) bars upon treatments on addition and starch gelatinization levels of pulse (lentil) flour

Hypothesis, Study 2:

I. Starch gelatinization level of the flour increases as the tempering period of the pulse (lentil) seeds increases

II. Increased addition level of pulse (lentil) flour is associated with increased product carbohydrate, more off-color, firmer and more cohesive texture, and less vulnerable to changes during storage

III. Increased starch gelatinization level of pulse (lentil) flour associates with firmer and more cohesive texture, and less vulnerable to lipid oxidation and changes by storage

2. Literature Review

2.1 Meat consumption and pulses

In 2019, a new version of the Canadian Food Guide was released, which replaced one of the four essential food groups, "meat and alternatives," with "protein foods," with specific instructions to promote the consumption of plant-based proteins such as beans, lentils, nuts, and seeds (Government of Canada, 2019). This shift towards plant-based protein sources signifies a change in dietary preferences from meat to plant-based proteins.

Meat is a valuable source of energy and a range of essential nutrients, including protein and micronutrients such as iron, zinc, and vitamin B12 (Godfray et al., 2018). Meat and meat products have become a staple component of many Western and non-Western diets, with perceptions of sensory enjoyment, satiety, and psychological satisfaction of affording meat (Font-i-Furnols & Guerrero, 2014; Godfray et al., 2018). However, despite global meat consumption continuing to rise, meat consumption in developed regions, particularly North America, has gradually decreased since 2005, driven by health, environmental, and animal welfare concerns (Godfray et al., 2018; Ngapo, 2022). This shift in consumer behavior has contributed to increased interest in alternative and plant-based proteins. It is expected that the global market revenue of plant-based protein foods to reach USD 33.99 billion in 2027 (Andreani et al., 2023).

Although not actively prioritizing it, consumers subconsciously prefer alternative proteins to resemble meat in terms of appearance, taste, texture, cost, and behavior (Joseph et al., 2020). This is likely due to the meat-based foundation of the Western diet, particularly in the US. Consequently, hybrid proteins that combine both meat and alternative plant-based proteins may gain more popularity than either meat-only or meat-free products (Joseph et al., 2020). One alternative protein that has garnered attention is peas, which belongs to the pulse family.

2.1.1 Pulses: a promising source of meat alternatives

Pulses are dry, edible seeds of plants in the legume family *Leguminosae*, characterized by long thin seed pods. They are only harvested as dried grains, and other plants in the legume family that are used for oil extraction, such as soybeans and peanuts, or harvested while still green, such as green beans and fresh peas, are not considered pulses (Pulse Canada, n.d.). Canada is one of the largest pulse producers in the world, and the four main types of pulses grown in Canada are dry peas, lentils, chickpeas, and beans (Pulse Canada, n.d.).

Pulses are rich in protein, containing twice the amount of protein of cereals, making them a good source of plant-based proteins and meat alternatives (Hall et al., 2017). They are also high in dietary fiber, resistant starch, protein, vitamins, and minerals, and low in fat, and hence their consumption has been associated with various health benefits, including lowering blood pressure, reducing the risk of heart disease, maintaining blood glucose levels, introducing antioxidant activities to reduce growth of cancer cells, and lowering cholesterol levels (Hall et al., 2017; Pulse Canada, n.d.).

However, pulse consumption among Canadians remains relatively low. Twenty percent of the interviewees were recognized as non-consumers. Namely, they had not consumed any types of pulse at home or at a restaurant during the past six months. Many of them identified that they disliked the taste of pulses, did not know how to cook them, or were not accustomed to including them in their usual meals, creating barriers to pulse consumption (Ipsos Reid, 2010). On the other hand, Tarrega et al. (2020) produced hybrid burgers with mixed proteins, beef and vegetable, reported higher consumer acceptance toward mixed products than meat-free products, indicating hybrid products as a reliable approach to increase plant-based proteins consumption. Consumers tend to choose meat-pulse products with similar texture, flavor, and appearance to traditional meat products, and marketing strategies on such products as a healthier and more sustainable alternative to meat is recommended (Joseph et al., 2020).

Education campaigns and recipe development programs can also help consumers overcome the barriers of pulse consumption by teaching them how to cook pulses, introducing them to new pulse-based meals, and emphasizing the health and environmental benefits of incorporating pulses into their diets. School intervention on the early exposure of various and healthy food items to children and teenagers have been shown as a useful tool to address issues on food neophobia and selectivity, also provide experience in healthy eating and cooking (Galpin et al., 2018).

2.1.2 Meat-pulse bars: Novel hybrid product

Burgers and nuggets, strips, and cutlets make up 48% of the alternative (and hybrid) protein market, likely due to their popularity in food service and quick service restaurants (Joseph et al., 2020). However, the majority of alternative or hybrid products are currently available only as refrigerated or frozen products (Joseph et al., 2020), with limited options for shelf-stable products. The importance of convenience and availability for consumers of meat alternatives (and hybrid) products suggests a potential market opportunity for shelf-stable hybrid products (Font-i-Furnols & Guerrero, 2014).

Shelf-stable meat products, such as jerky and sausage sticks, also known as meat snacks, are popular due to their high protein, low fat, and low-calorie content, as well as their savory taste. These meat snacks also have the advantage of being easily stored in small packages, making them convenient as appetite suppressors and energy boosters (Schafer, 2014; Transparency Market Research, 2021). Hybrid proteins could potentially serve as substitutes for these meat snacks, as their reduced meat protein content can be compensated by pulses protein, while maintaining the tastiness through the addition of a partial meat component. Hybrid products are also able to undergo the similar production procedures as those of conventional meat snacks, in order to achieve shelf-stability and appear as convenient products.

However, unlike jerky that can be produced from whole muscle, the incorporation of pulses into meat requires proper disintegration and mixing to form a stable mixed product. To overcome this challenge, a new snack form as a meat bar can be introduced.

The term "meat bar" was first introduced to the snack market by Epic Bar in 2013 (Solomon, 2015). While snack bars made from non-meat ingredients like granola and whey protein have been popular, many of them are high in sugar, calories, and synthetic additives and preservatives. Hybrid products with reduced carbohydrate, sugar, and fat, and increased protein content and reduced fat intake are gaining interest.

Snack bars with meat-only proteins can be simply developed by producing reconstructed jerky in a bar-like form. This would also allow for the incorporation of a wide variety of flavors and ingredients. The addition of pulses to the meat-only snack bar is easily accomplished and could create a meat-pulse bar that meets the aforementioned requirements.

2.2 Development of meat-pulse bars

To date, a standardized definition for meat bars has yet to be established. Incorporating pulses into jerky-like meat bar formulations seems both practical and uncomplicated. Various protocols exist for creating shelf-stable meat bars, differentiating between those produced from whole muscle and those from comminuted meat. Barbut (2015) outlined that meat products typically fall into one of four categories: whole muscle, restructured, ground, or coated. Among these, ground products appear most conducive to the incorporation of pulses and pulse ingredients.

2.2.1 Processing of meat-products: Comminution and salt addition

Comminution refers to the process of reducing particle size for the incorporation of raw meat materials into final food products (Aberle et al., 2012). This process is necessary for the production of all meat-containing food products, with the exception of whole-muscle products. Typically, equipment used for comminution, such as meat grinder and bowl chopper, generates a product flow of feeding raw meat materials, subsequently applying a shearing force to reduce the particle size. This yields product particles that are more consistent in size. Furthermore, this process allows a rapid distribution of ingredients and increases tenderness of meat materials (Aberle et al., 2012).

Comminution plays a critical role in meat processing as it facilitates the exposure of more salt-extracted soluble proteins, namely, myofibrillar proteins. These proteins are unique in their ability to gel and interact with other meat components and added ingredients. This interaction results in a gelatinous matrix that directly contributes to the structure building of meat products (Barbut, 2015). This structure then greatly influences several aspects of the production of meat bars and their end-product properties, such as forming, and texture, respectively.

2.2.1.1 Salt addition and structural myofibrillar proteins network

Salt, or sodium chloride, is the most common ingredients added to meat products, and considered as a Generally Recognized As Safe (GRAS) substance (Sebranek, 2009). Salt is highly water soluble and dissociates as sodium (Na^+) and (Cl^-) ions when dissolved in water, which then increase the ionic strength of the solution. When this solution is in contact with raw muscles (meats) and its ionic strength reaches 0.5 or higher, the myofibrils in muscles swell and disintegrate, and myofibrillar proteins (mainly myosin and actin) depolymerize and solubilize (Barbut, 2015; Sebranek, 2009). The solubilized myofibrillar proteins then show hydrophilic, namely, water-loving, instead of hydrophobic, namely, water-rejecting, properties, and prefer to bind and interact with water or other hydrophilic substances. This then extracts and brings solubilized myofibrillar proteins to the surface of raw meat cuts and provides sticky surfaces that bind with moisture. Further agitation of the raw meat materials and sodium chloride solution allows interaction between solubilized myofibrillar proteins from the surfaces of different meat particles, forming a structural network of the gel matrix (Barbut, 2015). Meanwhile, moisture, hydrophilic ingredients, such as soluble sugars, and fine particles, such as spice powders, can be bound or entrapped within the matrix, all of which influence the gelling structure and the dispersion of product ingredients, and hence show critical effect on multiple aspects after thermal treatment, for example, cook yield, texture, taste, color, of end-product properties. A salt concentration of 2% or more the formulations of most meat products will achieve the necessary ionic strength (Sebranek, 2009).

Salt is essential in meat processing because of its multiple functionalities. Besides structural contribution, salt is added in almost all, including whole-muscle ones, processed meat products, because of its distinctive salty flavor. The perception of saltiness is represented by the taste of sodium chloride (NaCl) and lithium chloride (LiCl), whereas no other salts have been found to show suitable substitution by providing additional flavors such as sweet and bitter (Barbut, 2015). However, LiCl is seldomly used, but potassium chloride (KCl) which made impart bitterness is widely used (Barbut, 2015). The sodium cation has been found to be responsible for the salty flavor, also serving as a flavor enhancer for other flavor components in food products (Sebranek, 2009).

Salt is widely used in meat products because it helps preservation. Many microorganisms have shown to be sensitive to high salt levels, possibly due to the occurrence of plasmolysis, by which cellular moisture is withdrawn from microbial cells due to the high salt concentration of surrounding environment (Barbut, 2015). Also, the water activity, namely, the moisture content available for chemical and biological activities, in a meat system is significantly reduced upon salt addition, and is recognized as one of the primary antimicrobial effect of salt in meat products (Sebranek, 2009). Anti-microbial properties become essentially important for products that are ready-to-eat or have extended shelf life, such as dried meat-pulse bars, to ensure consumer safety from perishable food products. Salt has to be added at high level (10 – 15%) to inhibit microbial growth and maintain shelf stability of a food product, but this might result in extremely intense saltiness and unpalatability (Barbut, 2015; Sebranek, 2009). To resolve, salt and nitrite are mostly added together to shelf-stable meat products, a process also knows as curing.

2.2.2 Processing of meat products: Curing

Curing generally refers to the addition of nitrite or nitrite-yielding ingredients, such as nitrate, to a meat system, but sometimes also involves the addition of salt, seasonings, or other ingredients

(Aberle et al., 2012). Nitrate has been used as a meat preservative for centuries, but it was not until the late 1800s that nitrite was determined to be the active compound (Sebranek, 2009). The most important reason for adding nitrite in meat products is to inhibit the growth of *Clostridium botulinum* spores. *C. botulinum* is a gram-positive, anaerobic, spore-forming rod bacteria that produces toxin that causes the disease botulism (Sindelar & Houser, 2009). While spores of *C. botulinum* can germinate, grow, and produce toxin in low-acid foods such as meat products, they are highly resistant to heat and can not be destroyed at temperature that most meat products are cooked, namely, lower than 100 ºC (Barbut, 2015; Sindelar & Houser, 2009). Botulism toxin prevent the proper functioning of specific nerves, leading to muscle paralysis and respiratory failure, and is attributed to be the most lethal of all food-borne diseases (20 – 50% mortality rate) (Sindelar & Houser, 2009).

Many studies have shown that nitrite is highly effective in controlling the growth of *C. botulinum*. Nitrite (sodium nitrite, $NaNO_2$) added to meat products dissolved in hydrophilic phase, yields nitrite ions (NO_2^-) to react with hydrogen (H^+) ions in the low acid (pH 5.5 - 6.0) environment (meat system) to produce nitrous acid (HNO_2) (equation 2.1, 2.2), which is in equilibrium with nitrogen trioxide (N_2O_3) that dissociates to form nitric oxide (NO) (equation 2.2, 2.3) (Sebranek, 2009). Nitric oxide then interferes with iron and sulphur enzymes of *C. botulinum* to prevent its adenosine-triphosphate (ATP) synthesis, and hence inhibit the emergence of vegetative cells from surviving *C. botulinum* spores and prevent the proliferation of vegetative cells that emerged from surviving spores (Barbut, 2015; Sindelar & Houser, 2009). It has been reported that increasing the nitrite level corresponds to an increase in the control of *C. botulinum* growth and toxin production (Sindelar & Milkowski, 2011). Also, salt has been found to show a synergistic effect. Lovenklev et al. (2004) found that sodium chloride at 2.5% (w / v) in the growth medium containing sodium nitrite (37.5, 75 ppm) completely inhibits the growth of *C. botulinum* (growth for treatments with salt addition at 0, 1.25%).

$$NaNO_2 \rightarrow Na^+ + NO_2^- \tag{2.1}$$

$$NO_2^- + H^+ \leftrightarrow HNO_2 \tag{2.2}$$

$$4HNO_2 \leftrightarrow 2N_2O_3 + 2H_2O \tag{2.3}$$

$$2N_2O_3 \leftrightarrow 2NO + 2NO_2 \tag{2.4}$$

Curing of meat products by nitrite have shown effective antimicrobial properties on other pathogens. Models analyzing the growth curves of *Listeria monocytogenes* in inoculated ready-to-eat meat products showed curing as a significant factor, whereas levels of salt concentrations (0.8 - 3.6% w / w) and product moisture contents (45.5 - 83.5% w / w) also showed impacts (Legan et al., 2004). It was previously believed that nitrite curing was not effective in controlling gram-negative pathogens such as *Escherichia coli* and *Salmonella* (Sebranek, 2009). However, recent studies using pathogen growth models have predicted a reduced growth rate of pathogens, including *E. coli O157:H7*, *Staphylococcus aureus*, and *Bacillus cereus*, when nitrite is present at levels used in cured meat products (Sindelar & Milkowski, 2011).

2.2.2.1 Nitrite curing and color of meat products

Curing is preferred in various meat products because it imparts the desired pink color in cured meat products. As aforementioned, when nitrite engages with moisture or raw meat materials, generally containing more than 60% moisture, it yields nitric oxide (NO). This NO reacts with the meat pigment, myoglobin, a protein that is water-soluble and has a heme group with a central iron (Fe) atom (Aberle et al., 2012). The heme group has the capacity to bind with various ligands including oxygen (O_2), carbon monoxide (CO), water, and NO. The specific ligand and the valence state of the Fe within the heme group dictate the color of the meat through the different chemical forms of myoglobin: deoxymyoglobin (purple), oxymyoglobin (bright red), carboxymyoglobin (bright red), and metmyoglobin (brown) (Figure 2.1) (King et al., 2023).

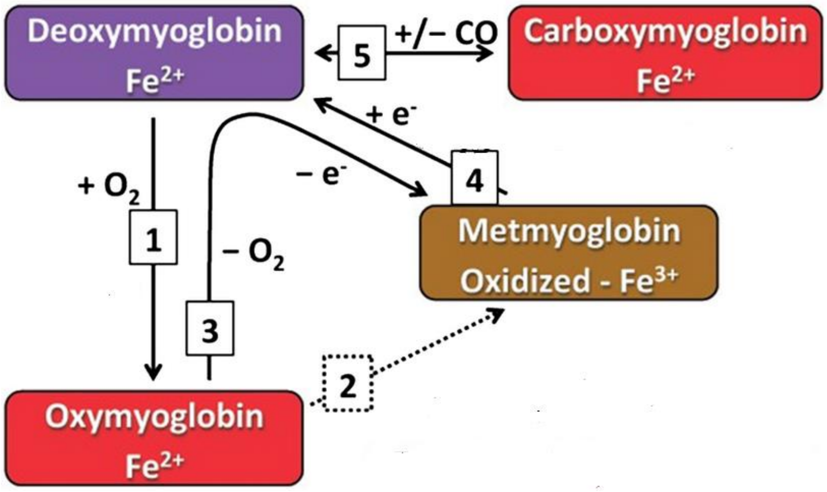


Figure 2.1 Schematic of the interconversions of different forms of myoglobin in meat, depending on ligands and valence state of iron.
Retrieved from (King et al., 2023)

As indicated in Figure 2.1, raw meat materials generally show a bright red color that is attributed to the pigment oxymyoglobin. This pigment emerges when atmospheric oxygen interacts meat myoglobin, with the iron in the heme group remains in its non-oxidized ferrous ion (Fe^{2+}) form. However, at the onset of the curing process, the highly oxidizing nature of nitrite transforms oxymyoglobin into metmyoglobin, the brown pigment form of myoglobin, with an oxidized ferric ion (Fe^{3+}) (King et al., 2023). This is sometimes noticed as the meat browning rapidly after nitrite addition (curing) (Aberle et al., 2012).

On the other hand, meat materials generally contain endogenous reductants such as metmyoglobin reductase, and exogenous reductants such as ascorbate and erythorbate, are generally added to meat materials while curing (Aberle et al., 2012). With the presence of reducing agents, the interaction between NO and myoglobin tend not to form the oxidized metmyoglobin, but a pigment complex, NO-myoglobin, instead, with a reduced Fe^{2+} ion showing a red color. This reaction is amplified in anerobic condition (King et al., 2023). Subsequent processing of cured meat products generally involves heating and acidifying, causing the NO-myoglobin to denature and yield the product NO-hemochrome. NO-hemochrom is the pigment responsible for the characteristic pink color in cured meat products (Barbut, 2015; King et al., 2023).

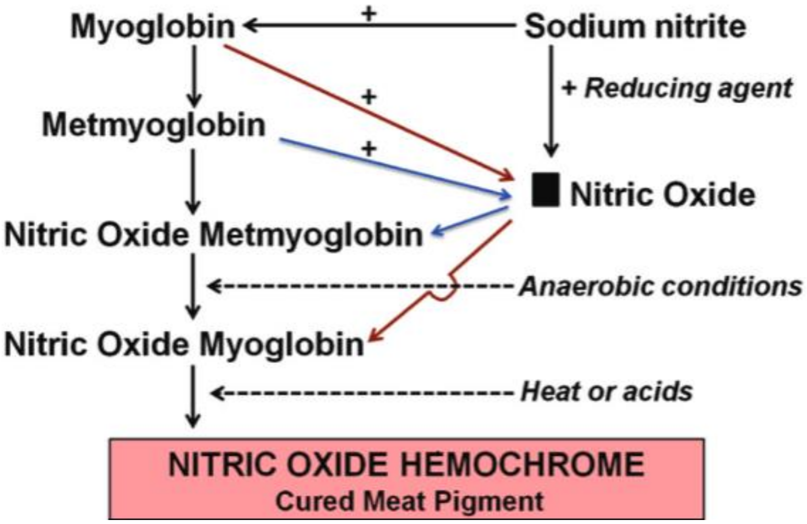


Figure 2.2 Reactions between nitrite and myoglobin to form nitric oxide hemochrome, cured meat color.
Solid arrows indicate reactions, dotted arrows indicate conditions, + indicates a reaction between, and the product is shown by the next arrow.
Retrieved from (King et al., 2023).

Additionally, curing is also extremely important to produce the distinctive cured meat flavor. In sensory studies, panelists were able to distinguish the flavor difference between cured and uncured products, and a nitrite level as low as 50 ppm was found to be sufficient to induce this unique cured meat flavor (Sindelar & Milkowski, 2011). Hundreds of volatile compounds have been identified in cured meats, and some of them were unique compared to uncured (Pegg & Shahidi, 2000; Sindelar & Houser, 2009). However, the principal mechanism and the primary compounds responsible for the unique flavor remain unknown. It is also believed that the inhibitory effect of nitrite on lipid oxidation aids in the development of the distinctive cured meat flavor (Pegg & Shahidi, 2000; Sindelar & Houser, 2009).

2.2.2.2 Lipid oxidation and inhibitory effect by curing

Lipid oxidation in food products refers to the non-enzymatic degradation of unsaturated fatty acids that produces free radicals, eventually leading to the production of off flavors, also known as rancidity (Aberle et al., 2012). It is considered as the major non-microbial cause of quality deterioration in meat products, and have shown impacts on product color, texture, nutritional value, taste, and aroma, that might relate to consumer rejection (Amaral et al., 2018).

Lipid oxidation is a free radical chain reaction that involves three primary steps: initiation, propagation, and termination. Free radicals are highly reactive species that contain one or more unpaired electrons, and can exist independently for a short period of time. Examples of free radicals such as hydroxyl radical ($HO^\bullet$) could be naturally produced in a biological system undergoing aerobic metabolism, such as mitochondria synthesis of ATP (Amaral et al., 2018). Initiation occurs when free radicals that have sufficient reactivity attack and remove a labile hydrogen atom from a fatty acid chain (RH), forming a lipid radical ($R^\bullet$) that quickly reacts with oxygen and generates peroxyl radical ($ROO^\bullet$). The peroxyl radical then abstracts hydrogen from another susceptible chain, such as adjacent RH, producing a lipid hydroperoxide (ROOH) and a new free radical ($R^\bullet$) that is able to continue the chain reaction, namely, propagation. Termination then occurs when two radicals react with each other or self-destruct to form non-radical products (Amaral et al., 2018; Min & Ahn, 2005). However, termination reactions are not always efficient and may result in new reactive compounds. Reactions that involve the decomposition of peroxyl and alkoxyl ($RO^\bullet$) radicals into secondary products such as alkanes, alcohols, and carbonyl compounds (equations 2.8 - 2.11 as examples) ensure the efficient termination of free radical chain reaction (Domínguez et al., 2019).

Initiation: $$\mathrm{RH} + \text{Initiator} \rightarrow \mathrm{R}^{\bullet} + \text{InitiatorH} \tag{2.5}$$

Propagation: $$\mathrm{R}^{\bullet} + \mathrm{O_2} \rightarrow \mathrm{ROO}^{\bullet} \tag{2.6}$$

$$\mathrm{ROO}^{\bullet} + \mathrm{RH} \rightarrow \mathrm{ROOH} + \mathrm{R}^{\bullet} \tag{2.7}$$

Termination: $$\mathrm{ROO}^{\bullet} + \mathrm{ROO}^{\bullet} \rightarrow \mathrm{ROOR} + \mathrm{O_2} \tag{2.8}$$

$$\mathrm{RO}^{\bullet} + \mathrm{RO}^{\bullet} \rightarrow \mathrm{ROOR} \tag{2.9}$$

$$\mathrm{RO}^{\bullet} + \mathrm{R}^{\bullet} \rightarrow \mathrm{ROR} \tag{2.10}$$

$$\mathrm{R}^{\bullet} + \mathrm{R}^{\bullet} \rightarrow \mathrm{RR} \tag{2.11}$$

Lipid oxidation primarily produces hydroperoxides that are odorless and tasteless and generally not interfering with product quality. However, hydroperoxides are not stable and tend to decompose into secondary compounds that contribute to sensory deterioration associated with lipid oxidation, such as off-color and rancid flavor (Domínguez et al., 2019). Many researchers have found that oxidative rancidity was inhibited by nitrite curing of meats. It was reported that nitrite addition at 156 ppm resulted in a significantly lower thiobarbituric acid reactive substances (TBARS), indicator of lipid oxidation level, value than uncured counterparts, in cooked meats (chicken, pork, and beef) stored at 4 ºC for two days (Pegg & Shahidi, 2000). This was also associated with increased sensory hedonic scores. The mechanism of nitrite on inhibiting lipid oxidation is still not clear, but some have suggested that the interaction between nitrite derivatives (NO) and metal, especially iron of heme myoglobin, prevent it from being liberated and denaturing heme. Liberated metal can easily attack fatty acids and initiate lipid oxidation. The nitroso compounds (such as N_2O_3) formed by interaction between nitrite and meat ingredients can also function as radical scavengers to stabilize unsaturated fatty acids and terminate lipid oxidation (Pegg & Shahidi, 2000)

2.2.2.3 Addition of cure accelerators: Reductants

As aforementioned, the curing by nitrite is generally accompanied with the addition of reductants, such as ascorbates and erythorbates. They play an important role in the meat curing process, functioning to facilitate the conversion of nitrite to nitric oxide (N_2O_3), in a liquid environment. Also, they are capable to convert the oxidized metmyoglobin, brown pigment, back the reduced myoglobin, red pigment, which later react with nitric oxide to produce the characteristic cured meat color. Acidic environment is favored for the formation of nitric oxide, and hence acidulants, such as glucono delta-lactone (GDL) and encapsulated citric acid (ECA) are generally added together with reductants to show a synergistic effect (Barbut, 2015).

2.2.3 Thermal processing of meat

Once the necessary ingredients for the production of a meat-pulse bar have been identified and their functions clarified, appropriate processing procedures can be considered. Fortunately, processing procedures for traditional meat snacks like jerky and summer sausages have been widely examined and can be modified for meat bar production. One applicable method is the production procedures for restructured jerky, which involves coarse grinding of the meat, adding other ingredients to the ground meat, mixing the meat mixture, stuffing and forming the meat bar (jerky) strip, heating and drying the formed meat bar strip, and properly packing and storing the finished meat bar product (American Association of Meat Processors [AAMP], 2004).

The transformation from inedible raw meat bar strip to a delicious finished meat bar is primarily achieved through the drying or cooking (heating) process. During this process, free water molecules located at or near the surface of the meat bar strip rapidly evaporate or migrate to the surface to evaporate, resulting in a reduction of product moisture. As the temperature continues to increase and more water molecules evaporate, the product drying rate, namely, the loss of product moisture over a constant period of time, quickly increases. This phase is commonly referred to as the constant drying rate phase (Barbosa-Cánovas et al., 1997; Barbut, 2015).

However, continued heating causes the denaturation of extracted myofibrillar proteins, which are the connecting structural proteins of the meat particle network. This denaturation increases the exposure of the hydrophobic groups of extracted muscle proteins, causing more proteins to closely align together. As a result, the space between extracted muscle protein molecules that used to trap water is reduced, forcing water molecules to escape and evaporate. Further protein denaturation under heating leads to the breakdown of the integral protein network into smaller molecules, further reducing the water entrapping space between muscle protein molecules and allowing a large amount of water to escape and evaporate. This process is typically accompanied by a constant or reducing drying rate of the product, indicating that there are not enough free water molecules on the product surface to evaporate, and the product drying rate is limited by the flow rate of internal water molecules migrating to the product surface (Barbosa-Cánovas et al., 1997; Barbut, 2015).

Heating and drying of the meat mixture eventually lead to the complete denaturation (crystallization) of the myofibrillar proteins, resulting in the solid state of the finished product. Additionally, other reactions related to meat components and ingredients, such as starch gelatinization, the Maillard reaction, and the development of product flavor profile through the formation of volatile compounds from lipid and protein degradation, occur and contribute to the final properties profile of the product (Sebranek, 2009).

2.2.3.1 Regulations of shelf-stable meat products

The Canadian Food Inspection Agency (2020) sets safety parameters for shelf-stable meat and poultry products stored at room temperature, commonly known as shelf-stable meat snacks. Such products must meet the following safety parameters to be considered shelf-stable: a) product water activity of 0.85 or lower, regardless of product pH, b) finished product pH of 4.6 or lower, regardless of product water activity, or c) product water activity of 0.90 or lower, while the pH of the finished product is 5.3 or lower. Water activity is an indicator of the moisture content in a food product available for chemical and biological reactions. Most bacteria cannot survive in an environment where water activity is ≤ 0.85. The lowest water activity level for growth of the known bacterial pathogen *Staphylococcus aureus* is 0.86 (Barbut, 2015).

Products that meet these safety parameters are considered to be shelf-stable and cannot support the growth of pathogenic and spoilage bacteria (Canadian Food Inspection Agency [CFIA], 2020). However, these parameters only limit bacterial growth and not the growth of yeast and mold, which can cause food-borne illness and reduce product shelf life. Vacuum packaging is generally used for dried meat products to prevent mold growth that occurs only under aerobic conditions. The cooking / heating process applied to almost all meat products, except raw muscle cuts, is highly effective in denaturing bacterial proteins and killing spoilage and pathogenic bacteria such as *Escherichia coli*. Ground meat products, including meat bars, should be heated until their internal temperature reaches 72°C before consumption (CFIA, 2020).

The pH parameter of 4.6 or lower still allows the growth of certain pathogenic bacteria, such as *Staphylococcus aureus* (minimum growth pH: 4) and *Listeria monocytogenes* (minimum growth pH: 4.39), but their growth can be limited by vacuum packaging and the addition of nitrite (Barbut, 2015).

2.2.3.2 Hurdle technology

The CFIA regulation also recognizes the concept of the hurdle effect, which suggests that the microbial stability and safety of a food product is determined by a complex interaction of several factors or hurdles, including temperature, water activity, pH, redox potential, and any other microbiological growth factors. While these parameters are commonly used to control the microbial safety and stability of a food product, the application of certain parameters may be too harsh on a food product and result in unpleasant organoleptic properties. For example, consumers generally do not like meat products with a pH as low as 4.6. Thus, depending on the hurdle effect, an intelligent mix of hurdles can be applied to preserve a food product that is microbially stable while also possessing good sensory properties and maintaining its nutritional and economic values (Leistner, 2000). This can be achieved through proper drying, namely, the removal of available product moisture, or the addition of acidulants, such as encapsulated citric acid, or a combination of both. Encapsulated citric acid is preferred over direct addition of citric acid to meat products because it allows for the slow release of citric acid during meat cooking, instead of immediately acidifying and denaturing the extracted muscle protein, which disrupts the formation of the extracted muscle protein network and results in defective product texture.

2.2.4 Other ingredients in processed meat products

2.2.4.1 Water

When producing shelf-stable snack meat bars, it is necessary to add water to the meat mixture. Water functions as a universal solvent that dissolves, delivers, and uniformly disperses functional ingredients, such as nitrite and salt, throughout the meat emulsion (Sebranek, 2009). Water is critical for the nitrite curing reaction and muscle protein solubilization and has a significant impact on the meat mixture stability, cooked product textural properties, and shelf stability of cured meat products. Moisture content is highly important for shelf-stable meat snack products because it strongly affects their water activity (Barbut, 2015). Water activity is defined as the vapor pressure of the product moisture at equilibrium and at a constant temperature relative to pure water at the same temperature. Water activity indicates the amount of water available for chemical and biological reactions and is used as a major factor in controlling spoilage and pathogenic bacteria, as well as influencing product shelf stability. Although meat snacks that are low in moisture content are generally low in water activity, these two parameters are not always directly associated. The water binding capacity of ingredients and meat components, such as salt, sugar, and proteins, also reduces product water activity.

2.2.4.2 Binders, extenders

Non-meat ccarbohydrates, such as starch, oligosaccharides, and dietary fiber, are widely utilized as binders in snack meat products. Carbohydrates that contain ample hydrophilic units, such as gelatinized starch, can bind with and stabilize entrapped free water molecules in the meat particle network, thus enhancing the stability of the meat mixture. Consequently, these binders contribute to product properties, including cooking yield and texture. Moreover, starch and oligosaccharides are introduced with salivary amylase during chewing, increasing the product's sweetness and improving its taste and flavor profile (Joly & Anderstein, 2009; Lamkey, 2009).

Humectants are commonly added to snack meat products with a high moisture content to improve safety and quality, especially in shelf-stable meat products (Msagati, 2012). Many countries require shelf-stable meat snacks to comply with specific regulation parameters concerning pH or water activity, or both, to ensure their safety and quality. Failure to meet these standards can render products vulnerable to spoilage and pathogenic microbiological activity, adversely affecting their quality and shelf life. Drying meat products to reduce water activity often results in an overly dry texture and unfavorable sensory attributes related to dryness and reduced chewability. To address these concerns, humectants, such as mono- and disaccharides like sucrose, and sugar alcohols like sorbitol, are added to meat products. These molecules contain a high concentration of hydrophilic hydroxyl (-OH) groups that can form hydrogen bonds with water molecules, attracting and immobilizing them. As a result, humectants reduce water activity, improve water-holding capacity, and increase softness by softening and smoothing the extracted muscle protein network by enhancing water entrapment. In particular, simple sugars, such as those found in humectants in dried meat products, may enhance aroma and flavor intensity by increasing product sweetness and promoting Maillard reaction (Msagati, 2012). Brown sugar, which is sucrose with added molasses, may also contribute additional flavor (Msagati, 2012; Sorapukdee et al., 2016).

Non-meat proteins, including soy and whey protein isolates, can be added to meat mixtures and function similarly to extracted myofibrillar protein when their protein hydrophobic groups are denatured, and their amphiphilic materials are exposed. This results in the development of the protein structure of the meat mixture, improving its water and fat holding capacity, stability, and other product properties (Egbert & Payne, 2009). Nonetheless, limited knowledge exists concerning the protein interaction between meat and other non-meat proteins.

2.3 Pulses & pulse ingredients

2.3.1 Pulse carbohydrates

Pulse carbohydrates, such as fiber and starch, have been incorporated into meat products. Similar to other plant carbohydrates, the fiber-rich fraction in pulses has the ability to interact with and absorb water molecules, which enhances the water-holding capacity of the extracted muscle protein matrix. Furthermore, the pulse fiber fraction can function as a thickening agent for the myofibrillar protein gel of the mixture, thereby increasing its stability (Bodner & Sieg, 2009). The addition of pulse fibers has been used to improve fat retention in extra high-fat beef patties and enhance textural properties, cook yield, and fat content in beef patties (Anderson & Berry, 2001).

2.3.1.1 Starch gelatinization of pulses

Pulse starch can also serve as a functional ingredient in meat products but only after undergoing starch gelatinization (X. Wang et al., 2022). Starch gelatinization is a process wherein starch granules are heated in the presence of water. Heating causes starch granules to absorb water, leading to swelling and water absorption of the starch granule, loss of the crystalline structure within the starch granule, and ultimately the breakdown of the starch granule and the leaching of amylose and amylopectin molecules, the two polysaccharides that comprise starch (Wang & Copeland, 2013). The interaction between the leaching amylose and amylopectin molecules and damaged starch granules contribute to the functionalities of pulse starch in meat products, such as pasting (due to swollen starch granules), water absorption, gelling (through the gelation of leached amylose and amylopectin molecules), and fat entrapping (due to the increased viscosity of the liquid phase and gelation) (Hoover et al., 2010; Singh, 2021).

Functions of pulse starch come into play during its gelatinization, which means the gelatinization characteristics of the starch can influence the properties of meat products when used.

These gelatinization characteristics can typically be measured using differential scanning calorimetry (Baks et al., 2007). This technique helps identify the starting temperature and the energy required for gelatinization, ensuring the correct conditions for starch gelatinization are achieved. Furthermore, assessing the extent of starch damage reveals how much amylose and amylopectin molecules have been released from a damaged starch granule. This gives an indication of the level of starch gelatinization level the pulses.

Research has shown that the thickening and setting qualities of pulse flour are heavily determined by the type of starch granule and the content of amylose and amylopectin. Specifically, flours with a higher amount of damaged starch often have a lower thickening quality and result in softer gels (Liu et al., 2020).

2.3.2 Pulse proteins

Upon denaturation and exposure of hydrophobic groups, pulse proteins have amphiphilic properties similar to other plant proteins. Therefore, they can be used as connecting proteins of the meat mixture, contributing to the strength and stability of the extracted protein network. Pulse proteins can function in conjunction with salt-extracted myofibrillar proteins, forming an amphiphilic pulse protein network within the myofibrillar network or acting as a thickening agent within the myofibrillar network gel to stabilize product structure (Kiosseoglou et al., 2021). These functions influence various product properties, including water and fat binding capacity, emulsifying properties, foaming properties, and gelation properties. Several characteristics of meat bars, such as texture, mouthfeel, and cook loss, depend on these functions (Boye et al., 2010).

2.3.3 Pulse antinutrients

Pulses contain many antinutritional compounds that interfere with the digestion process of monogastric species when seeds or flour are consumed. The presence of antinutritional compounds in pulses reduces their acceptability and nutrient bioavailability, with many of these compounds present in the hull of the seeds (Khattab & Arntfield, 2009). Dehulling can be done to remove the hull, which contains antinutritional factors such as tannins that impact protein digestibility and unfavorable color pigments, and also allows for ease of manufacturing (Rudraraju et al., 2021). Processing treatments, including germination, soaking, and heating, are commonly used to eliminate or reduce the effect of antinutritional compounds (Rudraraju et al., 2021). Infrared (IR) heating, also known as micronization, has been shown to be an effective treatment for pulses.

2.3.4 Micronization of pulses

Infrared red (IR) heating, also known as micronization, uses high-intensity infrared radiation with a wavelength of 1800 - 3400 nm as the source of energy. It has been shown to modify multiple functional properties of pulses and pulse ingredients, and suggested to exhibit benefits upon applications in food products (Pathiraje et al., 2023).

IR heating was found to inactive enzyme activities, including those related to undesirable anti-nutrient or oxidative activities. For example, IR heating of lentil flour (surface temperature 115 - 165 °C) reduced the activity of lipoxygenase (70 - 100%), peroxidase (32 - 100%), and trypsin inhibitors (up to 54%), an antinutritional factor that impacts protein digestibility (Pathiratne et al., 2015). Meanwhile, IR heating of chickpea seeds was associated with a decline in pasting temperature, increased oil absorption capacity, and increased degree of starch gelatinization, and increased digestible protein content of yellow pea (Pathiraje et al., 2023). This could be achieved solely by the IR heating of pulses, but tempering, or adjusting moisture content, of pulse seeds, is commonly performed before IR heating. The combined techniques of IR heating and tempering further encourage modifications that influence functional properties.

As mentioned, starch gelatinization is a factor for pulse functionalities. It has been reported that differing levels of starch gelatinization could be achieved by IR heating combining with tempering. Lentil flour with starch gelatinization levels from 17 to 24% were achieved by tempered and IR-heated lentils at different surface temperatures (115, 130, 150, and 165°C), while lentils with less than 23% moisture (non tempered) heated to the same surface temperatures, demonstrated no starch gelatinization (Pathiratne et al., 2015).

Protein solubility is another factor that could be influenced by IR heating and influence pulse functionalities. IR heating was found to reduce the nitrogen solubility and hence protein solubility of lentil flour by 33 - 64% across a pH range of 2 - 9, which was attributed to the heat-induced denaturation of proteins that exposed the hydrophobic regions, facilitating their aggregation in aqueous solutions (Bai et al., 2018). Such lower solubility may adversely affect the functional properties of proteins, such as the stabilization of foams, emulsions, or gels. Thus, employing mild heating conditions is recommended to achieve optimal functionality of pulse-derived ingredients.

Due to the influences on starch gelatinization and protein solubility, IR heating improve the water-holding capacity of the products. Bai et al. (2018) reported that tempered and IR-heated chickpea flour (20% moisture, 115 and 135°C) exhibited significantly higher water holding capacity (1.7 - 1.8 g / g) than untreated flour (1.1 g / g). This has been proposed to be due to the conformational changes of pulse proteins to expose hydrophilic proteins, and increased gelatinized starch, to interact and hence enhanced absorption of moisture (Ma et al., 2011). The moisture absorbing capacity and changes in flour-moisture interactions then influence various functioning properties, including gelling, pasting, and enzyme activities.

2.3.5 Pulses in meat products

Several applications of pulse flours or pulse fractions in meat products have shown beneficial properties. Whole-seed pulse flours and pulse proteins have been added to many high-fat sausage products such as bologna to reduce their fat content while maintaining mouthfeel and texture, creating a low-fat and value-added meat product (Boye et al., 2010). Utilization of pulse flours as extenders in meatball and burger patty formulations has been associated with higher product water-holding capacity than control product (no extender added), increasing the economic value of the meat products (Boye et al., 2010; Kiosseoglou et al., 2021). Incorporation of pulse flours into burger patties has also been reported to improve color stability and reduce lipid oxidation levels, both of which increase product shelf-life and potentially increase product economic value (Shariati-Ievari et al., 2016). Despite the many applications of pulses and pulse fractions in meat products, related research mainly focuses on examining the physicochemical and sensory properties of the end products. The interactions between pulses and pulse fractions and meat particles also remain unknown.

Specifically, pulse proteins or protein-rich fractions gained its popularity on applications in food industry, especially in meat products. Pulse proteins are applied to meat products in varying amounts (2 - 40% w / w) depending on the type of product. Traditional meat products with high fat content require more pulses or pulse proteins due to their fat-binding and emulsifying properties (Kiosseoglou et al., 2021). Fat is usually added to meat products with fine emulsion, such as bologna, to improve product juiciness. Reducing the fat content in meat products results in significantly higher purge losses and a firmer, chewier, and more rubbery texture (Egbert & Payne, 2009; Joly & Anderstein, 2009). To resolve, the incorporation of pulses and pulse proteins in low-fat formulations helps to maintain product textural properties and water-holding capacity (Boye et al., 2010; Kiosseoglou et al., 2021). On the other hand, pulse flours and pulse proteins may increase product toughness in comminut meat products such as meatballs due to their backbone functions. Limited research has been conducted on the use of pulses and pulse ingredients in shelf-stable meat snacks, such as meat bars. Shelf-stable dried meat snacks exhibit a different network structure of the extracted myofibrillar proteins, than homogenized meat products, such as summer sausages (Biscontini et al., 1996; Velinov et al., 1990). This likely lead to the different effects of pulses and pulse ingredients upon application in meat products.

2.4 Properties of snack bars: Instrumental and sensorial measurements

2.4.1 Textural profile

The development of novel meat-pulse bars requires an analysis of their physicochemical properties and sensory evaluation to determine consumer acceptability. In addition to regulatory parameters such as water activity and pH, other product properties including color, appearance, texture, taste and flavor, oxidation level, and storage stability also impact acceptability. Texture is especially important for alternative/hybrid protein products, which can differ significantly from traditional meat-only products in this aspect (Joseph et al., 2020). Textural analysis by Warner-Bratzler shear force, which measures the force required to cut through the sample by a thin blade, has been widely used for jerky textural profiling (American Meat Science Association [AMSA], 2016). However, since this test mainly measures the maximum stress required to completely shear through the sample, it is better suited for rigid than elastic samples, such as hard candy and whole-muscle jerky (Szczesniak, 1963; Szczesniak, 2002). Meat-pulse bars likely exhibit a viscoelastic textural profile due to the salt-extracted myofibrillar proteins network and pulses as functioning ingredients. Therefore, a three-point bending test may be considered to reveal the product's viscoelastic properties in addition to Warner-Bratzler shear force.

The three-point bending test is a classical experiment in mechanics, used to measure the Young's modulus of a material which indicates material elasticity in the shape of a beam. The beam of a certain length, rests on two roller supports and is subject to a concentrated loading force at its center (Figure 2.3). During this stress-strain behavior of the brittle materials, the deflection w_0 at the center of the beam is,

$$w_0 = \frac{PL^3}{48EI} \tag{2.12}$$

where E is the Young's modulus, I is the second moment of area defined by,

$$I = \frac{a^3 b}{12} \tag{2.13}$$

where a is the beam's depth and b is the beam's width. Hence, by measuring the central deflection w_0 and the applied force P, and knowing the geometry of the beam and the experimental apparatus, it is possible to calculate the Young's modulus of the material (Dupen, 2016).

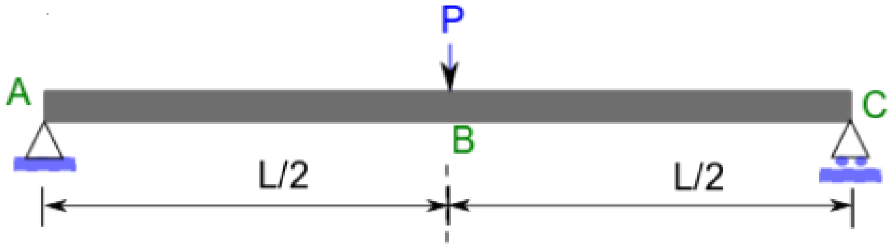


Figure 2.3 Demonstration of three-point bending test.
Retrieved from (Dupen, 2016).

When it comes to the characterization of food products with viscoelasticity, the bending testing is a very useful method. This is because, unlike rigid samples, viscoelastic samples like meat-pulse bars have the potential to deform without complete texture failure. In such samples, the resistance to applied force or compression depends more strongly on its size and modules of deformability, which is a term used to describe the non-linear stress-strain relationship of viscoelastic materials. The three-point bending test can reveal the modulus of deformability, namely, bending of a sample, which can be calculated by the initial slope of the stress-strain ratio graph (Figure 2.4). This is commonly referred to as the stiffness of a food sample, and is associated with characteristics such as elasticity or flexibility, cohesiveness or adhesiveness, and sample fracturability and bendability (*Lightweight 3-point bend*, n.d.), which reveals the strength of internal interactions between networking myofibrillar proteins and pulses.

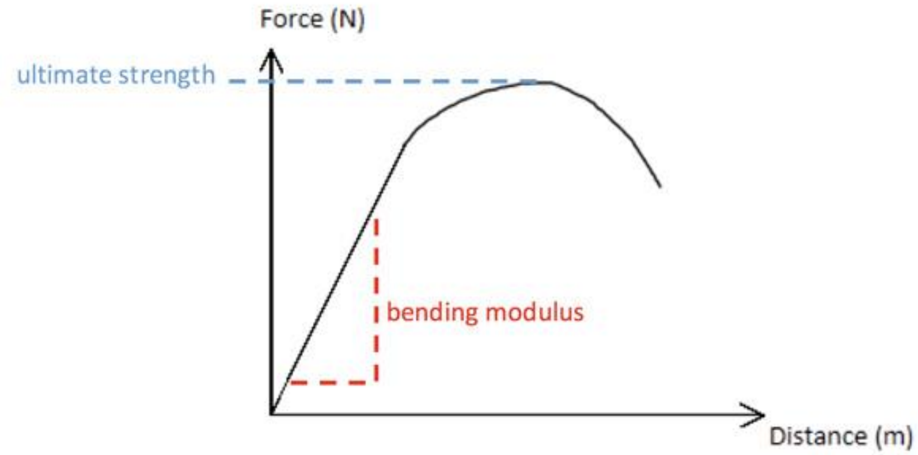


Figure 2.4 Stress-strain graph of three-point bending test.
Retrieved from (Smewing, 2018).

The ultimate strength, which refers to the highest force that a sample can withstand before fracturing or before the stress decreases if the sample does not fracture, can be used to indicate the sample resistant strength toward bending. This strength is determined by subjecting the sample to both compressive and tensile forces, making it a measure of the resistant strength of a more complex system. Unlike a perfectly homogeneous sample, which would have the same ultimate strength for both three-point bending and tensile testing, food products such as meat-pulse bars typically have voids and other defects that result in their tensile strength being lower than their bending strength. The area under the stress-strain graph indicates the energy absorbed by the sample, which could be used to indicate the energy requirement for sample texture failure (Smewing, 2018). However, it should be noted that this measurement only represents the first site of breakage and not complete texture failure. As meat-pulse bars are viscoelastic, they are likely to go through a gradual bending-to-breaking process, with the bending stress decreasing as partial sample breakage becomes more and more irreversible. Eventually, the bending stress rapidly drops to zero as complete texture failure occurs. The work absorbed upon complete texture failure by three-point bending might provide information about properties that could also be represented by Warner-Bratzler shear testing, such as whole-sample firmness and complete fracturability. Few studies exist on three-point bending as a measure of texture of meat snack bars.

2.4.2 Color profile

Meat color is an important attribute that greatly influences consumer perception of meat quality and freshness. As mentioned, the color of meat is primarily determined by the pigment myoglobin, after curing, the pigment nitrosohemochrome gives the product a pink (or red) color (depending on concentration) which is typically favored. Oxidation of nitrosohemochrome results in the formation of brown pigment.

Measurements of color properties of a food product is generally done by CIELAB color parameters L^*, a^*, b^*. The L^* parameter represents lightness, with higher values indicating lighter meat color, while the positive a^* and b^* parameters represent redness and yellowness, respectively (King et al., 2023).

2.4.3 Flavor and aroma

Flavor, a complex sensation more than just taste, but a combination of taste, smell, and even mouthfeel, dictating the overall acceptability of meats and meat products (Afzal et al., 2022). The characteristic meaty flavor and aroma is developed under thermal treatment, generally cooking, of meats and meat products, by which the responsible volatile compounds are produced through the oxidation of ribose phosphate (Mottram, 1998). Additionally, a series of reactions involving carbohydrate degradation, lipid degradation, and Maillard reaction involving thiamine (vitamin B1) degradation and Strecker degradation (converting amino acid to aldehyde) have been identified to produce volatile compounds that contribute to meat flavor (Afzal et al., 2022).

2.4.3.1 Lipid oxidation and flavor

Lipid oxidation is a complex chemical process that occurs in foods containing fats or oils, leading to the degradation of lipids and the formation of off-flavors, off-odors, and potentially harmful compounds (Aberle et al., 2012). One commonly used method to measure lipid oxidation is the thiobarbituric acid reactive substances (TBARS) assay, which quantifies the levels of reactive compounds produced during lipid oxidation. TBARS values serve as an indicator of oxidative deterioration in food products (Du & Bramlage, 1992). Lipid oxidation can be influenced by several factors, including temperature, light, presence of oxygen, and the presence of pro-oxidants or antioxidants.

2.4.4 Storage of snack bars

For shelf-stable products like meat or hybrid snacks, the long-term storage generally introduces unfavorable changes of product physicochemical properties, such as color darkening and off-flavor developed from lipid oxidation. Hence, the inherent capacity of the product to sustain its qualitative attributes and resist such storage-induced deteriorations become important. However, given the constraints of temporal and resource allocations, it is generally impractical to monitor the product throughout its entire storage duration. Hence, accelerated storage experiments emerge as an efficacious alternative. These methodologies are commonly conducted to simulate the effects of prolonged storage within a shorter time period, providing valuable insights into the stability and shelf life of food product. By subjecting samples to elevated temperatures and controlled storage conditions, researchers can observe and quantify the progression of defecting reactions, such as lipid oxidation and evaluate its impact on overall product integrity (Mizrahi, 2004).

2.4.5 Sensory evaluation

Over the past decades, businesses have come to realize that consumer demand is the critical driver of product success (Moskowitz et al., 2012). This realization then promoted the adoption of sensory analysis, a scientific discipline which utilizes human subjects as instruments to evoke, measure, analyze, and interpret subjects perception by senses of sight, smell, touch, taste, and hearing (Lawless & Heymann, 2010; Moskowitz et al., 2012). There are four major sensory attributes in a food item, appearance, odor / aroma / fragrance, consistency and texture, flavor (aromatics, chemical feelings, taste), and they are typically perceived in the mentioned order (Meilgaard et al., 2007). Sensory evaluation is of particular importance in fields that place significant value on consumer-relevant information, one of which is product development. Novel food products such as meat-pulse bars generally lack information on how it, for example, tasted upon consumption, and hence, sensory evaluation become a critical component for product development.

Sensory evaluation techniques could be generally divided into three categories: difference testing, descriptive analysis, and affective testing. Difference testing is the simplest sensory analytic tool, which compare the sensory attribute of two (or more) products that present together, to detect whether there is a difference. Results of this method generally yield as the statistics of frequencies and proportions (counting yes and no answers), producing quick, simple, but not so informative results. This also allows the difference testing become a popular method for the pre-steps of more comprehensive sensory evaluation, such as screening and training of descriptive analysis (Lawless & Heymann, 2010).

Descriptive sensory analysis is a technique used to evaluate products based on the intensity of perceived sensory attributes. This method is considered the most comprehensive and informative tool for sensory evaluation and is commonly used in product development to characterize product changes and address research questions. The information gathered from this analysis can be compared with information generated from consumer acceptance tests and instrumental analysis using statistical techniques such as multivariate regression and correlation. This helps to identify relationships and trends between sensory attributes and other product characteristics, allowing for a more complete understanding of the product (Lawless & Heymann, 2010).

Consumer affective tests are essential for understanding consumer preferences and behaviors towards a product. These tests help sensory scientists to determine the level of liking or disliking of a product by different consumer groups, which is crucial information for product development (Meilgaard et al., 2007; Moskowitz et al., 2012).

Quantitative data collected from consumers play a crucial role in product development. Various types of quantitative data can be obtained, including hedonic or acceptability measures, preference measures, attribute intensity or strength measures, and just-about-right (JAR) measures (Rothman & Jo, 2009).

2.4.5.1 Just-about-right (JAR) scale

While attribute intensity (descriptive) and hedonic (affective) scales provide information on the perceived strength and liking of sensory attributes, respectively, just-about-right (JAR) scale aims to combine attribute intensity and acceptability to understand how the perceived strength of specific attributes relates to the ideal level ("just-about-right") by the respondent. It is usually used alongside preference or acceptance tests, as a diagnostic tool to gain insights into the underlying reasons for hedonic responses. This approach helps identify specific attributes that can be adjusted, and the direction in which they should be modified to improve consumer acceptance. It also allows for shorter questionnaires by combining intensity and hedonic measures in a scale, minimizing respondent burden (Rothman & Jo, 2009).

Due to the combined design, JAR scales are bipolar scales that have an anchored midpoint representing "just about right.” Both ends of the scale are anchored with equal number of categories, to represent intensity levels of the attribute that are higher (or too much) and lower (or too little) than ideal (Moskowitz et al., 2012). The ideal midpoint then serves as the base for JAR scale interpretation, by which the attribute is indicated as too high, too low, or just about right by comparing product perception to ideal level (Rothman & Jo, 2009).

2.5 Meat-pulse bars: A healthier snack?

There have been increased incidences of obesity among children and teenagers, which have been be associated with their increased consumption of energy-dense foods with poor nutrition value (Oellingrath et al., 2011). Common examples of energy-dense foods such as soft drinks and candies account for the majority of snack consumption among young consumers, illustrating their unhealthy snacking behaviour (Gilbert et al., 2012). The development of a nutritious and healthier snack option like meat-pulse bars is expected to encourage students to choose healthier snack options. Additionally, the consumption of pulse-containing foods at lower ages helps consumers to develop healthier eating habits which may persist through their lives, as it reduces food selectivity, enhances the taste experience, and changes altitude toward pulses (Galpin et al., 2018; Nystrand & Fjørtoft, 2015). Overall, the development of meat-pulse bars not only satisfies the current trend of hybrid proteins consumption, but also offers a healthier snack option that bring economic and functional benefits to the food industry.

3. Study 1: Product development of meat-pulse bars for young consumers and examination of physicochemical and sensory properties

3.1 Abstract

Meat-pulse bars incorporating 12% pulse flours were formulated to assess the impact of various factors, namely, the type of pulse flours used (lentil vs. black bean), product water activity, and acidity, on processing parameters, physicochemical properties, storage behavior, and sensory attributes. No differences were observed in processing parameters, such as drying time and cook loss, water activity, and pH between meat-black bean and meat-lentil bars. Meat-black bean bars exhibited lower carbohydrate contents, darker color, and lower Warner-Bratzler (WB) shear values compared to their meat-lentil counterparts. Higher product water activity significantly ($p < 0.05$) reduced drying time and cook loss while altering the proximate compositions, although no such influence was detected on pH and color profile. WB shear values inversely associated with higher product water activity. The inclusion of acidifiers, glucono delta-lactone and encapsulated citric acid, led to increased product acidity but remained inconsequential on other processing and physicochemical attributes. Changes in product physicochemical properties (water activity, moisture content, color measurements, WB shear value) were slight by 180 days of ambient storage (20 °C), but samples of all treatments showed decreased water activity and moisture content, increased WB shear values, and altered color parameters (darker, less red, less yellow), by storage at elevated temperature (40 °C). Sensory evaluations conducted by target consumers, panelists from elementary-school (aged 9 - 14, n = 72) and high-school students (aged 16 - 17, n = 23), indicated a generally high acceptance of all treatments. No different hedonic scores were recorded across treatments for color, smell / aroma, taste / flavor, or overall acceptability by either group. Just-about-right scale ratings intimated that the younger panel might prefer softer and chewier products than the older group, although both groups showed a preference for moister texture and enhanced flavor. Consumers from both groups considered the meat-pulse bars as a healthy and nutritious snacking alternative.

3.2 Introduction

Pulses are not only valuable for their nutritional benefits but also possess functional properties in the food industry. Various pulse flours such as whole seeds, whole-pulse flour, protein fractions, starch fractions, or dietary fiber fractions have been utilized in different food products, exhibiting functionalities such as gelling, thickening, and emulsifying agents (Asif et al., 2013; Boye et al., 2010). Different pulse flours can provide distinct functional properties due to their compositional differences, with lentils, one of the largest crops in Canada, having a different carbohydrate content than lower-production pulses such as black bean (Asif et al., 2013; Tripathi et al., 2021). These differences could impact product properties when utilizing pulses in the food industry.

While meat is highly valued for its nutritional content and sensory appeal, its consumption has been declining due to concerns related to health, the environment, and animal welfare, especially in developed regions like North America (Godfray et al., 2018; Joseph et al., 2020). This has led to increased interest in alternative protein sources, with plant-based proteins gaining attention. However, while consumers show high acceptance for meat alternatives, they subconsciously prefer alternatives that resemble meat in terms of appearance, taste, texture, cost and behaviour (Joseph et al., 2020). This led to the preference of hybrid products that contain both meat and plant-based proteins than meat-only or meat-free products.

Shelf-stable meat snacks like jerky and sausage sticks are popular due to their high protein content, low fat, and convenient packaging (Transparency Market Research, 2021). Hybrid proteins can potentially serve as substitutes for these meat snacks by reducing the meat protein content and incorporating pulses as a partial replacement for the meat component. This allows for the maintenance of taste and savory qualities while also offering the convenience and longer shelf life associated with meat snacks.

The incorporation of pulses into a meat snack to create a hybrid protein product may offer additional advantages. The consumption of energy-dense snacks has been associated with the increased prevalence of obesity among children and teenagers (Liberali et al., 2020; Oellingrath et al., 2011). In response, a school intervention was implemented, which included the introduction of pulses into student diets during school hours. These interventions have been successful in improving the quality and quantity of students nutritional intake (Haileslassie et al., 2020). Meat-pulse bars could provide a complete nutrient profile and reduce hunger between classes due to its high protein content. Regulations have been established to produce meat snacks that limit food spoilage and are shelf-stable to consume, with requirements related to product internal temperature and time, and end-product water activity or pH below certain levels, or in combinations (CFIA, 2020).

The purpose of this study was to develop and produce meat-pulse bars that would appear as an acceptable snack for young consumers, and to examine how pulse flours and certain parameters, such as water activity and pH, influence the physicochemical and sensory properties of meat-pulse bars. Samples were also stored and tested to monitor their physicochemical changes over storage.

3.3 Materials

Beef outside rounds (*Biceps femoris*, replicate 1 & 2: Canada AAA, Cargill Foods, High River, AB, Canada, est. 93; replicate 3: Canada AAA, JBS, Brooks, AB, Canada, est. 38) were purchased from local retailers and stored at refrigerated temperature (≤ +4 °C) for 7 - 12 days post-mortem. Infrared heated (IR) black bean (lot no. 05619) and Eston lentil (lot no. 00219) flours were supplied by InfraReady Products Limited (Saskatoon, SK, Canada). Food-grade salt (sodium chloride), nitrite in the form of Prague powder (6.4% sodium nitrite in salt), and sodium erythorbate were obtained from Canadian Salt Company Ltd. (Pointe-Claire, QC, Canada), Innophos (Lowbanks, ON, Canada), and Unipack Packaging Products Ltd. (Edmonton, AB, Canada) respectively. Dry seasonings (black pepper (32-mesh), garlic powder, onion powder) were obtained from JB's Sausage Maker Supplies Ltd. (Saskatoon, SK, Canada). Brown sugar (dark brown) from Redpath Sugar Ltd. (Toronto, ON, Canada), and soy sauce (gluten-free, preservative-free) from Kikkoman Sales USA Inc. (San Francisco, CA, U.S.A) were purchased from local retailers. Encapsulated citric acid (ECA) (MeatShure, New Hampton, NY, U.S.A) containing 70.0 - 74.0% citric acid, and glucono delta-lactone (GDL) (Malabar, Burlington, ON, Canada) were sourced as acidulants. All materials used in meat-pulse bars production were food-grade and used in accordance with applicable regulations to be considered as generally recognized as safe (GRAS) components.

Concentrated sulphuric acid (Sigma-Aldrich Co., Oakville, ON, Canada), Kjet-Tabs (Fisher Scientific Ltd., Ottawa, ON, Canada), sodium hydroxide (Fisher), boric acid (Fisher), hydrochloric acid (EMD Chemicals Inc., Gibbstown, NJ, U.S.A), sodium carbonate (Fisher), N-point indicator (bromocresol green-methyl red mixed indicator, Fisher), petroleum ether (Fisher), and potassium chloride (Fisher), used for chemical analysis of meat-pulse bars were of ACS grade.

3.3.1 Preliminary production

Trial productions of jerky-like meat bars and meat-pulse bars were conducted using general production procedures and ingredient formulations based on guidelines from the American Association of Meat Processors [AAMP] (2004). Previous studies have shown that adding pulse flours, such as infrared-heated lentil and chickpea flours, at a 6% addition level in pork bologna and beef burgers improved textural properties and oxidative stability compared to meat-only products, also positive consumer acceptability (Kim & Shand, 2022; Shariati-Ievari et al., 2016). In preliminary production, pulse flours were added at different levels ranging from 0% to 24% (w / w), and mixed with other dry ingredients, with the sum of beef and pulse flour contents staying equal, partially replacing the protein content delivered by beef. However, higher addition levels of pulse flours, above 12%, resulted in difficulties in achieving proper mixing and forming of intact bars. Preliminary tastings of these meat-pulse bars revealed dryness, powdery texture, and lack of flavor, particularly at higher pulse flour additions. Additional ingredients including added moisture, soy sauce, brown sugar, and spices such as black pepper were incorporated to enhance product moistness, cohesiveness, and flavor. Oven schedules from (AAMP, 2004) were adjusted to fit the equipment capacity in this study, also to optimize product moistness and integrity. The smoking step typically used in jerky production was excluded. Additionally, various trials were conducted to determine the appropriate amount of acidulants required to meet pH requirements of the products.

3.4 Methods

3.4.1 Production of pulse flours

The procedures to attain infrared (IR) heated pulse flour were adapted from Pathiratne et al. (2015). Black bean and Eston lentil seeds were processed by infrared (IR) heating using a laboratory-scale grain micronizer (Micronizer: model A 156409-B0 FMC, Micronizing Company Ltd., Suffolk, U.K., vibrating conveyor, and feeder: Syntron Material Handling Co., Saltillo, MS U.S.A) to reach a surface temperature of 150 °C. The conveyor vibrating speed was adjusted to control the holding period of the black bean and lentil seeds under IR heating, and the surface temperatures of the seeds at the end of the vibrating conveyor was taken with an infrared thermometer to ensure the desired temperature was reached. The black bean and lentil seeds were then flaked by a roller mill (Apollo Machine and Products Ltd., Saskatoon, SK), then milled twice using a grain mill (The Kitchen Mill, model 91, Blendtec Co., Orem, UT, U.S.A). Granulation size of the flours were 300 µm or less. All procedures above were performed at InfraReady Products Ltd. (Saskatoon, Canada).

3.4.2 Initial grinding of meat

On the day of initial meat preparation, the pH of the meat blocks was tested (5.24 - 5.60) to ensure the meat was fresh and dark-cutting meat (pH > 6.0) was avoided. The fat and surface connective tissue of the outside rounds were then manually trimmed off, and the trimmed meat block was then cut into smaller cubes. These cubes were then put into the grinder (model AMHG-24, Biro Manufacturing Co., Lakeside Marblehead, OH, U.S.A.) and ground through a ½'' (6.35 mm), four-blades grinder plate once, yielding coarsely ground meat. The coarsely ground meat was then tumbled and well-mixed in a 150 L vacuum tumbler (The Turbo-Tumbler, model VSM 150, Glass GmbH & Co. KG, Paderborn, Germany) for 1 min under ambient pressure. The tumbled meat was then portioned for each treatment formation and packed in 406 x 508 mm (16'' x 24'') vacuum bags (polyethylene vacuum pouch, 3 µm thick, with oxygen permeability of 7.7 cc / m^2 / 24 h, supplied by MMIS Inc., Aurora, ON, Canada) at 100% vacuum with a vacuum packer (model 550A, Sipromac Co., Drummondville, QC, Canada), and stored in -30 °C freezer until ready for processing (14 - 21 days).

3.4.3 Formulation of meat-pulse bars

Four treatments of meat pulse bars: meat-black bean bar at water activity (A_w) < 0.85 (BB85) meat-lentil bar at A_w < 0.85 (L85), meat-lentil bar at A_w < 0.90 (L90), meat-lentil bar at A_w < 0.90 and pH < 5.3, achieved by adding encapsulated citric acid (ECA) (L90ECA), were produced from formulations shown in Table 3.1. The frozen coarsely ground meats were thawed in +1 °C cooler for approximate 24 h prior to processing. All ingredients except meat were weighed and stored at +4 °C the night before processing. Brown sugar and soy sauce were dissolved in water and well-mixed.

Table 3.1 Formulations of meat-pulse bars, infrared heated black bean & lentil flour. (% w / w).

Ingredients / Replicate	**BB85**	**L85**	**L90**	**L90ECA**
Beef, trimmed	72.55	72.55	72.55	72.55
IR black bean flour[1]	12	0	0	0
IR lentil flour[2]	0	12	12	12
Water	6.1	6.1	6.1	6
Brown sugar	6	6	6	6
Salt	1.5	1.5	1.5	1.5
Prague powder[3]	0.3	0.3	0.3	0.3
Soy sauce	0.6	0.6	0.6	0.6
Glucono delta lactone (GDL)	0.5	0.5	0.5	0.5
Encapsulated citric acid (ECA)	0	0	0	0.1
Sodium erythorbate	0.05	0.05	0.05	0.05
Black pepper	0.2	0.2	0.2	0.2
Garlic powder	0.1	0.1	0.1	0.1
Onion powder	0.1	0.1	0.1	0.1
Total	100	100	100	100

[1] IR: infrared heated, black ben flour (5.1% moisture, 4.1% ash, 1.1% fat, 23.3% protein, pH 6.44)
[2] IR: infrared heated, lentil flour (7.7% moisture, 2.3% ash, 1.0% fat, 23.5% protein, pH 6.30)
[3] Prague powder containing 6.4% nitrite.
BB85: beef-black bean bar, $A_w < 0.85$; L85: beef-lentil bar, $A_w < 0.85$; L90: beef-lentil bar, $A_w < 0.90$; L90ECA: beef-lentil bar, ECA added, pH < 5.3 & $A_w < 0.90$.

3.4.4 Production of meat-pulse bars

Upon processing, coarsely ground beef was passed through a 9.525 mm (3/8’’), four-blades grinder plate once; the finely ground meat was then added with salt and nitrite first and mixed for 1.5 min, then other ingredients were added and mixed for another 1.5 min (Standing Mixer, model BA-20, Berkel Sales & Service Inc., Houston, TX, U.S.A.). The mixture was then put into a stuffer (model EI-20, Mainca Inc., St. Louis, MO, U.S.A.) and stuffed out as meat bar strips using a jerky horn (5.5 x 25.5 mm) onto wire racks. Formed meat bar strips were then covered with plastic and left in +1 °C cooler overnight and subjected to thermal processing the next day.

3.4.4.1 Thermal processing of meat-pulse bars: Heating, Drying

Thermal processing of meat-pulse bars was performed by in a combined oven (SelfCooking Center, model SCC WE 101, Rational Inc., Mississauga, ON, Canada). The oven generates a heating stream that combines steam and hot air, and is able to control the temperature, humidity (steam) portion, and air flow rate of the stream. Though, thermal processing in this study mainly utilized hot-air drying, rather than steam or combined drying. Continuous thermal processing for heating (and drying) was performed as a step-by-step oven schedule (Table 3.2). Temperature and relative humidity inside oven chamber through thermal processing were monitored by measuring dry bulb and wet bulb temperatures. Both parameters were within reasonable range of the set-ups, in which temperature (dry bulb) did not exceed ± 5 °C and relative humidity did not exceed ± 10%. Internal temperature of raw meat-pulse bars was monitored to ensure it reached 71 °C for 15 sec before drying (step 4, Table 3.2) (Canadian Food Inspection Agency [CFIA], 2020). Dry bulb, wet bulb, and product internal temperatures were monitored using a digital thermometer (model TC-08, Omega Engineering Inc., St.-Eustache, QC, Canada), with thermocouples (type T, Omega Engineering Inc.) placed at the cold point of the oven, wrapped in a moist cloth placed at the bottom of the oven, and inserted in the geometric centre of one of the meat-pulse bars located close to the cold point, respectively. Because of the different treatments and hence moisture contents of the raw meat-pulse bars, the total drying time for the products varied. The dried products were then cooled in a +4 °C cooler for 1 hour, and the cook loss was then calculated as follows:

$$\text{Cook loss, \%} = \left(1 - \frac{\text{g dried,cooled strips}}{\text{g raw strips}}\right) \times 100\% \tag{3.1}$$

The dried and cooked product was then vacuum-packaged in 406 x 508 mm (16’’ x 24’’) vacuum bags (full vacuum, 0% air) and stored in +1 °C cooler to equilibrate for seven days then individually vacuum-packaged in 63.5 x 254 mm (2.5’’ x 10’’) (polyethylene vacuum pouch, 3 µm thick, with oxygen permeability of < 60.0 cc / m^2 / 24 h, water vapor permeability of < 10.0 cc / m^2 / 24 h at 38 °C, 100% RH, supplied by MMIS Inc.) and ready for analysis. In this study, a 6000 - 6500 g batch was produced for each treatment per replicate, total of three replicates.

Table 3.2 Set-ups of oven schedule, thermal processing of meat-pulse bars.

Step	Set-up temperature (dry bulb, °C)	Set-up relative humidity (%)	Set-up air flow rate	Set-up period (min)
1	55	20	3	15
2	55	0	3	15
3	55	10	5	30
4	80	45	3	30
5	80	20	5	30
6	80	10	5	*
Total drying period				120 + *

* meat-pulse bars dried to A_w below 0.85 or 0.90, drying times differed by treatments, replicates.

3.4.5 Sample preparation

The meat-pulse bars require sample preparation before analysis. For chemical analysis (pH, proximate composition), samples of meat-pulse bar products were prepared by cutting into small pieces (5 x 5 mm), then ground to fine powder by putting 50 g of sample pieces in a coffee grinder (Cuisinart Central Grinder, model DCG 12-BCC, Conair LLC, Woodbridge, ON, Canada) and ground for 3.5 - 5 min (sample preparation), with mixing and scraping after each minute (modified from AOAC 983.18, 2023). Samples subject to physical analysis (water activity, color, Warner Bratzler shear force) were also cut into pieces that suited equipment requirement (see later sections for details), and bar pieces from multiples positions, shelves etc. were selected to ensure representative sampling. Samples that were not analyzed immediately were vacuum packaged and stored at –30 °C then thawed on ice for approximate 1 h to room temperature prior to analysis. All analysis was done in duplicate per treatment per replicate.

3.4.6 pH

For pH determination, 20 g of prepared sample in a filter bag (Whirl-Pak Filter Sterilized Bags, supplied by VWR International LLC, Mississauga, ON, Canada) was diluted with 80 mL of distilled water, and well-mixed by a stomacher (Stomacher Lab Blender, model BA6021, Seward Ltd., West Sussex, U.K.) for 3 min. The pH of the yielding filtrate was measured by using a pH meter (Accumet Basic Benchtop Laboratory pH/mV meter, model AR15, supplied by Fisher) (AOAC 943.02, 2023). Analysis was performed in duplicate per treatment per replicate.

3.4.7 Proximate composition

The moisture content of samples (was measured by drying the weighted samples (3 - 4 g) in pre-dried aluminum dishes (57 mm, VWR) in air oven at 100 °C for 16 h (overnight). Sample moisture content was calculated per the following equation (AOAC 950.46, 2023):

$$\text{Moisture, \%} = \frac{\text{g sample} - \text{g dried sample}}{\text{g sample}} \times 100\% \tag{3.2}$$

Sample crude protein content was determined by Kjeldahl method (AOAC 981.10, 2023), using 6.25 as conversion factor from nitrogen to protein. Weighed samples (approximate 1.2 g) with two tablets of catalyst Kjel-Tabs were digested by 20 mL of concentrated sulfuric acid under heat treatment (Digestion Unit, Buchi Corp., Flawil, Switzerland) for 1.5 h or until all solutions were clear. The resulting solutions were cooled to room temperature then diluted with 50 mL of distilled water, and reacted with 90 mL of 30% sodium hydroxide solution. Then, the mixtures went through distillation using a steam distillation unit (model K355, Buchi Corp.). Distillate of each sample was collected in an Erlenmeyer flask containing 25 mL of 4% boric acid, and titrated with 0.173 N (standardized by sodium carbonate solution) hydrochloric acid under N-point indicator (bromocresol green and methyl red). End point of titration was indicated by a color change from green to pink. Sample protein content was calculated per the following equation:

$$\text{Protein, \%} = \frac{(\text{mL HCl of sample} - \text{mL HCl of blank}) \times 0.173\ \text{N} \times 0.014}{\text{g sample}} \times 6.25 \times 100\% \tag{3.3}$$

The crude fat content of each sample was determined by lipid extraction of the prepared sample by petroleum ether. Weighed samples (approximate 3 g) were put in cellulose thimbles (VWR International LLC) and mixed with acid-washed sand and pre-dried in an air oven at 100 °C overnight. The pre-dried thimbles were then placed in pre-dried, weighted beakers (Extraction Beaker, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) and extracted with 85 mL of petroleum ether that was added to the beakers, and the extraction was performed using a Gerhardt Soxtherm Extractor and Multistat (C. Gerhardt GmbH & Co. KG) for 7 h. Excess petroleum ether in beakers after extraction was removed by evaporation, and beakers containing extracted fat were cooled to room temperature and weighed. Sample crude fat content was calculated per the following equation (AOAC 960.39, 2023):

$$\text{Crude fat, \%} = \frac{\text{g crude fat}}{\text{g sample}} \times 100\% \tag{3.4}$$

Sample ash content was determined by the combustion method (AOAC 920.153, 2023). Weighed samples (approximate 2.5 g) put in pre-ashed crucibles were incinerated in a Isotemp

Muffle Furnace (supplied by Fisher) at 525 °C overnight. Crucibles containing sample ashes were then cooled to room temperature and weighed. Sample ash content was calculated as per the following equation:

$$\text{Ash}, \% = \frac{\text{g ash}}{\text{g sample}} \times 100\% \tag{3.5}$$

Analysis of proximate composition was performed in duplicate per treatment per replicate.

3.4.8 Water activity

For water activity, dried (and cooked) meat-pulse bars were cut into small pieces (3 x 3 mm) then put to fill half of a sample cup of water activity meter (AquaLab 4TE, Decagon Devices Inc., Pullman, WA, U.S.A). Cups containing sample pieces were placed in the meter chamber, and water activity measured by eletrosensing of water vapor. Saturated potassium chloride solution at room temperature (water activity: 0.84) was used as a standard to calibrate equipment every week, also to check equipment performance on reproducibility (AOAC 978.18, 2023). Each sample cup contained sample pieces from multiple strips, positions, and measurements were performed in at least three times per treatment per replicate.

3.4.9 Instrumental color measurement

Instrumental analysis of meat-pulse bars was performed using a colorimeter (HunterLab Colorflex, model A60-1010-615, Can-Am Instruments Ltd., Oakville, ON, Canada, measuring diameter: 10 mm). Samples strips were cut to pieces 40 mm in length, and placed such that the sample upper surface (opposite to surface in contact with drying rack) faced the sensor of the colorimeter. Sample color was measured as International Commission on Illumination (CIE) lightness, $L*$, redness, $a*$, and yellowness, $b*$, using illuminant A and 10º standard observer. A black cap covering sample pieces on top of the sensor was used to create a black environment, and standard black ($L* = 0$) and white ($L* = 100$) tiles were used to calibrate equipment every day before measurement. Equipment performance was checked by a standard pink tile ($L*$, $a*$, $b*$). Measurement on each sample piece was performed in duplicate, by taking the second reading on the sample after rotating it 90º, and at least four sample pieces from multiple strips, positions per treatment per replicate were measured.

3.4.10 Warner Bratzler shear force test

Samples of meat-pulse bars were analyzed using a TMS-Pro Texture Press (Food Technology Crop. [FTC], Sterling, VA, U.S.A., load cell no. 04-0083-10, 100 N) performing the Warner-Bratzler (WB) shear force test. Samples were cut into pieces 30 mm in length and placed at the center of the base sample holder of a heavy-duty blade set (FTC), with sample upper surface facing toward the blade. Blade movement appropriate to perform WB shear force test was controlled by a pre-set program (Texture Lab Pro (Force), version 1.18-407, FTC), which moved and cut through sample pieces at a constant speed at 200 mm per min. Distance of blade movement (x) and force applied to blade (y) were recorded, and the software produced a relationship graph after the test. The maximum force shown in the graph indicated the greatest force required to cut through samples, namely WB shear force value. Zero position was set 50 mm above the surface of the base sample holder, to allow space to put samples and to help the blade slide in guide ways of the shear slot to ensure samples completely cut. A blank test with no samples, namely, letting the blade to only travel, and cut nothing, through programmed movement was performed every day before measurement, to check equipment performance. Measurements were performed on at least 6 samples pieces from multiple strips, position, per treatment per replicate.

3.4.11 Storage stability

Samples of meat-pulse bars were stored at two different conditions to analyze storage stability. Half was stored at room temperature (RT, approximately 20 °C), and the other was stored in an incubator (Isotemp Incubator, model 550D, supplied by Fisher) with elevated temperature (HT, approximately 40 °C). To monitor physicochemical change over storage, samples were taken and tested on day 1, 30, 90, 180 of RT storage, and day 1, 30, 60, 90 of HT. Physicochemical measurements on day 1 samples were performed on the next day of the end of equilibration, namely, 8 days after the day of product drying (and cooking).

Moisture content, water activity, color, and WB shear values of meat-pulse bars were measured at each storage interval. Samples of HT storage was taken and kept in their vacuum package to cool to room temperature for approximate 30 min prior to analysis. Details for analytical methods were performed as explained above.

3.4.12 Sensory assessments

Due to school schedules and availability of schools participating the study, sensory evaluation of products in this study were performed in two trials. Consumers from elementary school students (9 - 14 years old, consumers E) were recruited and participated in spring 2019, analyzing samples of replicates 1 and 2. Consumers from high school students (15 - 19 years old, consumers H) were recruited and participated in winter 2019, analyzing samples of replicate 3.

Elementary school students from a total of eight classes, two different schools and high school student from two different schools were recruited, and trials were performed in a classroom setting in the schools. All students of the participating classes were provided with an in-class introductory presentation on terms and principles of sensory evaluation, then encouraged to participate in an in-class activity, voluntary tasting and rating of the products. Each consumer was assigned a package including a pen, a bottled water, a consent form, samples to be evaluated and corresponding scorecards, and a supplementary survey. Only data from consumers with parental consent to participate was collected, and a total of 72 consumers E (elementary school students) and 23 consumers H (high school students) received consent.

Meat-pulse strips were cut to pieces 20 mm in width and assigned and presented to consumers with 3-digit code and randomized order. Samples were presented in 76.2 x 101.6 mm (3” x 4”), transparent, resealable polyethylene bags (2 mm thickness, Staples ULC., Richmond Hill, ON, Canada), with each bag containing 3 sample pieces. Six-point hedonic scales (6: like very much, 1: dislike very much) for product color, smell / aroma, texture, taste / flavor, and overall acceptability, and just-about-right scales for product hardness, moistness, chewiness, saltiness, sweetness, and flavor were used. Participating consumers were asked to finish the scorecard quietly and independently, and drink water between samples. They were also asked to complete a supplementary survey on snacking behaviors after finishing tasting. The ethics application of this human-involved study was approved by the University of Saskatchewan behavioral ethics board, with approval from elementary and high schools, and consent and assent from parents and students, respectively (see Appendixes for scoresheet and survey).

3.4.13 Statistical analysis

The least square (LS) means and standard error of means were calculated with the three replicates of all samples. Observed data for drying parameters and end-product properties, water activity and pH, and proximate composition were fitted to a one-way ANOVA model, by factor as treatments, and variables that were measured for storage behaviour (water activity, moisture content, color parameters, and WB shear value) were fitted twice to different one-way ANOVA models, either by factor as treatment or storage time. Scoresheet ratings from sensory evaluation of the samples were collected, in which, due to the experimental design, separate trials were performed. Samples of replicates 1 and 2 were analyzed by consumers E and samples of replicate 3 were by consumers H. Hedonic ratings by consumers E were fitted to a four-way linear mixed model, by factors as treatments, school, gender, and age, and those by consumers H were fitted to a three-way linear mixed model, by factors as treatments, school, and gender. Ratings on just-about-right scale were expressed as the frequency of each rating, producing a frequency table. Responses from surveys were also expressed as frequency tables. R 4.2.2 (2022) was used to perform the analysis. Means were analyzed and separated with the Tukey's method. Significance was declared at p below 0.05.

3.5 Results and discussions

3.5.1 Meat-pulse bars: Processing parameters, regulatory standards

Samples of all treatments were dried until they reached target water activity. As shown in Table 3.3, target water activity was the determinant factor affecting product drying period, in which samples of lower target water activity ($A_w < 0.85$: BB85, L85) required significantly ($p < 0.05$) longer drying times than those of higher ($A_w < 0.90$: L90, L90ECA). This possibly resulted in the significantly higher dry (and cook) loss of BB85 and L85 samples than those of L90 or L90ECA (Table 3.3).

Table 3.3Processing parameters of meat-pulse bars, black bean & lentil.

Treatments	BB85	L85	L90	L90ECA
Drying period (min)	215^a	235^a	155^b	165^b
SEM	8.7			
Cook (and dry) loss (%)	42.1^a	42.9^a	36.7^b	36.7^b
SEM	0.85			
End-drying A_w	0.846^a	0.835^a	0.886^b	0.893^b
SEM	0.0038			
pH	5.49^b	5.46^b	5.45^b	5.22^a
SEM	0.016			

SEM: standard error of the estimated least-square means

^{ab} means within the same raw superscripted by a different letter are significantly different ($p < 0.05$)

BB85: beef-black bean bar, $A_w < 0.85$; L85: beef-lentil bar, $A_w < 0.85$; L90: beef-lentil bar, $A_w < 0.90$; L90ECA: beef-lentil bar, ECA added, pH < 5.3 & $A_w < 0.90$.

The drying periods in this study, necessary to fulfill the water activity regulatory standards, were typically shorter than those proposed in a production manual (AAMP, 2004). This is possibly attributable to the overall lower oven capacity, smaller production batch, higher target water activity, and differential oven settings and drying schedules (section 3.3.1 Preliminary production) applied in this study (AAMP, 2004).

As indicated, the produced meat-pulse bars did fulfill their respective requirements for water activity and pH (Table 3.3). As expected, BB85 and L85 reaching lower water activity (0.846, 0.835, respectively) yielded a higher cook (and dry) loss (~ 42%) than those reaching higher water activity, L90 (A_w 0.886, cook loss 36.7%) and L90ECA (A_w 0.893, cook loss 36.7%). Meanwhile, no marked disparities were identified between BB85 and L85 (or L90 and L90ECA) regarding these two parameters. The resulting dry (and cook) loss observed in this study paralleled findings reported by Kim, Kim, Kim, et al. (2021). They utilized hot air drying as a control in the production of pork jerkies, targeting a water activity below 0.85, and consequently documented a 40% dry loss.

As anticipated, the pH of L90ECA was significantly lower than that of the other treatments (Table 3.3). This confirms that the parameters of L90ECA (pH 5.22) satisfy the regulatory requisites for shelf-stability ($A_w < 0.90$ and pH < 5.3), while L90 (pH 5.45) does not. There was no variation in the pH values of the BB85 (5.49) and L85 (5.46) samples, suggesting that although the pH values for black bean and lentil flours differed (Table 3.1, pH 6.44, 6.30, respectively), they did not appear to modify the acidity of the product. Moreover, the different target water activities and corresponding product moisture content did not yield distinct pH values (L85 vs. L90), upon the same addition level of lentil flour. Comparable pH values (~ 5.4) for beef jerkies have been reported by Shazer et al. (2018), but without the addition of pulse flour and acidulants (glucono delta lactone [GDL], encapsulated citric acid [ECA]).

3.5.2 Meat-pulse bars: Proximate compositions

The proximate compositions of products of all treatments are shown in Table 3.4. As expected by target water activity, the moisture contents of BB85 and L85 were similar, and significantly lower than those of L90 (36.1%) and L90ECA (35.6%). Accordingly, the protein and ash contents of these two treatment samples were significantly higher than those of L90 and L90ECA. The addition of ECA to achieve lower sample pH did not appear to influence proximate composition, neither did different pulse flours, black bean vs. lentil. Meanwhile, L85 surprisingly showed a significantly higher carbohydrate content than that of BB85, while the carbohydrate contents between black bean (~ 66%) and lentil (~ 65%) flours were similar (Table 3.1).

Table 3.4 Proximate composition of meat-pulse bars, black bean & lentil. (%, wet weight basis)

Treatments	**Moisture**	**Protein**	**Carbohydrate**[1]	**Fat**	**Ash**
BB85	30.4^a	31.4^a	26.9^a	5.8^a	5.6^a
L85	28.4^a	32.1^a	28.5^b	5.6^a	5.4^a
L90	36.1^b	28.8^b	25.6^a	4.8^a	4.8^b
L90ECA	35.6^b	29.2^b	25.4^a	5.0^a	4.8^b
SEM	0.99	0.46	0.49	0.30	0.07

[1] Carbohydrate % = 100% - Moisture % - Protein % - Fat % - Ash %

SEM: standard error of the estimated least-square means

^{ab} means within the same column superscripted by a different letter are significantly different ($p < 0.05$)

BB85: beef-black bean bar, $A_w < 0.85$; L85: beef-lentil bar, $A_w < 0.85$; L90: beef-lentil bar, $A_w < 0.90$; L90ECA: beef-lentil bar, ECA added, pH < 5.3 & $A_w < 0.90$.

The most abundant carbohydrate in pulses is starch (Hoover et al., 2010). Both the black bean and lentil flours studied had undergone infrared (IR) heating. Interestingly, Hoover and Manuel (1996) reported that, while lentil starch granules showed deep surface cracks after heat exposure, black bean starch granules maintained smooth surface. The damage of starch granules in lentil flour might resulted in the release of amylose and amylopectin, the two molecules make up starch (Hoover et al., 2010), possibly increasing the levels of non-starch carbohydrates, poly- and oligosaccharides.. This might cause meat-lentil bars to retain more carbohydrates compared to meat-black bean bars, as the extracted myofibrillar proteins structure in meat bars might find it easier to trap the leaching amylose and amylopectin, than intact starch granules (see section 2:

Literature Review), from lentil flour. However, it's crucial to note that these observations are based on crude carbohydrate content by calculational difference, namely, approximate measurement. For a clearer understanding, direct carbohydrate analysis is recommended.

3.5.3 Meat-pulse bars: Physicochemical properties

Analyses were performed to determine the water activity, moisture content, color parameters, and WB shear force value of meat-pulse bars. These parameters were also monitored to demonstrate their behaviours over storage.

3.5.3.1 Water activity

Table 3.5 presents the water activity of meat-pulse bars over storage. As anticipated, L90 and L90ECA exhibited significantly ($p < 0.05$) higher water activity compared to BB85 and L85, through the storage period, under both conditions. Over a 90-days ambient storage (RT), the water activity remained fairly consistent for all treatments, although L90 water activity dropped by day 180. This agreed with findings from Yang et al. (2009), who observed consistent water activity in beef jerky made from *semimembranosus* over a 30-days storage at 25°C. However, when stored at elevated temperature (HT), the water activity of samples significantly declined after 90 days. This was likely due to excess moisture loss encouraged by elevated storage temperature (results discussed later).

Table 3.5 Water activity of meat-pulse bars, black bean & lentil, storing at ambient and elevated temperatures.

Treatments		Day1	Day30	Day60	Day90	Day180	SEM
BB85	RT[i]	0.846^{a1}	0.840^{1}	NA	0.830^{1}	0.820^{1}	0.0060
	HT[ii]	0.846^{a2}	0.812^{2}	0.755^{1}	0.712^{1}	NA	0.0114
L85	RT[i]	0.835^{a1}	0.830^{1}	NA	0.827^{1}	0.819^{1}	0.0087
	HT[ii]	0.835^{a2}	0.806^{2}	0.792^{12}	0.734^{1}	NA	0.0141
L90	RT[i]	0.886^{b2}	0.883^{12}	NA	0.887^{2}	0.869^{1}	0.0031
	HT[ii]	0.886^{b4}	0.872^{3}	0.854^{2}	0.837^{1}	NA	0.0025
L90ECA	RT[i]	0.893^{b1}	0.887^{1}	NA	0.885^{1}	0.873^{1}	0.0068
	HT[ii]	0.893^{b3}	0.871^{23}	0.849^{12}	0.821^{1}	NA	0.0091
SEM		0.0038					

[i] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 60% relative humidity

[ii] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 30% relative humidity

^{ab} means within the same column superscripted by a different letter are significantly different ($p < 0.05$)

^{12} means within the same row superscripted by a different number are significantly different ($p < 0.05$)

SEM: pooled standard error of the estimated least-square means

BB85: beef-black bean bar, $A_w < 0.85$; L85: beef-lentil bar, $A_w < 0.85$; L90: beef-lentil bar, $A_w < 0.90$; L90ECA: beef-lentil bar, ECA added, pH < 5.3 & $A_w < 0.90$.

3.5.3.2 Moisture content

Moisture content of the meat-pulse bars are shown in Table 3.6, and their values through storage were also recorded. Consistent with water activity results, L90 and L90ECA had a higher moisture content than BB85 and L85 across all storage periods and conditions. Except for L90, all samples retained their moisture during 180 days of ambient storage. However, storage at elevated temperature led to significant moisture loss, explaining the reduced water activity. This is expected since the elevated storage temperature encourage molecular movements and facilitate moisture evaporation from moister environment (meat-pulse bars, $A_w > 0.70$) to dryer (ambient air, relative humidity 60%) (Barbosa-Cánovas et al., 1997). Among treatments, BB85 (30.6 - 23.0) seemed to show a more pronounced moisture loss in HT storage compared to L85 (28.7 - 23.3). Similar difference among the storage behaviour of water activities of BB85 (0.846 - 0712) and L85 (0.835 - 0.734) was observed. This might be due to that samples of BB85 were observed to show a crumblier texture. Given that the addition level of pulse flours and the proximate compositions between BB85 and L85 were similar, this distinction may suggest that black bean flour has inferior gelling and water-holding abilities. As discussed, the black bean flour might have higher level of intact starch granules that contain lower levels of the hydrophilic compounds (poly- and oligosaccharides from leaching amylose and amylopectin), and hence reduced capacity to interact with water molecules and the structural myofibrillar proteins. On the other hand, treatments on product acidity did not influence product moisture and its behaviour over storage.

Table 3.6 Moisture content of meat-pulse bars, black bean & lentil, storing at ambient and elevated temperatures.

Treatments		**Day1**	**Day30**	**Day60**	**Day90**	**Day180**	SEM
BB85	RT[i]	30.6^{a1}	29.4^{1}	NA	28.7^{1}	27.9^{1}	0.63
	HT[ii]	30.6^{a3}	27.6^{2}	23.4^{1}	23.0^{1}	NA	0.55
L85	RT[i]	28.7^{a1}	27.4^{1}	NA	27.4^{1}	25.5^{1}	0.88
	HT[ii]	28.7^{a2}	26.9^{12}	23.8^{1}	23.3^{1}	NA	0.95
L90	RT[i]	36.1^{b23}	36.2^{3}	NA	34.3^{12}	33.0^{1}	0.40
	HT[ii]	36.1^{b2}	33.6^{2}	30.2^{1}	29.0^{1}	NA	0.59
L90ECA	RT[i]	36.9^{b1}	36.0^{1}	NA	35.2^{1}	33.8^{1}	1.17
	HT[ii]	36.9^{b2}	35.0^{2}	31.3^{12}	29.1^{1}	NA	1.26
SEM		0.63					

[i] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 60% relative humidity
[ii] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 30% relative humidity
^{ab} means within the same column superscripted by a different letter are significantly different ($p < 0.05$)
^{12} means within the same row superscripted by a different number are significantly different ($p < 0.05$)
SEM: pooled standard error of the estimated least-square means
BB85: beef-black bean bar, $A_w < 0.85$; L85: beef-lentil bar, $A_w < 0.85$; L90: beef-lentil bar, $A_w < 0.90$; L90ECA: beef-lentil bar, ECA added, pH < 5.3 & $A_w < 0.90$.

3.5.3.3 Instrumental color measurements

The color attributes of meat-pulse bars, were measured and delineated by CIE *L**, *a**, *b** values. Table 3.7 presents product lightness (*L**) of meat-pulse bars. Meat-pulse bars at lower water activity (and moisture content) (BB85, L85) showed significantly lower *L** values, namely darker color, compared to those at higher water activity, L90 and L90ECA. This is understandable, as moisture plays a pivotal role in light reflection. Reduced moisture translates to decreased light reflection and, consequently, a darker appearance (King et al., 2023). Different pulse flours (BB vs. L) and acidity levels (L90 vs. L90ECA) did not seem to affect product lightness.

Table 3.7 Instrumental *L (lightness) value of meat-pulse bars, black bean & lentil, storing at ambient and elevated temperatures.**

Treatments	*L**	**Day1**	**Day30**	**Day60**	**Day90**	**Day180**	SEM
BB85	RT^{i}	27.7^{a1}	27.6^{1}	NA	27.1^{1}	27.1^{1}	0.50
	HT^{ii}	27.7^{a1}	26.5^{1}	26.0^{1}	26.2^{1}	NA	0.39
L85	RT^{i}	28.2^{a1}	27.7^{1}	NA	26.7^{1}	26.5^{1}	0.64
	HT^{ii}	28.2^{a2}	26.0^{12}	25.4^{1}	25.0^{1}	NA	0.52
L90	RT^{i}	30.6^{b2}	30.2^{2}	NA	28.9^{12}	28.2^{1}	0.44
	HT^{ii}	30.6^{b3}	28.0^{2}	26.3^{12}	25.1^{1}	NA	0.54
L90ECA	RT^{i}	31.4^{b1}	31.2^{1}	NA	29.9^{1}	28.7^{1}	0.73
	HT^{ii}	31.4^{b3}	28.1^{2}	27.3^{12}	25.1^{1}	NA	0.62
SEM		0.35					

^{i} RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 60% relative humidity

^{ii} HT: samples stored in incubator at elevated temperature, approximate 40 °C, 30% relative humidity

^{ab} means within the same column superscripted by a different letter are significantly different ($p < 0.05$)

^{12} means within the same row superscripted by a different number are significantly different ($p < 0.05$)

SEM: pooled standard error of the estimated least-square means

BB85: beef-black bean bar, $A_w < 0.85$; L85: beef-lentil bar, $A_w < 0.85$; L90: beef-lentil bar, $A_w < 0.90$; L90ECA: beef-lentil bar, ECA added, pH < 5.3 & $A_w < 0.90$.

Samples of all treatments showed no significant change when stored at RT, except for L90. experienced no significant change on lightness through RT storage, except L90. In contrast, when stored under HT, meat-black bean bars retained their initial lightness, though L85 became significantly darker, lower *L** value, by day 60. Both L90 and L90ECA underwent a more pronounced color shift, exhibiting a darker color by day 30.

Table 3.8 presents product redness (*a**) of meat-pulse bars. Similar as lightness, meat-lentil bars at lower water activity (and moisture content) showed a significantly less red color, namely lower *a** value, than those at higher, L90 and L90ECA. This substantiates the earlier finding where products with lower moisture content led to decreased reflection, resulting in a darker color. Additionally, meat-black bean bars showed a less red color than that of meat-lentil bars (L85), which was likely due to the inherent greyish color of black bean flour. Interestingly, despite expectations, product acidity levels did not seem to exert an influence on product redness. It has been reported a more acidic environment is conducive to the conversion of nitrite to nitric oxide (N_2O_3), consequently catalyzing the formation of the characteristic cured meat pigment, nitroso hemochrome (Barbut, 2015; King et al., 2023). Validating this, Yu (2016) reported that at a pH range 3.4 - 5.7, the pigment, nitroso hemochrome, at lower pH, exhibited a redder color and lower extent of color fading over storage, namely, more stable color. This relationship between pH and redness was also substantiated when examining the storage behavior of L90 and L90ECA at RT.

Here, L90ECA displayed a reduction in redness, namely, lower *a** value, at a later point (day 90) than L90 (day 60). However, under HT storage, L90 and L90ECA exhibited similar storage behavior, suggesting the possibility that the storage condition, specifically temperature, may be a more influential factor on product color than acidity. Similar as lightness, BB85 showcased a no significant change and hence more stable redness than L85, especially during RT storage. The storage behavior between L85 and L90 were similar.

Table 3.8 Instrumental *a (redness) value of meat-pulse bars, black bean & lentil, storing at ambient and elevated temperatures.**

Treatments	*a**	**Day1**	**Day30**	**Day60**	**Day90**	**Day180**	SEM
BB85	RT[i]	12.8^{a1}	11.9^{1}	NA	10.8^{1}	9.0^{1}	0.91
	HT[ii]	12.8^{a3}	10.4^{2}	8.0^{1}	7.2^{1}	NA	0.52
L85	RT[i]	15.2^{b3}	13.7^{23}	NA	10.8^{12}	8.2^{1}	0.73
	HT[ii]	15.2^{b3}	9.3^{2}	7.0^{12}	5.8^{1}	NA	0.77
L90	RT[i]	17.9^{c2}	16.6^{2}	NA	12.6^{1}	10.2^{1}	0.78
	HT[ii]	17.9^{c3}	12.7^{2}	9.6^{12}	7.7^{1}	NA	1.01
L90ECA	RT[i]	17.3^{c2}	15.7^{2}	NA	13.5^{12}	9.8^{1}	0.90
	HT[ii]	17.3^{c2}	10.9^{1}	9.6^{1}	6.7^{1}	NA	0.94
SEM		0.46					

[i] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 60% relative humidity

[ii] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 30% relative humidity

^{ab} means within the same column superscripted by a different letter are significantly different ($p < 0.05$)

^{12} means within the same row superscripted by a different number are significantly different ($p < 0.05$)

SEM: pooled standard error of the estimated least-square means

BB85: beef-black bean bar, $A_w < 0.85$; L85: beef-lentil bar, $A_w < 0.85$; L90: beef-lentil bar, $A_w < 0.90$; L90ECA: beef-lentil bar, ECA added, pH < 5.3 & $A_w < 0.90$.

Table 3.9 presents product yellowness (*b**) of meat-pulse bars. L85 showed a significantly less yellow color, namely lower *b** value, than L90 and L90ECA. Given the inherent bright yellow color of lentil flour, this observation further corroborates the earlier finding related to lightness, wherein products with lower moisture content result in reduced reflection and thus a less yellow color. BB85 showed a less pronounced yellow color than L85, which could also be due to the inherent colors of black bean and lentil flour. Similar as lightness and redness, BB85 seemed to show no significant change and hence more stable yellowness than L85 through storage. The storage behavior between meat-lentil bars, L85, L90, and L90ECA on yellowness were similar.

Table 3.9 Instrumental *b (yellowness) value of meat-pulse bars, black bean & lentil, storing at ambient and elevated temperatures.**

Treatments	*b**	Day1	Day30	Day60	Day90	Day180	SEM
BB85	RT^{i}	7.3^{a1}	7.0^{1}	NA	7.0^{1}	6.3^{1}	0.60
	HT^{ii}	7.3^{a1}	6.8^{1}	5.9^{1}	6.3^{1}	NA	0.33
L85	RT^{i}	9.3^{b2}	8.6^{12}	NA	7.1^{12}	6.3^{1}	0.58
	HT^{ii}	9.3^{b2}	6.2^{1}	5.4^{1}	4.8^{1}	NA	0.52
L90	RT^{i}	11.5^{c2}	10.9^{12}	NA	9.6^{12}	8.3^{1}	0.70
	HT^{ii}	11.5^{c2}	8.7^{12}	7.1^{1}	5.8^{1}	NA	0.75
L90ECA	RT^{i}	11.2^{c1}	10.5^{1}	NA	9.8^{1}	8.5^{1}	0.76
	HT^{ii}	11.2^{c2}	8.0^{1}	7.2^{1}	5.4^{1}	NA	0.61
SEM		0.39					

[i] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 60% relative humidity

[ii] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 30% relative humidity

[ab] means within the same column superscripted by a different letter are significantly different ($p < 0.05$)

[12] means within the same row superscripted by a different number are significantly different ($p < 0.05$)

SEM: pooled standard error of the estimated least-square means

BB85: beef-black bean bar, $A_w < 0.85$; L85: beef-lentil bar, $A_w < 0.85$; L90: beef-lentil bar, $A_w < 0.90$; L90ECA: beef-lentil bar, ECA added, pH < 5.3 & $A_w < 0.90$.

In a study by Li et al. (2017), the incorporation of whole-seed lentil flour in beef burgers was shown to reduce myoglobin oxidation, during storage. This preservation of the preferred red pigment, oxymyoglobin, was attributed to the phenolic compounds inherent in lentil seeds. These compounds are known for their antioxidant properties which can delay oxidation processes, and subsequently, color changes in meat products. However, in the context of this study, the seeds of both black bean and lentil underwent dehulling prior to being processed into flour. This procedure essentially eliminates the seed coat, which contains the majority of phenolic compounds (Zhong et al., 2018). Considering this, the superior color retention observed in the meat bars with black bean than lentil might not be associated with its antioxidative properties but rather to the natural color of black beans. The inherent greyish color of black bean flour might mask the visual perception of color changes over time. In contrast, lentil flour, being lighter in color, might make any color alterations more noticeable.

The inclusion of lentil flour made meat-lentil bars appear more yellow compared to other beef and pork jerkies from other studies. The brightness levels (*L** values) for all samples in this study sat on the higher end when compared to other similar products (Shazer et al., 2018; Sorapukdee et al., 2016). These unique color attributes could stem from specific processing decisions like forgoing smoke color or the distinct pH levels in this study. Research has shown mixed results on how storage impacts dried meat products, mostly jerkies, colors. Yang et al. (2009) indicated that product lightness increased as storage increased, but Lim et al. (2013) stated that no major change was observed. It is generally agreed upon that prolonged storage tends to shift colors of dried meat (and hybrid) products to less preferred shades, affecting consumer appeal (Wongwiwat & Wattanachant, 2015).

3.5.3.4 Texture evaluation: Warner-Bratzler shear values

In this study, Warner Bratzler (WB) shear force was employed as a measure for product shear strain. WB shear force is essentially a reflection of the product peak resistance to shearing,, and have been widely studied in meats and meat products to be used as a general indicator for product firmness (AMSA, 2016). As shown in Table 3.10, different types of pulse flour, water activity (and moisture content), and product acidity all showed effects on the WB shear force values of products. L85 samples showed the highest WB shear force (64.5 N), while all the others yielded significantly lower values (44 - 49). Such data suggests that changing the pulse flour, from black bean to lentil, notably lessened the firmness of the product when using black bean flour. Meanwhile, BB85 was observed to show a crumblier texture, which might explain its diminished firmness and lower WB shear value. Considering the difference in carbohydrate compositions between the two, this difference in texture could be attributed to the black bean flour potentially having reduced quantities of the functioning hydrophilic poly- and oligosaccharides (from leaching amylose and amylopectin). This could limit the interaction between the flour and structural proteins in meat, undermining the strength of protein structural network robustness, as demonstrated by the observed crumbly texture.

Table 3.10 Warner-Bratzler shear value of meat-pulse bars, black bean & lentil, storing at ambient and elevated temperatures.

Treatments		Day1	Day30	Day60	Day90	Day180	SEM
BB85	RT[i]	47.9^{a12}	49.5^{12}	NA	46.9^{1}	56.3^{2}	2.02
	HT[ii]	47.9^{a1}	62.9^{2}	83.1^{3}	103.1^{4}	NA	2.60
L85	RT[i]	64.5^{b1}	67.5^{1}	NA	66.0^{1}	71.9^{1}	2.58
	HT[ii]	64.5^{b1}	81.3^{1}	97.5^{12}	123.9^{2}	NA	7.30
L90	RT[i]	44.0^{a1}	47.1^{1}	NA	41.0^{1}	44.2^{1}	3.27
	HT[ii]	44.0^{a1}	48.8^{1}	54.8^{1}	58.3^{1}	NA	3.38
L90ECA	RT[i]	44.7^{a1}	40.2^{1}	NA	45.9^{1}	48.2^{1}	2.90
	HT[ii]	44.7^{a1}	50.3^{12}	57.1^{12}	68.4^{2}	NA	5.05
SEM		1.67					

[i] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 60% relative humidity

[ii] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 30% relative humidity

^{ab} means within the same column superscripted by a different letter are significantly different ($p < 0.05$)

^{12} means within the same row superscripted by a different number are significantly different ($p < 0.05$)

SEM: pooled standard error of the estimated least-square means

BB85: beef-black bean bar, $A_w < 0.85$; L85: beef-lentil bar, $A_w < 0.85$; L90: beef-lentil bar, $A_w < 0.90$; L90ECA: beef-lentil bar, ECA added, pH < 5.3 & $A_w < 0.90$.

As anticipated, water activity and moisture content played a role in product texture. Products with a higher water activity, specifically L90 and L90ECA, showcased significantly lower WB shear values, suggesting a softer texture. This observation can be rationally attributed to the added moisture content expanding the spaces between the structural proteins network, thereby reducing its strength and yielding a softer product.

Contrary to expectations, product acidity seemed to have no impact on firmness. Earlier research by Ma et al. (2011) highlighted that pulse flours, including lentil, chickpea, and pea, exhibited the least protein solubility at a pH of 5 when exposed to heat treatment. Such a constraint on protein solubility from pulses might decrease the volume of hydrophilic components in the protein matrix, leading to a weakened proteins network and potentially softer texture. Yet, Ebert (2021) noted a pH of 5.7 as the isoelectric point for meat proteins extracted using salt, signaling the lowest protein solubility at this pH. A system like the meat-pulse bars in this study (L90ECA: pH 5.22, L90: 5.46), might decrease the solubility of pulse proteins but increase that of meat proteins. These combined factors could result in a uniform texture across products, even with varied pH levels.

Samples of all treatments showed no significant change on WB shear value by day 90, indicating minimal texture change. However, the HT storage appeared to significantly increased product firmness. By day 90 of HT storage, a significant increase was observed in the WB shear values across all treatments, underscoring a significant hardening and alteration in product textural properties. Of all the treatments, BB85 and L85 were the most affected, showing the greatest change in WB shear values. Such a pronounced change suggests that these treatments were particularly susceptible to textural alterations. This could be associated with to the notable moisture reduction in BB85 (Table 3.5).

Similar WB shear force values for beef jerky from *semimembranosus* jerky to that of L85 have been reported by Yang et al. (2009), though instead of increasing, they observed decreasing shear values during storage. In contrast, most other studies reported that the shear force value of meat-based jerkies increased as storage time increased (Lim et al., 2013; Wongwiwat & Wattanachant, 2015).

3.5.4 Meat-pulse bars: Sensory assessments

As mentioned, sensory evaluation of products in this study was performed in two trials, by consumers E (elementary school students) or consumers H (high school students).

3.5.4.1 Hedonic scores: Consumers E

A total of 72 students aged between 9 - 14 evaluated the four meat-pulse bar treatments. The means of hedonic scores for the sensory attributes of each treatment are shown in Table 3.11. Overall, there was no significant difference in the hedonic scores for color, smell / aroma, taste / flavor, and overall acceptability among the four products. However, hedonic scores of texture showed that L85 acquired a significantly ($p < 0.05$) lower rating than those of the other three products. Results of instrumental measurements for product WB shear force also showed significant differences between treatments. Hence, the significantly lower texture hedonic score of L85 from sensory evaluation was likely due to its significantly increased textural strength (firmness, chewiness etc.). Also, products with lower WB shear force values (BB85, L90, L90ECA) appeared to attain higher hedonic scores for texture, indicating that participants might favor the relatively lower-strength (less firm, less chewy etc.) texture of meat-pulse bars. However, as noted above, the lower shear value of BB85 was likely due to its crumblier texture, and hence reduced product textural strength (decrease in chewiness / cohesiveness) and likely higher hedonic texture rating. This also indicated that this group of consumers might favor softer, crumblier products over firmer, more cohesive products. As for color, the significantly darker, less red and yellow color of BB85 than other products, as indicated by instrumental measurement, was not illustrated by color hedonic scores. This might be due to the variations of the participants preferences. Also, this difference in appearance might not be apparent enough for this group of participants to pick up. Panelists appeared to show the most positive attitude toward taste / flavor among all sensory attributes of the products, by rating all products except BB85 above 5 (like moderately) on the six-point scale. Even though it was not significant, meat-lentil bars, especially L85 and L90ECA, showed a notably higher hedonic score for taste / flavor than that of meat-black bean bars (BB85), indicating that the black bean flavor might not be favored. The ratings of overall acceptability of all products were close to 5 (like moderately) (4.76 - 5.07) out of 6, indicating the participants generally liked all of the products. For this group of participants, no significant difference in hedonic ratings between schools, genders, and ages were observed (Table 3.12).

Table 3.11 Means of consumer (E: aged 9 – 14) ratings on the sensory attributes of meat-pulse bars, black bean & lentil. n = 72[1]

Treatments	Color ^{ns}	Smell / Aroma ^{ns}	Texture	Taste / Flavor ^{ns}	Overall acceptability ^{ns}
BB85	4.24	4.25	4.46^{ab}	5.01	4.84
L85	4.24	4.40	4.33^{a}	5.33	5.04
L90	4.32	4.28	4.81^{b}	5.08	4.89
L90ECA	4.35	4.42	4.72^{ab}	5.30	5.12
SE	0.191	0.206	0.204	0.183	0.176

[1] estimated least-square means of 23 responses on six-hedonic scales

[1] 1 = dislike very much, 6 = like very much

^{ns} means in this column do not show significant difference ($p < 0.05$)

^{ab} means within the same column superscripted by a different letter are significantly different ($p < 0.05$)

SEM: standard error of the estimated least-square means

BB85: beef-black bean bar, $A_w < 0.85$; L85: beef-lentil bar, $A_w < 0.85$; L90: beef-lentil bar, $A_w < 0.90$; L90ECA: beef-lentil bar, ECA added, $pH < 5.3$ & $A_w < 0.90$.

Table 3.12 Comparisons of means of hedonic ratings on sensory attributes of meat-pulse bars by school, gender, and age, consumers E, aged 9 – 14

Sensory attributes	School		Gender		Age		
	A (n = 52)	**B** (n = 20)	**Male** (n = 34)	**Female** (n = 38)	**9 – 10** (n = 21)	**11** (n = 25)	**12 - 14** (n = 25)
Color[1]	4.5 ± 0.16	4.0 ± 0.31	3.9 ± 0.248	4.5 ± 0.25	4.5 ± 0.27	4.6 ± 0.26	3.6 ± 0.35
Smell / Aroma[1]	4.6 ± 0.16	4.1 ± 0.32	4.0 ± 0.25	4.6 ± 0.26	4.5 ± 0.29	4.7 ± 0.27	3.8 ± 0.37
Texture[1]	4.7 ± 0.16	4.4 ± 0.31	4.4 ± 0.24	4.6 ± 0.25	4.5 ± 0.28	4.8 ± 0.26	4.3 ± 0.36
Taste / Flavor[1]	5.1 ± 0.13	5.1 ± 0.26	5.0 ± 0.20	5.3 ± 0.21	5.3 ± 0.23	5.2 ± 0.22	4.9 ± 0.30
Overall acceptability[1]	5.0 ± 0.13	4.9 ± 0.26	4.9 ± 0.20	5.0 ± 0.21	5.2 ± 0.23	5.1 ± 0.22	4.5 ± 0.30

ratings on six-point hedonic scales, wherein 1 = dislike very much, and 6 = like very much

[ns] means in this column do not show significant difference ($p < 0.05$)

[ab] means within the same column superscripted by a different letter are significantly different ($p < 0.05$)

SE: pooled standard error of least-square mean of 72 participants

BB85: beef-black bean bar, $A_w < 0.85$, L85: beef-lentil bar, $A_w < 0.85$, L90: beef-lentil bar, $A_w < 0.90$, L90ECA: beef-lentil bar, added ECA, $pH < 5.3$ and $A_w < 0.90$.

3.5.4.2 Hedonic scores: Consumers H

A total of 23 students aged between 16 - 17 evaluated the four meat-pulse bar treatments. The means of hedonic scales on the sensory attributes of each treatment are shown in Table 3.13. Overall, there was no significant difference in the hedonic scores for any of the attributes among the four products. Similar to the consumers E group, this group of panelists rated products of all treatments above 4.5 (4: like slightly) on a six-point scale for color, smell / aroma, and texture, whereas they appeared to favor the attribute, taste / flavor, the most, by rating all products above or close to 5 (like moderately). The ratings for overall acceptability of all products were above 5 (like moderately), indicating the participants generally liked all products. Also, hedonic scores by consumers H were generally higher than those by consumer E, indicating their higher acceptability toward the products than those by the lower age group. However, it should be noted that the standard errors of these hedonic results were relatively large, and the sample size was noticeably small. Hence, these results might not be representative of the larger population of consumers H.

Table 3.13 Means of consumer (H: aged 16 – 17) ratings on the sensory attributes of meat-pulse bars, black bean & lentil. n = 23[1]

Treatments	Color ns	Smell / Aroma ns	Texture	Taste / Flavor ns	Overall acceptability ns
BB85	4.90	4.93	5.10	4.99	5.07
L85	4.99	5.03	4.85	5.45	5.51
L90	4.88	4.63	5.09	5.41	5.41
L90ECA	4.82	4.82	5.33	5.43	5.33
SE	0.19	0.27	0.23	0.20	0.19

[1] estimated least-square means of 23 responses on six-hedonic scales

[1] 1 = dislike very much, 6 = like very much

[ns] means in this column do not show significant difference ($p < 0.05$)

[ab] means within the same column superscripted by a different letter are significantly different ($p < 0.05$)

SEM: standard error of the estimated least-square means

BB85: beef-black bean bar, $A_w < 0.85$; L85: beef-lentil bar, $A_w < 0.85$; L90: beef-lentil bar, $A_w < 0.90$; L90ECA: beef-lentil bar, ECA added, pH < 5.3 & $A_w < 0.90$.

The hedonic scores for product texture by consumers H showed no significant difference between treatments, even though the results of WB shear value did (Table 3.10). This may be due the variations of the participants' preferences, especially with a notably small sample group. However, even though no significant difference was observed, L90ECA showed relatively higher score on texture (5.33, notable difference in magnitude), whereas L85 showed relatively lower score (4.85). This lower hedonic score for L85 agreed with that from consumers E, which again was likely due to the increased textural strength (firmness) and dryness of L85. The not significantly lower hedonic score of L85 might also suggested the increased tolerance of a range of product textures by consumers of the higher age group. Panelists from the higher age group appeared to prefer the texture of a more acidic product (L90ECA) over other products, illustrating a opposite preference than consumers E who rated L90 texture higher than L90ECA. While results of WB shear value showed no difference between L90 and L90ECA, this difference in sensory texture acceptability might be due to the crumblier and dryer texture of L90ECA that was suggested by the "just-about-right" ratings from consumers E (discussed later), also illustrating the effect of product acidity on texture. However, this illustrated an opposite preference of consumers H than consumers E, who gave higher hedonic and "just-about-right" scores for L90, also suggesting that consumers from the higher age group showed higher acceptability for crumblier / dryer products.

No notable differences in hedonic ratings of color of all treatments were detected (neither in significance nor in magnitude), whereas L90 showed relatively lower hedonic score for smell /

aroma (not statistically significant but noticeable in magnitude). BB85 showed considerably lower scores for taste / flavor and overall acceptability than meat-lentil treatments (not statistically significant but notable in magnitude), indicating that black bean flour addition in meat-pulse bars, likely due to the introduction of black bean flavor, was associated with lower consumer acceptability. This also suggested the major impact of taste / flavor when consumers determine product overall acceptability, agreeing with results from (Holmer et al., 2012). However, little research has been conducted on the incorporation of pulse flours into dried meat (and hybrid) snacks. Given that taste / flavor seemed to show greater effect on product overall acceptability than other sensory attributes, further approaches to enhance product flavor are recommended, with the targeted group of consumers (consumers H, aged 16 - 17).

So far, little research has been conducted on the incorporation of pulse flours with dried meat (and hybrid) snacks at this percentage (12% at addition). The addition of pulse flours in meat sausages have been reported to yield acceptable products, but with a general low addition level of pulse flour (< 6%) (Kim & Shand, 2022; Shariati-Ievari et al., 2016). Holliday et al. (2011) was able to produce sausages with pulse addition up to 50%, but Tahmasebi et al. (2016) utilizing response surface methodology indicated a optimal additional level, 7.59%, of pulse flour (pea) in sausages. With the targeted group of young consumers (aged 9 - 17), further approaches to reduce product textural strength and improve product flavor are recommended.

3.5.4.3 Just-about-right ratings: Consumers E

The frequencies of just-about-right scale ratings for sensory attributes of the products by consumers E are shown in Table 3.14. More than 50% of the panelists rated the hardness of BB85, L90, L90ECA on “just about right,” while 44.3% of panelists gave that rating for L85. Also, ratings for hardness of L85 leaned toward “much too hard” / “slight too hard” direction (total 45.8%). This agreed with results from WB shear value measurements and texture hedonic scores. In contrast, ratings of hardness of L90ECA and BB85 leaned toward the “too soft” direction (total 29.1% and 29.1% respectively). L90 showed the highest number of responses at “just about right,” suggesting that the hardness of L90 was the most acceptable among the four treatments. Similarly, the chewiness of L90 appeared to be the most acceptable (78.9%), while products of all other treatments also attained more than 50% of the responses as “just-about-right”. Specifically, ratings for BB85 leaned toward “too crumbly” direction (total 29.1%), providing proof and explanation for its low WB shear value and texture hedonic score. Also, the number of responses at “just-about-right” for L90ECA (66.7%) was relatively lower than that of L90 (78.9%). This aligned with hypothesis that product with lower pH associates with decreased firmness, which was not revealed by results of WB shear values. As expected, even though all treatment products acquired more than 50% “just about right” responses for moistness, L90 (70.4%) and L90ECA (62.0%) showed much higher scores. Among treatments, L85 showed responses leaning toward “too dry” direction (total 38.6%). Though, it should be noted that L90ECA (total 29.5%, pH 5.22) yielded more responses toward “too dry” direction than L90 (total 15.3%, pH 5.45), suggesting that human perception of moistness differed by pH. Results of “just-about-right” scale for hardness, chewiness, and moistness agreed with other results in this study, also indicating that products with lower textural strength and moister texture were more acceptable among consumers E.

Table 3.14 Frequencies of consumer E (aged 9 - 14) ratings on just-about-right scale on the sensory attributes of meat-pulse bars, black bean & lentil. n = 72[1]

Treatments										
Hardness	Much too soft		Slightly too soft		Just about right		Slightly too hard		Much too hard	
	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]
BB85	7	9.7	14	19.4	39	54.2	10	13.9	2	2.8
L85	1	1.4	6	8.6	31	44.3	23	32.9	9	12.9
L90	2	2.8	8	11.3	49	69.0	11	15.5	1	1.4
L90ECA	3	4.1	18	25.0	39	54.2	10	13.9	2	2.8
Chewiness	Much too crumbly		Slightly too crumbly		Just about right		Slightly too chewy		Much too chewy	
	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]
BB85	6	8.3	15	20.8	43	59.7	8	11.1	0	0
L85	1	1.4	14	20.3	40	58.0	13	18.8	1	1.4
L90	0	0	6	8.4	56	78.9	9	12.7	0	0
L90ECA	3	4.2	7	9.7	48	66.7	12	12.7	2	2.7
Moistness	Much too dry		Slightly too dry		Just about right		Slightly too moist		Much too moist	
	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]
BB85	1	1.4	16	22.2	40	55.6	12	16.7	3	4.2
L85	6	8.6	21	30.0	36	51.4	6	8.6	1	1.4
L90	1	1.4	13	13.9	50	70.4	6	8.4	1	1.4
L90ECA	3	4.2	18	25.3	44	62.0	5	7.0	1	1.4
Saltiness	Not nearly salty enough		Not salty enough		Just about right		Slightly too salty		Much too salty	
	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]
BB85	3	4.2	19	26.8	43	60.6	6	8.4	0	0
L85	3	4.4	16	23.2	46	66.7	1	1.4	3	4.3
L90	0	0	13	18.6	53	75.7	3	4.3	1	1.4
L90ECA	4	5.6	14	19.7	45	63.4	7	9.9	1	1.4
Sweetness	Not nearly sweet enough		Not sweet enough		Just about right		Slightly too sweet		Much too sweet	
	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]
BB85	4	5.6	14	19.7	43	60.5	9	12.7	1	1.4
L85	4	5.8	10	14.5	46	66.7	8	11.6	1	1.4
L90	3	4.3	12	17.1	45	64.3	10	14.3	0	0
L90ECA	2	2.9	11	15.7	54	77.1	3	4.3	0	0
Flavor	Much too weak		Slightly too weak		Just about right		Slightly too strong		Much too strong	
	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]
BB85	4	5.5	13	18.0	45	62.5	6	8.3	4	5.6
L85	2	2.9	8	11.4	55	78.5	3	4.3	2	2.9
L90	1	1.4	11	15.5	51	71.8	6	8.4	2	2.8
L90ECA	0	0	10	13.9	56	77.8	4	5.6	2	2.8

[1] frequencies of just-about-right ratings from 72 responses, five-point scale

[2] #: the number of incidences of the rating

[3] %: the frequency of the incidences of the rating by percentage

[3] $\% = 100\% \times \frac{\text{the number of incidences,\#}}{\text{the number of total responses,72}}$

BB85: beef-black bean bar, $A_w < 0.85$; L85: beef-lentil bar, $A_w < 0.85$; L90: beef-lentil bar, $A_w < 0.90$; L90ECA: beef-lentil bar, ECA added, pH < 5.3 & $A_w < 0.90$.

All meat-pulse bar treatments acquired more than 60% responses of "just-about-right" for saltiness and sweetness. L90ECA yielded the highest score for sweetness (77%), and L90 for saltiness (76%). Considering that the salt and carbohydrate contents of L90 and L90ECA were similar, differences in perception might be due to pH effect (5.22 vs 5.45 respectively). Also, despite that the carbohydrate contents of L90 and L85 were significantly different, the number of responses on "just-about-right" for sweetness and saltiness of these two treatment products were similar, indicating that the differences in product physicochemical properties, such as moisture and carbohydrate differences, did not influence human perception of product basic taste. BB85 showed lowest number of responses as "just-about-right" on both of sweetness and saltiness, indicating the taste difference between black bean and lentil flours. Also, the crumblier texture of BB85, might interfere with the proper incorporation of flavoring agents to meat proteins network. Other treatments meanwhile, require a longer chewing time for consumption, which might be associated with better flavor perception. Hence, further examinations on the difference of flavor profile and the network structure between BB85 and other treatments are recommended. Similarly, all treatments showed more than 60% responses as "just-about-right" for flavor, whereas that of L85, L90, and L90ECA above 70%; responses of all treatments slightly leaned toward "too weak" direction, especially for that of BB85 (total 23.5%). Responses of saltiness and sweetness of all treatments leaned toward "not salty enough" and "not sweet enough" directions. The salt content in these products (1.8% at addition) was similar to those recommended in production manual and from other studies (1.5 - 2.0%) (AAMP,2004; Shazer et al., 2018). Considering the market trend that products low in salt and sugar are becoming more popular, adding more salt and sugars was not recommended. Overall, for consumers aged 9 - 14, the meat-pulse bars in this study were generally acceptable, with further improvements suggested to lower product textural strength and enhanced flavoring profile if needed.

3.5.4.4 Just-about-right ratings: Consumers H

The frequencies of just-about-right scale ratings for sensory attributes of the products by consumers H are shown in Table 3.15. Similar to results from consumers E, ratings for hardness of L85 leaned toward "much too hard" / "slight too hard" direction (total 43.5%), while the other three products attained more than 50% “just-about-right” rating. This then agreed with results of WB shear values, despite the not significant hedonic results. Hardness of BB85 leaned toward “too soft” direction (total 45.4%). L90ECA received the highest just-about-right rating for hardness (73.9%) and L90 received a slightly lower but still highly acceptable value (65.2%). Similarly, L90 and L90ECA showed higher just-about-right ratings for chewiness (both 73.9% “just-about-right”) than those of BB85 (39.1%) and L85, whereas L85 received 0 “just-about-right” responses but most “slightly / much too chewy” (total 82.6%). In contrast, BB85 slightly leaned toward “too crumbly” (total 43.5%) direction. As expected, L85 and BB85 received more responses for “slightly / much too dry” than those of L90 and L90ECA, whereas L90 and L90ECA again, received similar just-about-right ratings. Meanwhile, consumers H seemed to better distinguish product textural difference than consumers E, for example, only 29.1% consumers E, lower-ages indicated BB85 were “slightly / much too crumble” while 43.5% consumers H selected the same responses. It should be kept in mind though these results might be less representative due to the small sample size.

Table 3.15 Frequencies of consumer H (aged 16 - 17) ratings on just-about-right scale on the sensory attributes of meat-pulse bars, black bean & lentil. n = 23[1]

Treatments										
Hardness	Much too soft		Slightly too soft		Just about right		Slightly too hard		Much too hard	
	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]
BB85	1	4.5	9	40.9	10	45.5	2	9.0	0	0
L85	0	0	0	0	13	56.5	6	26.1	4	17.4
L90	0	0	6	26.1	15	65.2	2	8.7	0	0
L90ECA	0	0	5	21.7	17	73.9	1	4.4	0	0
Chewiness	Much too crumbly		Slightly too crumbly		Just about right		Slightly too chewy		Much too chewy	
	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]
BB85	0	0	10	43.5	9	39.1	4	17.4	0	0
L85	0	0	4	17.4	0	0	10	43.5	9	39.1
L90	0	0	3	13.0	17	73.9	3	13.0	0	0
L90ECA	0	0	5	21.7	17	73.9	1	4.4	0	0
Moistness	Much too dry		Slightly too dry		Just about right		Slightly too moist		Much too moist	
	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]
BB85	0	0	7	30.4	13	56.5	3	13.0	0	0
L85	2	8.7	7	30.4	14	60.9	0	0	0	0
L90	1	4.3	3	13.0	15	65.2	4	17.4	0	0
L90ECA	1	4.3	4	17.4	15	65.2	3	13.0	0	0
Saltiness	Not nearly salty enough		Not salty enough		Just about right		Slightly too salty		Much too salty	
	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]
BB85	1	4.3	7	30.4	11	47.8	3	13.0	1	4.3
L85	2	8.7	4	17.4	17	73.9	0	0	0	0
L90	0	0	4	17.4	16	69.6	3	13.0	0	0
L90ECA	0	0	9	39.1	12	52.2	2	8.7	0	0
Sweetness	Not nearly sweet enough		Not sweet enough		Just about right		Slightly too sweet		Much too sweet	
	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]
BB85	0	0	5	21.7	15	65.2	3	13.0	0	0
L85	0	0	4	17.4	16	69.6	3	13.0	0	0
L90	0	0	5	21.7	17	73.9	1	4.3	0	0
L90ECA	1	4.3	3	13.0	16	69.6	3	13.0	0	0
Flavor	Much too weak		Slightly too weak		Just about right		Slightly too strong		Much too strong	
	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]	#[2]	%[3]
BB85	0	0	4	17.4	13	56.5	6	26.1	0	0
L85	1	4.3	3	13.0	18	78.3	1	4.3	0	0
L90	0	0	5	21.7	18	78.3	0	0	0	0
L90ECA	0	0	5	21.7	15	65.2	3	13.0	0	0

[1] frequencies of just-about-right ratings from 72 responses, five-point scale

[2] #: the number of incidences of the rating

[3] %: the frequency of the incidences of the rating by percentage

[3] $\% = 100\% \times \frac{\text{the number of incidences,\#}}{\text{the number of total responses,72}}$

BB85: beef-black bean bar, $A_w < 0.85$; L85: beef-lentil bar, $A_w < 0.85$; L90: beef-lentil bar, $A_w < 0.90$; L90ECA: beef-lentil bar, ECA added, $pH < 5.3$ & $A_w < 0.90$.

Ratings for saltiness of BB85 and L90ECA appeared to lean toward the "not salty enough" direction (total 34.8% and 39.3% respectively) while L85 and L90 attained about 70% responses as "just-about-right". In contrast, all treatment products attained more than 60% response as "just-about-right" for sweetness. This indicated that for consumers H, difference in product water activity (and moisture content) and acidity did not influence sensorial flavor perception. Overall, all treatments showed more than 50% responses as "just-about-right" for flavor, while ratings for L85, L90, L90ECA slightly leaned toward "too weak" direction but, interestingly, ratings for BB85 slightly leaned toward "too strong" direction. Given that the formulations of L85 and BB85, except for lentil vs. black bean flour, were highly similar, the difference on their sensorial flavor perception could be due to the black bean flavor. This may also be associated with the relatively low hedonic score for flavor of BB85, as black bean flour might have introduced a noticeable beany flavor. Hence, further examinations of product flavor profiles and better distinction between "beany" / "lentily" and "meaty" are needed. On the other hand, just-about-right ratings of meat-lentil treatments suggested that a stronger, more intense flavor was favored. Methods to improve the product flavor profile might be achieved by increasing spices content or introducing stronger flavor, such as smoking. Overall, for consumers H aged 16 - 17, the meat-pulse bars in this study were generally acceptable, with further improvements on enhanced flavoring profile. The use of just-about-right scales clearly provided additional insight into consumer preferences than hedonic rating.

3.5.5 Snacking behaviors of young consumers: Survey responses

3.5.5.1 Snacking behaviors: Consumers E

Another purpose to develop these novel meat-pulse bars was to provide a healthier snack to young consumers. Participants were also asked to fill out a survey on their snack consumption behaviors, though not all participants completed the entire survey.

From the group of consumers E, a total of 56 participants completed the entire survey, whereas a total of 63 participants provided responses to certain questions in the survey but may not have completed it fully. Over 50% of the participants indicated that they consumed snacks two or more times per day, indicating that snacks play an essential role on their diet quality (Table 3.16).

Table 3.16 Consumption and purchase behavior of snacks by consumers E, aged 9 - 14

% Frequency [1]						
On average, how often do you consume snacks?						
2 or more times per day	1 time per day	5-6 times per week	3-4 times per week	1-2 times per week	2-3 times per month	1 time per month or less
55.6	17.5	9.5	12.7	0.0	4.8	0.0
On average, how often do you skip a meal but consume snacks?						
2 or more times per day	1 time per day	3-5 times per week	1-2 times per week	2-3 times per month	1 time per month	generally don't skip a meal.
3.2	11.1	7.9	17.5	4.8	9.5	46.0
On average, how often do you purchase snacks by yourself but not your parents?						
2 or more times per day	1 time per day	3-5 times per week	1-2 times per week	2-3 times per month	1 time per month	generally don't buy snacks.
0.0	1.8	8.8	12.3	29.8	19.3	28.1

[1] % frequency: $\% \text{ frequency} = \frac{\text{incidence of response}}{\text{total number of response}} \times 100\%$, n = 56 - 63

The most often consumed snacks for consumers E were "dairy products such as ice cream and yogurt" and "seeds, nuts, nut snacks and bars", followed by "bakery items such as crackers and cereal snack bars", "milk, fruit juices", "fruits", and "cookies, brownies", whereas the latter four categories (bakery, milk, fruits, cookies) did not show big differences in consumption frequency (Table 3.17). Meanwhile, consumers E group did not show noticeably high consumption frequencies for energy-dense snacks including fried potatoes, beverages such as soft drinks, and sweet snacks, but these categories accounted for the snacks the they purchased the most often (Table 3.16). This was likely due to that consumers E group had less access to purchasing than consuming, also indicated the strong preference of consumers E toward energy-dense snacks. This might also be due to that the generally higher cost of meat snack than others and the limited purchase behaviour by consumers E. The consumption of these energy-dense snacks has been considered as an important factor related to childhood obesity, and hence a healthier snack alternative is recommended (Galpin et al., 2018; Gilbert et al., 2012). Gilbert et al. (2012) also suggested the snack consumption period might also play roles in influencing diet quality. They reported that the after-school period (3 – 6 pm) was the most frequent time for snack consumption, agreeing with results of consumers E in this study (Table 3.18). It was also suggested that the excess consumption of energy-dense snacks might lead young consumers to skip meals and hence impact their diet quality (Kelishadi et al., 2017). However, this situation was not detected in present study as most particpants did not skip meals due to snacks (Figure 3.1).

Table 3.17 Snacks that consumers E usually consume and purchase, aged 9 - 14

% Frequency [1]	usually consume [2]	snacks you buy	snacks from family
Milk, fruit juices	41.7	10.0	61.7
Other beverages such as fruit drinks, soft drinks, and milk-based beverages	32.1	46.4	35.7
Fruits	39.0	18.6	57.6
Fried potatoes / chips	36.8	33.3	38.6
Cookies, brownies, snack cakes, doughnuts, muffins	39.0	37.3	42.4
Other bakeries such as crackers, bagels, pancakes, and cereal snacks and bars	42.1	22.8	57.9
Sweet snacks such as candies	32.1	53.6	33.9
Other dairy products	44.1	23.7	54.2
Seeds, nuts, nut snacks and bars	44.2	23.7	54.2
Meat snacks	29.1	25.2	61.8

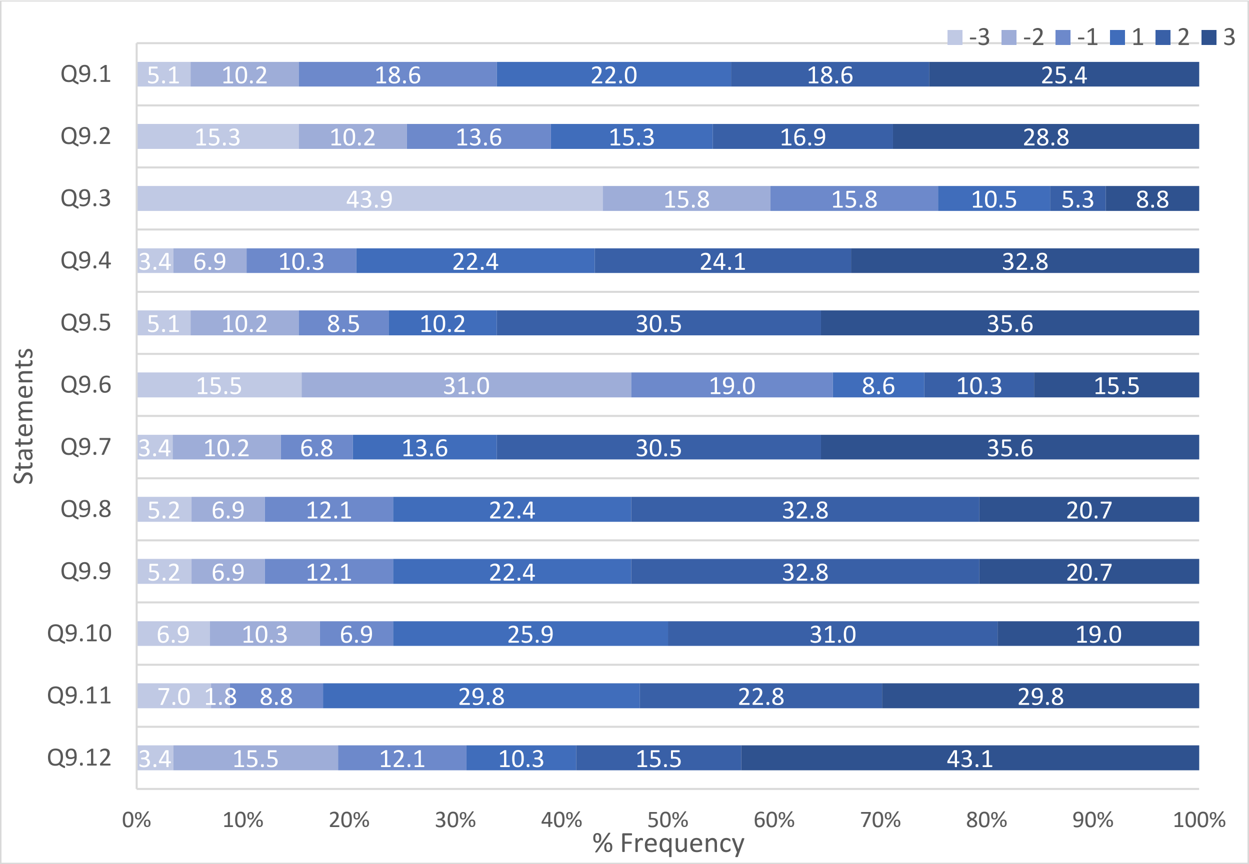
[1] % frequency: $\% \text{ frequency} = \frac{\text{incidence of response}}{\text{total number of response}} \times 100\%$, n = 56 - 63

[2] Sum of % usually consume, % snacks you buy, and % snacks from family might be larger than 100% as panelists were allowed to choose more than 1 answer

Table 3.18 Time slots that consumers E most often consume snacks, aged 9 - 14

9 - 11 a.m.		3 - 6 p.m.		After 8 p.m.	
incidence	% frequency [1]	incidence	% frequency	incidence	% frequency
15	23.8	46	73.0	11	17.5

[1] % frequency: $\% \text{ frequency} = \frac{\text{incidence of response}}{\text{total number of response}} \times 100\%$, n = 56 - 63



Q9.1. I only look for a snack when I am hungry.
Q9.2 I eat snacks whenever I want.
Q9.3 Sometimes I skip a meal because I am too filled with snacks.
Q9.4 Sometimes I feel hungry between meals.
Q9.5 I know that meat snacks are good sources of nutrients and complete protein.
Q9.6 When I am hungry, I prefer meat snacks over other snacks.
Q9.7 I know that pulses are rich sources of nutrients such as protein, fiber, vitamins, and antioxidants.
Q9.8 I believe that eating pulses is health-beneficial, and I would like to consume pulse-containing foods whenever they are available.
Q9.9 I generally don't like the taste / flavor / smell of pulses, so I tend not to consume pulses-containing foods.
Q9.10 I believe that the incorporation of pulses in meat snacks makes them more nutritious, healthier, and attractive to consumers.
Q9.11 I think that these meat-pulse bars are more nutritious, healthier options than the snacks I usually consume.
Q9.12 I would like to recommend these meat-pulse bars to my friends and parents.

Figure 3.1 Agreement scales on given statements, Q9.1 - Q9.12, by consumers E, aged 9 - 14.

% frequency: $\% \text{ frequency} = \frac{\text{incidence of response}}{\text{total number of response}} \times 100\%$

Six-point agreement scale on provided statements, -3: completely disagree, 3: completely agree.

The majority of the elementary participants did not often consume meat snacks (less than 1 times per week) but 80% of them indicated they had consumed meat snacks before (Table 3.19). This indicated that the group of consumers in this study have been exposed but not very familiar with meat snacks, especially novel meat and hybrid products such as meat-pulse bars. Although, consumers E showed knowledge of pulses being a good source of nutrients, and 75% of them indicated that they believed “the incorporation of pulses in meat snacks makes them more nutritious, healthier, and attractive to consumers” (Figure 3.1). Additionally, over 75% participants agreed that “sometime [they] feel hungry between meals”. This suggested the hybrid benefits of the meat-pulse bars being a novel snack option to young consumers, basing on the appealing and fulfilling properties and “complete protein” profile of meat component. However, most of the participants did not “prefer meat snacks over other snacks when [they are] hungry”. Overall, more than 80% of participants indicated that “these meat-pulse bars are more nutritious, healthier options than the snack [they] usually consume”, and 68% said they would recommend these products to others. Hence, the targeted consumer group (consumers E) agreed with the idea that the meat-pulse bars in this study could become an attractive snack alternative.

Table 3.19 Consuming behavior and familiarities of meat snacks and meat bars by consumers E, aged 9 - 14

% Frequency [1]						
Have you ever consumed meat snacks / meat bars? (n = 57)					yes	no
Meat snacks					79.3	20.7
Meat bars					39.7	58.6
If have consumed meat snacks, how often do you consume meat snacks? (n = 57)						
2 or more times per day	1 time per day	3-5 times per week	1-2 times per week	2-3 times per month	1 time per month	generally don’t buy snacks.
7.0	7.0	10.5	15.8	19.3	14.0	26.3

[1] % frequency: $\% \text{ frequency} = \frac{\text{incidence of response}}{\text{total number of response}} \times 100\%$

3.5.5.2 Snacking behaviors: Consumers H

A survey of snack consumption behavior by consumers H was also conducted. In this study, almost 80% of consumers H, aged 16 - 17, indicated they consumed snacks at least 1 time per day (Table 3.20), indicating the essential influence of snack consumption in their daily lives. Many researchers have related the increased incidences of childhood obesity to excess consumption of energy-dense snack (Liberali et al., 2020; Oellingrath et al., 2011). Energy-dense snacks including beverages such as soft drinks, sweet snacks such as candies, and “cookies, biscuits, cereal bars” have been reported to contribute the most energy of daily energy intake of consumers aged 4 - 18, while these three snack categories ranked as third to fifth most often consumed snacks (Gilbert et al., 2012). They also reported that young consumers most often consumed snack in after-school period, 3 - 6 p.m..

Table 3.20 Consumption and purchase behavior of snacks by consumers H, aged 16 - 17

% Frequency [1]						
On average, how often do you consume snacks?						
2 or more times per day	1 time per day	5-6 times per week	3-4 times per week	1-2 times per week	2-3 times per month	1 time per month or less
39.1	39.1	13.0	0.0	4.3	4.3	0.0
On average, how often do you skip a meal but consume snacks?						
2 or more times per day	1 time per day	3-5 times per week	1-2 times per week	2-3 times per month	1 time per month	generally don't skip a meal.
13.0	21.7	13.0	13.0	4.3	4.3	30.4
On average, how often do you purchase snacks by yourself but not your parents?						
2 or more times per day	1 time per day	3-5 times per week	1-2 times per week	2-3 times per month	1 time per month	generally don't buy snacks.
4.3	4.3	21.7	21.7	21.7	13.0	13.0

[1] % frequency: % frequency $= \frac{\text{incidence of response}}{\text{total number of response}} \times 100\%$, n = 23

In this study, almost 80% of the participating consumers indicated that they consumed snacks at least 1 time per day (Table 3.20), while over 60% of them most often consumed snacks in the after-school period (Table 3.21). Snack categories they most often consumed include "fried potatoes, chips", "other beverages such as soft drinks", "fruits", "dairy products such as ice cream", and "seeds, nuts, nut snacks and bars", most of them are energy-dense snacks (Table 3.22). Meanwhile, more than 30% of consumers H indicated they skipped a meal but consumed snacks at least 1 time per day (Figure 3.2). These results mainly agreed with the snacking behaviour of young consumers from other studies (Gilbert et al., 2012; Oellingrath et al., 2011), further confirming that the widely consumed energy-dense snacks greatly increases daily energy intake and impairs diet quality, and hence healthier, more nutritious snacks are recommended.

Table 3.21 Time slots that consumers H most often consume snacks, aged 16 - 17

9 - 11 a.m.		**3 - 6 p.m.**		**After 8 p.m.**	
incidence	% Frequency*	incidence	% Frequency	incidence	% Frequency
4	17.4	14	60.1	6	21.8

[1] % frequency: % frequency $= \frac{\text{incidence of response}}{\text{total number of response}} \times 100\%$, n = 23

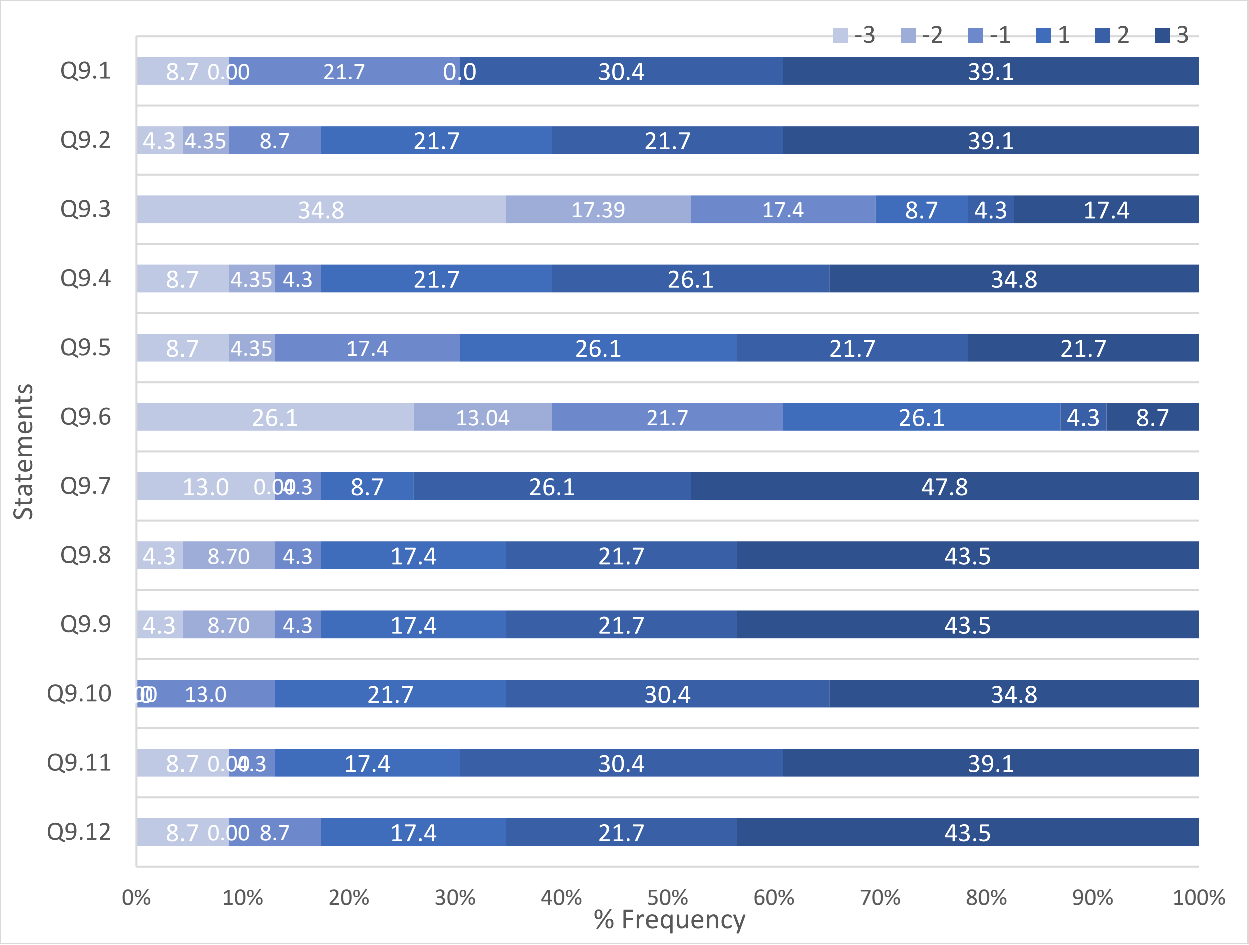
Table 3.22 Snacks that consumers H usually consume and purchase, aged 16 - 17

% Frequency [1]	**usually consume** [2]	**snacks you buy**	**snacks from family**
Milk, fruit juices	34.8	13.1	82.6
Other beverages such as fruit drinks, soft drinks, and milk-based beverages	45.0	50.0	55.0
Fruits	38.1	9.5	71.4
Fried potatoes / chips	47.6	33.3	71.4
Cookies, brownies, snack cakes, doughnuts, muffins	30.0	25.0	75.0
Other bakeries such as crackers, bagels, pancakes, and cereal snacks and bars	27.3	22.73	90.9
Sweet snacks such as candies	20.0	25.0	80.0
Other dairy products	38.1	14.3	81.0
Seeds, nuts, nut snacks and bars	38.1	14.3	81.0
Meat snacks	36.8	36.8	68.4

[1] % frequency: $\%\ \text{frequency} = \frac{\text{incidence of response}}{\text{total number of response}} \times 100\%$, n = 23

[2] Sum of % usually consume, % snacks you buy, and % snacks from family might be larger than 100% as panelists were allowed to choose more than 1 answer

More than 80% participants indicating they feel hungry between meals (Figure. 3.2) and many of them indicating they skipped meals but consumed snacks. Hence, meat snacks appeared to a better option than other snacks as it is rich in nutrients, provide complete proteins, and contributed to high satiety. More than 30% participants also agreed that they “prefer meat snacks when they are hungry” (Figure 3.2). On the other hand, more than 90% of participants consumed meat snack less than 2 - 3 times per month, whereas most of them are familiar with meat snacks including meat bars (Table 3.23). Given that barriers of pulse consumption in Canada includes “not knowing how to cook and prepare” and “don’t like the taste and flavor,” the introduction of meat-pulse bars to young consumers also resulted in their early exposure to pulse-containing foods. This likely reduce food neophobia and provide experience in eating various foods, both of which have been related to improve diet quality, and reduced risk of childhood obesity (Kaar et al., 2016; Miller et al., 2016). Participants of consumers H acknowledged the health benefits of the hybrid meat-pulse bars and believed “these meat-pulse bars are more nutritious, healthier options than the snack [they] usually consume” (Figure 3.2), indicating a wide acceptability of the hybrid meat-pulse bars.



Q9.1. I only look for a snack when I am hungry.
Q9.2 I eat snacks whenever I want.
Q9.3 Sometimes I skip a meal because I am too filled with snacks.
Q9.4 Sometimes I feel hungry between meals.
Q9.5 I know that meat snacks are good sources of nutrients and complete protein.
Q9.6 When I am hungry, I prefer meat snacks over other snacks.
Q9.7 I know that pulses are rich sources of nutrients such as protein, fiber, vitamins, and antioxidants.
Q9.8 I believe that eating pulses is health-beneficial, and I would like to consume pulse-containing foods whenever they are available.
Q9.9 I generally don't like the taste / flavor / smell of pulses, so I tend not to consume pulses-containing foods.
Q9.10 I believe that the incorporation of pulses in meat snacks makes them more nutritious, healthier, and attractive to consumers.
Q9.11 I think that these meat-pulse bars are more nutritious, healthier options than the snacks I usually consume.
Q9.12 I would like to recommend these meat-pulse bars to my friends and parents.

Figure 3.2 Agreement scales on given statements, Q9.1 - Q9.12, by consumers H, aged 16 -17.

% frequency: $\% \text{ frequency} = \frac{\text{incidence of response}}{\text{total number of response}} \times 100\%$

Six-point agreement scale on provided statements, -3: completely disagree, 3: completely agree.

Table 3.23 Consuming behavior and familiarities of meat snacks and meat bars by consumers H, aged 16 – 17

% Frequency [1]						
Have you ever consumed meat snacks / meat bars? (n=23)					yes	no
Meat snacks					69.6	30.4
Meat bars					52.2	47.8
If have consumed meat snacks, how often do you consume meat snacks? (n=21)						
2 or more times per day	1 time per day	3-5 times per week	1-2 times per week	2-3 times per month	1 time per month	generally don't buy snacks.
4.8	0.0	0.0	4.8	33.3	23.8	33.3

[1] % frequency: % frequency $= \frac{\text{incidence of response}}{\text{total number of response}} \times 100\%$

3.6 Conclusions

In conclusion, this study found that the use of different pulse flours, specifically black bean and lentil, did not modify the proximate compositions of the meat-pulse bars, aside from variations in carbohydrate content. However, achieving a lower target water activity did result in reduced moisture levels, leading, in turn, to higher contents of protein, carbohydrate, and fat. The addition of acidifiers led to a reduction in product pH, although this had no impact on factors such as cooking loss, water activity, or overall composition. Importantly, the acidifier addition was also found to have no measurable influence on product color, as demonstrated by L^*, a^*, b^* values, and firmness, as demonstrated by Warner-Bratzler (WB) shear values, through storage. Conversely, products with different pulse flours were associated with differences in color and texture measurements, suggesting the different extents of interactions between black bean and lentil flours with the structural, myofibrillar proteins network. Over the course of storage, products of all treatments experienced changes in color and texture, trending toward a firmer texture, and a color profile that was darker, less red, and less yellow.

Meat-pulse bars of all treatments in this study successfully attained generally high consumer acceptance among both the younger age group (consumers E, 9 - 14 years), and the slightly older demographic (consumers H, 16 - 17 years). Lower hedonic scores for texture were associated with drier, harder and chewier product (L85) by consumers E, but consumers H did not show difference on textural preference among treatments. Notably, while consumers E displayed a lower texture acceptance on drier (lower moisture content), and firmer (higher WB shear value) products (L85), consumers H did not exhibit any variations in textural preferences across treatments. Furthermore, no significant difference was detected, in the hedonic ratings for other sensory attributes including color, smell / aroma, taste / flavor, and overall acceptability, by both groups of panelists.

Evaluation using the just-about-right scales indicated that consumers E might lean toward a preference for softer, chewier texture, compared to consumers H. Conversely, both groups displayed a preference for a moister texture and a more pronounced flavor profile. Both groups presented snacking behaviors with potential implications for childhood obesity, with consumers H appearing to be particularly at risk. Nonetheless, for both groups, the meat-pulse bars in this study emerged as well-accepted alternatives offering a healthier and more nutritious snacking option.

3.7 Relation to next study

The differences in color, texture, and storage behavior between the meat-black bean and the meat-lentil bars were likely be, as discussed, attributed to variances in product carbohydrate content, which subsequently influences the extent of interactions between pulse flour and the structural, myofibrillar proteins network. Further examinations could be used to distinguish flour attributes, such as particle size, carbohydrate compositions, and starch gelatinization levels, to better understand these differences. Additionally, this investigation was limited to a singular level of pulse flour incorporation. A more systematic study should be performed, to attain a more nuanced understanding of the physicochemical changes induced by varying levels of pulse flour. Starch, being a predominant component in pulses, could significantly affect their functional characteristics, thereby altering the physicochemical properties of the resultant meat-pulse bars.

Factors like water activity, moisture content, and acidity appeared to exert minimal impact on the sensory attributes of the products in the current study, and therefore could be dismissed from immediate consideration. Interestingly, products with black bean flour were associated with unfavorable flavor profile, prompting their removal from subsequent experiments. Sensory evaluations by consumer panel indicated a preference toward more flavorful products, generating interest in conducting more rigorous, detailed analysis in flavor by trained panelists. Unfortunately, this aspect of the study was precluded due to the constraints imposed by the COVID-19 pandemic. Overall, the focus of the next study will be directed toward meat-lentil bars with treatments on the addition levels and starch gelatinization of lentil flours. A meat-only control will also be included to provide a comparative framework.

4. Study 2: Examination of functionalities of lentil flours in meat-pulse bars and effects on product quality

4.1 Abstract

A systematic study to examine the physicochemical changes of meat-pulse bars with different addition levels of infrared heated lentil flour and treatment on starch gelatinization of the lentil flour was conducted. Meat-lentil bars with lentil flour at addition level of 6%, 12%, 18% of non-tempered lentil flour, 6% and 12% of more gelatinized lentil flour, and 0% flour addition were produced. Gelatinized lentil flour was obtained by tempering lentil seeds for 24 h before going through infrared heating and milling, as indicated by the concentration of damaged starch in flour. The addition of lentil flour influenced meat bar properties, while the addition level and starch gelatinization of lentil flour also altered meat bar properties. Lentil flour addition did not show influence on the required drying period of the product and hence cook loss. The incremental addition level of lentil flour in this study did not affect the acidity of the meat-lentil bars. The proximate composition of meat-lentil bars differed among treatments, showing that carbohydrate content increased and fat content decreased, as lentil flour addition level increased. Samples of all treatments experienced decreased water activity, moisture content, increased textural strength (as shown by Warner Bratzler shear test and three-point bending test), increased lipid oxidation, and darkening color over storage, with high-temperature storage showing an accelerated effect than room-temperature storage. Samples with higher addition level and more-gelatinized lentil flour appeared to exhibit firmer, more cohesive texture than comparable controls, which might also be related with improved stability over storage. As for color, samples with higher addition level and more gelatinized lentil flour appeared to show lighter and more yellow color, also indicating improved color stability over storage. Improved product stability with regard to lipid oxidation over storage was also observed for meat-lentil bars, but only with sufficient addition levels (12% and above) of lentil flour and starch gelatinization content did not show effect. Consumers (n = 47) did not observe any differences among treatments with lentil flour addition with respect to sensory perceptions of smell / aroma, flavor, and overall acceptability, but meat-lentil bars at 18% addition

appeared to significantly alter color intensity on yellowness and texture intensity on dryness, and those at 12% on firmness. Properties change introduced by lentil flour addition to meat bars were generally acceptable, but consumers might prefer products with lower lentil flour addition (< 18%).

4.2 Introduction

Lentil, a legume characterized by its significant nutrient density and environmental sustainability (Pulse Canada, n.d.), present an innovative opportunity for enhancing conventional meat products. Canada, particularly Saskatchewan, is a global leader in lentil production, making it a popular ingredient in daily consumption (Pulse Canada, n.d.). While pulses such as chickpeas, black beans, and lentils have different compositional profiles, the study described in section 3 indicated that the addition of lentil or black bean flours at the same level (12%) did not result in differences in their proximate profiles. However, meat-lentil bars showed a higher shear strength that meat-black bean bars, suggesting that different pulse flours with the same proximate content might have different interactions with the ingredients in meat bars. Further examination of the effects of more specific pulse flours is warranted, beginning with the analysis of carbohydrate content, as it is the most abundant component (Hoover et al., 2010).

The behavior of pulse carbohydrates is heavily influenced by the levels of starch gelatinization, a process in which pulse starch granules release amylose and amylopectin molecules that exhibit pasting, swelling, and water- and fat-binding properties (Singh, 2021; Wang & Copeland, 2013), ultimately influencing the properties of meat-pulse bars. The level of pulse starch gelatinization depends on various factors, such as heating time, pressure, temperature, and added moisture content (Ma et al., 2011). Specifically, different levels of gelatinization can be achieved by tempering pulse flours for different time lengths, which affects the moisture absorption level and the degree of gelatinization as measured by the percentage of damaged starch granules over the total carbohydrate content of the product (Pathiratne et al., 2015). Lentil flour was chosen in this study, due to the higher acceptability ratings from the previous study. Also, Shariati-Ievari et al. (2016) found that lentil flour addition at 6% in beef burger was associated with favorable results for physicochemical properties and consumer acceptability. In this study, lentil flour was systematically added in increasing amounts, with a comparison to conventional meat-only samples, to examine the influence of pulses, particularly pulse starches, on the properties of meat-pulse bars.

Furthermore, it has been demonstrated that infrared heating of lentil flour can increase its nutritional value and reduce off-flavor production (Pathiratne et al., 2015). Liu et al. (2020) suggested that under infrared heating treatment, a longer tempering period and a smaller seed size of lentils lead to a greater degree of starch gelatinization and protein denaturation, which are associated with enhanced water holding capacity.

Clearly, the investigation of lentil flour as a pulse ingredient in meat-pulse bars provides an opportunity to understand the influence of carbohydrate content and the degree of starch gelatinization on the physicochemical and sensory properties of meat-pulse bars. The purpose of this study is to understand the effect of the addition and starch gelatinization levels of lentil (pulse) flour on the physicochemical properties, storage stability, and sensory characteristics of meat-lentil (pulse) bars as compared to the meat-only control sample.

4.3 Materials

Beef outside rounds (*Biceps femoris*, Canada AAA, Cargill Foods, High River, AB, Canada, est. 93) were purchased from a local retailer and stored at refrigerated temperature (≤ +4 °C) for 16 days post-mortem. Eston lentil seeds were infrared heated (IR) and supplied by InfraReady Products Ltd. (Saskatoon, SK, Canada). Food-grade salt (sodium chloride), nitrite in the form of Prague powder (6.4% sodium nitrite in salt), and sodium erythorbate were obtained from Canadian Salt Company Ltd. (Pointe-Claire, QC, Canada), Innophos (Lowbanks, ON, Canada), and Unipack Packaging Products Ltd. (Edmonton, AB, Canada), respectively. Dry seasonings (black pepper (32-mesh), garlic powder, onion powder) were obtained from JB's Sausage Maker Supplies Ltd. (Saskatoon, SK, Canada). Brown sugar (best brown) from Rogers by Lantic Inc. (Vancouver, BC, Canada) and soy sauce (organic gluten-free) from San-J Tamari (Henrico County, VA, U.S.A) were purchased from local retailers. Encapsulated citric acid (ECA) (MeatShure, New Hampton, NY, U.S.A) containing 70.0 - 74.0% citric acid, and glucono delta-lactone (GDL) (Malabar, Burlington, ON, Canada) were sourced as acidulants. All materials used in meat-pulse bars production were food-grad and used in accordance with applicable regulations to be considered as generally recognized as safe (GRAS) components.

Sodium acetate (Fisher Scientific Ltd., Ottawa, ON, Canada), calcium chloride (Fisher), sulphuric acid (Sigma-Aldrich Co., Oakville, ON, Canada), Kjet-Tabs (Fisher), sodium hydroxide (Fisher), boric acid (Fisher), hydrochloric acid (EMD Chemicals Inc., Gibbstown, NJ, U.S.A), sodium carbonate (Fisher), N-point indicator (bromocresol green-methyl red mixed indicator, Fisher), petroleum ether (Fisher), potassium chloride (Fisher), trichloroacetic acid (Fisher), phosphoric acid (Fisher), thiobarbituric acid (Sigma-Aldrich), and 1, 1, 3, 3-tetramethoxypropane (TMP) (Sigma-Aldrich) used for chemical analysis of meat-pulse bars were of ACS grade.

4.4 Methods

4.4.1 Production of lentil flours: Tempering

Procedures of seeds tempering were adapted from Liu et al. (2020). Lentil seeds were divided into four treatments respectively: a) non-tempered seeds, b) 8 h-tempered seeds, c) 16 h-tempered seeds, and d) 24 h-tempered seeds, with tempering level targeted to 25% moisture. Tempering of lentil seeds was done by adding required amount of distilled water calculated using the following equation:

$$\text{g required water} = \frac{\text{g seeds} \times (\text{\% traget moisture of seeds} - \text{\% initial moisture of seeds})}{100 - \text{\% initial moisture of seeds}} \tag{4.1}$$

Water was added to lentil seeds in plastic tubs with lid placed on. The seeds were allowed to absorb moisture and tempered at ambient temperature for stated time length, and water was added half-way through tempering to achieve the desired moisture content if needed. Moisture content of lentil seeds after tempering were determined as 19% (batch 1, replicate 1) and 23% (batch 2, replicate 2 and 3).

Lentil seeds from all tempering treatments were then further processed by infrared (IR) heating to reach a surface temperature of 150 °C, under the same procedures as described in section 3.4.1. Granulation size of the flours were 300 µm or less. Raw flours of lentil seeds from each batch that did not go through IR heating nor tempering were also prepared.

4.4.2 Production of lentil flours: Levels of starch gelatinization

An analysis kit for damaged starch (Starch Damage Assay Kit, Megazyme International Ltd., County Wicklow, Ireland) was used to determine the contents of damaged (or gelatinized) starch of above lentil flours, and hence their gelatinization levels, according to AACC Method 76-31.01 (2010). The raw flours only contained damaged starch, while the infrared-heated flours contained both damaged and gelatinized starch. Approximate 100 mg of flour of each sample was added with fungal α-amylase in sodium acetate buffer (pH 5.0, containing calcium chloride) then incubated at 40 °C for 10 min. Dilute sulfuric acid (0.2%, v / v) was then added to this mixture and well-mixed.

The resulting solution was centrifuged, and the supernatant was taken out and added with amyloglucosidase in sodium acetate buffer, and incubated at 40 °C. Glucose oxidase / peroxidase (glucose determination reagent) was then added, and the solution incubated at 40 °C for 30 min. The sample absorbance at wavelength 510 nm (Genesys 30 Visible Spectrophotometer, supplied by Fisher) was measured to indicate the concentration of generated glucose. The total amount of released glucose was applied to calculate the content of damaged (or gelatinized) starch of the sample on a dry basis.

4.4.3 Production of meat-lentil bars

The procedures of initial meat preparation were the same as described in section 3.4.2. The pH of meat blocks was tested (5.3 - 5.6), and dark-cutting (pH > 6.0) meat was avoided.

Formulations of each treatment of meat-lentil bars are shown in Table 4.1. After determining starch gelatinization level, only lentil flours at the lowest (non-tempered, 0.90% damaged starch in sample weight, NT) and highest (24-h-tempered, 27.48% damaged starch in sample weight, higher-gelatinized, HG) gelatinization levels were selected as different treatments of meat-lentil bars (see Results and Discussion). All ingredients except meat were weighed and stored at +4 °C overnight before processing. Brown sugar, soy sauce, and water were kept in a separate container and well-mixed together until dissolved just before processing.

Table 4.1 Formulations of meat-lentil bars, infrared heated lentil flour, non-tempered & 24 h-tempered. (% w/w)

Ingredients / Replicate	Con	NT6	HG6	NT12	HG12	NT18
Beef, trimmed	84.3	78.3	78.3	72.3	72.3	66.3
IR lentil flour, non-tempered[1]	0	6	0	12	0	18
IR lentil flour, 24h-tempered[2]	0	0	6	0	12	0
Water	6	6	6	6	6	6
Brown sugar	6	6	6	6	6	6
Salt	1.5	1.5	1.5	1.5	1.5	1.5
Prague powder[3]	0.3	0.3	0.3	0.3	0.3	0.3
Soy sauce	0.8	0.8	0.8	0.8	0.8	0.8
Glucono delta lactone (GDL)	0.5	0.5	0.5	0.5	0.5	0.5
Encapsulated citric acid (ECA)	0.15	0.15	0.15	0.15	0.15	0.15
Sodium erythorbate	0.05	0.05	0.05	0.05	0.05	0.05
Black pepper	0.2	0.2	0.2	0.2	0.2	0.2
Garlic powder	0.1	0.1	0.1	0.1	0.1	0.1
Onion powder	0.1	0.1	0.1	0.1	0.1	0.1
Total	100	100	100	100	100	100

[1] IR: infrared heated, lentil flour, non-tempered (7.7% moisture, 3.5% ash, 1.3% fat, 24.7% protein, pH 6.31)

[2] IR: infrared heated, lentil flour, 24 h-tempered (7.2% moisture, 3.5% ash, 1.5% fat, 24.6% protein, pH 6.31)

[3] Prague powder containing 6.4% nitrite.

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

Six treatments of meat-lentil bars: 6%, 12%, 18% of non-tempered lentil flour addition (NT6, NT12, NT18 respectively), 6%, 12% of 24 h-tempered lentil flour (HG6, HG12 respectively), and no lentil flour addition (Con) were produced as shown in Table 4.1. An additional treatment, HG18 was prepared, but the viscosity of this meat mixture was too high and unable to be extruded, so was dropped from the study. Prior to processing, the frozen coarsely ground meat was thawed in a +1 °C cooler for approximate 36 h. Procedures to produce meat-lentil bars were same as described in section 3.2.1. All samples were dried to target water activity below 9.0 using the set oven schedule (Study 1: Table 3.2). Dry bulb and wet bulb temperatures of the oven (SelfCooking Center, model SCC WE 101, Rational Inc., Mississauga, ON, Canada) during drying were monitored, and its relative humidity was within ± 10% of the set-up relative humidity and dry bulb temperature was within ± 5 °C of the set-up dry bulb temperature. The total cooking and drying times of the products were 150 - 180 min, depending on the different treatments (Figure 4.1). In this study, a 6500 – 7000 g batch was produced for each treatment per replicate, with a total of three replicates produced on different weeks.

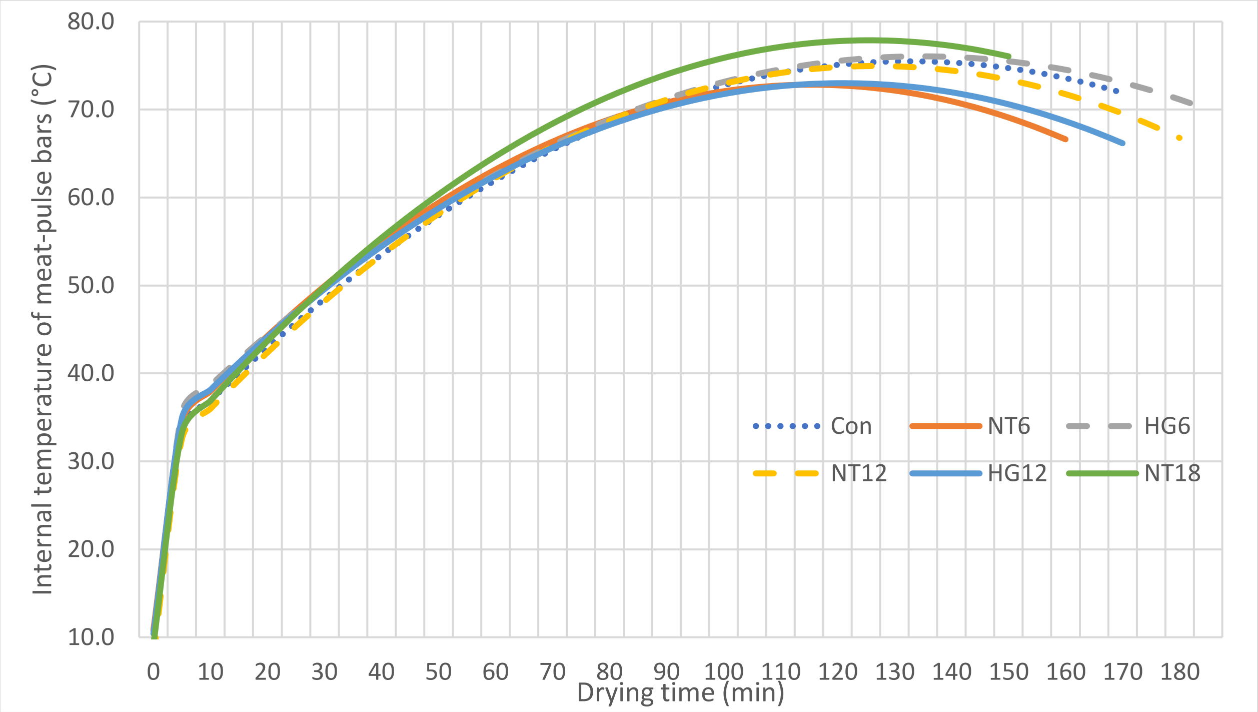


Figure 4.1 Heating curves of meat-pulse bars of all treatments during cooking / drying, shown as internal temperature of the products.

Curve of each treatment represents the temperature curves of three replicates.

Con: beef bar, no lentil flour addition, NT6: beef bar, 6% non-tempered IR lentil flour, NT12: beef bar, 12% non-tempered IR lentil flour, NT18: beef bar, 18% non-tempered IR lentil flour, HG6: beef bar, 6% 24h-tempered, IR lentil flour, HG12: beef bar, 12% 24h-tempered IR lentil flour.

4.4.4 pH and proximate composition of meat-lentil bars

Procedures for measurements on pH value and proximate content were same as section 3.4.6 and 3.4.7.

4.4.5 Water activity of meat-lentil bars

Procedures for measurements on water activity were same as section 3.4.8.

4.4.6 Instrumental color and texture measurements of meat-lentil bars

Procedures on instrumental measurements for color parameters $L*$, $a*$, and $b*$ were same as section 3.4.9. Average values of color parameters were obtained from at least eight readings per treatment per replicate (two readings per piece, at least four sample pieces), and a standard pink tile was used to monitor instrument performance.

Procedures for instrumental measurements of Warner-Bratzler (WB) shear force value were same as section 3.4.10. Average shear force values per treatment per replicate were attained from eight measurements.

Samples also went through three-point bending test for more comprehensive textural assessment. Samples were cut into pieces 80 mm in length, and placed on top of the two support arms of the support plate of a lightweight three-point bend set (Food Technology Crop., Sterling, VA, U.S.A., load cell no. 04-0083-10, 100 N), with sample upper surface facing opposite to the blade. Blade movement, appropriate for the three-point bending test, was controlled in a program modified from the WB shear test program, which similarly, moved and contacted samples at constant speed (200 mm per min). Similarly, a graph demonstrating the relationship between distance of blade movement, which equaled to the displacement of sample upon forced bending, and force applied to blade was generated (Figure 4.2). The maximum force (force a) shown in the graph indicated the greatest force required to induce sample breaking (break force). The accordance displacement at this point (displacement b) represented the greatest displacement, or deformability, that the sample resisted upon forced bending, and the slope (slope e), represented greatest resistance or deformability of the sample, also known as stiffness. The area under the curve until this point (area c) represented the energy required to induce sample breaking, also known as ultimate strength.

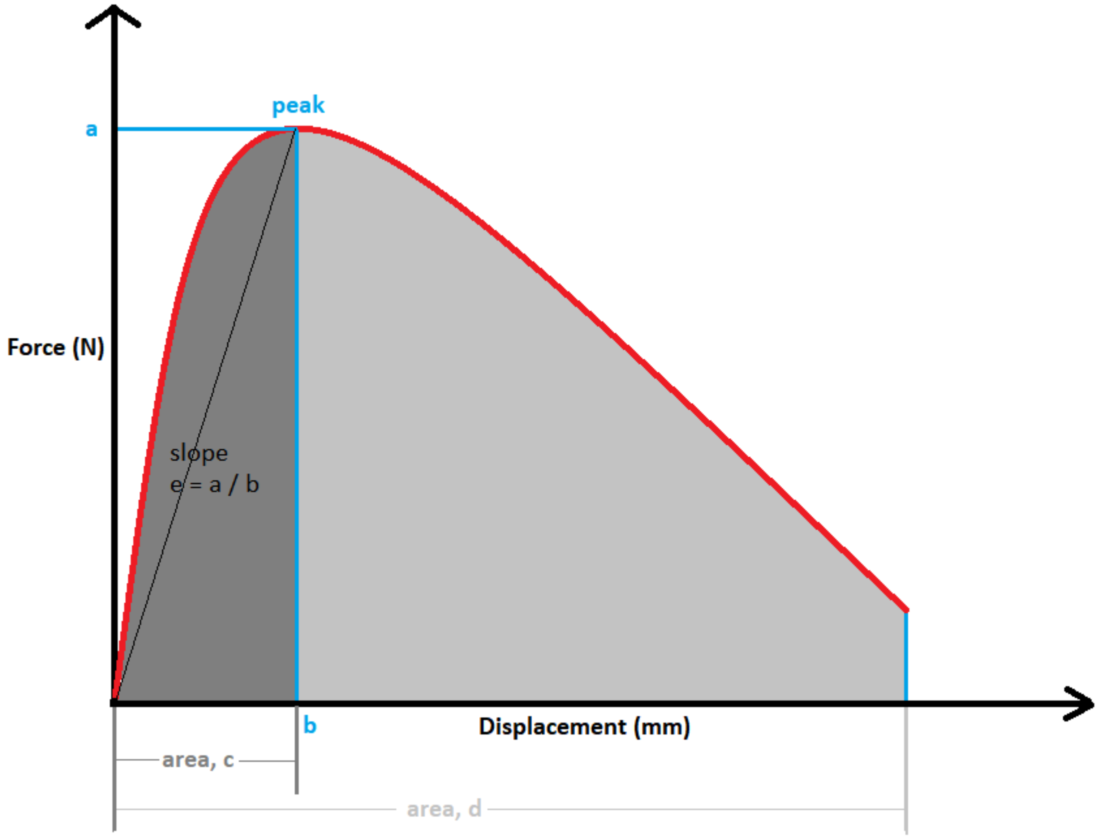


Figure 4.2 Example load-displacement curve of three-point bending test.

The zero force shown in Figure 4.2, except origin, indicated the loss of sample resistance upon forced bending, representing the complete textural failure of the sample. Energy required to cause complete textural failure could be demonstrated by the area (area d) under the curve from origin to the point at zero force, also known as total strength. Samples with different textural properties showed different displacements at zero force, namely, different endpoints to calculate total strength. With no available pre-set program, trial three-point bending test on preliminary products showed maximum displacement shorter than the longest possible displacement, 30 mm, which is limited by the lower space of the support plate. Hence, displacement at 30 mm was determined as the endpoint of the test, and sample total strength was calculated as area under curve until displacement at 30 mm. Distance between the supporting arms was set as 40 mm. Zero position was set 10 mm above the top of the support plate, to allow space to put samples and eliminate error-inducing sample pre-bending, and zero displacement was set upon the blade in contact with samples, so that blade displacement is in parallel with sample deformation. A blank test with no sample, namely, letting the blade to only travel, and bend nothing, through programmed movement was performed every time before measurement, to check equipment performance. Measurements were performed on at least seven sample pieces from multiple strips, positions, per treatment per replicate.

4.4.7 Lipid oxidation level of meat-lentil bars

The lipid oxidation of raw meat-pulse samples was measured as thiobarbituric acid (TBA) reactive substances according to Bedinghaus and Ockerman (1995) on previously frozen samples. Each sample (about 2.5 g) was extracted with 25 mL extraction solution (20% w / v trichloroacetic acid (TCA) with 1.6% v / v phosphoric acid), then blended with 25 mL of cold distilled water (Stomacher Lab Blender, model BA6021, Seward Ltd., West Sussex, U.K.). The slurry was filtered through filter paper (Whatman no.1, supplied by VWR) into a 50 mL volumetric flask. After bringing to volume, a 5 mL aliquot was mixed with 5 mL of 0.02 M TBA reagent, and heated in boiling water bath for 35 min to facilitate the formation of pink coloured thiobarbituric acid-malonaldehyde complex. After the mixture was cooled, the absorbance was measured at 532 nm (UV-1800 Ultraviolet-Visible Spectrophotometer, Shimadzu Corp., Kyoto, Japan). The extent of lipid oxidation was expressed as mg malonaldehyde / kg sample.

A standard curve was created by mixing 0.75, 1.5, and 2.25 mL of 2×10^{-7} mol / mL of 1, 1, 3, 3-tetramethoxypropane (TMP) with 10% TCA extracting solution (cold) and made to volume of 50 mL. The absorbance was measured with the mixture of 5 mL of TMP / TCA mixture and 5 mL of TBA reagent. The standard curve was plotted by absorbance as a function of TMP concentration. The recovery rate was around 80% with current experiment facilities. Analysis was performed in duplicate per treatment per replicate, and samples that were not analyzed on the day of packaging were frozen and stored in -80 °C until ready for analysis. Samples and reagents were kept cold (< +4 °C) at all times to limit oxidation during the experiment.

4.4.8 Storage stability of meat-lentil bars

There were two storage conditions of the vacuum-packaged, dried meat-lentil bars. One was room temperature (approximate 20 °C) and the other one was accelerated storage at high temperature (approximate 40 °C) (Isotemp Incubator, model 550D, supplied by Fisher). The timelines for storage stability testing were day 1, day 30, day 60, day 90 for both storage conditions.

Measurements for day 1 samples were performed on the next day after the end of equilibration, namely, 8 days after drying (and cooking), and day 30 as 30 days after equilibration and etc.

Analytical methods including moisture content, water activity, level of lipid oxidation, and color and texture of meat-lentil bars following the various storage times and conditions were as described above. Samples from high-temperature storage were allowed to cool at room temperature for approximate 30 min prior to analysis.

4.4.9 Sensory assessment of meat-lentil bars

Faculty, staff and students from the College of Agriculture and Bioresources, University of Saskatchewan were recruited as panelists for this study. A total of 47 panelists agreed to participate. Each participant was assigned a package including a pen, a bottled water, an instruction sheet, a consent form, samples to be evaluated and corresponding scorecards, and a supplementary survey. Meat-pulse strips were cut to 20 mm-width pieces and the product samples were assigned three-digit codes and presented to consumer panelists in randomized order. Samples were presented in 76.2 x 101.6 mm (3” x 4”) transparent resealable polyethylene bags (Bead Leading, supplied by Michaels, TX, U.S.A.), and each bag contained three sample pieces. A number of eight-point intensity and hedonic scales were used, including color intensity, color acceptability, firmness, dryness, overall texture acceptability, smell / aroma acceptability, flavor intensity, flavor acceptability, and overall acceptability (see Appendixes for scoresheet). Participants were asked to complete the scorecard independently, and drink water between samples. The ethics application for this human-involved study has been approved by the University of Saskatchewan Behavioral Ethics Board, and was conducted under Covid precautions. Participants took the package to a location of their choosing to perform the tasting and returned the ballots later.

4.4.10 Statistical analysis

The least square means (LS means) and standard error of means were calculated with 3 replicates of all samples. Observed data except sensory responses were fitted to a three-way (factor: treatments, storage time, storage condition) mixed linear regression model. Pearson correlation analysis was performed to analyze the correlations between variables. Sensory responses were collated and their LS means for treatments were attained (one-way ANOVA). R 4.2.2 (2022) was utilized to perform this analysis. Means were analyzed and separated with the Tukey’s method by R, lsmeans package. Significance was declared at $p < 0.05$.

4.5 Results and discussions

4.5.1 Production of lentil flours: seeds tempering and starch gelatinization

Prior research demonstrated that lentil flours subjected to infrared (IR) heating saw enhancements in their functional and nutritional profiles. IR heating has been shown to decreased the content of antinutrients, such as tannins and phytic acid, and deactivating enzymes that hinder digestion, such as trypsin inhibitor, on lentil and other pulses (Pathiraje et al., 2023; Pathiratne et al., 2015). It has also been associated with influences on multiple physicochemical and functional properties, such as reduced lightness, decreased protein solubility, and increased water- and oil-holding capacities (Liu et al., 2020; Pathiraje et al., 2023). IR heating of pulses (lentil) allows processors a degree of flexibility to balance between physicochemical and functional properties that optimally suit its intended food application, and hence is generally favored in product development and novel food production (Pathiraje et al., 2023).

Table 4.2 presents the damaged (or gelatinized) starch content in lentil flours that underwent different tempering periods. It was clear that the damaged (gelatinized) starch content of lentil flours was increased by tempering. Specifically, lentil flour that was tempered for 24 h exhibited the highest damaged (gelatinized) starch content at 27.28% (dry weight basis), whereas the non-tempered flour had the lowest content, 0.90%. These values align with the results from Pathiratne et al. (2015), given a similar seed moisture content (23%) and surface temperature (150 ºC). Additionally, Liu et al. (2020) reported a similar trend wherein the damaged starch content in lentil flours increased proportionally with the tempering periods, also at similar seed moisture content (25%) and surface temperature (130 °C and 150 °C). No differences in starch damage (gelatinization) were observed between raw and non-tempered flours.

To delve deeper into how starch gelatinization impacts the properties of meat-lentil bars, this study chose to use only those lentil flours exhibiting lowest, non-tempered (NT), and highest, 24 h-tempered (higher-gelatinized, HG), damaged (gelatinized) starch contents.

Table 4.2 Damaged starch contents of lentil flours at different tempering periods with subsequent infrared heating.[1]

Treatments (%)	**raw**	**non-tempered**	**8 h-tempered**	**16 h-tempered**	**24 h-tempered**	SEM
damaged starch, db	1.45^b	0.90^b	22.46^a	22.48^a	27.28^a	2.996
damaged starch, wb	1.33^b	0.85^b	21.54^a	22.01^a	27.24^a	3.23

[1] lentil seeds tempered for certain periods, then infrared heated at surface temperature 150 ºC

[ab] values with different letter within the same row are significant different, $p < 0.05$

SEM: standard error of the estimated least-square means

4.5.2 Processing parameters of meat-lentil bars

The drying period and cook loss of the meat-lentil bars are shown in Table 4.3. Samples of all treatments were dried to, at best practices, same target water activity (0.89). The drying period of samples of all treatments was approximate 170 min, and the cook (and dry) loss was about 37%. No significant ($p < 0.05$) difference were observed between the drying periods of samples of different treatments, possibly resulting in similar cook (and dry) loss. However, NT18 appeared to show relatively shorter drying period (150 min) and lower cook loss (29.1%) than other treatments, especially to Con. The cook loss values in this study were similar to those from Study 1 targeting the same product water activity (L90, L90ECA: 36.7%, 12% flour addition). This reinforced the finding that cook (and dry) loss was mainly determined by target water activity, and the production procedures developed in Study 1 showed replicability. Neither the addition level nor the starch gelatinization (pre-treatment) of lentil flour influenced the thermal processing parameters of meat-lentil bars. Such consistency is favored by manufacturers, as it indicates that the production procedures used for conventional meat snacks, such as jerkies, can be directly implemented when producing the hybrid meat-lentil bars. This ease of transition, combined with the potential benefits of pulses, could make meat-lentil bars an attractive proposition for the food industry.

Table 4.3 Processing parameters of meat-lentil bars.

Treatments	Con	NT6	HG6	NT12	HG12	NT18
Drying period (min) ^{ns}	170	160	185	180	170	150
SEM	10.2					
Cook (and dry) loss (%) ^{ns}	42.5	38.7	39.1	41.0	33.5	29.1
SEM	3.9					
End-drying A_w ^{ns}	0.884	0.889	0.888	0.888	0.894	0.902
SEM	0.0085					

^{ns} no significant different value between treatments

SEM: standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

4.5.3 Proximate composition and pH of meat-lentil bars

The proximate composition and pH of the meat-lentil bars are shown in Table 4.4. Samples of all treatments fulfilled requirements from Canadian Food Inspection Agency (2020) for being shelf-stable (A_w < 0.90 and pH < 5.30). Con appeared to show the lowest pH at 5.17, whereas samples of lentil treatments (HG6, NT12, HG12, NT18), except NT6, showed significantly ($p < 0.05$) higher pH. Addition levels and starch gelatinization of lentil flour did not show influence on product pH of meat-lentil bars. This indicated the strong buffering capacity of meat component in hybrid products. On the other hand, lentil flour addition significantly increased product acidity than Con, which was possibly caused by the higher pH of lentil flour (6.31, Table 4.1) than meat component (~ 5.4, section 4.4).

Table 4.4 Proximate composition and pH of meat-lentil bars. (%, wet weight basis)

Treatments	Moisture	Protein	Carbohydrate[1]	Fat	Ash	pH
Con	37.6^{a}	35.2^{a}	14.5^{a}	8.3^{a}	4.3^{a}	5.18^{a}
NT6	35.3^{ab}	33.1^{a}	20.4^{ab}	6.3^{b}	4.9^{a}	5.20^{ab}
HG6	36.1^{a}	32.7^{a}	20.6^{ab}	6.1^{b}	4.5^{a}	5.22^{b}
NT12	34.7^{ab}	33.3^{a}	21.9^{b}	5.4^{b}	4.6^{a}	5.22^{b}
HG12	34.6^{ab}	31.3^{a}	23.7^{bc}	5.5^{b}	5.0^{a}	5.22^{b}
NT18	31.1^{b}	33.0^{a}	28.1^{c}	4.3^{c}	5.0^{a}	5.22^{b}
SEM	0.94	0.87	1.29	0.21	0.20	0.006

[1] % carbohydrate = 100% - % moisture - % protein - % fat - % ash

^{ab} values from the same column with different superscripts significantly different, $p < 0.05$

SEM: standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

Proximate compositions of samples of meat-lentil bars are shown in Table 4.6. Even though samples of all treatments were dried to similar water activity, Con showed a significantly higher moisture than NT18. This is intriguing because a consistent water activity typically corresponds to a similar moisture content. With NT18 having less moisture, it possibly had more free water molecules, which are available for biological and chemical reactions presented in the products. This might be due to the presence of a large amount of hydrophilic lentil ingredients (polysaccharides, protein etc.) might adversely competes with the interactions between extracted myofibrillar proteins and their interactions with water molecules, causing a disrupted network by a bulking component, in which the large amount of lentil hydrophilic ingredients bind with moisture, reside between the interacting structural proteins. The inherent moisture-binding characteristics of lentil starch and polysaccharides might even pull moisture from the meat, particularly when lentil flour is used in large portion. This could cause loosely attached water molecules to be present in higher concentrations compared to the more secure moisture in Con. However, results from Table 4.8 (discussed later) indicates no major moisture differences across samples, suggesting other factors like meat consistency might have influenced the results.

Significantly lower fat contents were observed in samples of lentil treatments than Con. Additionally, samples with 6% and 12% lentil flour addition had higher fat contents than NT18. This could be attributed to meat having a higher fat content (7.3%) than lentil flour (NT: 1.3%, HG: 1.5%). For carbohydrate, Con showed a lower content than NT12, HG12, and NT18. The difference between NT12 and NT18 was also significant. This is likely because meat has a much lower carbohydrate content (1.5%) than lentil flours (63%).

On the other hand, the ash levels in different samples remained comparable, while lentil flour typically has a higher ash content (3.5%) compared to meat (1.1%). This was likely due to the negligible ash content in both ingredients. Similarly, the protein contents across samples were not significantly different. Samples with higher lentil flour addition were anticipated to have greater protein contents, given its low moisture (NT: 7.7%, HG: 7.2%) and high protein content (NT: 24.7%, HG: 24.6%). This could be attributed to that lentil proteins showed lowest solubility at pH 4 - 5 (Jarpa-Parra et al., 2014). As a result, the reduced solubility might cause lentil proteins to be lost during production or limit their interaction with extracted myofibrillar proteins. This could also account for the lower moisture compared of NT18 than Con, as the reduced protein solubility could impair lentil flour functionality, namely, reduced gelation capacity that help to entrap and stabilize moisture and reduce free water molecules.

Results of Study 1 indicated that, as shown by the different carbohydrate contents between meat-lentil and meat-black bean bars, the form and contents of starch granules, might also exhibit influence on meat-pulse bars properties. This led to further examinations on the starch component in lentil flour in this study. As suggested in Study 1, the presence of damaged starch granules might be the reason for the compositional and functional differences (Study 1: texture and flavor results).

Results from Study 1 suggested that the form and compositions of starch granules might impact the properties of meat-pulse bars. This was evident from the distinct carbohydrate contents between meat-lentil and meat-black bean bars. Consequently, this study delved deeper into the starch component in lentil flour. It was hypothesized, based on Study 1, that the presence of damaged starch granules might account for the observed differences in composition and functions, such as variations in texture and flavor.

Starch gelatinization of lentil flour showed no effect on samples proximate compositions, as no significant difference was observed between NT vs. HG samples at the same addition level. Overall, the addition of lentil flour to meat bars modified its proximate profile. As more lentil flour was added, fat content decreased and carbohydrate content increased. Moisture and protein contents only decreased, and ash content only increased, at highest addition level (18%).

4.5.4 Water activity, and water activity over storage of meat-pulse bars

In Table 4.5, the water activity values of samples of different treatments and their changes during storage are provided. As discussed, samples of all treatments were dried to similar water activity (0.89). These values, combined with the product pH, ensures compliance with the CFIA standards for shelf stability ($A_w < 0.90$ and pH < 5.30). However, it is important to note that this similarity does not necessarily reflect the effect of the treatments, but was rather a processing target. Statistical analysis on complete data set using three-way mixed linear models (factors: treatment, storage time, storage condition) identified significant treatment and storage time effects. No significant storage condition effect and interaction effects between factors was observed.

Table 4.5 Water activity of meat-lentil bar, with storage at ambient and elevated temperatures.

Treatments	Con	NT6	HG6	NT12	HG12	NT18
HT$^{i, ns}$						
Day1	0.884^{a2}	0.889^{a2}	0.888^{a2}	0.888^{a2}	0.894^{a1}	0.902^{a2}
Day30	0.848^{a1}	0.870^{a12}	0.858^{a12}	0.861^{a12}	0.866^{a12}	0.877^{a12}
Day60	0.838^{a1}	0.875^{b2}	0.853^{ab1}	0.852^{ab1}	0.862^{ab1}	0.863^{ab1}
Day90	0.829^{a1}	0.842^{a1}	0.834^{a1}	0.839^{a1}	0.838^{a1}	0.854^{a1}
RT$^{ii, ns}$						
Day1	0.884^{a2}	0.889^{a2}	0.888^{a3}	0.888^{a2}	0.894^{a3}	0.902^{a2}
Day30	0.861^{a12}	0.872^{a12}	0.874^{a23}	0.870^{a12}	0.877^{a23}	0.878^{a12}
Day60	0.837^{a1}	0.859^{a12}	0.847^{a12}	0.851^{a1}	0.855^{a12}	0.867^{a1}
Day90	0.832^{a1}	0.856^{a1}	0.840^{a1}	0.843^{a1}	0.846^{a1}	0.847^{a1}
SEM	0.0085					

[i] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 25% relative humidity

[ii] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 40% relative humidity

[ab] values within the same row with different superscript letters significantly different

[12] values under each storage condition, HT or RT, within the same column with different superscript numbers significantly different ($p<0.05$)

[ns] no significant different value only compared across storage conditions, HT vs. RT

SEM: pooled standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

Despite meeting processing requirements, the water activity values at day 1 (Table 4.5) did not unveil any distinctive treatments effect. Throughout storage, all treatments displayed a decline in water activity, consistent across both storage conditions. Meat-lentil bars in this study demonstrated a more pronounced reduction in water activity when compared to the meat-pulse bars from Study 1, which reported no such decline in water activity during room temperature storage. One possible explanation for this differential behavior might be the varying ambient relative humidity (RH) across the two studies (Study 2: 40%, Study 1: 60%). This larger difference between the moist environment of the meat-lentil bars ($A_w > 0.80$) and the drier ambient atmosphere could have accelerated the moisture movement. Such finding was reported by Wongwiwat and Wattanachant (2015), that chicken meat jerky stored at 33% RH for 30 days showed a greater reduction in water activity and moisture content than those stored at 75% RH. Additionally, no condition effect was identified. This suggests that, contrary to the findings in Study 1, the meat-lentil bars in this study exhibited consistent water activity behavior across different storage conditions.

In RT storage, Con showed significantly lower value in water activity by day 60, whereas lentil treatments except NT6 (HG6, NT12, HG12, and NT18) showed similar rate of change for water activity as Con. Samples of NT6 were slightly more resilient, with their water activity remaining stable until day 90. Despite these variances, all lentil treatments maintained comparable water activity values to Con throughout the RT storage. When under HT storage, samples of Con showed rapidly declined water activity, with significant decrease by day 30. In contrast, bars with lentil treatments started showing reductions by day 60, with NT6, in particular, recording a slower rate of decline. By day 90, however, water activity of NT6 aligned with Con. While there are minor differences in decline rates, particularly for NT6, no interaction effect between treatment and time was identified. These findings suggest that, lentil flour addition and starch gelatinization to meat bars do not substantially alter the storage behaviour of product water activity, namely, product stability to retain water activity and refrain moisture loss.

4.5.5 Moisture content over storage of meat-lentil bars

Table 4.6 presents the moisture content of samples of all treatments across different storage durations and conditions. Notably, when compared to results of proximate compositions in Table 4.4, there was no significant difference in the moisture content between treatments on day 1. This inconsistency could arise from systematic errors, such as variations in the meat composition, moisture alterations during frozen storage, or differing variances used in different datasets and analysis models. Yet, it is important to highlight that the day 1 values in Table 4.6, while not exactly matching, are relatively close to the moisture data in Table 4.4. This suggests that both datasets are likely accurate. Analysis model on the complete data set identified significant treatment, storage time, and storage condition effect, and no significant interaction effect.

Table 4.6 Moisture content of meat-lentil bar, with storage at ambient and elevated temperatures.

Treatments (%)	**Con**	**NT6**	**HG6**	**NT12**	**HG12**	**NT18**
HT$^{i, ns}$						
Day1	36.1^{a2}	35.0^{a2}	36.1^{a2}	36.5^{a2}	32.5^{a1}	33.0^{a1}
Day30	27.2^{a1}	32.4^{a12}	33.6^{a12}	33.0^{a12}	30.6^{a1}	30.2^{a1}
Day60	26.3^{a1}	31.4^{a12}	30.7^{a12}	31.5^{a12}	29.4^{a1}	28.2^{a1}
Day90	23.4^{a1}	28.0^{a1}	28.8^{a1}	28.9^{a1}	26.8^{a1}	26.7^{a1}
RT$^{ii, ns}$						
Day1	36.1^{a2}	35.0^{a1}	36.1^{a1}	36.5^{a1}	32.5^{a1}	33.0^{a1}
Day30	29.8^{a12}	34.3^{a1}	35.8^{a1}	35.6^{a1}	32.6^{a1}	31.4^{a1}
Day60	26.8^{a1}	33.9^{ab1}	34.8^{b1}	31.7^{ab1}	30.9^{ab1}	30.1^{ab12}
Day90	25.9^{a1}	30.8^{a1}	30.4^{a1}	32.3^{a1}	29.5^{a1}	29.6^{a1}
SEM	1.84					

[i] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 25% relative humidity

[ii] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 40% relative humidity

[ab] values within the same row with different superscript letters significantly different

[12] values under each storage condition, HT or RT, within the same column with different superscript numbers significantly different ($p<0.05$)

[ns] no significant different value only compared across storage conditions, HT vs. RT

SEM: pooled standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

Despite the detection of a significant treatment effect, moisture content measurements for all treatments were consistent at day 1. This corresponds with water activity outcomes where, due to processing prerequisites, day 1 values across treatments were intentionally kept similar. Over storage, samples of all treatments tended to lose moisture, across both conditions. This again was likely attributed to the moisture migration out of the packaging, as indicated in Study 1. Broadly, product moisture and related storage behaviour of meat-lentil bars, in this study, were analogous to those observed in Study 1.

Samples of Con seemed to lose moisture faster than those with lentil flour addition, especially under RT. By day 60, Con already showed a significant decrease in moisture content, while other treatments did not display such reduction. Moisture content of Con decreased even more rapidly under HT. Some meat-lentil samples, like NT6, HG6, and NT12, took until day 90 to show any significant moisture loss, while samples of HG12 and NT18 showed no significant moisture loss. This suggested meat bars with lentil flour addition retained moisture better, especially HG12 and NT18. However, given there was no interaction effect observed between treatments and storage time, we might conclude that treatments, lentil flour addition and starch gelatinization, had a minimal impact on storage behavior of product moisture, namely, product stability to retain moisture content.

Despite detecting a significant condition effect, there was not a clear difference in moisture content between samples stored at RT and those at HT at specific storage time. However, numerically, samples stored at RT tended to have slightly higher moisture content. The lack of significant differences based solely on storage condition might be due to the large variance (1.84% in ~ 30%).

Moisture content is an important factor on various properties of meat products, including texture, color, and overall acceptability, as highlighted by Lee and Kang (2003). Yet, there is limited understanding of these effects in shelf-stable, dried meat (and hybrid) products at moisture contents similar as those in this study. As in Study 1, the variations in moisture content among samples probably influenced the sensory characteristics of the meat-lentil bars.

4.5.6 Instrumental color measurements, and color over storage of meat-lentil bars

Instrumental color measurements of meat-lentil bars were delineated as *L** (lightness), *a** (redness), and *b** (yellowness) values. Table 4.7 presents the lightness of meat-lentil bars of different treatments, and their values through storage, under both conditions. Samples of HG12 and NT18 showed significantly higher *L** values than that of Con, namely, lighter color. This was likely due to the inherent bright yellow color of lentil flour. Treatment of starch gelatinization did not show influence on product lightness. Analysis model on the complete data set identified significant treatment, storage time, and storage condition effects, also significant treatments by time and time by condition interaction effects were detected.

Table 4.7 Instrumental *L (lightness) value of meat-lentil bar, with storage at ambient and elevated temperatures.**

Treatments	**Con**	**NT6**	**HG6**	**NT12**	**HG12**	**NT18**
HT[i]						
Day1	31.1^{a1x}	33.2^{ab2x}	33.3^{ab2x}	32.5^{ab2x}	34.4^{b2x}	34.9^{b2x}
Day30	29.7^{ab1x}	28.4^{a1x}	29.1^{a1x}	29.2^{a1x}	33.5^{c12x}	32.4^{bc12x}
Day60	29.7^{bc1x}	26.4^{a1x}	28.2^{ab1x}	27.8^{ab1x}	32.1^{c12x}	30.9^{bc1x}
Day90	29.2^{ab1x}	26.6^{a1x}	27.6^{a1x}	28.0^{ab1x}	31.1^{b1x}	29.7^{ab1x}
RT[ii]						
Day1	31.1^{a1x}	33.2^{ab3y}	33.3^{ab2x}	32.5^{ab2x}	34.4^{b1x}	34.9^{b2x}
Day30	31.4^{a1x}	31.3^{a23y}	31.7^{a12y}	31.0^{a12x}	33.2^{a1x}	33.3^{a12x}
Day60	30.4^{abc1x}	29.1^{a12y}	29.5^{ab1x}	29.9^{abc12x}	32.8^{c1x}	32.5^{bc12x}
Day90	29.8^{abc1x}	27.7^{a1x}	29.1^{ab1x}	29.0^{ab1x}	32.7^{c1x}	31.7^{bc1x}
SEM	0.992					

[i] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 25% relative humidity

[ii] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 40% relative humidity

^{abc} values within the same row with different superscript letters significantly different

^{123} values under each storage condition, HT or RT, within the same column with different superscript numbers significantly different ($p<0.05$)

^{xy} values only compared only across storage condition, HT vs. RT, with different superscript letters significantly different ($p < 0.05$)

SEM: pooled standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

Given that a significant interaction effect was identified, Figure 4.3 provides an interaction plot illustrating the combined effect of treatments and storage time on product lightness, *L** values. It is evident from the plot that as storage time progressed, the difference in *L** values between bars of NT treatment, non-tempered, and those of HG treatment, higher-gelatinized, lentil flours grew more pronounced. Meat-lentil bars with NT flour darkened more, namely, greater decrease in *L** values, over storage, compared to those with HG flour. This suggests an increased ability to retain color (lightness) by HG treatment, or starch gelatinization, upon lentil flour addition, illustrating a color stabilizing effect.

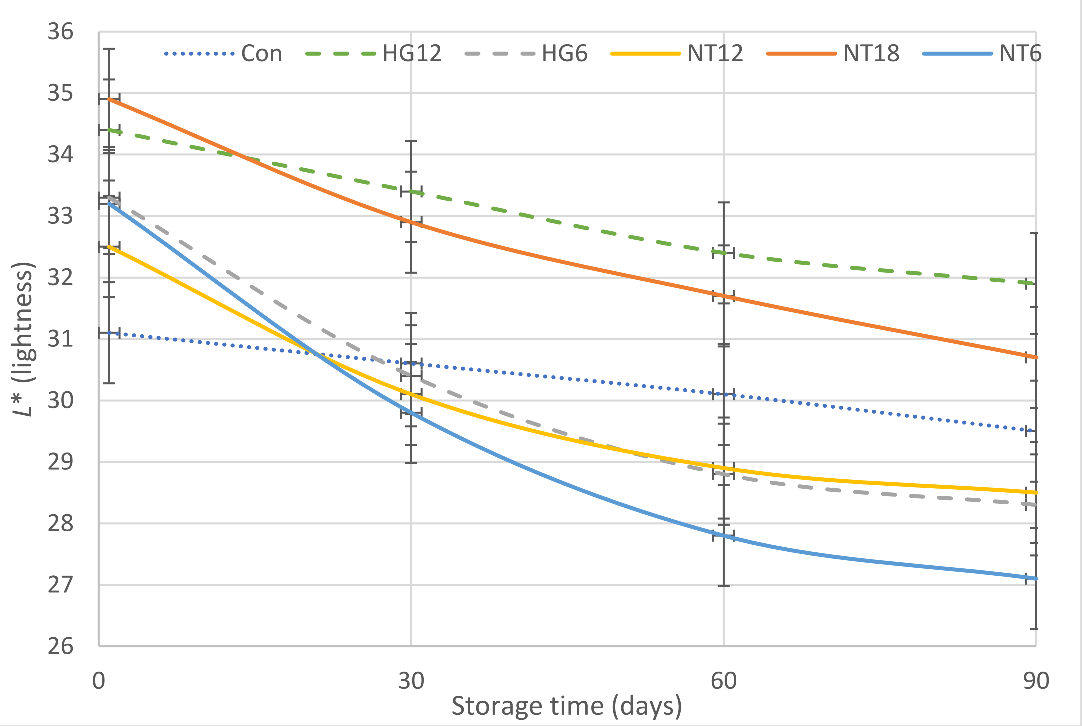


Figure 4.3 Interaction plots of treatment by storage time effect on color *L (lightness) values of meat-lentil bars.**

error bars representing SEM: pooled standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

However, among treatments, Con exhibited the most consistent and stable *L** (smoothest and least change of line) throughout storage, an observation that was surprising. Kim and Shand (2022) found out that lentil flour helped to stabilize the redness of hybrid pork bologna, indicating the stabilizing effect by pulse ingredient. Li et al. (2017) also reported that the oxidation of myoglobin was inhibited by the presence of lentil ingredients in beef burgers. However, both studies focused on hybrid products with relatively high meat component (lentil addition at 6%) and high moisture content (> 60%), and limited research focused on dried meat (and hybrid) products.

The decision to exclude HG18 treatment, which featured an 18% addition of lentil flour from seeds were 24 h-tempered, stemmed from the resulting meat-lentil mixture becoming overly sticky and unmanageable for further processing. This suggests that when lentil flour is introduced into dried meat products, as in this study, it tends to dominate the liquid phase of the system, leading to a final product that lacks in elasticity and flexibility. A possible reason behind this is the abundance of hydrophilic ingredients in lentil flour, such as proteins (24.6%) and poly- and oligosaccharides. These components have a strong water-interacting ability, likely outcompeting and binding with the structural, extracted myofibrillar proteins. This results in a dense filling amidst the structural proteins network, leading to a highly viscous but not very flexible mixture. On one side, this might strengthen product structural integrity, making it more resilient to external disturbance. However, on the other hand, it could delay the migration of reductants, such as the added erythorbates, to the meat proteins. No inhibitory effect and hence increased rate of myoglobin oxidation was shown.

A significant storage time by condition interaction effect was also identified (Figure 4.4). However, except for day 1 values, the line predicting the *L** values by time and condition interaction were quite similar (similar slope and rate of change). The significant interaction effect was likely due to the duplicated day 1 values, namely, no real HT effect yet and not a true interaction effect, and hence should not be considered in this study.

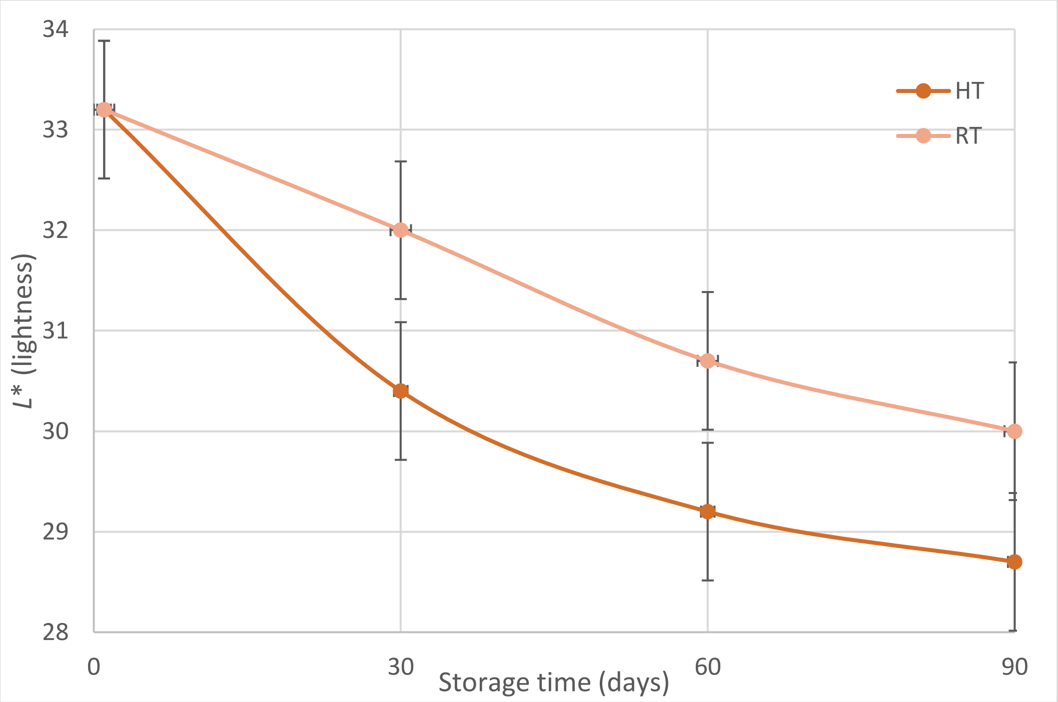


Figure 4.4 Interaction plots of storage time by storage condition effect on color *L (lightness) values of meat-lentil bars.**

error bars representing SEM: pooled standard error of the estimated least-square means
HT: samples stored in incubator at elevated temperature, approximate 40 °C, 25% relative humidity
RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 40% relative humidity

Table 4.8 presents product redness (*a**) of meat-lentil bars and their values over storage. Analysis model on the complete data set identified only significant time and condition showed effects, also significant time by condition interaction effect, but no treatments effect. This indicated that the lentil flour addition and starch gelatinization to meat bars showed no effect on product redness.

Table 4.8 Instrumental *a (redness) values of meat-lentil bar, with storage at ambient and elevated temperatures.**

Treatments	**Con**	**NT6**	**HG6**	**NT12**	**HG12**	**NT18**
HT[i]						
Day1	19.3^{a3x}	18.9^{a3x}	19.2^{a3x}	18.4^{a3x}	19.2^{a3x}	18.6^{a3x}
Day30	11.9^{a2x}	12.6^{a2x}	11.6^{a2x}	12.0^{a2x}	12.5^{a2x}	11.8^{a2x}
Day60	8.7^{a12x}	8.4^{a1x}	8.4^{a12x}	8.3^{a1x}	10.2^{a12x}	9.5^{a12x}
Day90	7.1^{a1x}	7.0^{a1x}	7.1^{a1x}	6.8^{a1x}	8.0^{a1y}	8.3^{a1x}
RT[ii]						
Day1	19.3^{a2x}	18.9^{a2x}	19.2^{a3x}	18.4^{a2x}	19.2^{a2x}	18.6^{a2x}
Day30	15.0^{a1y}	15.4^{a1y}	15.9^{a23y}	15.4^{a12y}	15.6^{a1y}	15.4^{a12y}
Day60	14.2^{a1y}	13.7^{a1y}	13.4^{a12y}	14.6^{a1y}	14.2^{a1y}	14.2^{a1y}
Day90	12.1^{a1y}	12.5^{a1y}	10.6^{a1y}	13.2^{a1y}	13.0^{a1y}	12.5^{a1y}
SEM	1.19					

[i] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 25% relative humidity

[ii] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 40% relative humidity

^{abc} values within the same row with different superscript letters significantly different

^{123} values under each storage condition, HT or RT, within the same column with different superscript numbers significantly different ($p<0.05$)

^{xy} values only compared only across storage condition, HT vs. RT, with different superscript letters significantly different ($p < 0.05$)

SEM: pooled standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

However, storage condition appeared to show detrimental effect on redness of the bars, with the *a** values of each treatment at each time point significantly different between storage conditions. High-temperature storage appeared to greatly decrease sample *a** values much more quickly than those under room-temperature storage. Meanwhile, samples of all treatments showed significantly decreased *a** values than day 1 at day 90, under both HT and RT. Specifically, all treatments showed significantly lower *a** values since day 30 at HT, whereas under RT, Con and NT6 showed significantly lower *a** values since day 30, but HG6, NT12, HG12, and NT18 did not show those until day 60. This was also illustrated as the stronger color defecting effect by HT storage (Figure

4.5). Also, the implication that HG6, NT12, HG12, and NT18 might be associated with smaller rate of change, and increased stability of color properties, agreeing with the results of *L**.

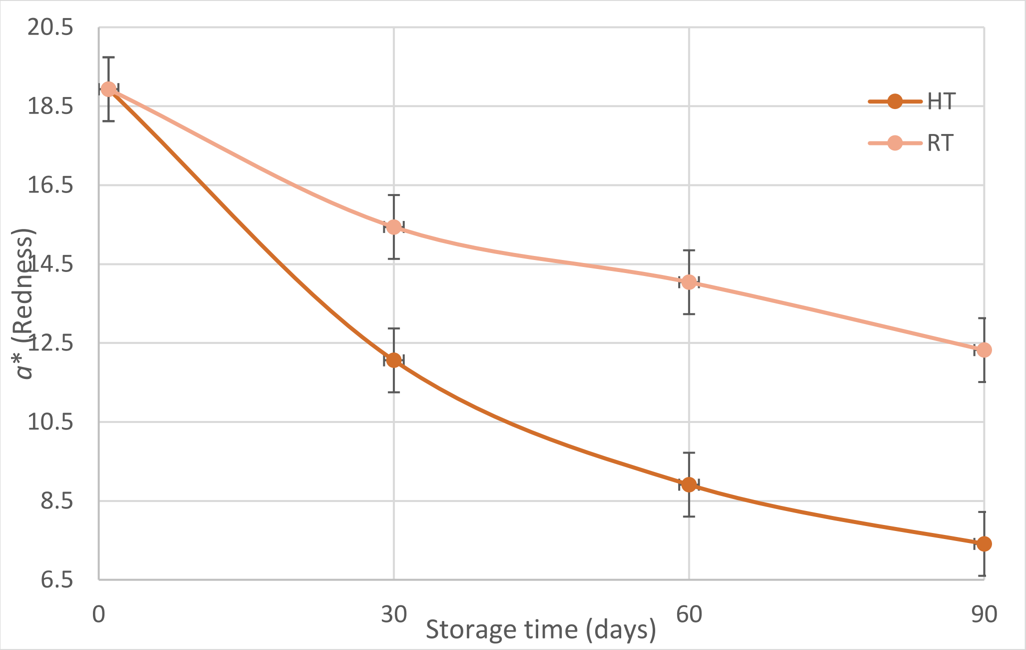


Figure 4.5 Interaction plots of storage time by storage condition effect on color *a (redness) values of meat-lentil bars.**
error bars representing SEM: pooled standard error of the estimated least-square means
HT: samples stored in incubator at elevated temperature, approximate 40 °C, 25% relative humidity
RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 40% relative humidity

On the other hand, storage conditions evidently had a pronounced impact on product redness (*a** values). Given that a significant time by condition interaction effect was identified, Figure 4.5 provides an interaction plot on product redness, *a** values. As demonstrated from the plot, meat-lentil bars under HT storage experienced a more rapid decline in their a* values, as compared to those stored at RT. By day 90, the *a** values for all treatments, under both conditions, had significantly reduced from their initial day 1 values. Breaking it down further, at HT, product redness of all treatments had decreased significantly by day 30. Conversely at RT, only Con and NT6 showed this decline by day 30. Treatments such as HG6, NT12, HG12, and NT18 maintained their redness till day 60. This phenomenon underscores the negative effects of HT storage on color (redness), as highlighted in Figure 4.5.

The interaction effect of time by condition on product redness, *a**, in the meat-lentil bars, offers valuable insights into how lentil incorporation affects product color. In cured meat and hybrid systems, as in this study, where pulses do not inherently product redness, the *a** value essentially indicant the level of nitroso hemochrome, the characteristic red cured meat pigment.

Previous results indicated that moisture content of all treatments was similar over storage, and no difference in product moisture was found between HT and RT storage. Under situations like this, the significant time by condition interaction on product redness suggests a greater decline in the red pigment under HT storage. This decline was likely due to chemical reactions, specifically the oxidation of the meat pigment myoglobin.

Furthermore, the distinct treatment by time interaction observed for product lightness (*L** values), alludes to the role of HG lentil flour to inhibit color changes. This idea finds support in the findings of Pathiratne et al. (2015). They observed that despite similar protein contents, lentil seeds exhibited reduced peroxidase activity as they were exposed to increased moisture levels through tempering. This aligns with the NT and HG lentil treatments in this study, experienced either none or extended tempering, respectively, and thus had higher seed moisture levels. While other studies have underscored the potential of micronized lentil to inhibit lipid and protein oxidation (Kim & Shand, 2022; Shariati-Ievari et al., 2016), this study stands out as is the first to reveal the intensified protective effect against myoglobin oxidation offered by lentil seeds tempering.

Table 4.9 presents product yellowness, *b** values, of meat-lentil bars and their values over storage. Analysis model on the complete data set identified significant treatments, time and condition effects, also significant time by condition interaction effect. Similar to lightness, color yellowness of HG12 and NT18 was significantly higher than that of Con, which was likely due to the inherent bright yellow color of lentil flour. Samples of meat-lentil bars except NT6 experienced reduction in yellowness under both storage conditions, and their storage behaviour on rate and extend of changes were similar. Con and NT6 maintained stable yellowness throughout the storage duration across both conditions, positioning them as the most stable treatments in terms of yellowness retention. By day 60, the yellowness values of meat-lentil treatments, excluding NT6, resembled those of Con. This suggests that the lentil flour addition and starch gelatinization might have a minor impact on product yellowness and its subsequent ability to retain yellowness, namely, stability, during storage. Even though a significant time by condition interaction was identified, the predictive lines for *b** values based on this interaction were quite similar. Consequently, this interaction may not have practical significance in this study and could potentially be overlooked.

Table 4.9 Instrumental *b (yellowness) values of meat-lentil bar, with storage at ambient and elevated temperatures.**

Treatments	Con	NT6	HG6	NT12	HG12	NT18
HT[i]						
Day1	10.7^{a1x}	12.1^{ab1x}	12.3^{ab3x}	12.6^{ab3x}	13.7^{b2x}	14.0^{b2x}
Day30	9.6^{a1x}	10.2^{a1x}	10.4^{a23x}	10.0^{a2x}	11.9^{a12x}	11.1^{a1x}
Day60	9.1^{a1x}	10.2^{a1x}	8.4^{a12x}	7.8^{a12x}	10.3^{a1x}	9.8^{a1x}
Day90	9.1^{ab1x}	10.0^{b1x}	7.7^{ab1x}	7.4^{a1x}	9.8^{ab1x}	8.9^{ab1x}
RT[ii]						
Day1	10.7^{a1x}	12.1^{ab1x}	12.3^{ab2x}	12.6^{ab2x}	13.7^{b2x}	14.0^{b2x}
Day30	9.9^{a1x}	11.4^{ab1x}	11.4^{ab12x}	11.7^{ab12x}	12.4^{ab12x}	12.6^{b12x}
Day60	9.7^{a1x}	10.9^{a1x}	10.1^{a12x}	10.1^{a1y}	11.1^{a1x}	11.9^{a12y}
Day90	9.3^{a1x}	10.8^{a1x}	9.5^{a1x}	9.3^{a1y}	11.1^{a1x}	10.5^{a1x}
SEM	0.82					

[i] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 25% relative humidity

[ii] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 40% relative humidity

[abc] values within the same row with different superscript letters significantly different

[123] values under each storage condition, HT or RT, within the same column with different superscript numbers significantly different ($p<0.05$)

[xy] values only compared only across storage condition, HT vs. RT, with different superscript letters significantly different ($p < 0.05$)

SEM: pooled standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

The color profile of samples of all treatments in this study shows a general similar *L** value (30 – 35), but slightly higher *a** (~18 vs. ~10) and *b** (10 – 15 vs. ~5) values than the beef jerkies with similar moisture content from other study (Konieczny et al., 2007). The difference was likely attributed to the lentil flour addition, imparts its intrinsic yellow hue, thereby increasing the *b** value. The higher *a** values observed in this study were possibly due to the shorter drying periods. Consequently, there was a diminished exposure to oxygen from the hot, dry air, resulting in a lower extent of myoglobin oxidation at the end of drying (and cooking). Overall, lentil flour addition to meat bars altered its color profile and was a negative influence on color stability, but treatments with higher addition level or more gelatinized flour might compensate for this negative effect as they showed increased color stability than hybrid products with low addition levels.

4.5.7 Instrumental texture measurements, and texture over storage of meat-lentil bars

Texture evaluation of meat products is important to control product quality and to ensure certain characteristics are met to produce an acceptable product. Instrumental tests on texture parameters such as shear are commonly used to predict sensory textural characteristics, which are major determinants of consumer acceptance of foods products (AMSA, 2016; Greve et al., 2010).

4.5.7.1 Warner-Bratzler (WB) shear test

A very common test used to determine the textural properties of meats and dried meat products is the Warner Bratzler (WB) shear test. This test measures the force required to shear through the sample by a perpendicular blade, and hence is a good indicator of bite force, firmness, toughness etc (AMSA, 2016)

Table 4.10 shows the WB shear force values of samples from different treatments, also the values over storage. Significant differences were observed between the WB shear force values of samples of different treatments, indicating the significant treatment effect on sample textural properties, even under the similar water activity (and moisture content). Con and HG6 showed the lowest (32.6 and 35.3 N respectively) shear values, which were significantly lower than those of NT12 (41.7 N), HG12 (44.5 N), and NT18 (46.8 N). NT6 showed the third lowest value (35.6 N), which was significantly lower than those of HG12 and NT18, but not NT12. It was clear that with the flour addition at sufficient level (12% in this case), the WB shear force values of meat-lentil bars increased as flour addition increased. No significant difference was observed between the NT and HG treatments at the same addition level, suggesting the little to no effect of flour gelatinization level on WB shear force values of the samples. However, while NT6 and NT12 showed similar values, the WB shear force values of HG6 was significantly lower than that of HG12. This suggested that the higher gelatinization level of lentil flour might be associated with a greater, but possibly not significantly, increase in WB shear force values, since an addition difference at only 6% for HG treatments (versus 12% for NT) was statistically different. Results of WB shear values in this study were generally lower than those reported in other studies (Shazer et al., 2018; Wongwiwat & Wattanachant, 2015). This was likely due to of the higher (0.88 - 0.90 vs. 0.78 - 0.80) moisture content (and water activity) in this study might be associated with higher elastic modules, as reported by Ioffe et al. (2002). Analysis model on the complete data set identified significant treatment, storage time, and storage condition effects, also with significant treatment by storage time and storage time by storage condition interactions effects.

Table 4.10 Warner-Bratzler shear values of meat-lentil bar, with storage at ambient and elevated temperatures.

Treatments (N)	**Con**	**NT6**	**HG6**	**NT12**	**HG12**	**NT18**
HT[i]						
Day1	32.6^{a1x}	35.6^{ab1x}	35.3^{a1x}	41.7^{bc1x}	44.5^{c1x}	46.8^{c1x}
Day30	39.2^{a2x}	40.0^{a12x}	39.4^{a12x}	48.3^{b2y}	52.3^{b2y}	53.6^{b2y}
Day60	42.8^{a23x}	41.3^{a2x}	43.1^{a23x}	52.4^{b23x}	54.9^{bc2x}	58.9^{c23y}
Day90	47.7^{a3y}	43.1^{a2x}	45.7^{a3x}	57.0^{b3x}	57.0^{b2x}	64.5^{c3y}
RT[ii]						
Day1	32.6^{a1x}	35.6^{ab1x}	35.3^{a1x}	41.7^{bc1x}	44.5^{c1x}	46.8^{c1x}
Day30	37.9^{a2x}	39.3^{a1x}	37.3^{a12x}	43.5^{ab12x}	46.1^{b12x}	49.3^{b12x}
Day60	41.0^{a2x}	40.1^{a1x}	41.0^{a2x}	48.9^{b23x}	51.5^{b23x}	53.0^{b23x}
Day90	43.1^{a2x}	41.0^{a1x}	42.6^{a2x}	53.8^{b3x}	53.2^{b3x}	57.8^{b3x}
SEM	1.54					

[i] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 25% relative humidity

[ii] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 40% relative humidity

[ab] values within the same row with different superscript letters significantly different

[12] values under each storage condition, HT or RT, within the same column with different superscript numbers significantly different ($p<0.05$)

[xy] values only compared only across storage condition, HT vs. RT, with different superscript letters significantly different ($p < 0.05$)

SEM: pooled standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

WB shear force values of the samples increased under both room-temperature (RT) and high-temperature (HT) storage. Under RT, all treatments except NT6 started to show significantly increased WB shear force values by day 60, whereas NT6 showed no significantly different values through RT storage. Additionally, NT12, HG12, and NT18 showed a further increase in WB shear force values at day 90, as compared to those at day 60. Under HT, Con, NT12, HG12 and NT18 showed significantly increased WB shear force values by day 30, whereas NT6 and HG6 showed by day 60. Con, HG6, NT12, and NT18 continued to show a further increase in WB shear force values at day 90. Both temperatures demonstrated the increasing firming effect during storage, which could be associated with the moisture loss. Moisture could take up the space between the meat proteins network and function as smoothing and softening agents in meat-lentil bars, and hence moisture loss during storage likely results in increased strength of structural proteins network, demonstrated by the increased WB shear value.

Given that significant interaction effects were identified, interaction plots between treatment and storage time effect (Figure 4.6) were produced. Unlike results of water activity and moisture content, the significant interaction effect between treatment and storage time on sample WB shear value indicated that as storage time increased, treatment significantly influenced product behavior, and hence, stability. Stability, is defined as product ability to maintain its physicochemical properties as similar to its day 1 value, under storage conditions. Product physicochemical changes over storage such as moisture loss contributes to lack of stability.

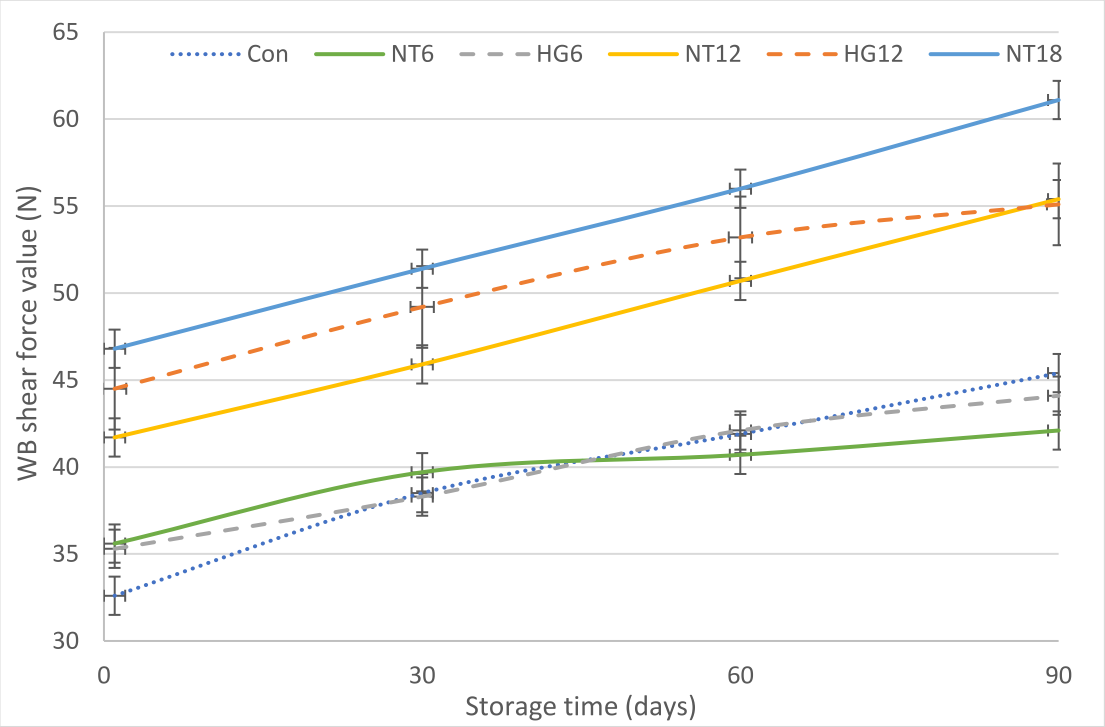


Figure 4.6 Interaction plots of treatment by storage time effect on Warner-Bratzler shear values of meat-lentil bars.

error bars representing SEM: pooled standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

From Figure. 4.6, NT6 showed the least and slowest (small slope value) change, while HG6 and Con showed slower change than other treatments. This suggested that meat bars with 6% addition of lentil flour were able to maintain a more stable WB shear force value, or, instrumental texture profile, over storage, especially for treatment with non-gelatinized flour. However, when the addition level of lentil flour was higher (12, 18%), this stabilizing effect on product texture (WB shear force value) was no longer demonstrated, basing on the greater and quicker change of WB values of NT12, HG12, and NT18. This might again be due to that moisture loss of meat-lentil bars could lead to the formation of a more rigid proteins structure that showed increased strength and WB shear force value. Treatment with more-gelatinized lentil flour might be associated with more stable WB shear values, but this trend was not illustrated until longer storage (HG12 more stable line than NT12, HG6 similar slope as NT6, after day 60)

As mentioned, the WB shear force value indicated the greatest force required to shear through a sample. Properties of a meat product such as the location of muscle proteins, the structure of the muscle proteins network, and the homogeneity of the product, influence its structural strength to resist shear, and hence influence WB shear force values (AMSA, 2016). Additionally, factors that influence the structural strength of a meat product, such as moisture content, influence WB shear force values. The WB shear force values of meat-lentil bars in this study were relatively higher to jerkies at similar moisture content (36%), reported by Sorapukdee et al. (2016). This was likely due to the enhanced structural strength by the functioning hydrophilic ingredient introduced by lentil flour addition to myofibrillar proteins matrix, and hence firmer texture and increased WB shear values.

4.5.7.2 Three-point bending test: Break force

While the Warner Bratzler shear test is commonly applied to dried meat products such as jerky, the meat-lentil bars in this study represents a hybrid product that is expected to possess certain properties of snack bars. With current snack bars generally being a reconstructed products containing multiple ingredients that are glued to a base (ex. granola bar), textural properties relating to sample flexibility, such as bendability, are usually of interest. One of the tests to determine these properties is the three-point bending test (Moss et al., 2023).

The three-point bending test was applied, and the force required to induce the first-sight of sample textural failure (breakage) under bending was measured. Sample values of bending force at first-sight breakage (break force) for different treatments are shown in Table 4.11, also their changes over storage. Similar as WB shear force, a significant treatment effect was identified for break force values. Meat bars with no lentil flour addition showed the lowest break force values (5.1 N). Meat-lentil bars at 12% addition showed significantly higher break force values than Con (NT12: 10.1 N, HG12: 9.9 N), but not toward those at 6% addition. NT18 then showed the highest break force value (11.7 N), which was significantly higher than those of Con, NT6, and HG6. It was clear that with the sufficient addition level (12% in this case), break force values of meat-lentil bars increased as the flour addition level increased. On the other hand, starch gelatinization of lentil flour showed no effect on break force values. This results generally agreed with results of WB shear force values, except that no significant difference was found between HG6 and HG12. Analysis model on the complete data set identified significant treatment, storage time, and storage condition effect, but no interaction effects.

Table 4.11 Break force values, by three-point bending test, of meat-lentil bar, with storage at ambient and elevated temperatures.

Treatments (N)	**Con**	**NT6**	**HG6**	**NT12**	**HG12**	**NT18**
HT[i]						
Day1	5.1^{a1x}	6.1^{ab1x}	7.1^{ab1x}	10.1^{bc1x}	9.9^{bc1x}	11.4^{c1x}
Day30	6.7^{a12x}	8.5^{ab12x}	9.3^{abc12x}	13.6^{c12x}	11.5^{bc12x}	13.2^{c1x}
Day60	7.3^{a12x}	8.9^{ab12x}	9.7^{ab12x}	14.8^{c2x}	12.6^{bc12x}	15.2^{c1x}
Day90	9.4^{a2x}	10.5^{a2x}	12.3^{ab2x}	17.1^{c2x}	15.2^{bc2x}	19.3^{c2y}
RT[ii]						
Day1	5.1^{a1x}	6.1^{ab1x}	7.1^{ab1x}	10.1^{bc1x}	9.9^{bc1x}	11.7^{c1x}
Day30	5.9^{a1x}	7.1^{ab1x}	8.4^{abc1x}	11.9^{cd12x}	11.2^{bcd1x}	12.9^{d12x}
Day60	6.1^{a1x}	7.5^{a1x}	8.2^{ab1x}	12.6^{bc12x}	12.0^{bc1x}	13.8^{c12x}
Day90	7.6^{a1x}	8.8^{a1x}	9.6^{ab1x}	15.0^{c2x}	13.3^{bc1x}	15.7^{c2x}
SEM	1.11					

[i] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 25% relative humidity

[ii] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 40% relative humidity

^{ab} values within the same row with different superscript letters significantly different

^{12} values under each storage condition, HT or RT, within the same column with different superscript numbers significantly different ($p<0.05$)

^{xy} values only compared only across storage condition, HT vs. RT, with different superscript letters significantly different ($p < 0.05$)

SEM: pooled standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

Break force values of the meat-lentil bars increased under both storage conditions. Under RT, only NT12 and NT18 showed significantly increased break force values by day 90. Under HT, all treatments except NT12 showed significantly increased break force value by day 90, whereas NT12 showed by day 60. The increasing bending force with storage also agreed with the general trends of previous results (water activity, moisture content, WB shear force values). On the other hand, this might imply that treatments had an effect on the stability (rate of change) of break force values. NT12 appeared to show the quicker change on break force values and hence lower stability than other treatments under both storage conditions, whereas NT18 also showed lower stability than other treatments under RT. Although, it is noted that NT18 still experienced a greater increase on break force value under HT than RT. The suggested stabilizing effect of NT18 was likely due to the more rapid change of other treatments under HT. However, no significant interaction between treatment and storage time was found.

Only NT18 showed a significantly higher break force value than that of RT, at day 90, HT. Together with discussion above, this demonstrated the accelerating effect on altering product texture by HT storage, especially for treatments at high flour addition level (18%).

As mentioned, the three-point bending test is used to assess the elasticity and flexibility of a product. It measures the tendency of a material to fracture, crumble, crack, or fail upon the application of force, and hence it is a good indicator for hardness, crumbliness, crunchiness, and brittleness. Specifically for the bending force value at first-sight breakage, it measures the rupture force that induces the first crack, or first-sight texture defect, of the product (Greve et al., 2010). Meanwhile, the WB shear force test shears through the sample and provides WB shear force value that indicates the whole-sample shear resistance upon complete textural failure. Therefore, even though surface strength also show influence, WB shear force values are usually used to describe the whole-sample textural properties, such as firmness (AMSA, 2016). On the other hand, when the break force value could also be influenced by the strength of internal structural tissue, it shows better representation on the sample surface strength, especially for non-homogenous products such as meat-lentil bars. It also focuses more on sample flexibility by allowing certain degrees of texture defect such as cracks or bending deformation, while samples subjected to WB shear force test seldom moves then deforms (Luyten et al., 1992). This also explains the slightly different treatment effect on sample break force than WB shear force values. In this study, the break force value was expected to associate with sample surface strength, surface cohesiveness, and overall texture acceptability.

4.5.7.3 Three-point bending test: Stiffness

Sample stiffness under three-point bending test was obtained as the slope of the force - displacement relationship until first-sight breakage. Stiffness, a constant modulus of the sample that determines sample deformability under applied force, represents the highest potential of product resistance to deformation, bending in this case, before texture defect (first-sight breakage).

Hence, it becomes a good indicator of textural properties relating to the strength of internal interactions, such as cohesiveness and crumbliness (Greve et al., 2010).

Table 4.12 shows the stiffness of samples of different treatments, also the values over storage. Con showed the lowest stiffness value (0.44), while NT18 showed a significantly higher stiffness value (1.04). This indicated an increasing effect on sample stiffness at the highest level of lentil flour addition (NT18), whereas treatments with flour addition at lower levels showed no influence. No significant difference was identified by the treatment on starch gelatinization. Analysis model on the complete data set identified significant treatment, storage time, and storage condition effects, also significant time by condition interaction effect.

Table 4.12 Stiffness, by three-point bending test, of meat-lentil bar, with storage at ambient and elevated temperatures.

Treatments (N / mm)	**Con**	**NT6**	**HG6**	**NT12**	**HG12**	**NT18**
HT[i]						
Day1	0.44^{a1x}	0.52^{ab1x}	0.59^{ab1x}	0.85^{ab1x}	0.91^{ab1x}	1.04^{b1x}
Day30	0.67^{a12x}	0.78^{a1x}	0.91^{ab12x}	1.32^{b12x}	1.33^{b12x}	1.14^{ab1x}
Day60	0.73^{a12x}	0.83^{a1x}	0.95^{ab12x}	1.50^{c2y}	1.38^{bc12x}	1.46^{bc1x}
Day90	0.95^{a2x}	0.97^{a1x}	1.16^{ab2x}	1.76^{c2y}	1.68^{bc2y}	2.03^{c2y}
RT[ii]						
Day1	0.44^{a1x}	0.51^{ab1x}	0.59^{ab1x}	0.85^{ab1x}	0.91^{ab1x}	1.04^{b1x}
Day30	0.55^{a1x}	0.62^{ab1x}	0.79^{abc1x}	1.08^{abc12x}	1.14^{bc1x}	1.18^{c1x}
Day60	0.56^{a1x}	0.64^{a1x}	0.80^{ab1x}	1.09^{ab12x}	1.21^{b1x}	1.26^{b1x}
Day90	0.78^{a1x}	0.82^{a1x}	0.91^{ab1x}	1.36^{bc2x}	1.28^{abc1x}	1.53^{c1x}
SEM	0.143					

[i] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 25% relative humidity

[ii] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 40% relative humidity

[ab] values within the same row with different superscript letters significantly different

[12] values under each storage condition, HT or RT, within the same column with different superscript numbers significantly different ($p<0.05$)

[xy] values only compared only across storage condition, HT vs. RT, with different superscript letters significantly different ($p < 0.05$)

SEM: pooled standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

Sample stiffness increased under both storage conditions. Under RT, only NT12 showed significantly increased stiffness than by day 90, while other treatments maintained similar values to Con through storage. Under HT, Con, HG6, HG12, and NT18 showed significantly increased stiffness by day 90, while NT12 showed that by day 60, and NT6 maintained within similar values through storage. Also, the stiffness value of NT18 at day 90 was significantly higher than its day 30 and 60 values. This agreed with the general trends of previous results, especially with break force values. NT12 appeared to show quicker change and hence reduced stability than other treatments, under both conditions. Con did not appear to show quicker change in stiffness than other treatments (except NT6), indicating no effect of lentil flour addition to improve stability of sample stiffness. However, unlike results of break force values, NT6 appeared to show the highest stability of stiffness under both conditions, while no difference was observed between Con or HG for break force values. On the other hand, NT6 showed more stable stiffness than HG6, but reverse for NT12 vs. HG12. Hence, we can conclude on the effect of starch gelatinization on the stability of product stiffness, and further examinations are required.

Significant time by condition interaction was identified, and the interaction plot was produced as Figure 4.7. Th plot clearly demonstrates an accelerating effect by HT storage to increase product stiffness. Specifically, NT12 showed significantly higher stiffness values than RT at day 60 and 90, while HG12 and NT18 showed that at day 90, HT. This indicates that the condition effect to increased product stiffness by HT is more pronounced by lentil flour addition especially at high addition level (12 and 18%).

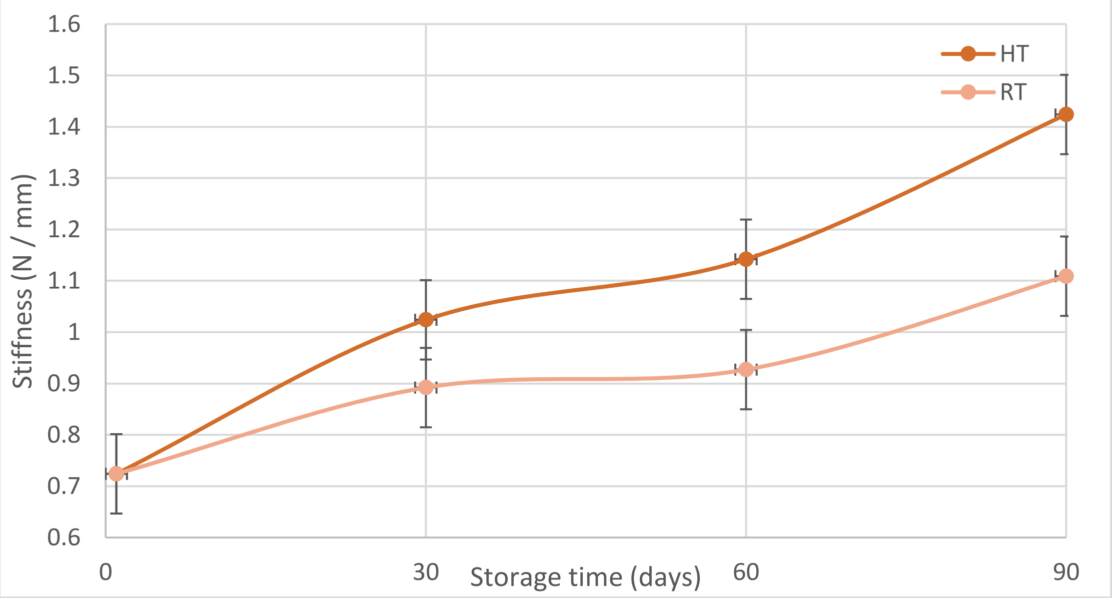


Figure 4.7 Interaction plots of storage time by storage condition effect on stiffness by three-point bending test of meat-lentil bars.
error bars representing SEM: pooled standard error of the estimated least-square means
HT: samples stored in incubator at elevated temperature, approximate 40 °C, 25% relative humidity
RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 40% relative humidity

4.5.7.4 Three-point bending test: Ultimate strength, and total strength by bending

Table 4.13 shows the work absorbed by samples of different treatments under the three-point bending test, either upon the first-sight of texture defect (breakage) or upon complete textural failure (end of test). These values were defined as the ultimate strength and sample total strength by bending, and their values over storage also recorded in Table 4.13. For both parameters, analysis model on the complete data set identified significant treatment, storage time, and storage condition effects, and no significant interaction effect.

Table 4.13 Ultimate and total strength, by three-point bending test, of meat-lentil bar, with storage at ambient and elevated temperatures.

Ultimate strength						
Treatments (N x mm)	**Con**	**NT6**	**HG6**	**NT12**	**HG12**	**NT18**
HT[i, ns]						
Day1	39.9^{a1}	46.7^{ab1}	57.2^{abc1}	82.5^{c1}	71.7^{bcd1}	89.6^{d1}
Day30	45.9^{a1}	62.5^{a12}	65.1^{a1}	96.9^{bc12}	68.8^{ab1}	103.1^{c12}
Day60	49.8^{a1}	64.8^{a12}	66.3^{a1}	98.0^{b12}	77.3^{ab1}	107.2^{b12}
Day90	64.9^{a1}	78.2^{a2}	81.7^{a1}	112.8^{bc2}	90.2^{ab1}	125.5^{c2}
RT[ii, ns]						
Day1	39.9^{a1}	46.7^{ab1}	57.2^{abc1}	82.5^{cd1}	71.7^{bcd1}	89.6^{d1}
Day30	41.0^{a1}	55.2^{ab1}	59.7^{abc1}	89.5^{cd1}	74.2^{bcd1}	94.2^{d1}
Day60	45.8^{a1}	58.4^{ab1}	64.5^{ab1}	99.2^{c1}	79.7^{bc1}	102.2^{c1}
Day90	49.8^{a1}	64.7^{ab1}	69.9^{ab1}	107.4^{c1}	82.8^{bc1}	111.5^{c1}
SEM	7.44					

Total strength						
Treatments (N x mm)	**Con**	**NT6**	**HG6**	**NT12**	**HG12**	**NT18**
HT[i, ns]						
Day1	106^{a1}	128^{ab1}	134^{ab1}	176^{bc1}	208^{c1}	199^{c1}
Day30	129^{a12}	171^{ab12}	149^{ab1}	184^{bc1}	229^{c1}	225^{c12}
Day60	143^{a12}	171^{ab12}	167^{ab1}	201^{bc1}	243^{c1}	250^{c2}
Day90	172^{a2}	180^{a2}	182^{a1}	222^{ab1}	248^{b1}	255^{b2}
RT[ii, ns]						
Day1	106^{a1}	128^{ab1}	134^{ab1}	176^{bc1}	208^{c1}	199^{c1}
Day30	120^{a1}	145^{ab1}	151^{ab1}	180^{bc1}	224^{c1}	207^{c1}
Day60	126^{a1}	156^{ab1}	153^{ab1}	189^{bc1}	230^{c1}	225^{c1}
Day90	141^{a1}	165^{ab1}	163^{ab1}	204^{bc1}	238^{c1}	235^{c1}
SEM	13.0					

[i] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 25% relative humidity

[ii] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 40% relative humidity

[ab] values within the same row with different superscript letters significantly different

[12] values under each storage condition, HT or RT, within the same column with different superscript numbers significantly different ($p<0.05$)

[ns] no significant different value only compared across storage conditions, HT vs. RT

SEM: pooled standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

All treatments at both storage conditions experienced significant increases in ultimate strength and total strength over time. These represent sample resistance toward first deformation and complete textural failure, respectively. Significant differences were observed between any values of NT12, HG12 and NT18 vs. Con, any of NT12, and NT18 vs. NT6, and NT18 vs. HG6. Clearly, with sufficient addition level (12%), the ultimate strength of meat lentil bars increased as the flour addition level increased. No effect was shown by treatments with flours at starch gelatinization. These results agreed with those of WB shear force and break force values. However, considering that HG6 vs. HG12 showed no significant difference but NT6 vs. NT12 did, treatment with flour at higher gelatinization level might be associated with lower increasing effect. Given that sample ultimate strength considers only the first sight of sample breakage, but not complete texture failure, it should show a relation with break force. The significantly different values between NT6 and NT12 that was not observed from break force results, was possibly caused by differences in slope or x value that both determine the area under the force-displacement curve, also known as stiffness and displacement.

For total strength, significant differences were observed between any values of NT12, HG12 and NT18 vs. Con, and any of HG12, and NT18 vs. either NT6 or HG6. This clearly indicated the increasing sample total strength with lentil flour addition, whereas under sufficient addition level (6% for HG and 12% for NT), sample total strength increases as addition level increased. No significant effect was found between treatments with starch gelatinization. However, similar as WB shear value, HG12 showed a significantly higher total strength than HG6, while NT12 did not. This again suggests the possibly greater increasing effect by treatments with lentil flour at higher gelatinization level. Both WB shear value and total strength obtain measurements upon complete texture failure, and hence both are good representations of the total sample resistance against deformation (to texture failure).

4.5.7.5 Correlation between variables of instrumental texture measurements

Figure 4.8 indicates the correlation coefficients among the texture parameters measured by the two instrumental tests. A strong ($r > 0.85$, adjusted correlation coefficient indicated 85% of data points were on the best-fit linear line between the variables), significant relationship has found between any two of the textural parameters. This indicated that the test results between WB shear value and three-point bending were associated with each other. Given that three-point bending test was included in this study in order to reveal a more complex product texture profile, the results did not suggest a new aspect. However, three-point bending test has been interested and widely applied in plant-based bar-like products (Greve et al., 2010; Kim et al., 2009), and is still recommended for plant-based and hybrid products.

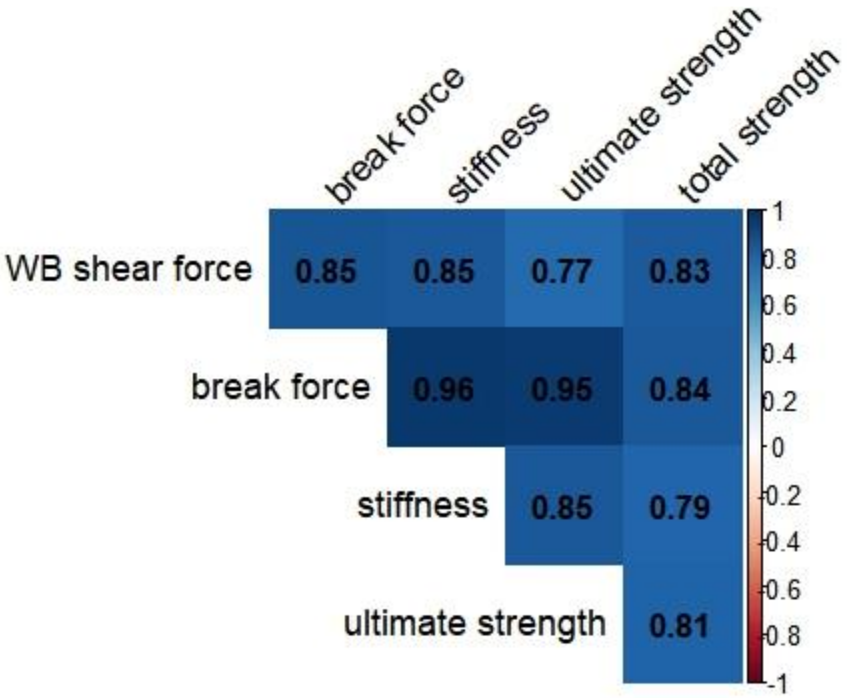


Figure 4.8 Correlogram of correlation coefficients (r) among instrumental measurements on texture, Warner-Bratzler shear test, and three-point bending test. n = 144
significant, $p < 0.05$, positive correlations displayed in blue boxes and negative correlations in red, correlations with $p > 0.05$ not shaded.
correlation coefficients shown by the legend at the right, color intensity proportional to the correlation coefficients.

While the break force value focused more on product surface tension and stiffness than on internal strength (discussed above), it was interesting to see that the two variables showed a pretty high correlation (0.96). Similar result was also shown as WB shear value showed a same correlation between break force and stiffness values. This indicated that when measuring, the determinant factor of stiffness was likely the maximum force (break force value), but not displacement. This then suggested the low resistance of product toward deformation, and hence low flexibility (or bendability). This agreed with results of Study 1 suggesting that product bendability and cohesiveness increased at higher moisture content (magnitude difference in this study).

As mentioned, ultimate strength reveals product surface tension, and total strength reveal overall (surface and internal) strength. Ultimate strength showed a pretty high correlation with break force value (0.95), and a relatively low correlation with WB shear value (0.77). This was reasonable as ultimate strength measured only until surface breakage, also suggesting low product bendability. Total strength showed similar correlations with other texture measurements. This indicated that the energy required to cause texture failure by bending (total strength) was similarly correlated to that of surface tension (break force), internal strength (stiffness), or texture failure by cutting (WB shear), suggesting a relatively consistent product and low product bendability. Overall, the three-point bending test provided more useful indicators on texture parameters than the WB shear force test, including break force, stiffness, and total strength values. On the other hand, when time and effort are limited, products low in bendability, such as meat-pulse bars in this study, might utilize WB shear test as a possible representation for bending parameters.

4.5.8 Thiobarbituric acid reactive substances (TBARS) values, and TBARS over storage of meat-lentil bars

Measurements on TBARS, thiobarbituric acid reactive substances, values offer valuable insights into the properties and shelf stability of products. TBARS (thiobarbituric acid reactive substances) are secondary metabolite of lipid oxidation in food products, and is commonly used to represent its level of lipid oxidation that generally leads to the production of off-flavors (Aberle et al., 2012; Domínguez et al., 2019). TBARS values under storage, both RT and HT, indicated an additional factor affecting product acceptability and shelf stability, especially in meats and meat products (Sun et al., 2001).

Table 4.14 presents the (TBARS) values of samples of all treatments, captured both initially and throughout storage. As indicated, NT18 showed significantly lower TBARS value than Con, which might suggest the inhibitory effect on lipid oxidation by lentil flour addition. Similar trend was also reported by Li et al. (2017) and Shariati-Ievari et al. (2016). However, NT12 showed a significant higher TBARS value than TBARS, contradicting the finding. The lentil flour addition and starch gelatinization did not show effect on product lipid oxidation level. The TBARS values of samples detected in this study was around 3 mg malonaldehyde per kg sample (30 – 35% moisture), which was similar, on a dry basis, as values from other studies that utilized similar production procedures to produce restructured jerkies (Lim et al., 2013; Yang et al., 2009).

Table 4.14 TB Thiobarbituric acid reactive substances (TBARS) values of meat-lentil bar, with storage at ambient and elevated temperatures.

Treatments (mg / kg sample)	**Con**	**NT6**	**HG6**	**NT12**	**HG12**	**NT18**
HT[i]						
Day1	3.18^{b1x}	3.13^{ab1x}	3.16^{ab1x}	3.21^{b1x}	3.00^{ab1x}	2.91^{a1x}
Day30	3.40^{b1y}	3.27^{ab1x}	3.23^{ab1x}	3.36^{ab12x}	3.21^{ab12x}	3.09^{a12x}
Day60	3.67^{b2y}	3.54^{ab2x}	3.60^{b2y}	3.56^{ab2x}	3.43^{ab23x}	3.31^{a2x}
Day90	3.99^{b3y}	3.81^{ab3y}	3.82^{ab2y}	3.88^{ab3x}	3.61^{a3x}	3.65^{a3x}
RT[ii]						
Day1	3.18^{b1x}	3.13^{ab1x}	3.16^{ab1x}	3.21^{b1x}	3.00^{ab1x}	2.91^{a1x}
Day30	3.13^{a12x}	3.28^{a12x}	3.13^{a12x}	3.21^{a1x}	3.14^{a12x}	3.05^{a12x}
Day60	3.39^{a2x}	3.45^{a23x}	3.40^{a23x}	3.37^{a1x}	3.31^{a23x}	3.24^{a2x}
Day90	3.68^{a3x}	3.55^{a3x}	3.63^{a3x}	3.73^{a2x}	3.54^{a3x}	3.56^{a3x}
SEM	0.075					

[i] HT: samples stored in incubator at elevated temperature, approximate 40 °C, 25% relative humidity

[ii] RT: samples stored in ambient temperature as at typical room temperature in Saskatoon, Canada, approximate 20 °C, 40% relative humidity

^{abc} values within the same row with different superscript letters significantly different

^{123} values under each storage condition, HT or RT, within the same column with different superscript numbers significantly different ($p<0.05$)

^{xy} values only compared only across storage condition, HT vs. RT, with different superscript letters significantly different ($p < 0.05$)

SEM: pooled standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

Thermal processing, inherent in production of dried meat (and hybrid) products like meat-lentil bars, is known to foster lipid oxidation. The heat disintegrates fats and oils, resulting in the generation of free radicals, which in turn initiate lipid oxidation (Aberle et al., 2012; Domínguez et al., 2019). The heated surroundings also speed up molecular motion, further promoting lipid oxidation. Restructured products, like jerkies and meat bars, might be even more susceptible to oxidation compared to whole-muscle meats. This is because the extensive surface area of restructured products is readily exposed to oxygen, creating a conducive environment for oxidation.

Analysis model on the complete data set identified significant treatments, storage time, and storage condition effects, also a significant time by condition interaction was identified.

During HT storage, samples of all treatments showed significantly increased TBARS values, indicative of lipid oxidation. Remarkably, Con consistently exhibited greater oxidation than NT18. While the initial 30 days of storage showed minimal changes, a surge was observed post this period. On the other hand, RT storage presented subtler differences between treatments, in which by day 90, all treatments saw a rise in lipid oxidation. It seems that the rate of lipid oxidation for Con, NT12, and NT18 was faster than other treatments, suggesting an inhibitory effect on lipid oxidation by starch gelatinization of the lentil flour, particularly under more intense storage condition (HT) and prolonged storage duration. However, no significant treatment by time interaction effect was identified, and further examination and statistical analysis are required to corroborate the inhibitory effect of lentil flour addition in meat bars.

Even though a significant interaction time by condition effect was identified, the predictive lines based on this interaction were mainly parallel. Hence, this significant interaction effect might not be considered.

4.5.9 Sensorial assessments of meat-lentil bars

A total of 47 panelists aged between 18 - 69 years old evaluated the six formulations of the meat-lentil bars. The mean scores for the intensity and hedonic scales of the sensory attributes of each treatment are shown in Table 4.15. The mean values for smell / aroma on an eight-point hedonic scale (8 = like extremely, 1 = dislike extremely) of samples of all treatments ranged from 5.5 - 6, namely, “like slightly” to “like moderately”. No significant differences in smell / aroma hedonic scores of any treatments were found, indicating no effect by the addition and starch gelatinization level of lentil flour on the smell / aroma acceptability of dried meat bars. Similarly, samples of all treatments achieved a hedonic score on flavor acceptability within the range 5.5 - 6 (“like slightly” to “like moderately”), and no significantly differences were found between the flavor acceptability of any two treatments. This was likely associated with the flavor intensity scores, in which samples of all treatments showed values from 5 - 6 (“slightly intense” to “moderately intense”), with no significantly different values shown between treatments. This indicated that the addition and starch gelatinization level of lentil flour showed no influence on panelists perception of the flavor profile of meat-lentil bars, both on intensity and acceptability. Meanwhile, Ipsos Reid (2010) reported that given the dislike of pulse taste and flavor is one of the most common barriers for pulse consumption. The lack of difference flavor perception in meat bars with lentil flour addition indicate little impact in product flavor, which was likely masked by the seasonings and meat, and hence generally acceptable hybrid products.

Table 4.15 Means of consumer ratings on the sensory attributes of meat-lentil bars. n = 47

Treatments	**Color intensity**[1]	**Color acceptability**[2]	**Smell / aroma acceptability**[2]	**Firmness**[3]	**Dryness**[4]	**Overall texture acceptability**[2]	**Flavor intensity**[5]	**Flavor acceptability**[2]	**Overall acceptability**[2]	**Purchase intention**[6]
Con	6.94^{ab}	6.15^{ab}	5.63^{a}	4.13^{a}	4.52^{a}	5.76^{ab}	5.54^{a}	5.72^{a}	5.83^{a}	3.59^{a}
NT6	6.98^{ab}	6.07^{ab}	5.91^{a}	4.09^{a}	4.43^{a}	5.91^{a}	5.63^{a}	5.89^{a}	5.91^{a}	3.70^{a}
HG6	7.16^{a}	6.33^{a}	5.76^{a}	3.98^{ab}	4.26^{ab}	5.80^{ab}	5.36^{a}	5.93^{a}	5.93^{a}	3.72^{a}
NT12	6.96^{ab}	6.07^{ab}	5.54^{a}	3.20^{c}	3.80^{bc}	5.61^{ab}	5.50^{a}	5.85^{a}	5.65^{a}	3.50^{a}
HG12	6.78^{ab}	5.84^{ab}	5.85^{a}	4.15^{a}	3.85^{bc}	5.39^{ab}	5.17^{a}	5.67^{a}	5.59^{a}	3.35^{a}
NT18	6.50^{b}	5.70^{b}	5.78^{a}	3.46^{bc}	3.57^{c}	5.20^{b}	5.35^{a}	5.67^{a}	5.52^{a}	3.20^{a}
SEM	0.159	0.179	0.168	0.181	0.163	0.195	0.148	0.189	0.193	0.155

[1,2,3,4,5] Estimated least-square means of 47 responses on eight-point intensity or hedonic scales

[1] 8 = dark red, 1 = dark yellow, [2] 8 = like extremely, 1 = dislike extremely, [3] 8 = extremely soft, 1 = extremely firm, [4] 8 = extremely moist, 1 = extremely dry, [5] 8 = extremely intense, 1 = extremely bland

[6] Estimated least-square means of 47 responses on five-point intention scale, 5 = definitely would buy it, 1 = definitely would not buy it

^{ab} means within the same column superscripted by a different letter are significantly different ($p < 0.05$)

SEM: standard error of the estimated least-square means

Con: beef bar, no addition lentil flour, NT6: beef-lentil bar, 6% addition non-tempered IR lentil flour, HG6: beef-lentil bar, 6% addition 24 h-tempered IR lentil flour, NT12: beef-lentil bar, 12% addition non-tempered IR lentil flour, HG12: beef bar, 12% 24 h-tempered IR lentil flour, NT18: beef-lentil bar, 18% addition non-tempered IR lentil flour.

Samples of HG6 showed the highest score on color intensity (7.17, “brownish red”) and NT18 showed a significantly lower value (6.59, “purplish red”) (Table 4.15). Other treatments showed color intensity within this range and were not significantly different. Even though no significant treatment effect was found on sample redness by instrumental color measurements, the sensory results was reasonable and likely caused by the lighter and yellower color of NT18 from instrumental color measurements. Also, it could be hard to distinguish between yellowness and redness within an overall brown product (meat-lentil bars) by vision perception. Difference in color intensity may be associated with the higher color acceptability of HG6 (6.32, “like moderately”) and lower acceptability of NT18 (5.66, “like slightly”), with other treatments showed not significantly different hedonic scores within the range.

Firmness, the force necessary to attain a given deformation with a bite through the product by the front teeth in the case, is one of the most common and important determinants of food behaviors in the mouth, and hence is considered an important texture property (Szczesniak, 1963). The intensity of sample firmness showed different values among treatments. NT12 showed a significantly lower (3.20, “moderately firm”) value (firmer) than HG12, HG6, NT6, and Con (~ 4, “slightly firm”), and NT18 showed a slightly higher value (3.48) that was significantly different than HG12, NT6, and Con. This indicated the increasing effect of NT treatments at higher addition level (≥ 12%) on sample firmness. This generally agreed with results of WB shear values, which showed increased values as the flour addition level increased. As mentioned, WB shear value test measures the whole-sample resistance against applied force (shear), and hence could be used as indicator of firmness. However, the highest firmness values by NT12 and NT18 were never indicated by WB shear values, but the ultimate strength of sample under three-point bending test. This might be due to that firmness can also be defined as the work required to fracture the product, which might be more associated with ultimate strength (Guinard & Mazzucchelli, 1996). Though, both instrumental parameters showed properties relating to sensory firmness and could be used as indicators, especially when WB shear force value and ultimate strength showed a strong correlation ($r = 0.77$).

Dryness in this study was defined as the degree to which the sample felt dry in the mouth (Szczesniak, 1963). NT18 showed the lowest dryness score (3.57, “moderately dry”) that was significantly lower than those of Con, NT6, HG6 (~ 4.5, “slightly moist”). NT12 and HG12 showed slightly higher but not significantly scores than NT18 at approximate 3.8 (“moderately dry” to “slightly dry”), which were significantly lower (dryer) than those of Con and NT6. The highest dryness of the NT18 bars also agreed with results of product proximate compositions, in which NT18 contained the lowest moisture and lowest fat content and also highest non-meat solids content. Even though no hedonic scale on sample dryness was used, many panelists provided comments on samples being too dry and showed favor of moister products. Additionally, NT18 showed a significantly lower hedonic score (5.11, “like slightly”) on overall texture acceptability than other treatments (5.39 - 5.70, “like slightly” to “like moderately”). This might imply the determinate effect of sample dryness on the overall texture acceptability of meat-lentil bars, given that samples with lowest hedonic score also showed driest texture (NT18) but not firmest (NT12).

Despite the treatment differences in color and textural perceptions, samples of all treatments showed similar overall acceptability (5.43 - 5.80, “like slightly” to “like moderately”) and purchase intention (3.14 - 3.57, “might or might not buy it”). Again, no difference on overall acceptability and purchase intention by treatments with lentil flour addition might indicate consumers acceptance (willingness to try) on hybrid meat-lentil bars, as these products scored as high as the meat-only control. However, the purchase intention of all samples was relatively low in general, requiring product improvements for sensory attributes such as moister texture.

4.6 Conclusions

The addition of lentil flour influenced meat bar properties, while the addition level and starch gelatinization level of lentil flour also altered meat bar properties. Water activity was not influenced by lentil flour treatments, and the addition of lentil flour to meat bars increased product pH than meat-only control. Proximate compositions of meat-lentil bars differed between treatments, in which carbohydrate content increased and fat content decreased, as lentil flour addition level increased. Samples of all treatments experienced decreased water activity, moisture loss, darkening color, firming texture, loosing bendability, and greater lipid oxidation over storage, and all changes were expectedly accelerated by high-temperature storage. Samples added with higher levels of and more-gelatinized lentil flour appeared to exhibit firmer, more cohesive texture, and perhaps improved textural stability over storage. As for color, samples added with higher level and more gelatinized lentil flour showed lighter and more yellow color, also improved color stability over storage. Improved product oxidative stability over storage was also observed for samples with added lentil flour, but only under sufficient addition levels (12%), and difference in starch gelatinization level did not show effect. Treatments with lentil flour addition showed differences in color and textural perceptions under sensory evaluation, but were equally as acceptable in flavor, smell / aroma, and overall acceptability as the meat-only control. All meat-lentil bars appeared as acceptable products that might be promoted to the market.

5. General Discussion

The meat-pulse bars developed and produced in the present study appeared to be acceptable hybrid proteins products. Young consumers (aged 9 - 17) rated the overall acceptability of meat-pulse bars (infrared-heated black bean and lentil flours, addition level 12%, water activity < 0.85 or 0.90, pH < 5.5 or < 5.3) from 4.8 to 5.1 on a six-point hedonic scale (6: like very much), and adult consumers (aged 18 - 69) rated that of meat-lentil (infrared-heated flour, treatments on addition (6 - 18%) and starch gelatinization levels) bars from 5.4 to 5.9 on an eight-point hedonic scale (8: like extremely). Specifically, the control meat bar with no pulse ingredient received an overall acceptability rating at 5.8 by adult consumers (eight-point hedonic scale), which was within the similar range of those of lentil-treated samples. This indicated that consumers expressed a general liking (six-point hedonic: 5 like moderately; eight-point hedonic: 5 like slightly) of the meat-pulse bars, and hence novel hybrid proteins products were developed and study objective was achieved.

One of the biggest advantages of hybrid protein products is that it retains the meaty taste and flavor that is favored by consumers. While sensory factors, including color and texture, all plays essential roles on the consumer acceptance of meat and meat products, taste appeared to be the biggest concern for people willing to try meat alternatives, as many of them believed that they would not like the taste of meat alternative and hence not trying (Clark & Bogdan, 2019; Joseph et al., 2020). For cooked meat products, Maillard reaction and the degradation of lipid are the main contributor of meat flavors, by which the savory and meaty flavor and fatty aromas are produced, respectively (Mottram, 1998). Also, the curing agent (nitrite) produces the "cured" flavor (Sebranek, 2009). The meat-pulse bars developed in present study received relatively high hedonic rating on their flavor profile (≥ 4.9 six-point scale, > 5.5 eight-point scale), while pulse-treated samples showed no difference than that of meat-only control sample, indicating consumers acceptance of it. Compared to studies on other meat alternatives product that related to lower acceptance than those of conventional meat products, results from present study well illustrated the benefit of hybrid proteins product (Tarrega et al., 2020). It was also hypothesized that young consumers prefer products that are sweeter, more flavorful, and softer (Moskowitz et al., 2012), agreeing with just-about-right results from this study and consumers comments. On the other hand, due to limited experimental resources and quarantine policy by Covid, little research was done on the flavor aspect in the present study. With young consumers indicated they would prefer more flavorful products and adult consumers rated products flavor slightly intense, future improvements of meat-pulse bars might be to enhance its flavor profile. Also, sensory evaluation by trained panelists to better understand the influential level of certain ingredients, such as lentil flour, to product flavor is highly recommended.

In the present study, the impact of pulse flours on the color profile of meat-pulse bars was found to be relatively minor, providing an additional advantage for these hybrid protein products. Color and appearance are crucial sensory factors that influence the acceptance of traditional meat and meat products, with red being the preferred color and pink for cured meat products containing added nitrite (Font-i-Furnols & Guerrero, 2014; Sebranek, 2009). In the case of cured meat products such as jerky-like meat bars, a stable pink color is typically desired, resulting from the reaction between myoglobin and nitric oxide (derived from nitrite) to form the pigmented pink compound known as nitroso hemochrome (King et al., 2023). It was hypothesized that the addition of pulse (lentil) helped to reveal and stabilized the red (or pink) color that are favored by consumers (Kim & Shand, 2022; Li et al., 2017), but results in this study did not agree with this hypothesis. In addition to the mentioned reason, reason of this unexpected results might be that processed meat products such as jerkies and sausages, generally contains reducing sugars (for example, glucose) that reacts with amine (from proteins) to form a brown pigment during heating (Barbut, 2015). This possibly explains, even for cured products, the dark red color of meat-pulse bars in present study, especially when reducing sugars, for example damaged starch of HG flour, were introduced. Reasonably, the amount and availability of reducing sugars might influence the extent of Maillard reaction. Products added with more-gelatinized pulse (lentil) flour likely contained higher amount of oligo- and polysaccharides that are available for Maillard reaction, but no major color difference were found between samples with treatments on gelatinization level. Similarly, the carbohydrate profile of pulse flours (different pulses or different levels of starch gelatinization) did not show substantial influence on product color, suggesting the extent of Maillard reaction between pulses carbohydrate and meat proteins might mainly depends on overall carbohydrate content. However, Maillard reaction might also be an important factor for color changes during storage. Given that meat-only control sample showed the least amount and the most stable rate of color changes during storage, the accelerated darkening, as compared to control, color of meat-lentil bars was clearly due to the introduction of lentil flour, and hence browning by Maillard reaction or other means (results of Study 2, color). It was also reported that Maillard browning did not only occur under heating (and cooking), but also occurred during storage, especially under higher temperature (Barrett et al., 2000).

Another benefit of hybrid proteins products is that products with both meat and plant-based proteins might show a more favorable texture profile than that of conventional meat products. Consumers generally want a “soft” and “smooth” texture for hybrids and meat alternatives, which could correspond to the tenderness and juiciness and moistness of conventional meat products (Joseph et al., 2020). Generally, tenderness and juiciness and moistness of restructured, as compared to whole-muscle, processed meat products are improved by the addition of binders that hold or retain the moisture portion of products and hence stabilize its structural protein network. Plant-based carbohydrates, such as starch and fiber, and proteins have shown to be good binders in meat products (section 2: Literature Review). This has been related with softer and moister texture in hybrid products such as burgers with lentil flour (Lim et al., 2013), and was hypothesized to also contribute to a softer and moister (more favorable) texture than conventional meat products, of products in this study, dried hybrid bars.

However, adding pulse flours to meat bars seemed to show ambiguous effect on texture, which showed an adverse direction as was hypothesized. The shear and bending forces of meat-lentil bars appeared to increase as the addition level of lentil flour increased, showing a, instead of softening, hardening effect to the samples (results of Study 2, texture). Also, many consumers from Study 1 indicated that they considered the products “too hard” or “too dry”, especially for those of lentil treatments (results of Study 1, sensory). This indicated an adverse effect on the texture profile of meat bars by the addition of pulse flours, which was unexpected and also unfavored. This was likely due to that the bulk components (lentil flour interacting components, not completely dissolved brown sugar as observed etc.) present in present study restrained the solubilized meat proteins to form a rigid, stable structure, and hence, instead of stabilizing, disrupting product structure. Specifically, the large amount of pulse carbohydrate present in present study showed potential to form a secondary network, in which oligosaccharides and starch granule interact with each other to result in bulk component or to form a carbohydrate network within the meat proteins network, further disrupting the product.

On the other hand, the increased stiffness of meat-pulse bars as the addition level of lentil flour increased seemed to be an advantage, agreeing with the hypothesis that pulse addition helps to improve product bendability. Stiffness, measured by the slope of applied stress (tensile or compression) and sample deformation in a stress-strain curve, indicate product structural strength to resist deformation, also known as Young's modules and sometimes elasticity for elastic products. In food products, this is generally used to indicate flexibility, cohesiveness or adhesiveness of the products. This property is generally used to evaluate the textural profile of plant-based snack (Greve et al., 2010; Kim et al., 2009). Compared to Warner-Bratzler shear force and breaking force values, stiffness provides a more comprehensive demonstration on both the external and internal strength of the product. This is very useful when examine the binding effect of binders in complex food system such as hybrid proteins products. Barbut (2015) reported that for chicken proteins network (gels), their Young's modulus (stiffness) increased as the volume fraction of binder (rice barn wax particle and glass bead) in the system increased, while the size of the binder and binding properties (strongly or weakly bound) also influence product Young's modules. They also indicated that the higher Young's modulus was associated with a favorable more rigid and stable texture.

Including both three-point bending and Warner-Bratzler shear force test of hybrid proteins products better illustrates their textural properties. Reasonably, a better instrumental textural analytical method is the one that better mimic human mastication. Breaking force measured by three-point bending test is considered to be the most common method to determine the fracture properties of solid foods, also the most reliable one as it depends very little on equipment design, geometry, and sample dimensions (Kim et al., 2009; Luyten et al., 1992). However, the bending action does not mimic human mastication. Warner-Bratzler shear force in this case seemed to show better representation of product texture, by which it mimics the cutting action during processing and the biting down during human mastication. Specifically, Warner-Bratzler shear force test is widely applied for meat and meat products (AMSA, 2016). With the blade cutting through the entire sample, Warner-Bratzler shear force test is also believed to better represent whole-product rigidity than only surface fracture by three-point bending. Though, three-point bending also yields a very useful parameter, stiffness (Young's modulus) for product internal interaction such as elasticity. It has been reported that stiffness was the best indicator for some of product textural attributes including cohesiveness and adhesiveness (Kim et al., 2009).

One of the major advantages of these dried hybrid meat products is that they are convenient for consumers and generally have a longer shelf life than their all-meat counterparts. Conventional products such as beef jerkies generally indicate a shelf life of more than 90 days, which would require a long testing period when researching new formulations. Hence, accelerated storage is generally involved. Accelerated storage, defined as the storage of the food products at controlled environments to accelerate deterioration, is useful in predicting the shelf life of ambient-stable products having long shelf lives. However, this should be performed without altering the mechanisms or order of changes of the product under normal storage conditions. Samples from both studies were stored under high-temperature (HT) conditions for accelerated storage. However, data of Study 1 (180 days room-temperature (RT) vs. 90 days HT) did not show similar results, suggesting the interaction effect of treatment differing between storage conditions. This interference prevented the use of HT results, of Study 1, to predict product properties (moisture content, color, WB shear value) behaviour over longer term RT storage. Hence, the interaction effect was further analyzed in Study 2. Surprisingly, time by condition interaction did not show significant effect on product instrumental measurements (moisture content, color, texture parameters, lipid oxidation level), and time by condition interaction only showed significant effect on product stiffness and redness (a^*). This suggested that storage period also become a factor of the controlled environment for accelerated storage and shelf-life testing. While temperature is the most commonly used accelerating factor, other factors including humidity, light, and pH have also been identified as accelerating factors (Mizrahi, 2004), but their influence was limited in this study. The storage behaviour of product properties except stiffness and redness in longer term ambient storage could be predicted by the HT results in this study. Though, further analysis of data is needed to determine the kinetic constant of storage.

Combined results of Study 1 and 2 further revealed some of the mechanisms of product storage behaviour. It has been suggested that the color changes of samples through storage was not influenced by pH (L90 vs. L90ECA, Study 1) and moisture content (L85 vs. L90, Study 1), and hence, the color darkening of products through storage was mainly by chemical reaction, namely, myoglobin oxidation. Analysis on the interaction effect on the color parameters of Study 2 samples also supported this finding (results of Study 2, color). However, further examination is needed to identify the oxidative state of product myoglobin through storage. The surprisingly low hedonic, and not so favorable just-about-right ratings on the texture of meat-black bean bars than meat-lentil ones (Study 1) suggested that there were texture differences other than that shown by WB shear values. Results of three-point bending test (Study 2) further revealed differences in product bendability and cohesiveness among the samples which may have influenced consumer acceptability in Study 1. No significant treatment by time interaction was identified for product stiffness, suggesting that the internal structural stability of the products was not influenced by pulse addition nor starch gelatinization (Study 2). Hence, the difference in the textural stability by different types of pulses (black bean vs. lentil, Study 1) was likely due to the physical properties of pulse flour, such as particle size.

This study expressed an interest in determining whether pulse flour treatments, such as tempering and micronization, exert influence on their applications in meat products. It has been documented that flour derived from tempered pulse seeds demonstrated an increase in gelatinized starch content and enhanced pasting, gelling, and moisture-absorbing capacities (Liu et al., 2020). Consumers assessment results from Study 1 indicated a preference for softer, more succulent, cohesive, and flavorful products, which might be achieved by the pre-tempering of pulse seeds. However, results of Study 2 contradicted this hypothesis, revealing that pre-tempering lentil seeds yielded more cohesive (desirable), but also firmer (not desirable) products.

Specifically, the incorporation of 18% pre-tempered, or HG, lentil flour into raw meat resulted in a batter mixture that was excessively sticky and bulky, thereby impeding further processing into meat-pulse bars. This observation aligns with those in literature suggesting that pulse flours with elevated levels of starch gelatinization demonstrate increased gelling and water absorption properties. Meanwhile, it implies that these enhanced functionalities of pulse flours may not always be advantageous in meat and hybrid products.

In a complex meat system characterized by limited moisture but high moisture absorbers (also known as binders or extenders), such as the meat-pulse (18%) bars in this study, pulse flour likely became the primary ingredient to interact with water molecules and extracted myofibrillar proteins. This interaction resulted in a stable myofibrillar protein-pulse flour-myofibrillar protein network, as opposed to a myofibrillar protein "moisture (with pulse flour)" myofibrillar protein network. This change likely enhanced product internal strength and subsequently, cohesiveness and firmness. Such a property may be more desirable in hybrid products that require a sturdier structure, or those that are more susceptible to changes leading to defected properties. Examples of such products may include products with a higher fat content (such as hybrid sausages), a coarser texture (whole-seed pulses), or those undergoing treatments encouraging oxidation and enzymatic activity (such as tumbling). Generally, in dried meat-pulse system, products added with lentil flour with higher starch gelatinization level, were associated with more stable color and texture properties. However, it might not justify the additional expenditure of time, effort, and cost required for pre-gelatinizing flours through tempering and infrared heating.

The products developed in this study showed a generally higher carbohydrate, and lower fat content than those of commercial meat snack and meat bar products (see Appendixes for sample commercial products). This is due to the introduction of pulse flours, which generally contains much higher carbohydrate (60 - 65%) and lower lipid contents (< 2%) than meat (raw meat: carbohydrate < 1%, fat 5 - 10%) (Aberle et al., 2012; Tripathi et al., 2021). Also, dietary fiber is introduced to the hybrid system. This could imply the possible health benefits over conventional meat snacks, with lower fat content. Additionally, product protein contents in this study were high. Market potential of this hybrid meat-pulse bars might rely on claims on reduced fat content and increased fiber content, also higher contents of minerals, such potassium, and vitamins, such as folate, that generally do not present in meats and meats products.

6. Future Approaches

Novel technologies could be used to better improve meat-pulse bars properties. Hot-air drying utilized in the present study has been associated with long drying time, off-flavor, and tough texture, which could be minimized by advanced drying techniques (Kim, Kim, Cha, et al., 2021). It has been reported that steam and vacuum drying could produce jerky-like products that showed better capacity for moisture retention and decreased hardness, as demonstrated by shear force value (Kim, Kim, Cha, et al., 2021; Kim, Kim, Kim, et al., 2021). Also, as discussed, the presence of high concentrations of non-meat solids disrupted the meat proteins network, which might be attributed to some of the unfavorable properties of meat-pulse bars. Because of this, methods that facilitate the interaction between meat particles and other ingredients might be favored, so that a more homogeneous raw meat batter is made. Possible approaches might include comminuting meat particles to even smaller pieces, pre-hydrating pulse flour and other ingredients in the water and dissolved salts with meat particles, or tumbling and mixing meat particles and brine (pulse flours, seasonings, etc.) for longer time. However, some of these methods encourages oxidation and enzymatic activities in products, and their effects might not always be favored.

Specifically for hybrid and meat alternative products, extrusion has gained recent interest. The high temperature and high pressure of extrusion provide sufficient energy to cook, dry, and shape plant-based ingredients to a jerky- or sausage-like shape, also the long screwing process allow sufficient time and mixing for plant-based ingredients to interact to form a meat proteins-like network. Also, the high pressure induced by extrusion generally denature enzymes, possibly inhibiting unfavorable enzymatic activities. High-moisture extrusion has been widely applied to plant-based proteins to successfully produce products with meat-like fibrous conformation, but were mostly focused on soy or cereal proteins (Joshi & Kumar, 2015). Jerky-like hybrid products with beef and plant materials at high carbohydrate content, such as potato, could also be produced by extrusion, and were associated with lower extent of firming during storage, namely, increased texture stability, than no-meat counterpart (Barrett et al., 2000). This indicates the possible approaches of producing hybrid meat-pulses products by, especially high-moisture, extrusion.

More advanced technology such as 3D printing has also gained interest to produce hybrid products due to its flexibility on designing internal food structure and hence texture (T. Wang et al., 2022).

7. Overall Conclusions

To summarize, meat-pulse bars of all treatments (Study 1: infrared-heated black bean, lentil flours, 12% addition w / w, water activity < 0.85,0.90, pH < 5.5, < 5.3; Study 2: infrared-heated lentil flour, different tempering periods (0, 24 h) and addition levels (0, 6, 12, 18% w/w) successfully gained generally high consumer acceptance. The harder texture of samples with lower moisture content might be perceived by young consumers, but this did not influence product overall acceptability. The harder and drier texture of samples with higher addition level of lentil flour was also noticed by adult consumers and rated less liked, but again no influence on overall acceptability. Adult consumers were also able to distinguish the color difference with higher addition levels of lentil flour. For young consumers, ratings of just-about-right scales suggested that they would like products to be softer, moister in texture and more flavorful. Most young consumers showed positive attitudes toward the meat-pulse bars, and they agreed that these meat-pulse bars provide a healthier option for snacks. This could be used as a marketing campaign to promote the consumption of these products.

The effect of pulse flours on the properties of meat-pulse bars depends on the addition level and moisture content of the product. Different types of pulses and starch gelatinization show minor effects, and pH does not significantly alter the properties of the bars. As the addition level of pulse flour increased and product moisture content decreased, the product became firmer, more resistant to bending, and darker (less red in color). Meat-pulse bars at 12% (w / w) flour addition appeared to show most desirable properties with highest possible addition level. The level of lipid oxidation varied between samples but was inhibited at samples with high addition of lentil flour (18%). Samples with lentil flour with starch gelatinization were associated with better storage stability as there was less textural and color change during storage, up to 90 days at 40 ºC. Also, during storage, samples with black bean flour showed greater textural and color changes than those with lentil flour. All the samples with lentil flour showed lower incremental changes in lipid oxidation than the meat-only control sample. All samples experienced unfavorable changes (getting firmer, darker color, increased lipid oxidation level) during storage, with high-temperature storage clearly show greater effect than room-temperature storage. Pre-tempering or gelatinization is only preferred when products demand more stable properties, such as subjected to dryer, higher temperature storage, or upon modification resulting in less stable structure, such as higher fat and large ingredients (whole-seed pulses) or harsher treatments. Storing samples under elevated temperature was useful to suggest their storage behaviour under ambient temperature with a longer, but generally commercially available shelf life.

This is the first attempt to add high amounts of pulse flours to dried meat snacks. Further research and development are needed to improve the meat-pulse bars. Analyzing the flavor profile of the product can provide insights on how to enhance the taste and aroma of the bars. Evaluation of sensory attributes by trained panelists can help identify the specific characteristics that can be improved, such as texture, appearance, and flavor. Advanced processing technologies like high-moisture extrusion and 3D printing can also be explored to improve the production process and product quality. These methods may offer new ways to optimize the texture, appearance, and taste of meat-pulse bars. Overall, continued research and development can help to enhance the appeal and nutritional value of meat-pulse bars, making them a more attractive and viable snack option for consumers.

8. References

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Appendix A: Evaluation scoresheet for sensory evaluation by young consumers, Study 1

Panelist #: ………….

Sample Code: **712**

PART I: Sensory Evaluation of Meat-Pulse Bars

Instruction: Please evaluate the samples in the order that the samples are presented to you. Please take a drink of water **before** and **between** samples.

1) For each sensory characteristic, check **ONE** descriptor along the 6-point scale that best describe your impression.

	6 like very much	5 like moderately	4 like slightly	3 dislike slightly	2 dislike moderately	1 dislike very much
Color						
Smell/Aroma						
Texture						
Taste/Flavor						
Overall Acceptability						

2) For each sensory characteristic, check **ONE** descriptor along the 5-point scale that best describe your impression.

Hardness	much too soft	slightly too soft	just about right	slightly too hard	much too hard
Chewiness	much too crumbly	slightly too crumbly	just about right	slightly too chewy	much too chewy
Moistness	much too dry	slightly too dry	just about right	slightly too moist	much too moist
Saltiness	not nearly salty enough	not salty enough	just about right	slightly too salty	much too salty
Sweetness	not nearly sweet enough	not sweet enough	just about right	slightly too sweet	much too sweet
Flavor	much too weak	slightly too weak	just about right	slightly too strong	much too strong

Please provide comments on what you liked and didn't like about this product:

I liked . . .	I didn't like . . .

Appendix B: Survey on snacking behaviour for young consumers, Study 1

Panelist #:

PART II: Student Survey about Snacks

Please answer the questions below. The information will be treated with strict confidence and all information collected will not be identified with your name.

Snack defined in this survey: any foods and drinks that are consumed between meals.

1. On average, how often do you consume snacks?

2 or more times per day	1 time per day	5-6 times per week	3-4 times per week	1-2 times per week	2-3 times per month	1 time per month or less

2. When do you most often consume snacks?

between breakfast and lunch (9 – 11 a.m.)	after school but before supper (3 – 6 p.m.)	after supper (after 8 p.m.)

3. On average, how often do you skip a meal but consume snacks?

2 or more times per day	1 time per day	3-5 times per week	1-2 times per week	2-3 times per month	1 time per month	I generally don’t skip a meal.

4. What kinds of foods do you usually consume as snacks? What snacks do you buy by yourself? What snacks are usually purchased by your parents and available from your family? (check all that apply) (usually consume: ≥ 3 – 5 times per week)

	usually consume	snacks you buy	snacks from family
milk, fruit juices			
other beverages include fruit drinks, soft drinks (pops), sports or energy drinks, and milk-based beverages such as chocolate milk, milkshakes, but **exclude water, tea, and coffee**			

fruits (fresh, canned, frozen, dried, or cooked)			
fried potatoes, corn chips, tortilla chips, popcorn			
cookies, brownies, snack cakes, dough-nuts, sweet or Danish rolls, Pop-Tarts, and other pastries such as fruit strudel, sweet muffins, dessert breads (such as banana bread, lemon loaf)			
other bakeries such as crackers, biscuits, bagels, pancakes, croissants, and cereal snacks and bars such as granola bars, Quaker oat bites, Nutri-Grain			
sweet snacks, confectionaries, sugars, candies, jams, jello, pudding, and sugars added to food, such as syrups and white sugar			
other dairy products such as ice cream, yogurt, cheese and any other solid dairy products			
seeds, nuts, nut snacks and bars, energy bars, and high- protein bars such as Clif energy, Solo Gi, Kelloggs Vector, SunRype energy, PowerBar, Pure Protein, Simply Protein, Atkins protein			
meat snacks such as jerkies, meat / sausage sticks, meat bars such as Epic, Meatbar, and any form of meat-based snacks			

5. On average, how often do you purchase snacks by yourself, but not by your parents?

2 or more times per day	1 time per day	3-5 times per week	1-2 times per week	2-3 times per month	1 time per month	I generally don’t buy snacks.

6. Have you ever consumed meat snacks or meat bars?

meat snacks		meat bars	
yes	no	yes	No

7. If yes to Q6- on average, how often do you consume meat snacks / bars?

2 or more times per day	1 time per day	3-5 times per week	1-2 times per week	2-3 times per month	1 time per month	I generally don’t eat meat snacks.

8. What are the types of meat snacks that you have consumed? If you have tasted meat bars before, what are the names of the meat bars that you have consumed?

meat snacks:……………..……………… ……………………………………………………

meat bars:…………………… ……………………………………………………………

9. Below is a list of statements relating to snack consumption habits, lifestyle, and attitude toward meat-pulse bars. For each, please indicate how much you agree or disagree on the scale provided.

Statement	completely agree ◄──► completely disagree					
	1	2	3	4	5	6
I only look for a snack when I am hungry.						
I eat snacks whenever I want.						
Sometimes I skip a meal because I am too filled with snacks.						
Sometimes I feel hungry between meals.						
I know that meat snacks are good sources of nutrients and complete protein.						
When I am hungry, I prefer meat snacks over other snacks.						
I know that pulses are rich sources of nutrients such as protein, fiber, vitamins, and antioxidants.						
I believe that eating pulses is health-beneficial, and I would like to consume pulse-containing foods whenever they are available.						

I generally don’t like the taste / flavor / smell of pulses, so I tend not to consume pulses-containing foods.						
I believe that the incorporation of pulses in meat snacks makes them more nutritious, healthier, and attractive to consumers.						
I think that these meat-pulse bars are more nutritious, healthier options than the snacks I usually consume.						
I would like to recommend these meat-pulse bars to my friends and parents.						

10. What are the features of these meat-pulse bars that you like and don’t like? How do you think we can make these meat-pulses bars better?

……………………………………..……………………………………………………………….

……………………………………………..…………………………………………………………

……………………………………………………..…………………………………………………

Appendix C: Evaluation scoresheet for sensory evaluation by consumers, Study 2

Sample Code: **192**

Panelist #: …………………

PART I: Sensory Evaluation of Meat-Lentil Bars

Instruction: Please evaluate the samples in the order that the samples are presented to you. Please take a drink of water **before** and **between** samples.

3) For each sensory characteristic, check **ONE** descriptor along the 8-point scale that best describe your impression.

Color	dark red	brownish red	purplish red	bright red	slightly yellow brown	moderately yellow brown	brownish yellow	dark yellow
Color / Appearance Acceptability	like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
Smell / Aroma Acceptability	like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
Firmness	extremely soft	very soft	moderately soft	slightly soft	slightly firm	moderately firm	very firm	extremely firm
Dryness	extremely moist	very moist	moderately moist	slightly moist	slightly dry	moderately dry	very dry	extremely dry
Overall Texture Acceptability	like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely

Flavor Intensity	extremely intense	much intense	moderately intense	slightly intense	slightly bland	moderately bland	much bland	extremely bland
Flavor Acceptability	like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely
Overall Acceptability	like extremely	like very much	like moderately	like slightly	dislike slightly	dislike moderately	dislike very much	dislike extremely

(continue to next for the same sample)

4) For each sensory characteristic, check **ONE** descriptor along the 7-point scale that best describe your impression.

Color Intensity	much too light	moderately too light	slightly too light	just about right	slightly too dark	moderately too dark	much too dark
Firmness	much too soft	moderately too soft	slightly too soft	just about right	slightly too firm	moderately too firm	much too firm
Dryness	much too moist	moderately too moist	slightly too moist	just about right	slightly too dry	moderately too dry	much too dry
Cohesiveness	much too crumbly	moderately too crumbly	slightly too crumbly	just about right	slightly too cohesive	moderately too cohesive	much too cohesive
Saltiness	much not salty enough	moderately not salty enough	slightly not salty enough	just about right	slightly too salty	moderately too salty	much too salty
Sweetness	much not sweet enough	moderately not sweet enough	slightly not sweet enough	just about right	slightly too sweet	moderately too sweet	much too sweet

Meaty Flavor Intensity	much too weak	moderately too weak	slightly too weak	just about right	slightly too strong	moderately too strong	much too strong
Overall Flavor Intensity	much too weak	moderately too weak	slightly too weak	just about right	slightly too strong	moderately too strong	much too strong

Please provide comments on what you liked and didn't like about this product:

....

....

5) If this product is commercially available, what would be your purchase intention?

Definitely would buy it	Probably would buy it	Might or might not buy it	Probably would not buy it	Definitely would not buy it

Appendix D: Nutritional Compositions of Commercial Meat Bars

Brand [1]	**Protein, %** [2]	**Carbohydrate, %** [2]	**Fat, %** [2]
Chef's Cut	~ 33	~ 15	~ 29
DNX	25 - 33	16 - 21	~ 23
Epic	20 - 35	10 - 20	10 – 16
Krave	18 - 28	10 - 35	11 - 20
Mighty	~ 28	10 - 14	9 - 12

[1] products shown were available in retailer stores at the time of purchase, 2018 – 2022, in Saskatoon, SK, Canada, products shown do not represent all commercial products available to the market

[2] compositional values attained from Nutrition Facts tables on product packages, best efforts were made to provide most recent information, but products might be upgraded or modified by manufactures.