OPTIMIZATION OF HUMECTANTS DURING PREPARATION OF BUFFALO JERKY TYPE PRODUCT AND ITS STORAGE STABILITY

by

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This dissertation was submitted to the Central Department of Food Technology, Institute of Science and Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of M. Tech in Food Technology.

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Approval Letter

This *dissertation* entitled *Optimization of Humectants during Preparation of Buffalo Jerky Type Product and its Storage Stability* presented by Anil Basnet has been accepted as the partial fulfillment of the requirement for the M. Tech. degree in Food Technology.

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Abstract

The present study optimized the concentration of different humectants in pretreatment infusion by response surface methodology. The concentration of salt, sucrose, brown sugar, maltodextrin and citric acid used were 0 to 9 g, 0 to 9 g, 0 to 3 g, 0 to 3 g, and 0 to 3 g respectively. The best-optimized sample obtained through DE® and sensory analysis was compared with the control sample (without pretreatments of humectants) concerning storage stability for 30 days in 5 days intervals at 5% level significance.

From graphical and numerical optimization, the optimized combination obtained was 4 g salt, 2 g sucrose, 2 g brown sugar, 1.5 g maltodextrin, and 0.5 g citric acid giving buffalo jerky with moisture content, water activity, fat content, crude protein, ash content, pH of 10.96%, 0.63 at 20.5°C, 4.51%, 85.47%, 8.4% and 5.62 respectively. The sensory and microbial quality of the buffalo jerky prepared from pretreated humectants mixture meat was found significantly superior to untreated (control) samples, with comparable proximate compositions. Salt concentration was more effective in reducing the water activity of the jerky than other humectants used in the present study. Crude protein content and pH were not significantly different ($P>0.05$) between the optimized sample and control sample whereas moisture content, fat, ash content, and water activity were significantly different ($P<0.05$). There was a significant difference ($P<0.05$) between the optimized and control samples in terms of storage stability. The optimized jerky sample was stable for 30 days at room temperature without any decrease in sensory quality and microbiological spoilage to the control jerky sample which lasted for only 20 days at room temperature.

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List of abbreviations

Abbreviation	Full-Form
ANOVA	Analysis of variance
a_w	Water activity
HDPE	High-density polyethylene
IMF	Intermediate moisture food
IMM	Intermediate moisture meat
MSG	Monosodium glutamate
RH	Relative humidity
RSM	Response surface methodology
TBA	Thiobarbituric acid
T-BARS	Thiobarbituric acid reactive substances
TPC	Total plate count
USDA	United States Department of Agriculture
YMC	Yeast and mold count

Part I

Introduction

1.1 General introduction

Nepal is an agricultural country that contributes 33% of the nation's GDP and livestock contributes almost 11% of GDP. Buffalo is one of the major livestock species for milk and meat production in Nepal. Buffalo meat is abundantly found in the market and is relatively cheaper than other meat animals (Kharel *et al.*, 2010). Buffalo meat production is potentially increased and preferred by consumers due to its lipid composition (e.g. low in cholesterol content and saturated fatty acids), rich in iron content, and awesome sensory attributes (e.g. more tender, flavorful, and more succulence). Also, some of the important characteristics of buffalo meat have the advantage of having low fat and cholesterol aids in superiority over beef (Giordano *et al.*, 2010).

Nepal is a Hindu country; slaughtering and consumption of beef are banned. So, beef can be replaced with buffalo which resembles many more characteristics of beef. Also, Shumaker and Kendle (2017) highlighted that traditionally jerky were prepared from big animals such as beef, horse, water buffalo, pork, and other game animals. *Sukuti* is Nepal's only indigenous low moisture dried meat product, which is generally prepared from buffalo meat. But, it generally has a hard texture which makes a negative impact on chewiness perception. Also, mold growth is the primary factor limiting the shelf life of traditional dry meat in Nepal which is generally occurred due to unhygienic handling during processing and primitive method of preservation (Kharel *et al.*, 2010; Subba, 2018).

Jerky is one of the popular and favorite meat product in the United States of America which is made using different humectants in a combination of salting, smoking, and drying (Allen *et al.*, 2007; USDA, 2019). Alternatively, smoking and curing are done to enhance the flavor of the product. Jerky is generally prepared by: lean meat is cut into thin strips and marinated in humectants solution followed by drying. Jerky may be prepared from the restructured or minced meat and flesh of big animals such as buffalo, horses, cattle, pork, etc (Buck *et al.*, 2020; Shumaker and Kendle, 2017). The main raw materials for the preparation of the jerky are salt, sugar, citric acid, brown sugar, soy sauce, and Worcestershire sauce whereas nitrite curing, monosodium glutamate, maltodextrin, and smoke flavor are optional (Shumaker and Kendle, 2017). Due to recent evidence of

foodborne illness in the consumption of jerky, the United States Department of Agriculture has recommended destroying pathogens either by post- drying heating or pre-cooking the meat (Allen *et al.*, 2007).

Humectants are those food additives that are used mostly in food for controlling viscosity and texture. Also, humectants aid in retaining moisture, reduce water activity and perform the important function of improving food softness (Chen *et al.*, 2002; Fennema, 1996). Humectants may be divided into two classes: natural and synthetic. Examples of natural humectants include tartrates, glycerin, and its triester, invert sugars, salt, liquid glucose, egg yolk, honey, brown sugar, maltodextrin, citric acid, vinegar, isolated soy protein (ISP), whole egg powder (WEP), hydrolyzed vegetable protein (HVP), sodium lactate, etc. Synthetic humectants include mono propylene glycol, sorbitol, and mannitol (FritoLay, 2020; McCabe, 2019; Sritongtae *et al.*, 2011). The basic principle for the preservation of intermediate moisture meat is the application of humectants. It helps in the reduction of water activity and microbiological growth is retarded which results in increasing the shelf-life of the product. Most of the traditional intermediate moisture meat is prepared using humectants in the world. Most of the traditional IMM such as *jerky, biltong, tasajo, khilsi, cecina, charqui,* etc are prepared by marination with salt and sugar (Leistner, 1992; Subba, 2018).

To overcome such type of problem, Intermediate moisture meats like jerky are suitable in term of chewiness perception and mold growth can be prevented by the addition of humectants which lowers the water activity of the product and increase its shelf life. In this research, the concentration of different humectants was optimized by design expert software. Ten best formulations in terms of desirability were further investigated. Sensory, proximate analysis, microbiological examination, and storage stability were studied.

1.2 Statement of the problem

Drying meat is one of the most practical ways to preserve meat in developing nations. Worse cold storage facilities, climatic conditions, and its nature of being highly perishable meat in rural areas are not stable for long period (Kumar *et al.*, 2019; Leistner, 1992; Prabhakar, 1999; Subba, 2018).

Due to the highly perishable nature of meat, meat cannot be kept for a longer time in rural areas. Major quality problems of sukuti are a poor dark color, very tough product, shrinkage, poor mouthfeel, lack of juiciness, poor rehydration, mold spoilage, and oxidative rancidity

in high fat dried meat (Kharel *et al.*, 2010; Subba, 2018). Such types of problems can be prevented by the preparation of buffalo jerky using intermediate moisture technology. Buffalo meat in Nepal can be flourished as jerky is a nutrient-dense and shelf-stable IMM product at room temperature for several months.

Thus, to overcome such major quality problems of sukuti and proper utilization of buffalo meat in jerky preparation, combinations of different humectants are essential to minimize the above quality problems of sukuti. Chewiness perception can be enhanced, and mold growth can be prevented by additions of humectants which lowers water activity and increase the shelf-life of the jerky.

1.3 Objectives of the study

1.3.1 General objective

The general objective of this work was to optimize the best mixture of humectants during the preparation of buffalo jerky and evaluation of its quality and shelf-life.

1.3.2 Specific objectives

The following were the specific objectives of this work:

i. To optimize the mixture of humectants for the preparation of buffalo jerky.
ii. To select the best jerky in terms of sensory evaluation.
iii. To analyze the storage stability of the best sensory evaluated product at room temperature.

1.4 Significance of the study

Over the different corners of the world, there are different traditional IMM. Their processing varies with the climatic conditions and the economic and technological status of the country. Thus, the use of salt, sugar and other humectants act as further preservative agents gradually evolved in combination with drying. As it is a low-cost preservation technique in tropic countries like Nepal can benefit from such type of preservation technique with the use of humectants and it is stable for some months where a refrigeration facility is not available. As it is a useful food during various natural calamities as it can be consumed without cooking.

Jerky plays a vital role in supplying nutrients to military personnel at high altitudes, mountaineers, astronauts, and persons on an expedition to far-reaching areas like Antarctica and even some of the highest peaks of Nepal (Shumaker and Kendle, 2017). In addition, these products prove very useful during natural calamities such as cyclones, floods, and earthquakes as instant food (Kumar *et al.*, 2019). In tropical countries like Nepal, India, other African countries where cold storage facilities are absent or deficient, IMM like jerky can be of great importance as they can be stored at room temperature conveniently without refrigeration and consumed without further processing.

Buffalo meat is cheaper than beef and is consumed high in Nepal. Also, it has low marble fat and cholesterol than beef (Giordano *et al.*, 2010). With a scientific approach, the product and process can be developed into a way for economic growth for the meat process industry in Nepal. Mold growth is the primary factor in limiting the shelf life of traditional dry meat in Nepal (Subba, 2018). Such problem of mold growth can be minimized by using humectants and lowering water activity beyond mold growth value (i.e.0.60-0.70 a_w) (Srinivas, 1996). As Jerky is manufactured from beef which is strictly not consumed in Nepal. Traditional low moisture meat of Nepal, Sukuti has a very much tough texture which affects consumers' chewiness perception.

Hence, a study was planned to prepare jerky from buffalo meat with humectants and to study certain quality parameters to help in the development of IMM products like jerky, which have greater acceptability and may promote the tourism sector as jerky is very much popular worldwide. Also, buffalo meat has the same characteristic in terms of chemical composition and low content of cholesterol. Therefore, in the present work, the intermediate moisture technology principle for the preparation of jerky was utilized on buffalo meat by using a different humectants mixture.

1.5 Limitations and delimitations of the work

i. Instrumental measurement of the texture of the prepared jerky could not be carried out.
ii. Microbiological analysis was limited to yeast and mold count.
iii. Lipid oxidation analysis was limited to peroxide value determination.

Part II

Literature review

2.1 Trends of production and consumption of buffalo meat in Nepal

Meat is the most valuable livestock product which helps in enhancing human nutrition. Buffalo meat is the flesh of the water buffalo, a large bovid, farmed for its milk and meat in many South Asian countries, some parts of Europe, and the Rest of the African countries (Giordano *et al.*, 2010; Kandeepan and Rajkumar, 2009). Buffalo meat is a specifically valuable source of omega-3 fatty acids, vitamin B_{12}, vitamin B_6, niacin, and bioavailable iron, zinc, and selenium (Williams, 2007).

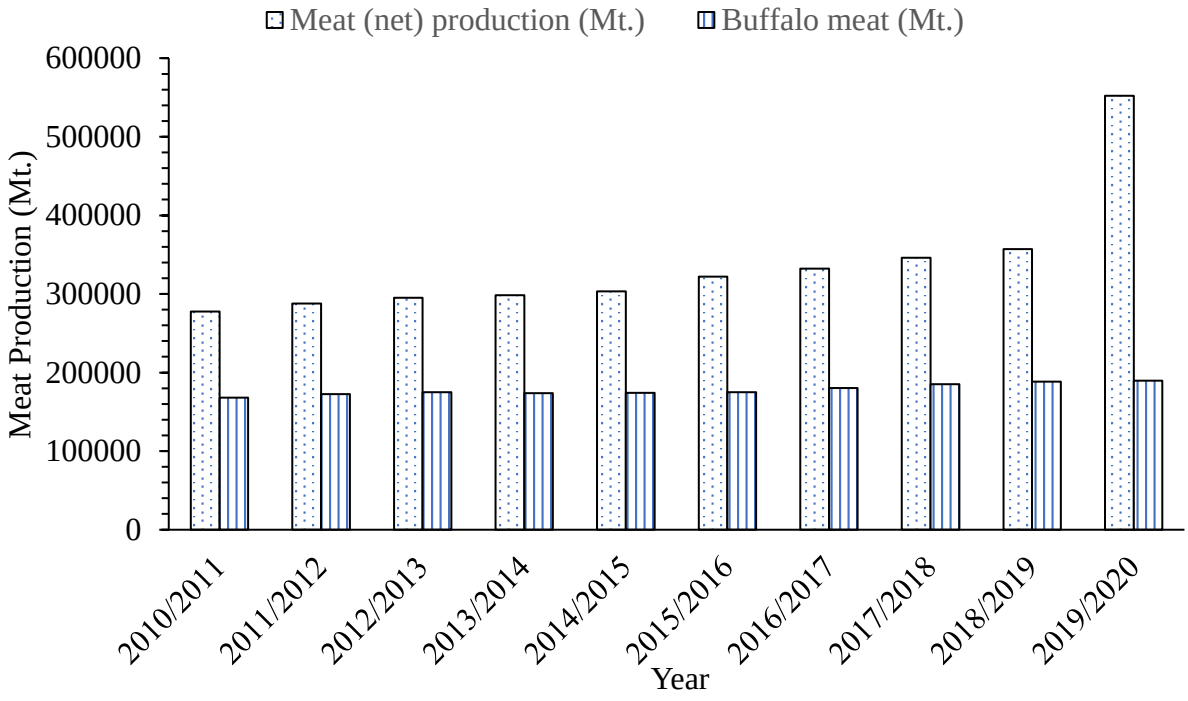

Fig. 2.1 Meat (net) with buffalo meat production in Nepal

Tamburrano *et al.* (2019) reported that buffalo meat is so much like beef in terms of composition, quality, and organoleptic sensory characteristics and has an added merit of low-fat content, cholesterol, and calories. The chemical composition of buffalo meat is presented in Table 2.1. Buffalo meat has been reported to have a low concentration of total lipids (1.37 g/100 g) (Naveena and Kirant, 2014). Buffalo meat from 2 years old male calves showed a fat percentage of 1 to 3.5 (Rao and Kowale, 1991; Thomas *et al.*, 2008). Lapitan *et al.* (2007) reported that moisture content (wb), protein content (wb), fat content (wb), and ash content

(wb) values for crossbreed water buffalo meat as 71.74%, 20.82%, 6.82%, and 1.14% respectively pre-slaughter factors such as species, breed, size, and age of the animal affect the texture and quality of the meat (Toldrá, 2017).

Recently, the livestock sector in developing countries is one of the fastest-growing sectors, with its share of gross domestic product at 33% and is quickly increasing (FAO, 2007; Thornton, 2010). MOALD, (2021) data (Fig. 2.1) shows that buffalo meat has a higher demand in Nepal, followed by goats, chicken, and pork. The enhancing global meat market provides a significant opportunity for livestock farmers and meat processors in these least developed countries. Also, increasing livestock production and the lack of safe processing and marketing of hygienic meat and meats products aid a big hurdle (FAO, 2019). *Sukuti, momo*, keema curry, *choyla*, *kachila*, meat balls, and sausages are the major products made from buffalo meat. In the Newari community, *kachila*, a popular Newari meat item is eaten raw which may be contaminated with pathogens and there will be a potential risk of food poisoning (Adhikari, 2006).

Table 2.1 Chemical composition of buffalo meat (value per 100 g raw, lean meat)

Parameters	Value (g)
Water	76.30
Protein	20.39
Total fats	1.37
Ash	0.98
Saturated fatty acids	0.460
Monosaturated fatty acids	0.420
Polyunsaturated fatty acid	0.270
Cholesterol	46

Source: Naveena and Kirant (2014)

2.2 Preservation of meat by Intermediate Moisture technology

As fresh meat is an ideal medium for the growth of microorganisms and is subject to rapid spoilage, the interior of the animal is virtually free of organisms except for the lymph nodes

and excluding the gastrointestinal and respiratory tracts. Other factors include environmental conditions before slaughter and during processing affect the degree of contamination on the surfaces of the meat (FAO, 2007). Bacteria such as *Staphylococcus, Micrococcus, Pediococcus, Streptococcus, Lactobacillus, Clostridium,* and *Bacillus have* been isolated from cured meat whereas yeasts and molds are not usually associated with freshly made meat products, but after aging, sausages and IMM may evident the growth of such fungi (Huang and Nip, 2001; Taoukis and Richardson, 2020).

Intermediate moisture meats are the meat products that are stabilized by water activity a_w in the range of 0.60 to 0.90, although additional hurdles, such as heating, preservatives, pH, and E_h are also important parameters too. Such foods need no refrigeration during storage (Kumar *et al.*, 2019; Leistner, 1992). IMMs have a soft texture, consumable without further processing and preparation with shelf stability of several months assured without thermal sterilization, freezing, or refrigeration (Taoukis and Richardson, 2020).

Jerky is a popular meat snack due to its simple preparation procedure, nutrient density, lightweight, shelf stability at room temperature as well as wide variations in taste and aroma. Traditionally it is prepared from the thin slices of the whole muscle of beef, horse, buffalo, whale, and game animals. Recently jerky is prepared from meat trimming or poor meat cuts by restructuring or by comminuted meat. The shelf life of jerky is more than 1 year attributed to its low moisture (less than 15%), water activity (0.55-0.70), and pH of less than 4 (Buck *et al.*, 2020; Kumar *et al.*, 2019). Addition of salt, sugar and sodium nitrite during jerky preparation aid in pathogens destruction, but the combination of different humectants is more effective than single humectants to achieve necessary lethalities (Nummer *et al.*, 2004).

Biltong is a ready-to-eat product widely consumed as a snack in South Africa. Biltong can be made using several similar approaches. The traditional method involves marinating followed by air-drying at low temperatures, around 35ºC, for one week. Beef is commonly used meat. Lean meat strips are prepared from intact muscles (common hindquarter is used) which can be up to 400 mm long (commonly cut along the grain) and 25 to 50 mm thick and marinated in a seasoning mixture commonly containing salt, black pepper, coriander, brown sugar, vinegar, nitrate or nitrite which is followed by low-temperature drying. The typical composition of biltong has a moisture content (20 to 30%); salt (3 to 8%); pH (5.6 to 5.9); water activity (0.7 to 0.75) (Huang and Nip, 2001; Kumar *et al.*, 2019; Subba, 2018).

Charqui is native to South America with much produced in Brazil. A traditional approach to making *charqui* has many similarities to that used in the dry curing of bacon. A fresh side of beef is cut into three pieces that are butchered open, cut into strips like biltong, and then hung at ambient temperature for about an hour. The strips are immersed in brine for a further hour, drained, dipped in coarse dry salt, stacked 1-1.5 m high, covered in salt, and left overnight. The piles are turned daily for 4 days with strips from the top going to the bottom and vice-versa and the piles are recovered with salt. Drying begins on the 5^{th} day when meat is hung over-the drying tray and exposed to the sun for no longer than 1-2 h. This drying and curing are repeated 5-7 times until the meat has lost 40% of its fresh weight. Charqui is fattier product. The best grade final product contains 20-35% fatty tissue (Huang and Nip, 2001).

Pastirma is a meat product made of salted and dried beef, highly esteemed in Turkey and Egypt as well as other Muslim countries. Meat from 5 to 6 years old beef cattle is used, taken from the hind-quarter within 6 to 12 h of slaughter. The meat is cut into long strips (500 to 600 mm) with a diameter, of not more than 50 mm. The strips are rubbed and covered with salt-containing potassium nitrate and several slits are made in the meat to aid salt penetration. The strips are piled 1 m high and kept for one day at room temperature. The process is repeated, turning the pile from top to bottom. The strips are then washed and air-dried for 2 to 3 days in summer or 15 to 20 days in winter. After drying, the strips are piled up to 300 mm high and pressed with heavy weights for 12 h. They are dried for a further 2 to 3 days and pressed again for 12 h (Burfoot *et al.*, 2010).

Tasajo is a salted meat-based product made in Cuba and has similarities with charqui. Traditionally, the meat is salted then sun-dried, a process that takes at least three weeks. Industrially, wet salting in saturated salt brine (1%) for 8 h, dry salted, and finally hot air-dried at 60ºC until a 50% weight loss is achieved. Three basic processes are used to prepare dry meat slices, dry meat cubes or strips, or shredded dried meat. Products vary according to the species of meat, technology, and spices used. Water activity can lie between 0.6 and 0.9. Some have even less than 0.6 (Low Moisture Food) (Burfoot *et al.*, 2010; Huang and Nip, 2001).

Lup Cheong is a popular Chinese product like dry sausage. The hind leg is selected for this product, which is cut in short strips. Back fat is cut into 10 mm cubes and mixed with

meat. After the addition of spices, it is left for marinating for several hours and then filled in pork small intestine and dried over burning charcoal for 3 days. *Tsire* or *Suya* is made from beef, mutton, or goat meat. The lean meat is cut into 1 cm thick slices and after seasoning with salt, spices, groundnut flour, and oil roasted over a smokeless fire to a moisture content of 23% (Huang and Nip, 2001).

Cecina is intermediate moisture meat produced and consumed to a large extent in Mexico. There is a large variation in the formulation and processing of *Cecina* and accordingly, its quality varies considerably (Reyes-Canoa *et al.*, 1994). *Kilishi* is a tropical intermediate moisture meat product prepared in African countries. It is prepared essentially from beef slices, infused in a slurry of defatted groundnut paste and spices, and sundried. *Kilishi* keeps well without refrigeration for several months (Ogunsola and Omojola, 2008)

Sukuti is a popular Nepalese dried meat. Commercial *sukuti* is made almost exclusively from buffalo meat. Preferentially round muscle is used for making *sukuti.* There are some process variations but generally, the meat is cut in 1 to 1.5 cm x 1 to 1.5 cm thick strips or 1 cm approx. thin slices and dried by the sun, air, or over the smokeless fire to 10 to 20% moisture content. It may be spiced with salt and red chili powder (Kharel *et al.*, 2010).

2.2.1 Production of Intermediate Moisture food products

Several manufacturing techniques are applied for producing IMF products. They can be classified into four main categories (Taoukis and Richardson, 2020):

- Partial drying can be used in the production of IMFs only if the starting materials are naturally rich in humectants. This is the case with dried fruits (e.g., raisins, apricots, prunes, dates, apples, and figs) and syrups (e.g., maple syrup). The final a_w of these products is in the range of 0.6 to 0.8.
- Moist infusion, or osmotic dehydration, involves soaking solid food pieces in water–humectants solution of lower a_w: This technique has also been defined as dewatering impregnation soaking. The difference in osmolality forces water to diffuse out of the food into the solution. Simultaneously, the humectants diffuse into the food, usually more slowly than the water diffuses out. Salt or sugar solutions are usually employed. This is the method to produce candied fruits. In addition, novel meat and

vegetable IMF products have been produced by infusion in solutions of salt, sugar, glycerol, or other humectants.

- Dry infusion consists of first dehydrating solid food pieces and then soaking them in water–humectants solution of the desired a_w. This process is more energy-intensive, but it results in high-quality products. It has been used extensively in the preparation of IMFs for the National Aeronautics and Space Administration (NASA) and the U.S. military. The latter is a major developer and user of IMF products.
- The process of direct formulation involves weighing and direct mixing of food ingredients, humectants, additives, followed by cooking, extrusion, or other treatment, resulting in a finished product with desired a_w. This method is fast and energy-efficient and offers great flexibility in formulation. It is used for both traditional IMF (e.g., confections, preserves) and novel IMF (e.g., pet foods, snacks) products.

2.3 Jerky

Jerky is one of the most versatile and popular intermediate moisture meats in the United States of America and Canada. It has been made traditionally with sliced whole muscle or restructured meat of various animals or poultry and has enhanced preservation through curing and drying with a reduction in water activity (a_w) (Buck *et al.*, 2020; Chen *et al.*, 2002a; Kumar *et al.*, 2019; Shumaker and Kendle, 2017; USDA, 2019). Jerky is a convenience, high protein, light-weight, shelf-stable meat snack popular for campers, hikers, and many general consumers (Huang and Nip, 2001; Lee and Kang, 2003). Kumar *et al.* (2019) has concluded that the shelf-life of jerky is more than 1 year due to its low moisture content (less than 15%), Water activity a_w in the range of 0.60 to 0.75, and pH value of less than 4. If jerky is made safely, it is nutrient-dense, shelf-stable, and lightweight (Park, 2009). Shumaker and Kendle (2017) state that once jerky is dried, a pound (0.45 kg) of meat is usually reduced to about 4 ounces (0.11 kg) during processing. Beef jerky water activity ranged from 0.66 to 0.74 on 20 days storage at ambient temperature (Lim *et al.*, 2012).

Homemade jerky is associated with several foodborne illness outbreaks due to meat sources becoming contaminated with pathogenic microorganisms and the pathogen survives the jerky-making procedure (Buck *et al.*, 2020; Burfoot *et al.*, 2010). In the jerky-making process, huge safety concern is the risk of allowing bacteria that may cause human food

poisoning to increase to high levels in the warm, dry environments of a meat dehydrator or oven drying process (Shumaker and Kendle, 2017). Marinade of different humectants is treated with citric acids for their effectiveness in aiding destruction of pathogens during jerky preparation (Albright *et al.*, 2002). Also, citric acid as a humectants plays an important role to enhance flavor, adjust the pH of the meat, and aids in the inversion of the sugar (Belitz *et al.*, 2009). Traditional and modern drying method during beef jerky preparation method ranged 57.8% and 64.3% respectively protein content on dry basis (Shi *et al.*, 2020).

Many researchers have concluded that traditionally prepared jerky in which raw meat is dried at temperatures of about 60ºC to 68ºC, does not kill pathogens if present in the meat (Buck *et al.*, 2020). The consumption of homemade and commercially made jerky leads to gastroenteritis outbreaks in the USA and Mexico. Between the years 1966 and 1955, 8 outbreaks occurred in the USA, causing over 250 illnesses. Among them, two outbreaks were associated with *Staphylococcus aureus* and 6 were associated due to contamination of *Salmonella.* These cases were primarily associated due to consumption of local commercially prepared jerky whose processing times and temperatures never reached a level to destroy pathogens. In the year 1995, *E. coli* O157:H7 occurred in Oregon due to the consumption of home-processed deer jerky. Many researchers have suggested that the traditional home-drying jerky process was inadequate to kill food poisoning pathogens (Allen *et al.*, 2007; Buck *et al.*, 2020; Chen *et al.*, 2002; Prabhakar, 1999). Buck *et al.* (2020) report that for foodborne illness to occur, a certain fault must take place:

1. Meat source becomes contaminated with a pathogenic microorganism.
2. Foodborne pathogens survive the jerky-making processes.
3. When the jerky is consumed.

Allen *et al.* (2007) suggestions for methods for destroying foodborne pathogens are described in brief in the following paragraphs:

- Post-drying heating: in this method, meat strips are placed on trays of drier or oven preheated to 135°C and then heated for 10 min effectively destroy or reduce pathogens. This method produces the most traditional jerky with a tough texture.
- Pre-cooking the meat: In this method meat slices or strips are dipped into a marinade and heated long enough to heat the meat strips or slices to 70°C which destroys pathogens that may present. This method is currently recommended by the USDA.

Also, it has the advantage that meat pre-cooked will minimize the time to dehydrate and will result in jerky with less tough texture and juiciness.

The preparation of jerky as outlined by Allen *et al.* (2007) and Shumaker and Kendle (2017) is given in the paragraphs that follow:

- Meat selection: Generally, jerky is made from any animal meat, birds, or poultry. Meat selected for jerky preparation must contain less than 10% fat as meat cut with high-fat content would be rancid quickly.
- Preparation of the meat strip: Preparation of the meat strip is a challenging thing to get correct when preparing jerky is cutting the meat into perfect long thin strips. As fresh meat is usually flexible, it is recommended to freeze the meat before cutting it into strips or slices. Also, it is essential to trim as much fat from the surface as possible before slicing it into jerky strips or slices. For best results, strips or slices must be about 1 inch to $1^{1/2}$ inches wide.
- Preparation of jerky marinade: Marinade recipes include mixtures of humectants, soy sauce, Worcestershire sauce, food additives, and spices. Prepared meat strips or slices are placed into shallow utensils and covered with marinade. Generally, meat strips are marinated for several hours or overnight, according to the choice of the manufacturer.
- Destroying pathogens in jerky: It is discussed above about the method of destroying pathogens in jerky.
- Drying the meat strips or slices: Jerky can be dried in an oven or an electric dehydrator. But, sun drying is not recommended for jerky making due to the risk of contamination and unsteady heat transfer across meat surfaces.
- Storage of jerky: When all the procedure is completed, jerky is pressed gently between paper towels or napkins to absorb any excess fat presently. Jerky is cooled to room temperature and packaged in air-tight containers. Some examples of air-tight containers include plastic zipper bags, glass jars, and vacuum-sealed plastics. Properly dried and treated jerky can be safely be stored for up to 2 weeks at room temperature or three to six months when packed in a nitrogen-filled package.

2.4 Effect of water activity (a_w) on the microbiological quality of meat

Water in muscle and meat is held in three forms viz. bound, immobilized, and free water in which a major portion is made up of free water. The thermodynamic approach to the influence on the rate of microorganisms in food is known as water activity (a_w). a_w is the fundamental factor that affects most of the microbial growth requirements such as nutrients, temperature for growth, pH, redox potential, etc. Water activity (a_w) is defined as the ratio of the water vapor pressure of food to that over pure water at a given temperature (Belitz *et al.*, 2009; Thomas *et al.*, 2008).

The effect of a_w on microorganism growth is very essential in IMM. At the usual temperatures allowing microbial growth, most bacteria require a_w in the range of 0.9-1.00. the minimum a_w below which most important foodborne bacteria will grow is about 0.90, depending on the specific bacteria (Potter and Hotchkiss, 1995). Some halophilic bacteria may grow at an a_w of 0.75 and certain osmophilic yeasts can grow even at lower a_w, but these microorganisms seldom cause food spoilage as compared to most bacteria, molds are more resistant to drying conditions. Molds can grow on foods having an a_w of about 0.80 and can show slow growth at room temperature for several months on foods with a_w as low as 0.70. Mold growth is completely inhibited at an a_w of about 0.65. However, such low a_w is generally not applicable in the fabrication of IMM, many of which have below 20% moisture (Park, 2009; Taoukis and Richardson, 2020).

Water activity is a major factor in preventing or limiting microbial growth. In several cases, a_w is the primary parameter responsible for food stability, modulating microbial response, and determining the type of microorganisms encountered in food. The factors affecting microbial growth, death, and survival in food products are temperature, oxygen, nutrient availability, acidity and pH, presence of natural or added inhibitors, etc. The influence of a_w on vegetative microorganisms and spores is one of the most complex and fascinating. Adverse environmental conditions, such as a_w changes that cause osmotic stress, can enhance the sporulation response in spore-forming microorganisms, but bacterial endosperms and some fungal spores have special requirements, such as optimum a_w values for initiating germination and outgrowth. In addition, the production of secondary metabolites (toxins) is affected by a_w. Therefore, sporulation, germination, and toxin

production are affected by a_w along with other environmental factors (Tapia *et al.*, 2007). The minimum a_w required for the growth of microorganisms is given in Table 2.2.

Table 2.2 Minimum a_w required for the growth of microorganisms

Water activity values	Microorganisms inhibited by the lowest a_w in this range
1.00-0.95	*Pseudomonas, Escherichia, Proteus, Shigella, Klebsiella, Bacillus, Clostridium perfringens, C. botulinum* E, G, some yeasts
0.95-0.91	*Salmonella, Vibrio parahaemolyticus, Clostridium botulinum (*A, B), *Listeria monocytogenes, Bacillus cereus*
0.91-0.87	*Staphylococcus aureus* (aerobic), many yeasts (*Candida, Torulopsis, Hansenula), Micrococcus*
0.87-0.80	Most molds (mycotoxigenic *Penicillium*), *Staphylococcus aureus, Saccharomyces, Debaryomyces*
0.80-0.75	Most halophilic bacteria, mycotoxigenic *Aspergillus*
0.75-0.65	Xerophilic molds (*Aspergillus chevalieri, A. candidus, A. Wallemiasebi), Saccharomyces bisporus*
0.65-0.61	Osmophilic yeasts (*Saccharomyces rouxii*), a few molds (*Aspergillus echinulatus*)
<0.61	No microbial proliferation

Source: Tapia *et al.* (2007)

2.5 Effect of humectants on drying kinetics of dried meat

Meat drying is the oldest preservation technique that preserves meat and thus increases the shelf-life of raw meat. It reduces storage and transportation costs and handling stress-free by reducing size, weight, and risk of microbial contamination (Mishra *et al.*, 2017). Meat drying characteristics such as shrinkages, rehydration ratio, apparent or effective diffusion coefficient, or diffusivity affects the drying behavior of the meat (Akthar and Pandey, 2015).

Dried meats are those meat products with whole muscle or ground-and-formed meat products that have been dehydrated (i.e. removal of water) which results in unique sensory properties and increased stability. The different types of dried meat products are presented in Table 2.3. Taoukis and Richardson (2020) stated that depending on the degree of dehydration, dried meats can be conveniently classified as:

A. Low moisture meats: products that contain less than 25% moisture and have a water activity (a_w) level of <0.60
B. Intermediate moisture meat: products that contain less than 50% moisture and have a water activity (a_w) level of 0.60 to 0.85

Table 2.3 Types of dried meat products

Dried-meats	Process involved	Typical products
Dry and semi-dry sausages (mainly fermented)	Fermentation and ripening	*Pepperoni*, *Genoa*, Italian and Greek *salami*, summer sausage, *Lebon bologna*, etc.
Dried whole muscle products (mainly dry-cured)	Curing (i.e. addition of salt, nitrite etc.) and drying	Parma & country hams, *Prosciutto, Pancelta, Cappocola*, etc.
'Whole muscle' meat snacks	Drying (occasionally marinated)	*Jerky*, *biltong*, *pastruma* or *bastruma, tasajo*, *pemmican*, *sukuti*, etc.

Source: Skandamis and Goundaki (2009)

Humectants are a substance that attracts water to themselves; they can retain water in foodstuffs, reduce water activity and perform the important function of improving food softness (Sorapukdee *et al.*, 2016). Maltodextrin are generally a polysaccharide produced from corn, tapioca or wheat starch that helps as a thicker or filler in jerky product (FritoLay,

2020; Shaefer, 2018). Chicken jerky made with sucrose and mixed sugar stored in 33% RH condition had maximum quality changes in textural and rancidity spoilage that were unaccepted after 7 days of storage (Wongwiwat and Wattanachant, 2015).

Chen *et al.* (2000) found that the shear force value of Chinese-style pork jerky was reduced by increasing glycerol and sorbitol. The most commonly used osmotic agents are sucrose and sodium chloride. Osmotic dehydration in meat consists of immersing meat in aqueous solutions of high osmotic pressure such as sucrose, salt, etc., and allowing water to transfer from the meat surface into the solution by osmosis (Chen *et al.*, 2002). Chen *et al.* (2002) also studied that a high level of sucrose is more effective than a low level of sucrose in lowering moisture content and water activity a_w. The effect of different levels of sucrose on Chinese style pork jerky is shown in Fig. 2.2

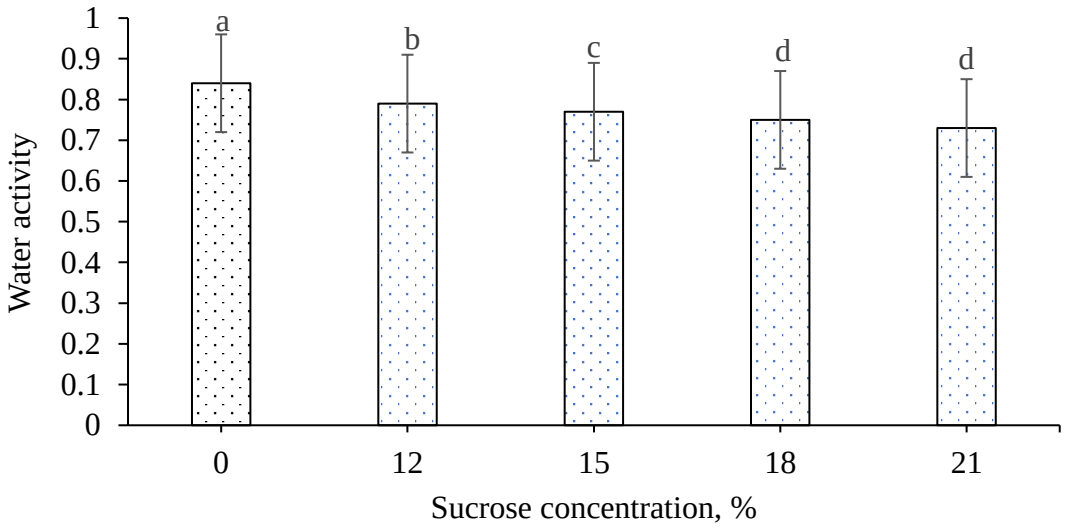

Fig. 2.2 Effect of different levels of sucrose on water activity in Chinese- style pork jerky

2.6 Effect of humectants on the storage stability of meat

Humectants are those food additives that include nonionic polyols such as sucrose, glycerol, salt, honey, etc. Their main role is decreasing moisture content and water activity (a_w). They also perform an important function of improving food softness. Also, it has been recognized that humectants are important to improve processing yield and sensory properties such as tenderness and juiciness of jerky products (Jang *et al.*, 2014). Humectants are classified into two classes, viz., natural and synthetic. Examples of natural humectants are tartrates,

glycerin and its trimester, invert sugar, honey, etc. synthetic humectants including mono-propylene glycol, sorbitol, mannitol, etc. (Msagati, 2013). Brown sugar is a mixture of white sugar and molasses, which are responsible for color development and aids to slightly increase nutritive of the product due to its mineral content (McCabe, 2019).

As with all foodstuffs, dried meat may spoil by microbial, enzymatic, and chemical deterioration. Dried meat is normally processed to $a_w < 0.50$ during storage at ambient temperature. With the addition of humectants, a decrease in water activity causes osmotic stress to microbial cells and subsequently leads to cell damage and death (Frazier and Westhoff, 2003). Obanu (2001) stated that enzymes of widespread occurrence are completely inactivated at water activities as low as 0.85 or less. Non-enzymatic reactions of aldehyde amino condensation i.e. Millard reaction are strongly water-dependent and reach a maximum at 0.60 to 0.70 which causes several effects such as darkening development of meat surface with off-flavors and odors, toughening in texture, loss of nutrient value especially lysine destruction (Chen *et al.*, 2002)

Chen *et al.* (2002) suggested that pork jerky with a high level of sucrose has negative effects on protein stability and extractability of myosin heavy chain due to non-enzymatic browning during storage. Jamhari and Lakshmiwati (2018) concluded that interaction between sugarcane levels and drying methods on the protein content and tenderness of ground beef *dedeng* resulted due to addition of sugarcane at the levels of 40% yielded *dedang* with the best tenderness during storage.

2.7 Effect of humectants on the microbiological quality of IMM

As fresh meat is an ideal medium for bacterial growth and is subject to rapid spoilage, the interior of the animal is virtually free of organisms except for the lymph nodes and excluding the gastrointestinal and respiratory tracts. Other factors include environmental conditions before slaughter and during processing affect the degree of contamination on the surfaces of the meat (Frazier and Westhoff, 2003). Bacteria such as *Staphylococcus*, *Micrococcus*, *Pediococcus*, *Streptococcus*, *Lactobacillus, Clostridium,* and *Bacillus* have been isolated from cured meat whereas Yeasts and molds are not usually associated with freshly made products, but after aging, sausages and IMM may evident the growth of such fungi (Huang and Nip, 2001).

Jerky is generally stabilized by a_w in the range of 0.60 to 0.85, also the addition of hurdles such as heating, preservatives, pH, and E_h are important parameters to be considered. Such types of meat need no refrigeration during storage (Leistner, 1992). IMMs have a soft texture, are consumable without further preparation, and with shelf stability of several months assured without thermal sterilization, freezing, or refrigeration (Taoukis and Richardson, 2020). Buck *et al.* (2020) reported that a recent outbreak of foodborne illness due to *Salmonella* spp in beef jerky and *E. Coli* O157:H7 in venison jerky raise a great concern over the safety of homemade jerky. Some of the important factors to be considered during the preparation of IMM drying are listed:

1. Water content
2. Water activity
3. Acidity or pH
4. Chemical preservatives and additives
5. Oxygen availability
6. Temperature of storage

The main spoilage organisms associated with dried meat as well as IMM are molds and yeast requiring low a_w. Many pathogenic bacteria like *Salmonella*, *Staphylococcus aureus*, *Listeria Monocytogens*, *Clostridia*, etc. (FAO, 2019). Generally, IMM-like jerky is obtained from pre-cooked meat is generally lower in bacterial counts and absence of coliforms during the storage as compared to raw dehydrated meat products. Pathogenic bacteria cannot grow below a water activity of 0.85-0.86, whereas yeast and molds are more tolerant of a reduced aw of 0.80, but usually, no growth occurs below aw of about 0.60 (Kharb and Ahlawat, 2010). According to Chukwu and Imodiboh (2009), *Khilishi*, an IMM of Africa, treated with potassium sorbate was stable for 21 days storage in terms of yeast and mold count. Chen *et al.* (2000) investigated a slow increase in yeast and mold count in Chinese-style pork jerky with different levels of glycerol and sorbitol during storage at 28°C.

Sorapukdee *et al.* (2016) studied that jerky made with spent hen meat with 15% glycerol addition showed superior quality in which total aerobic bacteria, yeast, and mold & *Staphylococcus aureus* were not detected in ambient temperature storage. The microbial count (log CFU/g) of spent hen meat and jerky products from 15% glycerol at ambient temperature is given in Table 2.4.

Table 2.4 Microbial count of spent hen meat and jerky with 15% glycerol at ambient temperature

Microbiological analysis	Raw meat	Jerky formulation with 15% glycerol
Total aerobic bacteria count	2.79	Not detected
Yeast and mold count	2.56	Not detected
Staphylococcus aureus	2.77	Not detected

Source: Sorapukdee *et al.* (2016)

Sureshkumar *et al.* (2006) studied that buffalo meat sausage made by adding humectants VIZ salt, sugar, isolated soy protein (ISP), whole egg powder (WEP), hydrolyzed vegetable protein (HVP), sodium lactate, and enough heat treatment was acceptable up to 3 days whereas untreated samples spoiled 1st day of storage at room temperature. Chen *et al.* (2000) suggested that the replacement of sucrose with sorbitol, glycerol, and xylitol provide partially positive effects on the quality and microbiological characteristics of semi-dried jerky, and replacement with 5% xylitol could be an optimal replacement level. Das *et al.* (2006) found that aerobic mesophilic count and psychotropic counts of goat meat patties were not influenced by the addition of soy proteins as humectants at ambient temperature.

Karthikeyan *et al.* (1999) reported that with humectants, isolated soy protein (ISP) is most effective in chevron meat keema of water activity of 0.88. He also found that humectants treated keema is safe and well acceptable up to 3rd day and fairly acceptable up to 5th day during storage at 35ºC. Sahoo and Anjaneyulu (1997) reported that based on physio-chemical, sensory, and microbiological quality parameters, vacuum packaging improved the quality of nuggets and is stable and acceptable for 30 days refrigerated storage compared to 10 days in control samples.

Chen *et al.* (2000) studied that the addition of 6% to 9% glycerol or sorbitol resulted in a decrease in mold and yeast counts during storage. Further increase in glycerol and sorbitol content showed a gradual inhibition of mold and yeast growth. Gailani and Fung (1989) studied that Sharmoot, Sudanese dry meat, made using sorghum flour and okra flour as a humectant showed a positive response against yeast & molds, *Staphylococcus aureus*, and *Clostridium perfringens.* Choi *et al.* (2008) concluded that low microbial activity was

observed due to fact that microbial growth is retarded by low water activity a_w due to the addition of different humectants. As drying temperature is one of the important parameters for the safe processing of homemade jerky. Buck *et al.* (2020); Shumaker and Kendle (2017) reported that drying must be performed at 145-155 °F for 15-30 min as guided by USDA FSIS meat and poultry jerky compliances. The correlation between minimal a_w and minimal temperatures for the growth of microorganisms are given in Table 2.5

Table 2.5 Correlation between minimal a_w and minimal temperature for growth of microorganisms

Organisms	Minimal a_w	Minimal temperature required
Bacteria	0.91	-10ºC
Yeasts	0.88	-12ºC
Molds	0.84	-18ºC

Source: Tapia *et al.* (2007)

2.8 Measurement of water activity (a_w) using Paw kit device

The paw kit device (Fig. 2.3) is manufactured by Decagon Devices, Inc. Pullman, WA, USA. It is specially designed for government inspectors, jerky and IMM manufacturers, snack food manufacturers, and production-line QA managers. The paw kit devices measure the accuracy of ±0.02 a_w. Its range of measurement is 0.10 to 1.00 in room temperature of 15ºC to 40ºC. The sample types for measurement are powder, liquid and solid. It is operated with an in-built battery. To make a measurement, flip back the sensor cover and place the sample in small quantity over the sample cup. A push of a button brings an accurate reading within five minutes with 5 times beep (Anonymous., 2007).

Fig. 2.3 Paw kit water activity meter

2.9 Lipid oxidation in meat and IMM products

Lipid oxidation in meat and meat products serves as an essential parameter that assesses the quality of processed meat and meat products. Lipids in meat and meat products are one of the most chemically unstable food components that participate in oxidative reactions supported by several factors through quite complex mechanisms (Lima *et al.*, 2013). Lipids are important components of all types of meat and are responsible for most of the desirable characteristics of meats. They are important for the flavor and aroma profile of meats and contribute to tenderness and juiciness (Min and Ahn, 2005). Lipid oxidation affects color, texture, nutritional value, taste, and aroma leading to rancidity, which is responsible for off-flavors and unacceptable taste (Lima *et al.*, 2013).

The development of oxidative rancidity in meat begins at the time of slaughter, when blood flow is interrupted, the metabolic processes are blocked (Lima *et al.*, 2013). It is a rather complex process in which unsaturated fatty acids react with molecular oxygen via free radical chain-forming peroxides. The first auto-oxidation is followed by a series of secondary reactions, which lead to lipid degradation and the development of oxidative rancidity products (Lima *et al.*, 2013).

Peroxide value serves as a useful indicator of the extent of oxidation of lipids, fats, and oils. The peroxide value gives the degree of peroxidation and measures the number of total peroxides in the substance. As, double bonds found in fats and oils play a role in autoxidation (Chakrabarty, 2003). Kong *et al.* (2011) studied the control of lipid oxidation in extruded salmon jerky snacks in which the addition of rosemary oleoresin was effective against lipid oxidation whose Peroxide value was less than 6 meq/kg after 16 weeks of storage at room temperature with nitrogen packaging.

The thiobarbituric acid (TBA) assay is regarded to be the most method for measuring the oxidation deterioration of lipids in meat. As TBRAS value remains higher in dehydrated meat products as compared to fresh and smoked meat. During the storage period, lipase action in meat products causes a gradual increase in Free Fatty Acids in IMMs (Chukwu and Imodiboh, 2009).

2.10 Sensory characteristics of jerky type meat

The organoleptic quality of IMM is an integral factor determining its quality and plays a vital role in meat marketability. The drying methods have a significant effect on the lightness (L), redness (a), and yellowness (b) values, which reflect the degree of browning during drying as well as being a cause of variation in light scattering from the surface of the meat (Mishra *et al.*, 2017). Sucrose acts as humectants, which helps the formation of texture and tenderness, gives flavor through browning, and gives sweetness to *dendeng* (Jamhari and Lakshmiwati, 2018). Brown sugar aids superior humectants in enhancing humectants in chicken breast jerky (Nam *et al.*, 2017b). Salt, sugar, brown sugar, maltodextrin, and citric acid are the main humectants ingredients during the preparation of jerky that influence color, taste, texture, flavor, and overall acceptability of jerky (Allen *et al.*, 2007; FritoLay, 2020; Nam *et al.*, 2017; Shumaker and Kendle, 2017).

Part III

Materials and methods

3.1 Materials

3.1.1 Chemicals and apparatus required

All the chemicals, glassware, and equipment used for the study were of lab-grade quality and collected from the Central Department of Food Technology (CDFT), Quality control department of Natural fish and meat center Pvt. Ltd., Jhapa, and Zest Laboratories, Bhaktapur. The major apparatus, chemicals, and equipment required are listed in Appendix A.

3.1.1.1 Dryer and weighing machine

Tray dryer of internal dimension 82×41 cm with digital temperature controller (manufactured by Sanjeev Scientific Udyog, India) with a motor fan and an exhaust on side of the dryer with drying range up to 200ºC. Weighing machine (Accuracy Grade: II class, Non-linearity:<0.01%, Operating temperature: 0-40ºC, <90% RH, Manufactured by prince scale industries)

3.1.2 Sample collection and preparation

Buffalo meat from the round of the hindquarter of the carcass was purchased from the local market of Dharan (VRC meat mart and organic foods). Buffalo meat slaughtered around 3 hours earlier was collected in the morning with a zipper plastic bag and transported immediately to the CDFT laboratory. Excess fat if the present was chopped off to prevent rancidity during storage. Meatloaves were then cleaned by removing bones fractions and excess sinew and stored in a refrigerator at 0°C to 2°C for 24 h before experiments for all jerky samples to be prepared. Meatloaves have frozen enough overnight to obtain enough consistency for cutting into strips. The meat was cut along the direction of its muscle fiber bundle into thin strips of 10-12 cm long, 2 cm wide, and 1 cm in thickness. Fat, Protein, Moisture content, ash content, pH of raw meat was analyzed.

3.2 Methodology

3.2.1 Experimental design

Mixture design is a specialized form of response surface methods (RSM). Experimenters chose a standard mixture design called a simplex lattice as this design is augmented with axial check blends

and the overall centroid. All components must be entered in the same units of measure and each run must sum to the same total.

A mixture design with an I-optimal design type was employed. The independent humectants mixture component for the experiments were salt, sugar, fructose, brown sugar, and citric acid. Response variables will be water activity a_w. The five components of the mixture design were coded as was shown in Table 3.1

Table 3.1 Five components of the mixture design along with its response to water activity

	Component 1	Component 2	Component 3	Component 4	Component 5	Response 1
Run	A: Salt	B: Sucrose	C: Brown Sugar	D: Maltodextrin	E: Citric acid	Water activity
	g	g	g	g	g	
1	1.80	2.74	1.29	3	1.17	
2	5.11	0	1.59	1.51	1.79	
3	6	0	0	3	1	
4	4	0	3	0	3	
5	6	1	3	0	0	
6	2.13	2.49	3	1.02	1.36	
7	2.56	0	2.06	2.97	2.40	
8	5.66	2.47	0	0	1.87	
9	2.74	1.99	0.05	2.23	3	
10	0.55	6	0	2.10	1.35	
11	3.38	2.37	1.41	0	2.84	
12	0	1	3	3	3	
13	3.11	4.33	0	2.56	0	
14	2.13	2.49	3	1.02	1.36	
15	0	5.25	2.66	2.09	0	
16	5.11	0	1.59	1.51	1.79	
17	2.59	6	1.21	0	0.2	
18	0	5.15	1.66	0.31	2.88	
19	5.17	2.55	0.64	1.64	0	
20	4	0	3	3	0	

3.2.2 Buffalo jerky preparation

Using design expert V11 software, 10% humectants were optimized and other ingredients (listed in Table 3.2) were made constant in every sample. The sample size was 100 g initially with different humectants infusion of 10%. A total of 20 runs were given by design expert version 13.0.5.0 (design type I-optimal coordinate exchange, randomized, no blocks, with quadratic design model). The range used was 0 to 6 g for salt and sucrose and 0 to 3 g for brown sugar, maltodextrin, and citric acid. Most of the experimental range for different humectants used and food additives were selected by taking the references from the DFTQC manual on food processing technology (DFTQC, 2010) and several homemade jerkies making recipes (Allen *et al.*, 2007; Anonymous, 2021; Buck *et al.*, 2020; FritoLay, 2020; Shumaker and Kendle, 2017).

Table 3.2 Total mixture of humectants infusions along with other food additives

Ingredients	Quantity
Humectants	10%
Soy sauce	12%
Monosodium glutamate (MSG)	1%
Nitrite curing salt	1%
Ascorbic acid	1%
Cinnamon powder	3%
Meat masala	2%
Vinegar	10 ml
Water	5 ml
Lean Meat	70%

The general experimentation procedure is presented in Fig. 3.2

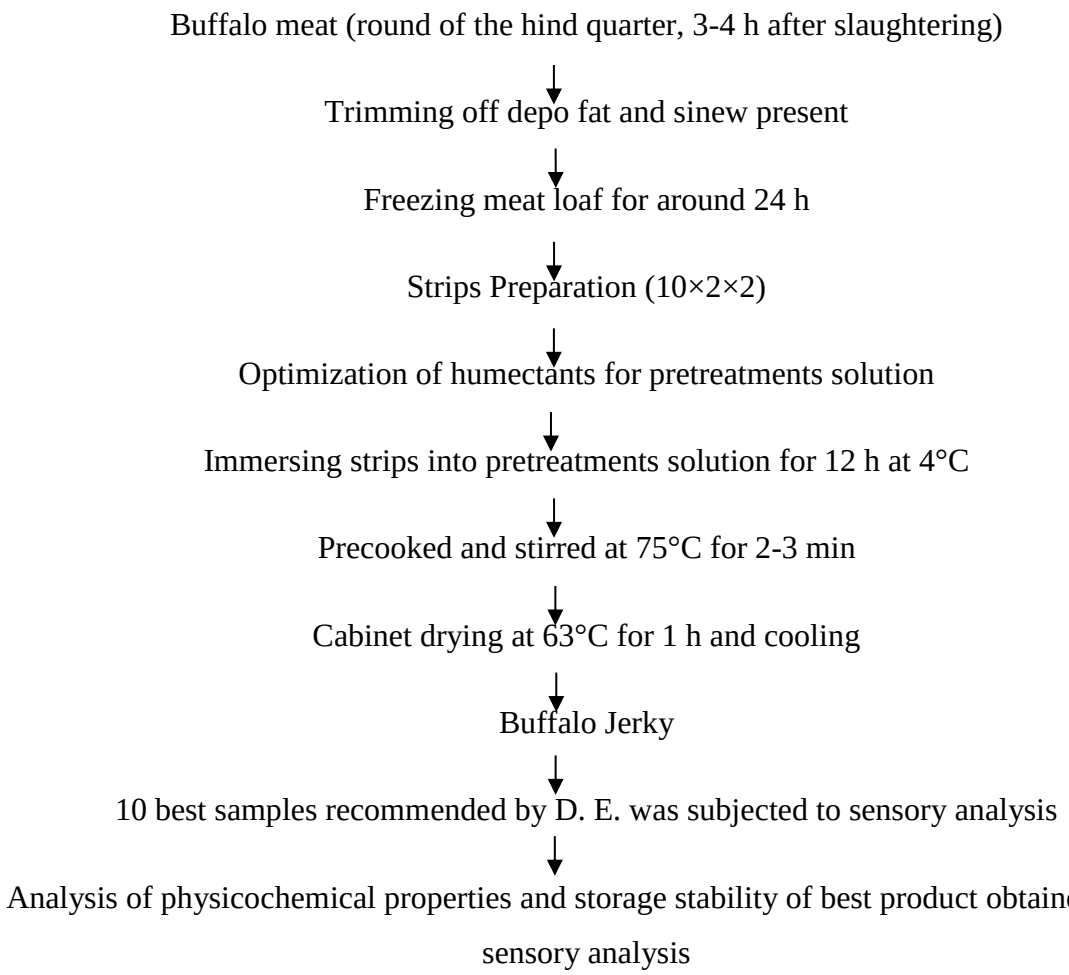

Fig. 3.2 Experimental flow diagram for sample pretreatments and optimization

Twenty samples were suggested by D. E. for optimization of different humectants viz., salt, sugar, brown sugar, maltodextrin, and citric acid were prepared. The meat strips were immersed in humectants solution along with food additives (Table 3.2) were stored for 12 h at 4ºC with regular turning and piling for even and homogenous distribution. Before drying, the pretreated meat strips were precooked and stirred at 75ºC for 2-3 min. After that, strips were then placed on a wire mesh tray in a single layer and placed into the drying chamber. The strips were cabinet dried at 63ºC for 1 h and allowed to cool inside the drier. Finally, packed into low-density polyethylene (LDPE) bags. Then, the samples were subjected to different sensory analyses, physiochemical and storage stability of the best product obtained from the sensory analysis.

3.3 Sensory evaluation

The sensory evaluation was performed according to the acceptance method. The best desirable formulations given by D E® were taken to sensory analysis shown in Table 3.1. Several panels consisting of semi-trained panelists were asked to evaluate the acceptability of the product based on the taste, texture, color, juiciness, and overall acceptability using the 9-point hedonic scale (Pimental *et al.*, 2016). Ten panelists (consisting of teachers and students of Central Campus of Technology and Central Department of Food Technology), were asked to circle on the respective description for their likes and dislikes, 9 for extremely liked, and 1 point to the extremely disliked sample. Coded samples were presented to all the panelists separately in a clean and silent environment between 1:00-3:00 pm under adequate light at room temperature. 10 samples labeled as A, B, C, D, E, F, G, H, I, & J were the samples of buffalo jerky suggested by D E.

3.4 Analytical procedure for physicochemical properties

3.4.1 Moisture content

Moisture content was determined by the hot-air oven method as per AOAC (2005). Approximately around 10 g of jerky sample was minced and spread over the petri-dish and placed in a hot air oven previously set at 103±2°C. the decline in the weight of the plate was noted every hour until the two consecutive weights becomes constant.

3.4.2 pH

The value of raw and dried meat samples was determined by using the method suggested by Subba (2010). Approximately around 10 g of minced meat sample was homogenized with 100ml distilled water. The pH value was calculated using a hand pH meter, a HANNA instrument made in Mauritius. The pH meter was calibrated with buffer 7.0 and 4.0.

3.4.3 Crude fat

The crude fat content of the samples was determined by the Soxhlet extraction method as described in AOAC (2005) using petroleum ether solvent. Approximately 10 g of sample was taken and packed in a thimble.

3.4.4 Crude protein

Crude Protein was determined by the Kjeldahl method as described in AOAC (2005). 5 g sample was digested with Conc. H_2SO_4 and nitrogen content estimated by the Kjeldahl method was multiplied by conversion factor 6.25 to compute protein content.

3.4.5 Ash content

The ash content was evaluated according to AOAC (2005). 10 g of fat-free sample was taken in a crucible and the samples were charred over a low Bunsen burner flame to volatilize as much organic matter. The crucible was then transferred to a muffle furnace set at 500 °C for 3-4 h.

3.4.6 Water activity

The water activity of the samples was determined by Instrument, AMTAST WA-60A water activity meter, USA (Anonymous, 2007). The sample was chopped with a knife finely. The sample was kept in a full, the device was placed upon it. After 5 min, the device beeps 5 times which indicated the completion of the analysis. Finally, water activity along its corresponding temperature was noted.

3.4.7 Peroxide value

Peroxide value was determined based on the method described by KC and Rai (2019). 5 g of ground meat was weighed accurately (by difference) in the Iodine flask. 25 ml of solvent was added. 1 ml of KI solution was added and allowed to stand for 1 min (with gentle shaking). 35 ml of distilled water was added, and a few drops of the starch indicator were added. The appearance of blue color on the addition of starch indicates the presence of free iodine. Liberated iodine was titrated with 0.01N or 0.1N sodium thiosulphate until the blue color vanished. Blank determination was carried out simultaneously. The peroxide value was calculated using the following equation,

$$P.V = \frac{N \times (\mathrm{Vs} - \mathrm{VB}) \times 1000}{\text{Wt. of sample (g)}}$$

Where,

N = normality of Sod-thiosulfate, V_s= Sod-thiosulfate consumed by sample (ml), and VB = Sodium-thiosulfate consumed by blank (ml).

3.4.8 Microbiological analysis

Buffalo jerky samples were taken for microbiological analysis in terms of Yeast and mold count as a part of the shelf-life study. The results were expressed as Log_{10} colony-forming units per gram (log_{10} cfu/g) of jerky.

3.4.8.1 Yeast and mold count.

Yeast and mold were determined using potato dextrose agar as described by Adhikari (2006). The jerky sample (10 g) was put in sterile glass jars containing 90 ml of sterile distilled water and mixed for approximately 2 min. Decimal serial dilutions were prepared in triplicates. Counting was done after incubating the plates at 35-37°C for 12-24 h.

3.5 Statistical analysis

The experiment was conducted in triplicates and all measurements were made in triplicates. Data obtained was statistically processed by one-way ANOVA using IBM SPSS 20 (IBM Corporation, Malborough, MA, USA). Tukey's HSD post hoc test was used to separate means for the case of significant difference. All other calculations were performed in Microsoft Office Excel 2016.

Part IV

Results and discussion

The present study was carried out to optimize buffalo jerky with a combination of different humectants and study the storage stability of the best sample with the control sample. The whole experimental procedure was divided into two parts. At first meat, strips were pretreated with an infusion of different humectants. The various combination of humectants was received from a design expert. The water activity (a_w) of each sample was optimized using RSM. The best desirable samples obtained from optimization were subjected to sensory evaluation. Finally, the best product formulated from the sensory evaluation was further subjected to a storage stability study. The main storage stability parameters were moisture content, water activity (a_w), peroxide value, and yeast & mold count for 30 days in 5 days intervals.

4.1 Proximate analysis of buffalo meat

The proximate and physiochemical properties of the raw buffalo meat used for the study was shown in Table 4.1

Table 4.1 Proximate composition of raw buffalo meat

Parameters	*Values (mean ±SD)
Moisture (wb)	74.73± 0.50
Protein (db)	80.85± 0.68
Fat (db)	12.06± 0.66
Ash content (db)	6.09± 0.88
pH	5.13± 0.10
Water activity (a_w)	0.98± 0.01 at 20.90 °C ± 0.50

*each value is the mean ± standard deviation of three replicates.

A similar type of results was reported by Lapitan *et al.* (2007), Naveena and Kirant (2014), Rao and Kowale (1991) and Thomas *et al.* (2008). There are many factors such as animal itself, including breed or breed crosses, age, body size, sex, age, and body weight during slaughtering, diet, management (rearing system, exercise, weather conditions, etc.), stress, preslaughter condition, and slaughtering practice that affects carcass status and meat quality (Toldrá, 2017).

4.2 Effect of individual humectants on water activity (a_w) of jerky

4.2.1 Effect of salt on the water activity (a_w) of jerky

The effect of salt on water activity (a_w) of the jerky is given in the box plot figure in Fig. 4.1.

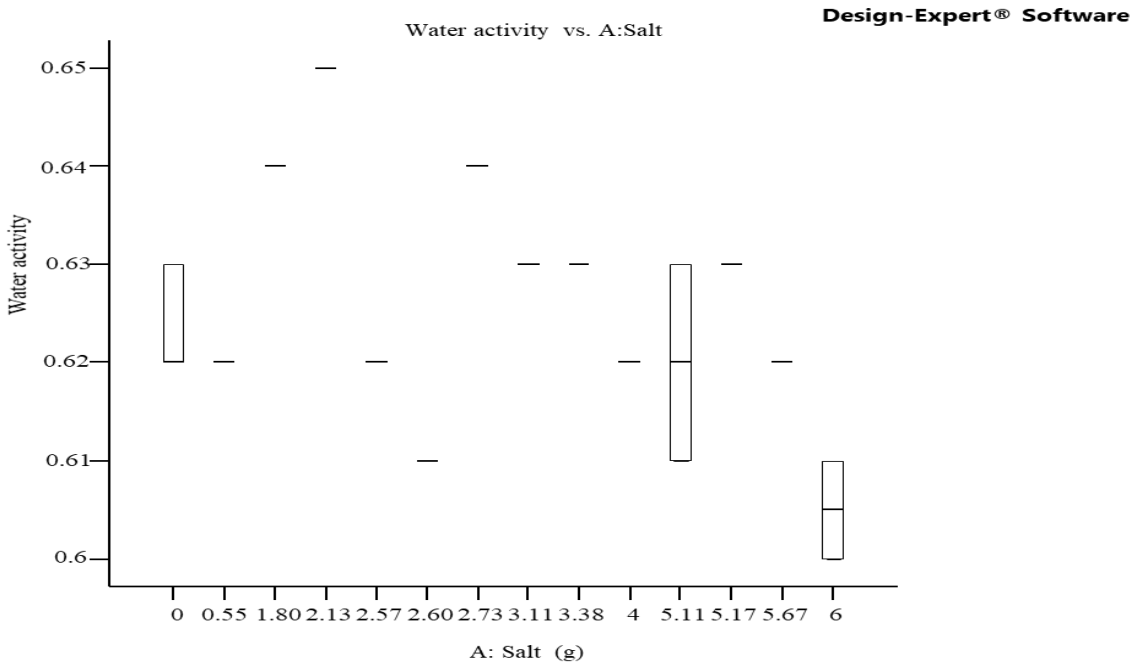

Fig. 4.1 Box plot diagram for water activity vs salt (g)

In the above Fig. 4.1, the box plot between water activity and salt (g) showed that there were 3 response regions in water activity vs salt viz. 0 g, 5.10559 g, and 6 g. At salt = 0 g, low water activity value lay at 0.62, 1st quartile water activity values lay at 0.62, median water activity value lay at 0.62, 2nd quartile water activity value lay at 0.63 and high-water activity value lay at 0.63. At salt = 5.10559 g, low water activity value lay at 0.61, 1st quartile water activity values lay at 0.61, median water activity value lay at 0.62, 2nd quartile water activity value lay at 0.63 and high-water activity value lay at 0.63. At salt = 6 g, low water activity value lay at 0.6, 1st quartile water activity values lay at 0.6, median water activity value lay at 0.61, 2nd quartile water activity value lay at 0.61 and high-water activity value lay at 0.61. At salt = 0 g, water activity ranges were from 0.62 to 0.63. At salt = 5.10559 g, water activity ranged from 0.61 to 0.63. At salt = 6 g, water activity ranged from 0.6 to 0.61. This indicated that water activity (a_w) deceased with an increase in salt concentration.

Yang *et al.* (2012) found that water activity of the 10% salt solution pretreated samples showed the lowest value for up to 8 h drying in pork jerky. Gu *et al.* (2021) also reported that high salt levels in restructured products result in lower a_w values and better preservative

effects. Ziegler *et al.* (1987) found a linear relationship between water activity and moisture content on some commercial sausages made by using salt and sucrose. From the above evidence, it was concluded that salt concentration decreases the water activity of jerky.

4.2.2 Effect of sucrose concentration on water activity (a_w) of jerky

The effect of sucrose on water activity (a_w) of the jerky is given in the box plot figure in Fig. 4.2.

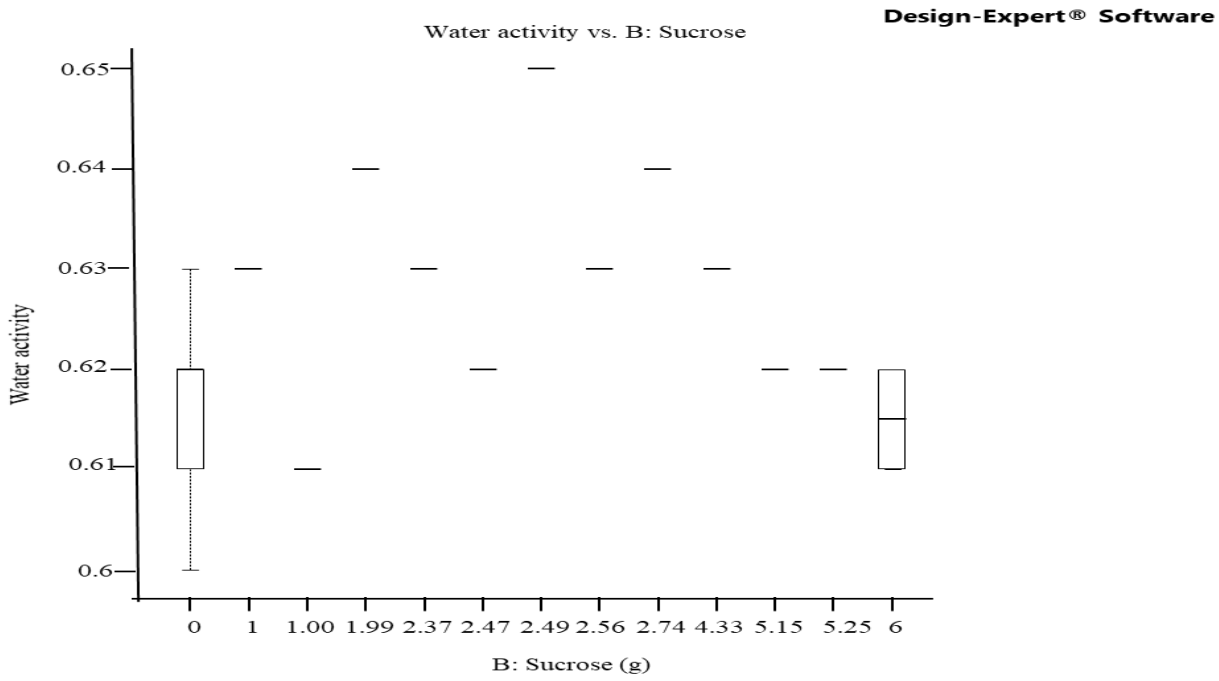

Fig. 4.2 Box plot diagram for water activity vs sucrose

In the above Fig. 4.2, the box plot between Water activity and Sucrose (g) showed that there were 2 response regions in water activity vs sucrose viz. 0 g and 6 g. At sucrose = 0 g, low water activity value lay at 0.6, 1st quartile water activity values lay at 0.61, median water activity value lies at 0.62, 2nd quartile water activity value lies at 0.62 and high-water activity value lies at 0.63. At sucrose = 6 g, low water activity value lay at 0.61, 1st quartile water activity values lay at 0.61, median water activity value lies at 0.615, 2nd quartile water activity value lies at 0.62 and high-water activity value lies at 0.62. At salt = 0 g, water activity ranges were from 0.60 to 0.63. At sucrose = 6 g, water activity ranged from 0.61 to 0.62. This indicated that water activity (a_w) prop. had no linear relationship between an increase or decrease in sucrose concentration of humectants. As there is no relationship between water activity and sucrose concentration at the level of 6 g which could be due to

the presence of other humectants brown sugar and fructose that act like sucrose in decreasing water activity of jerky type products.

Chen *et al.* (2002) concluded that sucrose concentration between 18 and 21% has no significant difference in water activity value and more than 21% sucrose concentration could only achieve water activity below 0.73 to have any effect on sucrose concentration. Our study also assisted the same conclusion as above, as water activity was high at 0 g concentration as well as with 6 g concentration value was 0.61 to 0.62. Chen *et al.* (2002) also stated that higher levels of sucrose concentrations in pork jerky had negative effects on protein stability and extractability of myosin heavy chain due to non-enzymatic browning.

4.2.3 Effect of brown sugar on water activity (a_w) of jerky

The effect of brown sugar on the water activity(a_w) of the jerky is given in the box plot figure in Fig 4.3.

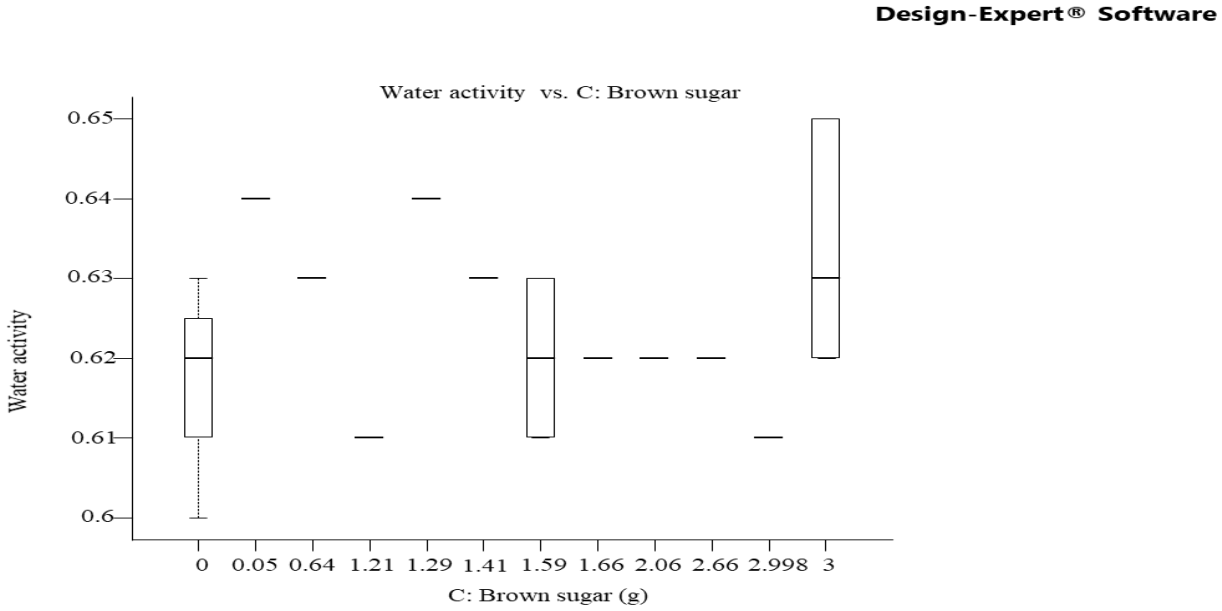

Fig. 4.3 Box plot diagram for water activity vs brown sugar

In the above Fig. 4.3, the box plot between Water activity (prop.) and brown sugar (g) showed that there were 3 response regions in water activity vs salt viz. 0 g, 1.59 g, and 3 g. At brown sugar = 0 g, low water activity value lay on 0.6, 1st quartile water activity values lay on 0.61, median water activity value lay on 0.62, 2nd quartile water activity value lay on 0.63 and high-water activity value lay on 0.63. At brown sugar = 1.59 g, low water activity value lied on 0.61, 1st quartile water activity values lay on 0.61, median water activity value lay on 0.62, 2nd quartile water activity value lay on 0.63 and high-water activity value lay on

0.63. At brown sugar = 3 g, low water activity value lied on 0.62, 1st quartile water activity values lay on 0.62, median water activity value lay on 0.63, 2nd quartile water activity value lay on 0.65 and high-water activity value lay on 0.65. At brown sugar = 0 g, water activity ranges were from 0.61 to 0.63. At brown sugar = 1.59 g, water activity ranged from 0.61 to 0.63. At brown sugar = 3 g, water activity ranged from 0.62 to 0.65. This indicated that water activity (a_w) prop. had no linear relationship between increased or decreased brown sugar concentration.

McCabe (2019) reported that brown sugar is a mixture of white sugar and molasses, which is responsible for color development and aids in slightly increasing the nutritive value of the product. As most of the properties of sucrose and brown sugar were similar. As 0 to 3 g was incorporated in the experiment for its color development purpose. FritoLay (2020) reported that brown sugar is produced by adding molasses to refined white sugar (sucrose) which could be used interchangeably with sucrose in the baking jerky.

4.2.4 Effect of maltodextrin on water activity (a_w) of jerky

The effect of maltodextrin on water activity(a_w) of the jerky is given in the box plot figure in Fig. 4.4.

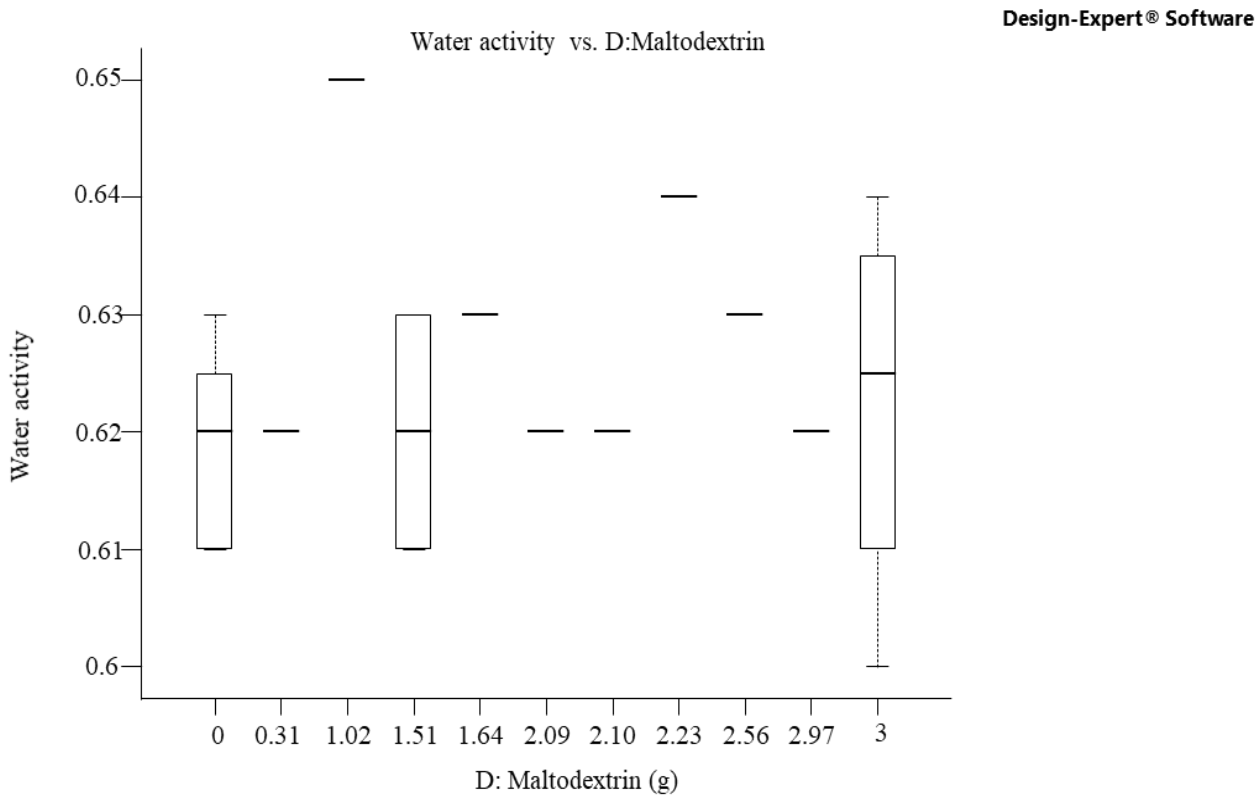

Fig. 4.4 Box plot diagram for water activity vs maltodextrin (g)

In the above Fig. 4.4, the box plot between Water activity (prop.) and maltodextrin (g) showed that there were 3 response regions in water activity vs salt viz. 0g, 1.51 g, and 3 g. At maltodextrin = 0 g, low water activity value lied on 0.61, 1st quartile water activity values lay on 0.61, median water activity value lay on 0.62, 2nd quartile water activity value lay on 0.625 and high-water activity value lay on 0.63. At maltodextrin = 1.51 g, low water activity value lied at 0.61, 1st quartile water activity values lay at 0.61, median water activity value lay at 0.62, 2nd quartile water activity value lay at 0.63 and high-water activity value lied on 0.63. At maltodextrin = 3 g, low water activity value lied on 0.60, 1st quartile water activity values lay on 0.61, median water activity value lay on 0.625, 2nd quartile water activity value lay on 0.635 and high-water activity value lay on 0.64. At salt = 0 g, water activity ranges were from 0.61 to 0.63. At maltodextrin = 1.51 g, water activity ranged from 0.61 to 0.63. At maltodextrin = 3 g, water activity ranged from 0.60 to 0.64. This indicated that water activity (a_w) prop. had no linear relationship between increased or decreased maltodextrin concentration.

Shaefer (2018) reported that maltodextrin is generally used as a filler or thickener or to raise the volume in processed foods like jerky. In our experiment, we had used maltodextrin in the range of 0 to 3 g concentration which didn't have any effect on the water activity of jerky. FritoLay (2020) stated that maltodextrin was a polysaccharide usually produced from corn, tapioca, or wheat starch that aids as a thickener in the jerky products in low quantity.

4.2.5 Effect of citric acid on water activity (a_w) of jerky

The effect of citric acid on water activity(a_w) of the jerky is given in the box plot figure in Fig. 4.5.

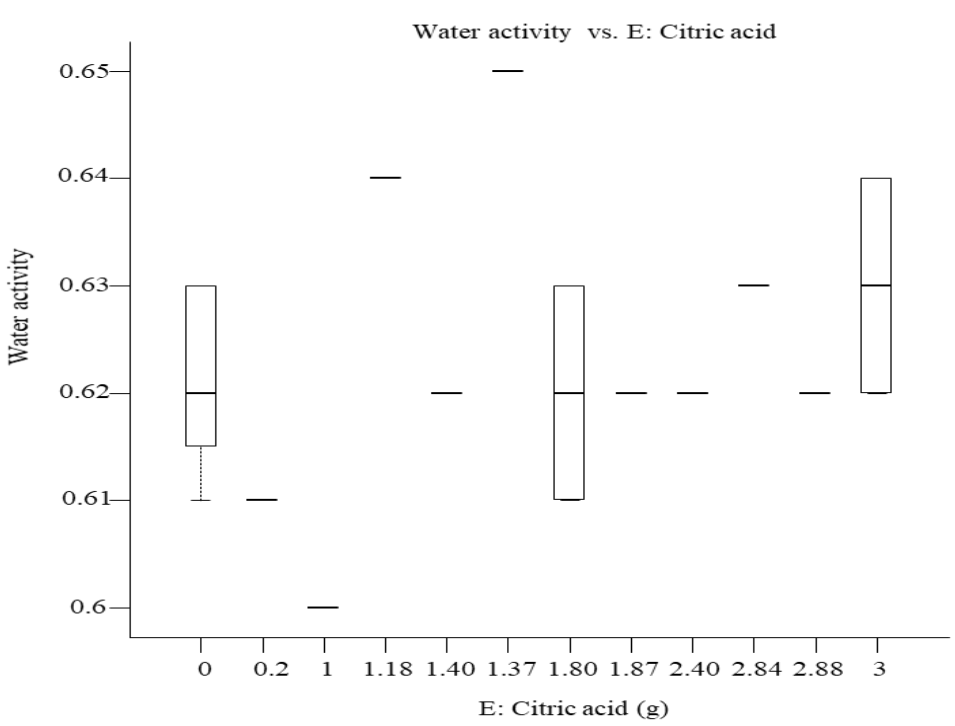

Fig. 4.5 Box plot diagram for water activity vs citric acid (g)

In Fig. 4.5, the box plot between Water activity and citric acid (g) showed that there were 3 response regions in water activity vs salt viz. 0 g, 1.80 g, and 3 g. At citric acid = 0 g, low water activity value lay on 0.61, 1st quartile water activity values lay on 0.615, median water activity value lay on 0.62, 2nd quartile water activity value lay on 0.63 and high-water activity value lay on 0.63. At citric acid = 1.80 g, low water activity value lay at 0.61, 1st quartile water activity values lay at 0.61, median water activity value lay at 0.62, 2nd quartile water activity value lay at 0.63, and high-water activity value lay on 0.63. At citric acid = 3 g, low water activity value lay on 0.62, 1st quartile water activity values lay on 0.62, median water activity value lay on 0.63, 2nd quartile water activity value lay on 0.64 and high-water activity value lay on 0.64. At citric acid = 0 g, water activity ranges were from 0.61 to 0.63. At citric acid = 1.80 g, water activity ranged from 0.61 to 0.63. At citric acid = 3 g, water activity ranged from 0.62 to 0.64. This indicated that water activity (a_w) prop. had no linear relationship between increased or decreased citric acid concentration.

Belitz *et al.* (2009) reported that the main role of citric acid in meat and meat product is to enhance flavor, adjust the pH of the meat, and aid in the inversion of sucrose.

4.3 Effect of a mixture of humectants on the water activity (a_w) of the jerky

According to Buck *et al.* (2020); Shumaker and Kendle (2017), the drying temperature for preparation is 63.8°C for 1 h. When the meat strips were fragile enough after 1 h of drying, drying was terminated which was 64°C for 1 h. Thus, humectants infused meat strips were dried for 1 h and the water activity (a_w) of the strips was computed by a Paw kit water activity meter. The water activity of the jerky was found to be influenced by the pretreatment of the 10% humectants mixture applied before drying.

The final water activity (a_w) values of the jerky samples varied from 0.60 to 0.65 at 20ºC. Appendix I shows the coefficient of the model and other statistical attributes for the final product response. The response water activity (a_w) was described by the best fitted quadratic model as suggested by fit statistics having higher R^2. The regression model fitted to experimental results of water activity (a_w) showed that the model F-value of 4.32 was significant ($p<0.0001$) and the lack of fit test was not significant ($p>0.05$). The model F-value of 4.73 indicated that the model was significant and there was only a 4.80% chance that F-value could increase due to noise. P-values less than 0.0500 indicated model terms were significant and value less than 0.1000 indicate the model terms are not significant. Lack of fit F-value of 0.11 implies the lack of fit is not significant relative to the pure error. There was a 94.95% chance that a lack of fit F-value could increase due to noise.

The fit of the model was also expressed by the coefficient of determination R^2, which was found to be 0.9298, indicating that 92.28% of the variability of the response could be explained by the model. The Adjusted R^2 was 0.7333 and the predicted R^2 -0.6690 is insensible understanding with the Adjusted R^2. Adequate precision (8.5179) showed adequate power. A negative Predicted R^2 implies that the overall mean was a better predictor of the response than the current model. It was suggested that in some cases a higher-order model could also predict better. Adeq. Precision values were 8.5179 which measures the signal-to-noise ratio. A ratio greater than 4 was desirable and its value obtained indicates an adequate signal that was used to navigate the design space.

The final equation in terms of coded components

For the effect of variables on the response (water activity) of the jerky samples, model eq. 4.1 was obtained. The quadratic model fitted for water activity (a_w) with coded values of the variables is presented in eq. 4.1

Water activity (a_w) = 0.5337×A + 0.5128×B + 0.6960×C + 0.3162×D + 0.4737×E + 0.3532×AB + 0.0387×AC + 0.556×AD + 0.3174×AE + 0.0805×BC + 0.6373×BD + 0.4298×BE + 0.3422×CD + 0.1275×CE + 0.6168×DE ……. (4.1)

Where, A, B, C, D, & E are coded values for salt (g), sucrose (g), brown sugar (g), maltodextrin (g), citric acid (g) respectively during pretreatments of the jerky with a combination of humectants.

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the mixture components are coded as +1 and the low levels are coded as 0. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

The final equation in terms of actual components

For the effect of variables on the response (water activity) of the jerky samples, model eq. 4.2 was obtained. The quadratic model fitted for water activity (a_w) with actual values of the variables is presented in eq. 4.2

Water activity (a_w)= 0.0533659×A + 0.0512779×B+0.0695953×C + 0.0316233×D + 0.0473716×E + 0.00353181×AB + 0.000386645×AC + 0.0055603×AD + 0.00317364×AE + 0.000805201×BC + 0.00637326×BD + 0.00429754×BE + 0.003422×CD + 0.00127525×CE + 0.00616762×DE ……. (4.2)

Where, A, B, C, D, & E are coded values for salt (g), sucrose (g), brown sugar (g), maltodextrin (g), citric acid (g) respectively during pretreatments of the jerky with a combination of humectants.

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space. The positive coefficient value of the linear mixture of humectants indicates that an increase in the combination of humectants increases the water activity (a_w) of the jerky samples. ANOVA table also indicated that a linear mixture of humectants has significant effects on the final water activity of the jerky after drying at 63°C

for 1 h. Fig. 4.6 shows the effect of a linear mixture of humectants on final water activity (a_w).

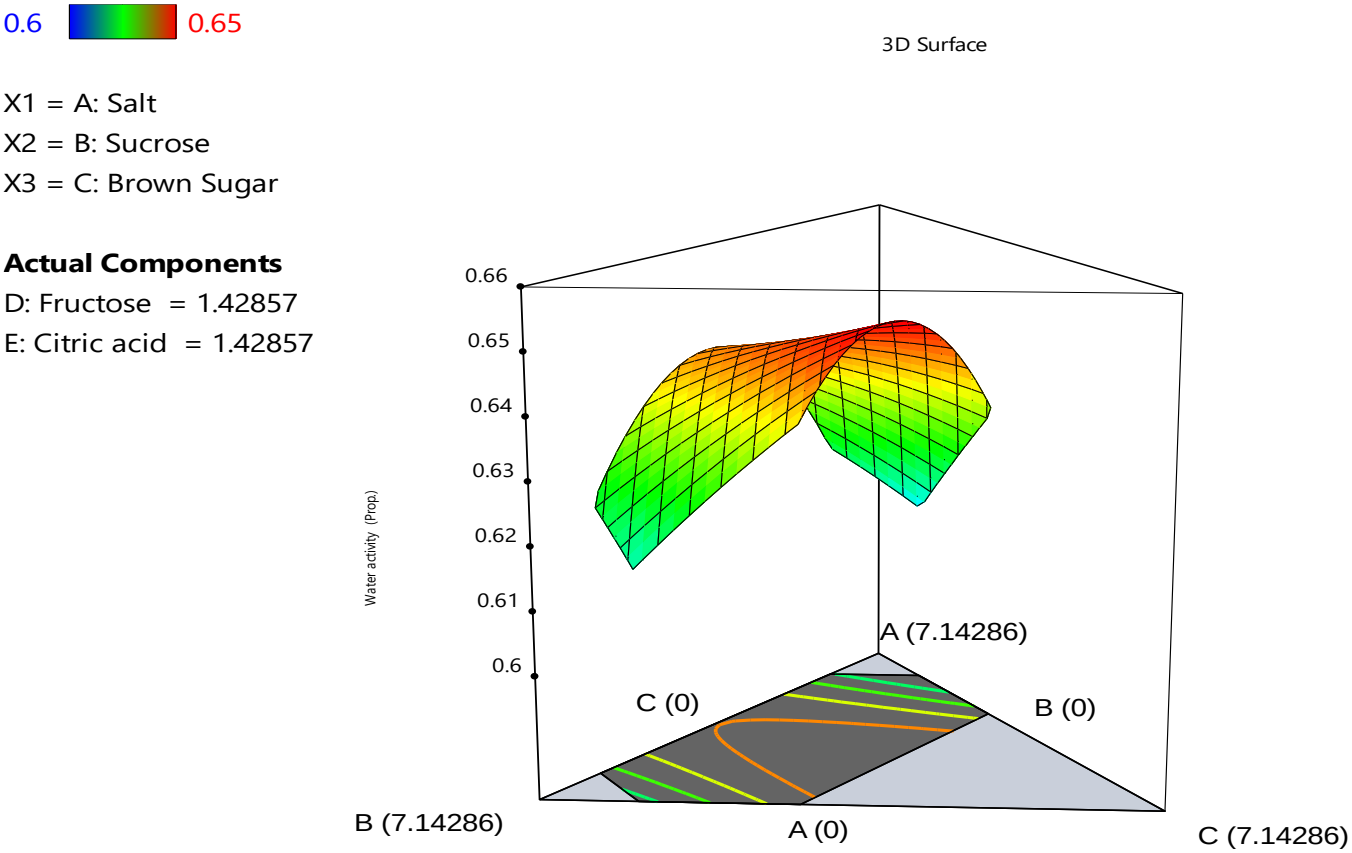

Fig. 4.6 3-D Response surface curve for the interaction of linear mixture of different humectants on water activity (a_w)

The water activity of jerky addressed to different combinations of humectants did not differ greatly. Nummer *et al.* (2004) reviewed that in many studies, the addition of salt, sugar, and sodium nitrite can enhance pathogens' destruction in meat jerky preparation, but combinations of different humectants were more effective than single humectant to achieve necessary lethalities. Albright *et al.* (2002) noticed that marinade recipes were modified with citric acids for their effectiveness in enhancing the destruction of pathogens during jerky. Sodium chloride (NaCl) is an essential ingredient that provides different features and has been used as a preservative to prevent spoilage and to increase the shelf life of processed meat, in addition to providing characteristic flavor, color and tenderness. The major function of salt is to bind water molecules and to reduce the water activity (aw) of the product causing the dehydrating effect. A high level of salinity may impair the conditions under which pathogens can survive (Mishra *et al.*, 2017). Also, Fructose and brown sugar aid in the same preservative action as salt and sugar i.e. reducing the water activity of the meat and retarding

the growth of microorganisms. Wongwiwat and Wattanachant (2015) reported that chicken jerky made sucrose and mixed sugar stored in 33% RH condition had the highest quality changes in textural and rancidity features that were unacceptable in sensory evaluation after 7 days of storage.

4.4 Optimization of best formulations of humectants based on the desirability

The samples of buffalo jerky were prepared with a linear mixture of different humectants as shown in Table 3.2. Samples were coded as 1 to 20 for each run. The maximum and minimum values for a linear mixture of humectants were obtained from a laboratory trial study. The number of different humectants i.e. salt, sucrose, brown sugar, fructose, and citric acid ranges from 0 to 6 g, 0 to 6 g, 0 to 6 g, 0 to 3 g, and 0 to 3 g respectively. According to literature, the amount of acetic acid used for the curing was usually in the range of 10-100 ml of meat. Hence, 10 ml acetic acid per 100 g of meat was used for curing purposes. Nitrite curing salt, ascorbic acid, and MSG were applied at 1% on the infusion of humectants. The combination of citric acid, ascorbic acid, and nitrite curing salt aids in color development and retards microorganisms' growth (Belitz *et al.*, 2009). The range and amount of mixture of humectants and other food additives used were suggested by DFTQC (2010) and a packet of beef jerky marketed by Frito-Lay, INC, USA, which is also by many studies on IMM products around the world (FritoLay, 2020).

The effect of a mixture of humectants on the preparation of jerky on water activity (a_w) of the jerky samples was observed. A second-order polynomial model for the dependent variable was tested to fit the experimental data and model validation was measured by using the coefficient of determination (R^2), Adj. R^2, model F-value, Adeq. Precision and lack of fit F-value. Numerical and graphical optimization was done with the help of a design expert to find the best desirable combinations of the factors. Computed effects of factors on responses were shown in Table 4.3. Constraints of setting goals for optimization was shown in Table 4.4.

These experimental plans were to create a suitable combination of humectants for the pretreatments of raw meat strips to develop buffalo jerky with water activity between 0.60 to 0.65 with the help of D E®. After analyzing the data of the initial actual design, 10 samples were given by D E® among the best formulations of the linear mixture of humectants with its desirability and predicted value which is given in Table 4.3.

Table 4.3 Responses for a mixture of humectants concentrations in the treatment solution

Run	A: Salt g	B: Sucrose g	C: Brown sugar g	D: Fructose g	Citric acid g	Predicted water activity (a_w) Prop.
1	1.80	2.74	1.29	3	1.17	0.64
2	5.10	0	1.59	1.512	1.79	0.63
3	6	0	0	3	1	0.6
4	4	0	3	0	3	0.62
5	6	1	3	0	0	0.61
6	2.13	2.49	3	1.02	1.36	0.65
7	2.56	0	2.06	2.97	2.40	0.62
8	5.66	2.47	0	0	1.87	0.62
9	2.74	1.99	0.05	2.23	3	0.64
10	0.55	6	0	2.10	1.35	0.62
11	3.38	2.37	1.41	0	2.84	0.63
12	0	1	3	3	3	0.63
13	3.11	4.33	0	2.56	0	0.63
14	2.12	2.49	3	1.02	1.37	0.65
15	0	5.25	2.66	2.09	0	0.62
16	5.10	0	1.59	1.51	1.79	0.61
17	2.59	6	1.21	0	0.2	0.61
18	0	5.15	1.66	0.31	2.88	0.62
19	5.17	2.56	0.64	1.64	0	0.63
20	4	0	3	3	0	0.62

Table 4.4 Response optimization constraints for a mixture of humectants concentration in the treatment solution

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Salt	is in range	0	6	1	1	3
B: Sucrose	is in range	0	6	1	1	3
C: Brown Sugar	is in range	0	3	1	1	3
D: Fructose	is in range	0	3	1	1	3
E: Citric acid	is in range	0	3	1	1	3
Water activity	is in range	0.6	0.64	1	1	3
StdErr (Water activity)	none	0.00442036	0.00675216	1	1	3

The responses predicted by D E® based on the goal-setting Table 4.4, different optimized constraints obtained were shown in Table 4.5

Table 4.5 Different optimized linear mixtures predicted by Design Expert®

Samples	Salt	Sucrose	Brown Sugar	Maltodextrin	Citric acid	Water activity	StdErr (Wate activity)	Desirability
A	0.55	2.78	1.58	2.34	2.76	0.64	0.005	1.000
B	0.00	1.00	3.00	3.00	3.00	0.63	0.006	1.000
C	1.80	2.74	1.29	3.00	1.17	0.63	0.006	1.000
D	3.38	2.37	1.41	0.00	2.84	0.63	0.005	1.000
E	5.11	0.00	1.59	1.51	1.80	0.62	0.004	1.000
F	4.00	0.00	3.00	0.00	3.00	0.62	0.006	1.000
G	0.00	5.25	2.66	2.09	0.00	0.62	0.007	1.000
H	5.66	2.47	0.00	0.00	1.87	0.62	0.007	1.000
I	2.59	6.00	1.21	0.00	0.20	0.61	0.007	1.000
J	4.00	2.00	2.00	1.50	0.50	0.62	0.006	1.000

10 solution with 1 desirability was selected as referred by the software. The best humectants optimized for linear mixture with its predicted water activity (a_w) is given in Table 4.5 with its predicted water activity (a_w).

4.5 Verification of the results

The desirability of the model developed for predicting the optimum response values was computed using the recommended optimum conditions of the variables and was also used to confirm the experimental and predicted values of the response. For this meat, strips were pretreated with a recommended combination of different humectants following the method described in Fig 3.2. The result for the expected responses and experimental responses following the optimized condition was compared with deviation (%), presented in Table 4.6.

Table 4.6 Predicted and actual values of the response at the optimized condition

Sample	Predicted a_w	*Actual a_w	Deviation (%)
A	0.64	0.65 ± 0.021	1.56%
B	0.63	0.63± 0.017	0%
C	0.64	0.63± 0.015	1.56%
D	0.63	0.63± 0.029	0%
E	0.62	0.62± 0.015	0%
F	0.62	0.62± 0.021	0%
G	0.62	0.62± 0.010	0%
H	0.62	0.62± 0.015	0%
I	0.61	0.61± 0.012	0%
J	0.62	0.62± 0.000	0%

*Real values are mean ± SD of triplicate determination.

There was only very little variation (maximum of 1.5625% in water activity) in responses as predicted by the model and on actual conditions. Water activity was only slightly (<1.56%) deviated from the predicted values by the model. Due to less deviation of the values, the 10 formulations obtained through D E® was subjected to sensory analysis, and the best product formulation was identified for further storage stability of the jerky.

4.6 Sensory analysis of the jerky prepared

For the sensory analysis of the 10 best formulations recommended by D E®, 10 semi-trained panelists were taken. They were teaching staff and students of CDFT, M. tech (Food Technology). The panelist was selected based on their ability to recognize the sensory parameters and they were well known about the characteristics of jerky and beef jerky was taken as a reference.

In a 9-point hedonic rating (Appendix A-1), panelists were asked to score the samples based on their perception of likes and dislikes of the samples. Major sensory parameters for the dried meat are; appearance/color, flavor, taste, texture, and overall acceptability. These parameters were compared between 10 samples; A, B, C, D, E, F, G, H, I, and J. Values on the top of the bars bearing similar superscripts are not significantly different at a 5% level of significance. Vertical error bars represent the ± standard deviation of scores given by

panelists. The mean sensory value for each sensory parameter i.e. color, taste, texture, juiciness, and overall acceptability is given in Fig. 4.7, 4.8, 4.9,4.10, and 4.11 respectively.

4.6.1 Color

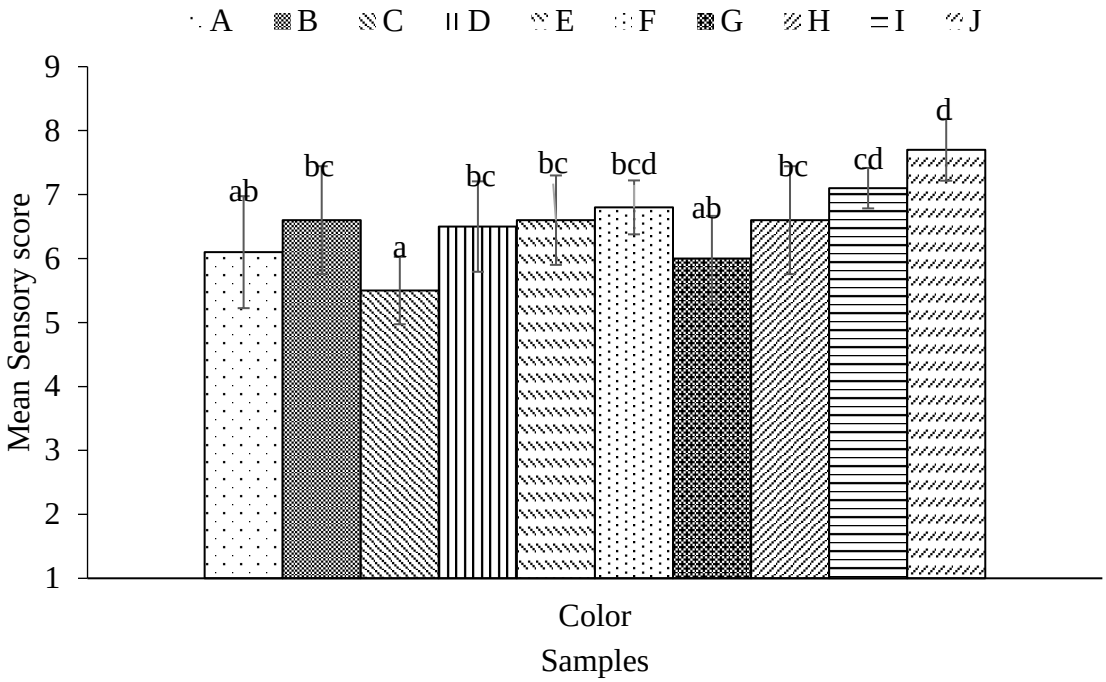

Fig. 4.7 Average mean sensory score for different jerky samples in terms of color

The mean sensory scores for color were 6.10, 6.60, 5.50, 6.50, 6.60, 6.80, 6.00, 6.60, and 7.10 for the 10 jerky samples. There were 10 panelists for color, among them 94% of respondents noticed that dark brown color was best than 6% with light brown color. From the hedonic sensory analysis and preference test, it was seen that significant numbers of panelists liked to sample J more than other samples of jerky. Sample J was preferred because of its dark brown color. The scores for the color of the jerky samples ranged from 5.50 (sample C) to 7.10 (sample J) which was significantly higher. As samples A, C, and G, samples B, D, E, F, and H, and samples F, I, and J were significantly similar ($p>0.05$) while others were significantly different ($p<0.05$) in terms of the mean score for different jerky samples in terms of color.

Allen *et al.* (2007) recommended that USDA FSIS meat and poultry jerky compliance guidelines recommended liquid immersion of humectants mixtures pasteurization step before drying. Shumaker and Kendle (2017) also recommended that a mixture of humectants

with salt, sugar, brown sugar, maltodextrin, and citric acid in the recipe of beef jerky making process which aids in color development of dark brown, adjusting of pH, and ultimately reducing water activity of the jerky. Sample F, I, and J were preferred by most of the panelists due to their equal proportion of humectants mixture with better dark brown color as most of the jerky prepared worldwide.

4.6.2 Taste

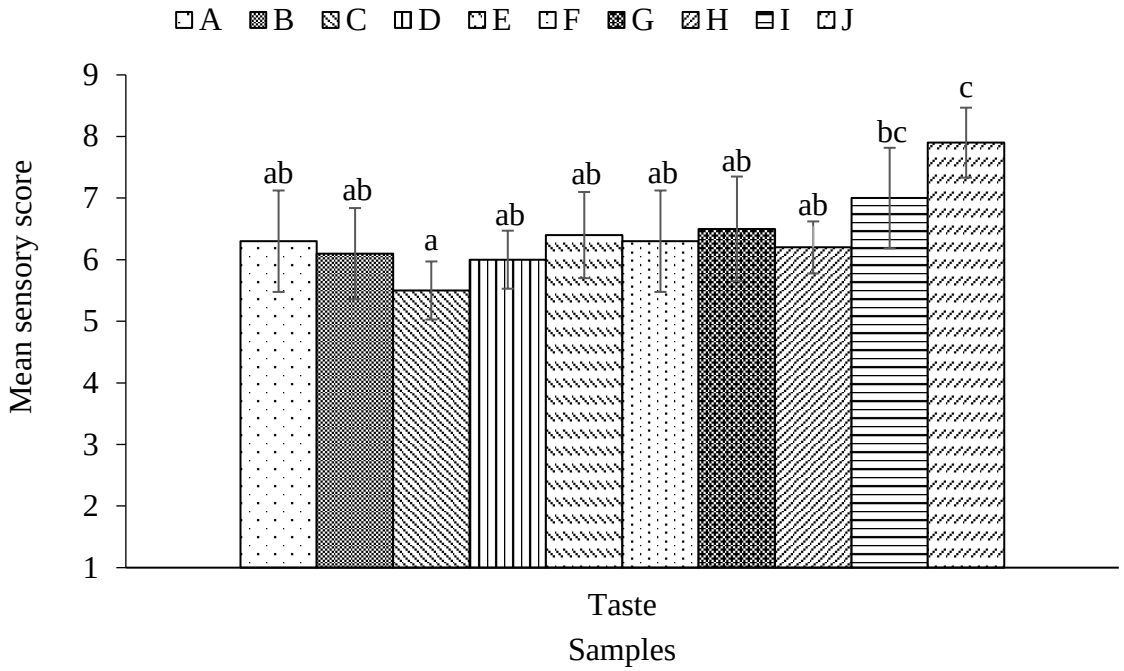

Fig. 4.8 Average mean sensory score for different jerky samples in terms of taste

The mean sensory scores for taste were 6.30, 6.10, 5.50, 6.00, 6.40, 6.30, 6.50, 6.20, 7.00, and 7.90 for the 10 jerky samples (Fig 4.8). There was no significant difference between the mean sensory score for the taste. There were 10 panelists for color, among them, 99% of respondents noticed that a salty and sour taste was superior to 1% with a sweet taste. From the hedonic sensory analysis and preference test, it was seen that significant numbers of panelists liked to sample J more than other samples of jerky. Sample J was preferred because of its equal proportion of every humectant. The scores for the color of the jerky samples ranged from 5.50 (sample C) to 7.90 (sample J) which was significantly higher. As most of the samples were significant to each other samples of jerky. Among them, Samples H, I, & J were significantly similar ($p>0.05$) while others were significantly different ($p<0.05$).

Most of the research suggested that sugar is an important ingredient in the preparation of jerky worldwide, which is generally sweet. Chen *et al.* (2002) reported that 21% sugar-incorporated pork jerky was superior in terms of hardness, sweetness, and overall acceptability of the jerky. Most American, Chinese, Japanese, and Korean people are fond of sweet tastes but in the context of Nepal, most of the population like salty and sour tastes in comparison to sweet tastes in processed meat.

4.6.3 Texture

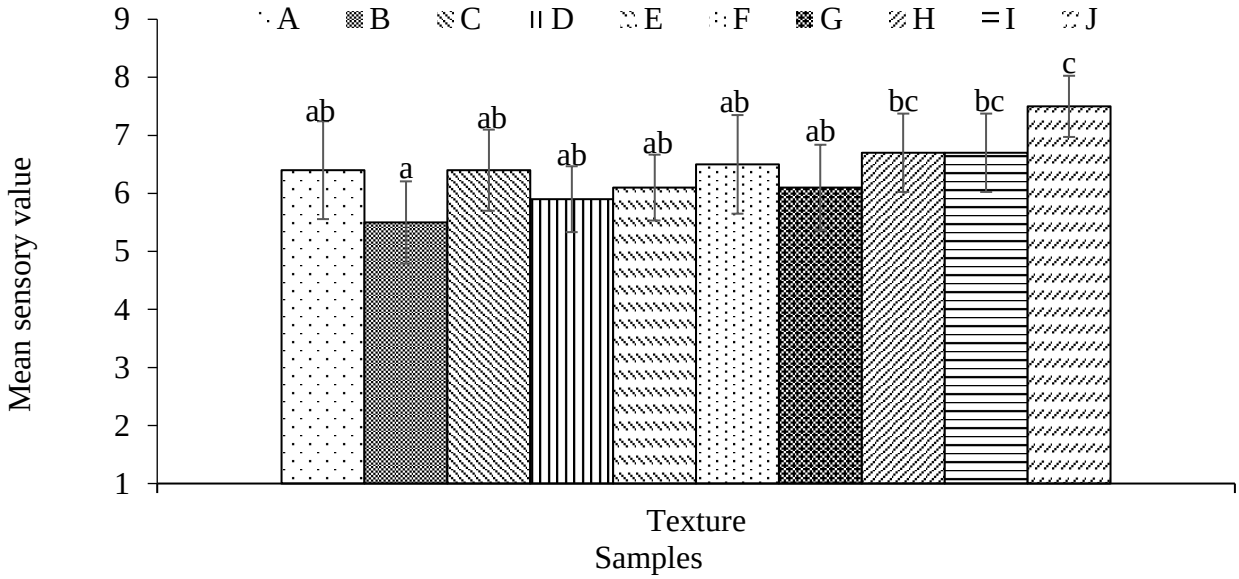

Fig. 4.9 Average mean sensory score for different jerky samples in terms of texture

The mean sensory scores for texture were 6.40, 5.50, 6.40, 5.90, 6.10, 6.50, 6.10, 6.70, 6.70, and 7.50 for the 10 jerky samples (Fig 4.9). There was no significant difference between the mean sensory score for the texture. There were 10 panelists for texture, among them 100% of respondents noticed that moist and tender jerky. From the hedonic sensory analysis and preference test, it was seen that significant numbers of panelists liked to sample J more than other samples of jerky. Sample J was preferred because of its equal proportion of every humectant and softer in texture than other samples of jerky. The scores for the texture of the jerky samples ranged from 5.50 (sample B) to 7.50 (sample J) which was significantly higher. As most of the samples were significant to each other samples of jerky. Among them, Samples A, B, C, D, E, F, & G were significantly different ($p<0.05$) from J, Sample B was significantly different ($p<0.05$) than samples H, I & J. Samples H, I, and J were significantly similar ($p>0.05$) while others were significantly different ($p<0.05$).

As each sample was not significantly different in terms of texture as water activity ranges from 0.62 to 0.65. Jang *et al.* (2014) reported that the addition of humectants greater than 5% increases the shear force of jerky which is responsible for the increase in the tenderness of jerky. Jamhari and Lakshmiwati (2018) reported that the sugarcane level of 20% was superior in tenderness (kg/cm^2) of ground beef *dendeng*. As in our present study, combinations of humectants used was 10% in every sample. We could conclude that there was no significant difference in texture with the samples of jerky.

4.6.4 Juiciness

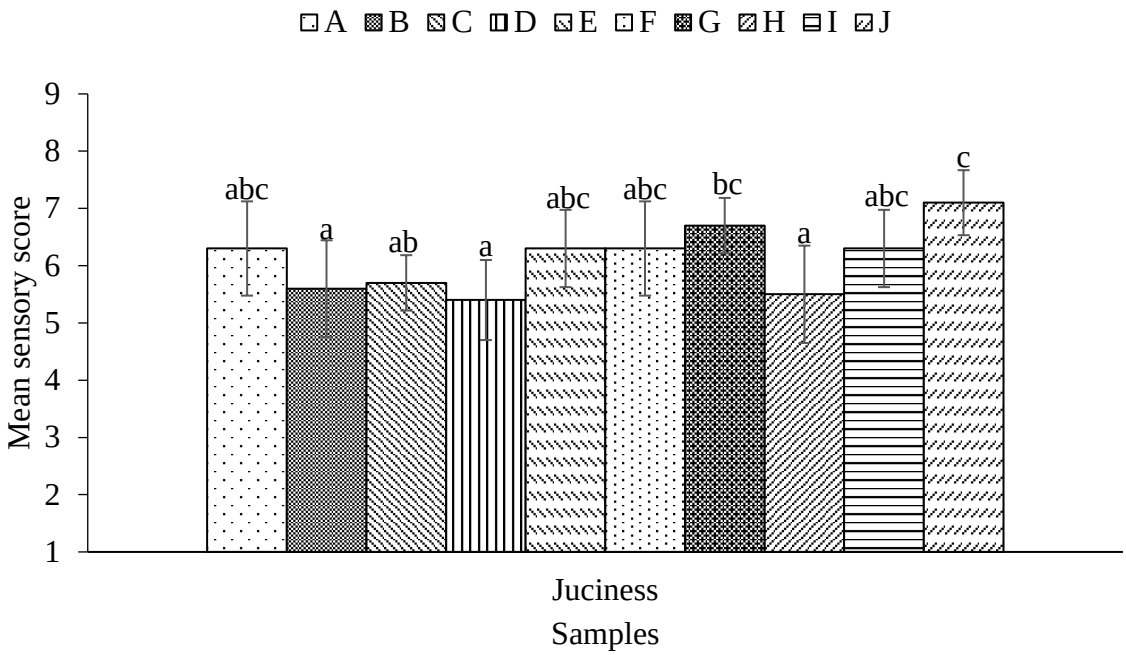

Fig. 4.10 Average mean sensory score for different jerky samples in terms of juiciness

The mean sensory scores for juiciness were 6.30, 5.60, 5.70, 5.40, 6.30, 6.30, 6.70, 5.50, 6.30, and 7.10 for the 10 jerky samples (Fig. 4.10). There was no significant difference between the mean sensory score for the texture. There were 10 panelists for texture, among them 100% of respondents noticed that moist and tender jerky. From the hedonic sensory analysis and preference test, it was seen that significant numbers of panelists liked to sample J more than other samples of jerky. The scores for the juiciness of the jerky samples ranged from 5.40 (sample D) to 7.10 (sample J) which was significantly higher. As most of the samples were not significant to each other samples of jerky. Among them, Samples A, E, F, G, I & J were significantly similar ($p>0.05$) while others were significantly different ($p<0.05$). D-G. Nam *et al.* (2017b) reported that brown sugar was superior in enhancing

chewiness in chicken breast jerky. As it was clear that juiciness perception was less in sample H, whereas there was no significant difference between the samples.

4.6.5 Overall acceptability

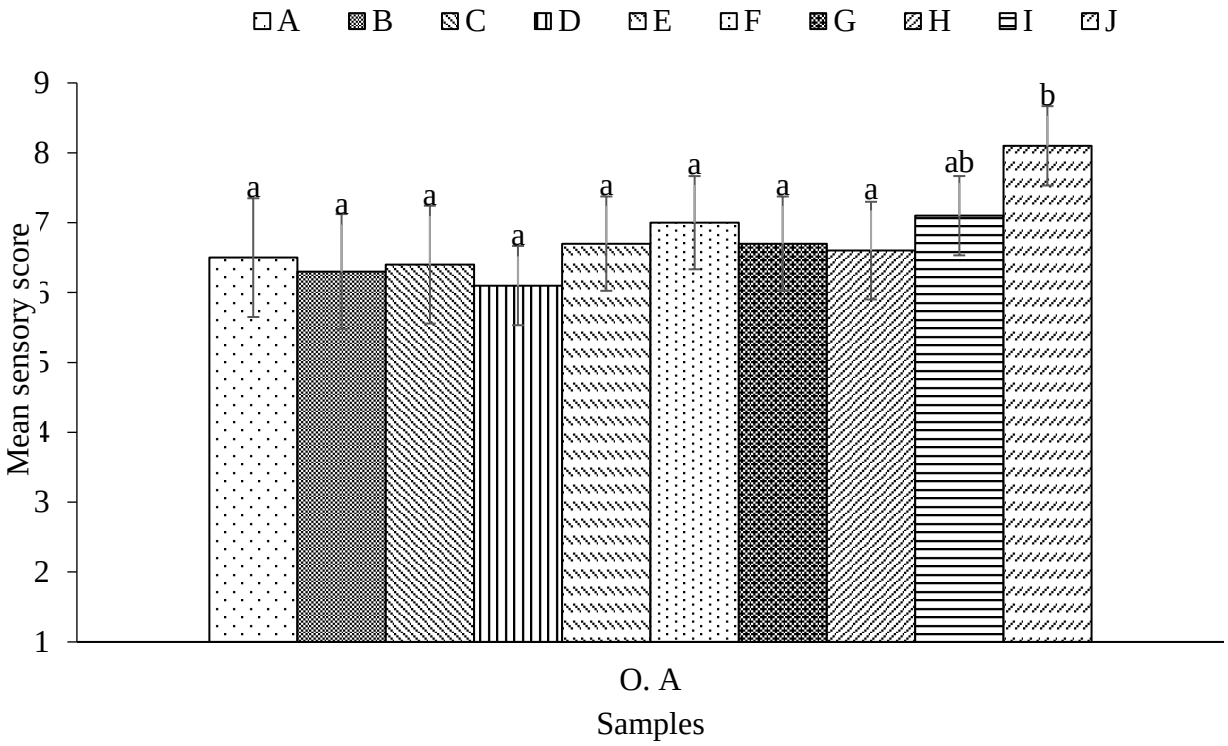

Fig. 4.11 Average mean sensory score for different jerky samples in terms of O. A.

The mean sensory scores for overall acceptability values were 6.50, 6.30, 6.40, 6.10, 6.70, 7.00, 6.70, 6.60, 7.10, and 8.10 for the 10 jerky samples (Fig. 4.11). There was a significant difference between the mean sensory score for the texture. From the hedonic sensory analysis and preference test, it was seen that significant numbers of panelists liked to sample J more than other samples of jerky. Sample J was preferred because of its equal proportion of every humectant and softer in texture than other samples of jerky. The taste was also superior with a combination of salty, sour, and sweet tastes. The scores for overall acceptability of the jerky samples ranged from 5.40 (sample D) to 8.10 (sample J) which was significantly higher. As most of the samples A, B, C, D, E, F, G & H are significantly different($p<0.05$) from sample J whereas Sample I and Sample J were not significantly different ($p>0.05$).

4.7 Physiochemical properties of buffalo jerky

The best 10 optimized linear mixtures of humectants were subjected to sensory analysis and among 10 samples, sample J was found to be best in terms of different sensory parameters.

A control sample was prepared without infusion in humectants mixture. But, the meat cut, heating time, and temperature were the same for both samples. The proximate composition of buffalo jerky and control samples is shown in Table 4.7.

Table. 4.7 Physicochemical properties of buffalo jerky compared with the control sample

Parameters	Control (mean ±SD) (n=3)	Buffalo jerky (mean ±SD) (n=3)
Moisture	15.58 ± 0.78^{a}	10.96 ± 0.81^{b}
Fat (db)	6.00 ± 0.92^{a}	3.87 ± 0.59^{a}
Protein (db)	86.59 ± 0.56^{a}	64.34 ± 0.76^{b}
Ash (db)	7.29 ± 0.93^{a}	16.35 ± 0.87^{b}
pH	5.88 ± 0.21^{a}	4.98 ± 0.78^{a}
Water activity	0.71 ± 0.01^{a}	0.63 ± 0.01^{b}

*: Values are the means of three determinations. The figures in the parentheses are standard deviations. Figures in the row bearing different alphabets in superscripts are significantly different at $p<0.05$.

As there is no legal definition and standard for buffalo jerky and even for Nepali indigenous dry meat *Sukuti*. Most of the results of the proximate analysis agreed with various literature (Allen *et al.*, 2007; Chen *et al.*, 2002; Chen *et al.*, 2000; Kharel *et al.*, 2010; Shi *et al.*, 2020; Sorapukdee *et al.*, 2016). It was found that the control sample had a significantly higher moisture content (14%) than optimized samples (15%) prepared in the laboratory. As moisture content in the final product is directly proportional to the shelf life of the product. As the control sample was prepared without the application of humectants, the moisture content was high in the control sample.

The pH of the control sample was 5.88 followed by jerky prepared with a linear mixture of humectants had a pH of 5.62. This variation in pH could be due to the addition of a linear mixture of humectants and the addition of vinegar lowers the pH of the jerky sample. Denaturation of protein could affect the pH of the jerky which is due to the formation of amines from meat proteins during storage at room temperature (Das and Jayaraman, 2003). Jang *et al.* (2014) reported the mean pH ranged from 5.84 to 5.86 with sorbitol, glycerol, and xylitol humectants in the preparation of semi-dried jerky. (Nam *et al.*, 2017) found that the

mean pH value of pH was ranged from 5.20 to 5.90 with different humectants prepared chicken breast jerky.

The amount of fat content in the optimized jerky was less than in the control sample. In both samples, meatloaves were trimmed if excess fat was present. So, the meat strips were similar for both the control and optimized sample. So that fat content in control and optimized samples were not significantly different ($P>0.05$) from each other. Higher fat content in the final product is susceptible to off-flavor in IMM (Mishra *et al.*, 2017). The increased value of ash content in the pretreated jerky was due to the addition of humectants during pretreatments of the meat strips.

Fat content and pH values were significantly similar because lean meat with excess fat was trimmed off and lean meat for jerky preparation was the same cut. Moisture content, crude protein content, ash content, and water activity were significantly different from each other because optimized samples were made by a combination of humectants whereas control samples were made without combinations of humectants followed by drying at the same temperature and time for both samples.

4.8 Storage stability of the best optimized jerky sample

The optimized as well as control samples of jerky type product were placed in 40 μm LDPE pouches and locked with rubber bands and stored at ambient condition at 27±2°C. Change in moisture content, water activity, peroxide value and yeast and mold count were noted on 5 days interval up to 30 days for optimized sample whereas up to 20 days for control sample because it spoiled earlier than optimized sample.

4.8.1 Physiochemical and lipid oxidation jerky during storage

Moisture content and water activity (a_w) were the important physio-chemical parameter to evaluate the shelf life of jerky. Changes in moisture content and a_w of optimized and control sample for 30 days and 20 days respectively were shown in the Table (Appendix H)

4.8.1.1 Changes in moisture content during storage of optimized jerky and control jerky

In the two samples i.e. optimized and control buffalo jerky type product, moisture content was recorded over time interval. The obtained values are tabulated in Appendix J. The trend of change in moisture content is presented in Fig. 4.12.

The control sample exceeded the legal threshold 25% in 20 days which was unacceptable while the optimized sample was stable for 30 days at room temperature. Significant difference ($p<0.05$) was found among the moisture content for optimized and control sample except at 5th day of storage. In the Fig. 4.12, on the 5th day optimized sample and control sample had a similar effect on the moisture content of the product. The moisture content of the control sample had a significant difference ($p<0.05$) between 0, 10th, 15th, 20th, 25th & 30th days with optimized samples. 5th day had no significant difference ($p>0.05$) in the moisture content of the jerky between optimized and control samples.

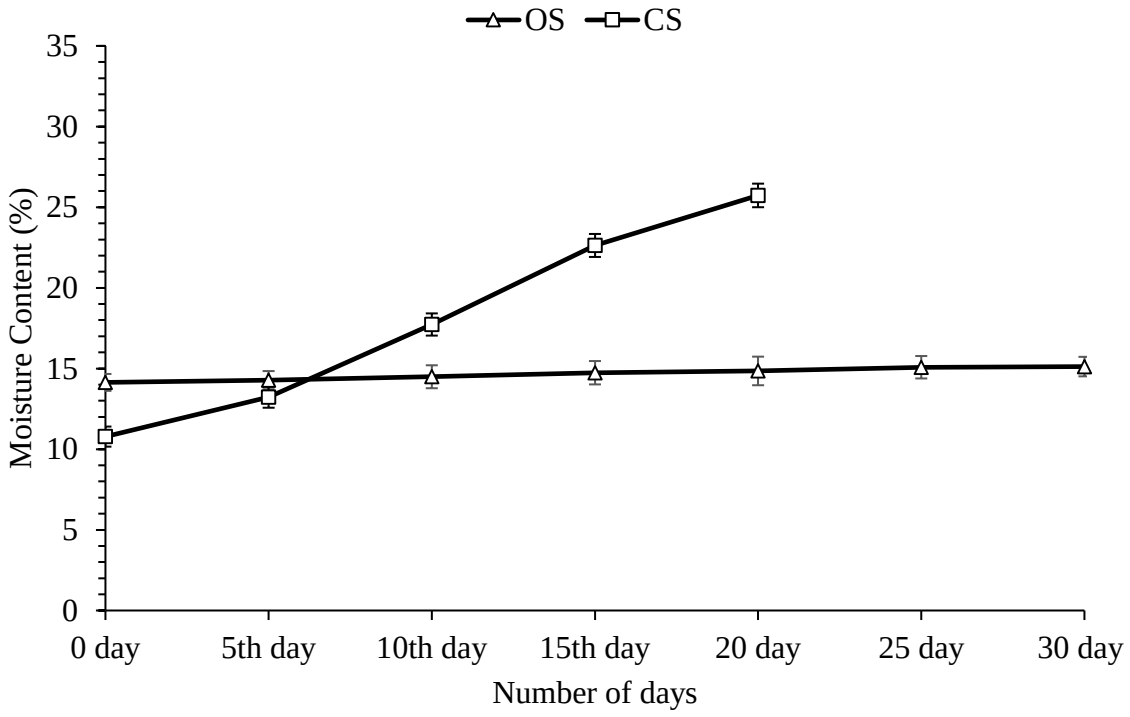

Fig. 4.12 Effect of storage in moisture content of the jerky

The data points are the mean values of triplicate observations. Error bars represent standard deviation from the mean. Significance testing results are presented in Appendix H.

There was significant similar ($p>0.05$) between the optimized sample stored from 0 to 30 days on 5 days interval whereas significant difference ($p<0.05$) between the control sample stored from 0 to 20 days on 5 days interval. The increase in moisture content was found to incline gradually within several days. At 0 to 5 days, there was overlap between optimized and control samples and gradually increased till the 30th day. Optimized sample moisture

content was high initially, but its increment was less than the control sample because of the addition of humectants to the optimized sample (Chen *et al.*, 2002) .

4.8.1.2 Changes in water activity (a_w) during storage of optimized jerky and control jerky

In the two samples i.e. optimized and control buffalo jerky type product, water activity was recorded over time interval. The obtained values are tabulated in Appendix J. The trend of change in moisture content is presented in Fig. 4.13.

The effect of storage days on the water activity (a_w) of both samples is shown in Table (Appendix J1). The initial water activity (a_w) of the optimized sample and control was found to be 0.63 and 0.61 at 20.5ºC in 0 days of storage. Statistical analysis showed that water activity (a_w) was increased significantly during the 30 days storage period for both the jerky samples (Appendix G2 and H2). The final water activity (a_w) of the optimized sample and control sample was found to be 0.70 at 30 days of storage and 0.82 at 20 days of storage as shown in fig. 4.13. The interaction effect of jerky samples and storage period on water activity (a_w) was significant ($p<0.05$) (Appendix H2).

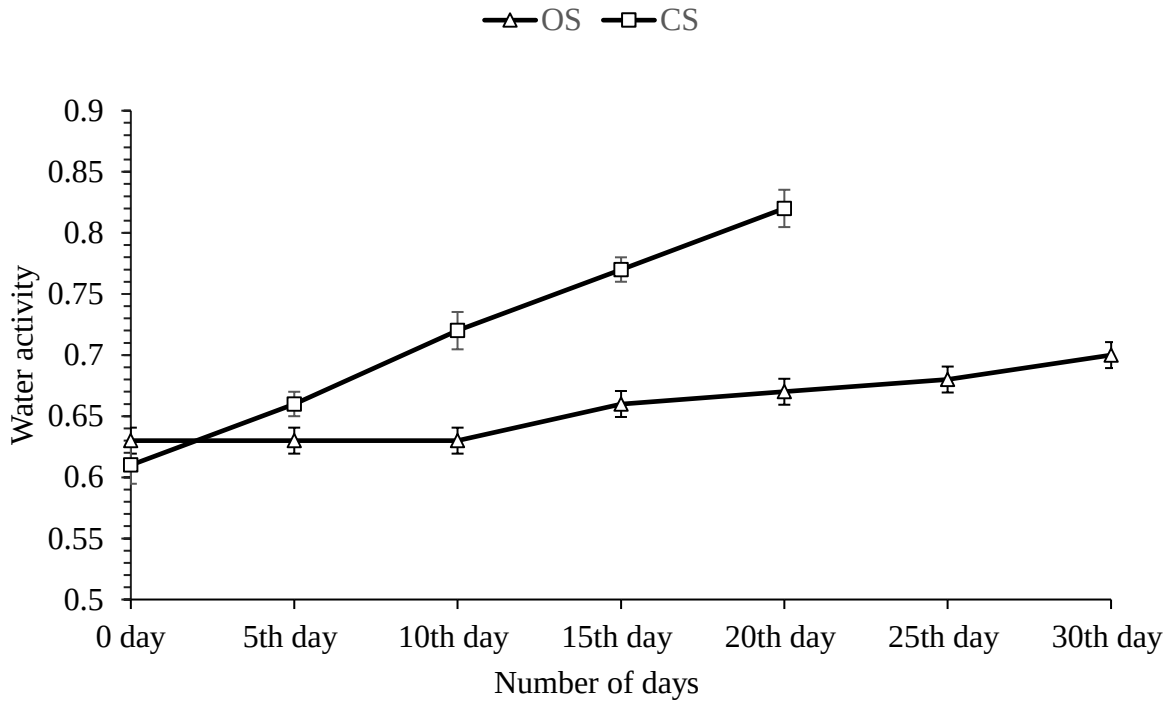

Fig. 4.13 Effect of storage in water activity (a_w) of the jerky

The data points are the mean values of triplicate observations. Error bars represent standard deviation from the mean. Significance testing results are presented in Appendix H.

Optimized and control samples had significant differences ($p<0.05$) in terms of the water activity of the jerky. In Fig. 4.13, optimized samples' water activity at 0 and 5th days were significantly different ($p<0.05$) whereas 20th and 25th day were significantly similar ($p>0.05$) during storage at ambient temperature. Control sample water activity were significantly different ($p<0.05$) on 30 days storage at room temperature in 5 day interval.

It was clear from the Fig. 4.13 that the water activity of the optimized sample increased from 0.63 to 0.70 at 20.5ºC for 30 days in comparison to the control sample. The water activity of the control sample increased gradually in comparison to the optimized due to the absence of humectants that reduce water activity and moisture (Msagati, 2013). Both the samples were treated at 72ºC for 2 h with similar packaging material. Lim *et al.* (2012) reported that during storage of beef jerky, water activity increased from 0.66 to 0.74 on 20 days.

4.8.1.3 Changes in peroxide value during storage of optimized jerky and control jerky

In the two samples i.e. optimized and control buffalo jerky type product, peroxide value was recorded over time interval. The obtained values are tabulated in Appendix J. The trend of change in moisture content is presented in Fig. 4.14.

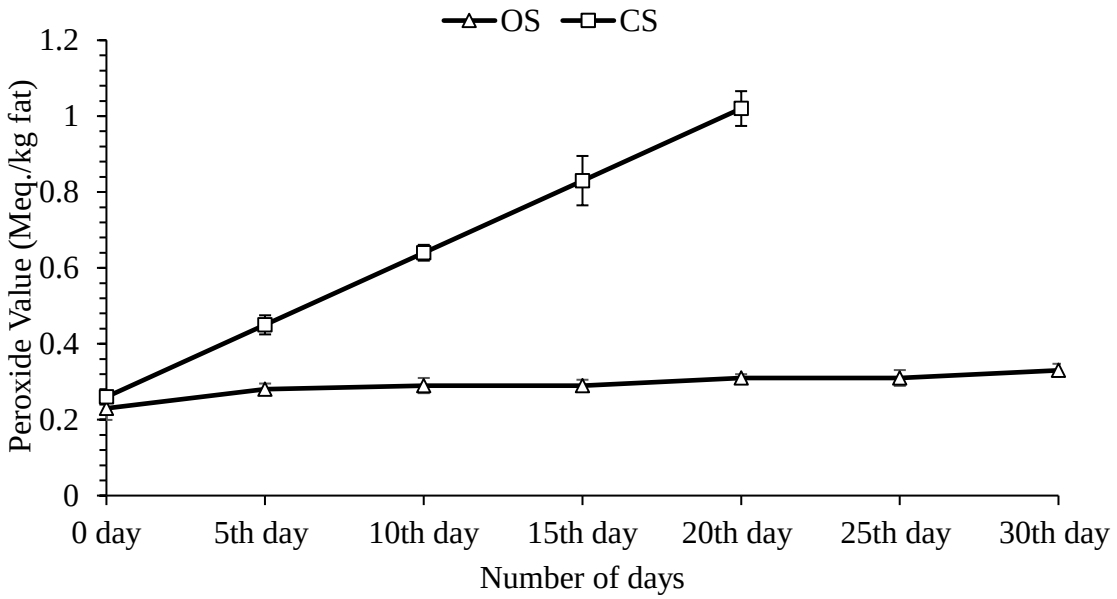

Fig. 4.14 Effect of storage in peroxide value of the jerky

The data points are the mean values of triplicate observations. Error bars represent standard deviation from the mean. Significance testing results are presented in Appendix H.

PV kept on increasing with the progress of the storage period for both the optimized sample and control sample. There was a significant increase in PV on both the sample each 0^{th}, 5^{th}, 10^{th}, 15^{th}, 20^{th}, 25^{th}, and 30^{th} days (Appendix G4 and H4). There was a significant difference in PV between the optimized sample and the control sample in the storage period of 30 days (Appendix I4). PV of optimized jerky and control jerky increased from 0.23 and 0.26 Meq peroxide/kg fat in 0 day storage to 0.33 and 1.02 Meq peroxide/kg fat in 30 days of storage at room temperature conditions as shown in Fig. 4.14. The optimized sample was significantly different ($p<0.05$) than the control sample on peroxide value during storage from 0 to 30 days. In the Fig. 4.14, optimized samples on 0^{th}, 20^{th}, 25^{th} and 30^{th} were significantly different ($p<0.05$) with no. of days during storage whereas 5^{th}, 15^{th} and 10^{th} day were significantly similar ($p>0.05$) with no. of days during storage. Control samples on 20 days storage at 5 days interval were significantly different ($p<0.05$) with no. of days during storage.

A similar increasing trend in peroxide value during the storage period was observed by Kong *et al.* (2011) for extruded salmon jerky snacks. Kong *et al.* (2011) also reported that the initial peroxide value increased 10 times greater at the 4^{th} week of storage.

4.8.2 Microbial analysis of the jerky during storage

Yeast and mold count were done in every 5 days for 30 days period to evaluate shelf life through microbiological analysis of the optimized samples and control samples. The yeast and mold count of the optimized sample and control sample were shown in the table (Appendix J4).

4.8.2.1 Yeast and mold count of jerky during storage

In the two samples i.e. optimized and control buffalo jerky type product, peroxide value was recorded over time interval. The obtained values are tabulated in Appendix J. The trend of change in moisture content is presented in Fig. 4.14.

The changes in yeast and mold count (log CFU/ml) of the optimized sample and control sample for 30 days storage period was presented in the Table (Appendix J2). The yeast and mold count varied significantly with the progress of the storage period up to 30 days for both the optimized and control beverages (Appendix G5 and Appendix H5) but for the optimized beverage, there was no significant difference ($p>0.05$) in yeast and mold count during 30 days of storage. The yeast and mold count of both the optimized sample and control sample

was found to be increased from 0.23 log CFU/ml and 0.26 log CFU/ml to 0.33 log CFU/ml and 1.34 log CFU/ml at 20 days as shown in Fig. 4.15. The optimized sample was significantly different ($p<0.05$) than the control sample on yeast and mold count during storage from 0 to 30 days. In the Fig. 4.15, optimized samples on 0^{th}, 20^{th}, 25^{th} and 30^{th} were significantly different ($p<0.05$) with no. of days during storage whereas 5^{th}, 15^{th} and 10^{th} day were significantly similar ($p>0.05$) with no. of days during storage. Control samples on 20 days storage at 5 days interval were significantly different ($p<0.05$) with no. of days during storage.

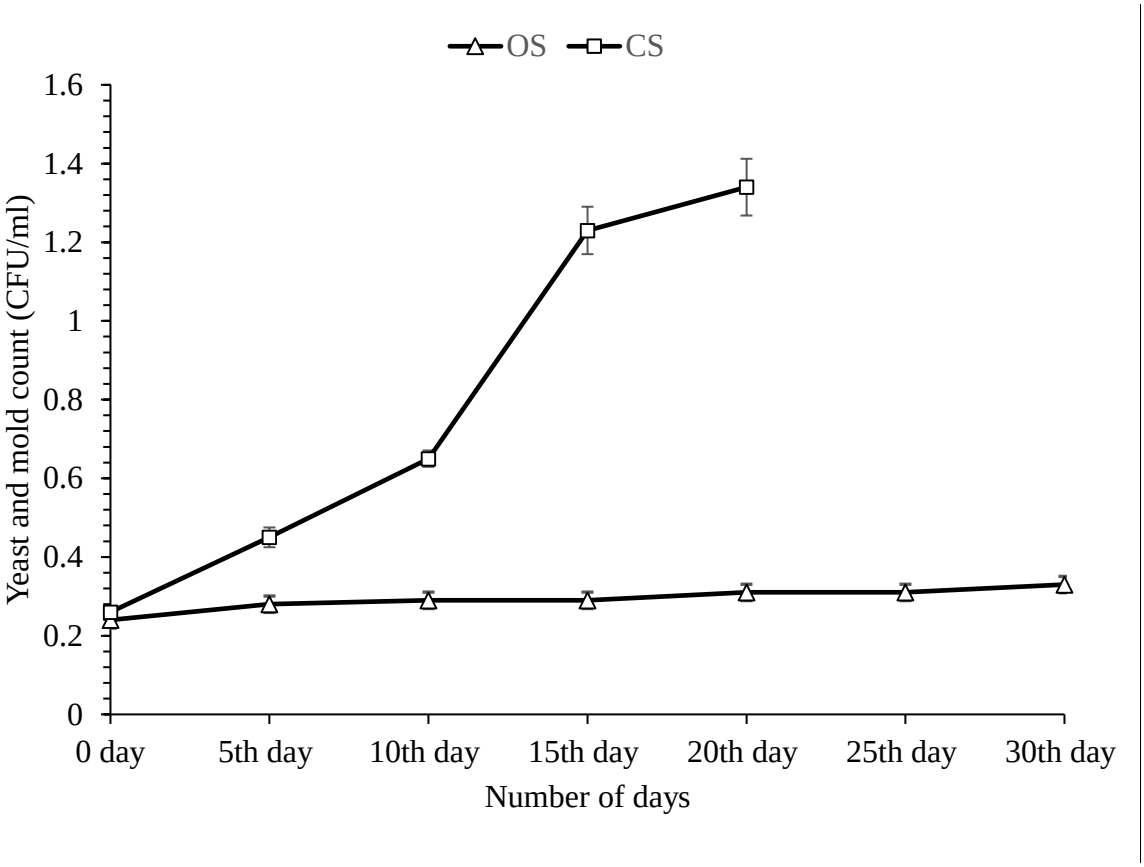

Fig. 4.15 Effect of storage in yeast and mold count of the jerky

Since the increase in yeast and mold count was slow in the optimized sample than in the control sample. Since, the addition of humectants reduces water activity and suppresses microbial growth (Sorapukdee *et al.*, 2016). A similar climbing trend in yeast and mold count during storage was observed by Chukwu and Imodiboh (2009) for potassium sorbate treated *khilishi* during 21 days of storage and Chen *et al.* (2000) for Chinese style pork jerky with different levels of glycerol and sorbitol during storage at 28 °C.

Part V

Conclusions and recommendations

5.1 Conclusions

As per the objectives, the materials and methods stated in this research were carried out for the results. Based on the obtained results, the following conclusions have been drawn:

1) An increase in the concentration of salt content in the linear mixture of humectants, lowers water activity and enhances the overall acceptability of the buffalo jerky-type product.
2) The observed models accurately predicted the response parameters (water activity) having R^2 and minimum error.
3) The physio-chemical properties of optimized jerky sample and control jerky sample (prepared without humectants mixture) differ significantly in terms of moisture content, protein, ash & a_w.
4) Sensory analysis showed that optimized sample with 4 g salt, 2 g sucrose, 2 g brown sugar, 1.5 g maltodextrin & 0.5 g citric acid was better than other samples due to its higher average sensory score on taste, texture, color, juiciness & O. A.
5) The humectants optimized sample inhibited lipid oxidation significantly which was studied in terms of PV when compared with control sample on 30 days storage at room temperature.
6) The humectants optimized sample exhibited significant antimicrobial activity study in term of yeast and mold count when compared with control sample on 30 days storage at room temperature.

5.2 Recommendations

1) Humectants concentration can be raised from 10% to 20% during the preparation of buffalo jerky type meat product.

2) Buffalo meat can be replaced by chicken and pork meat for the preparation of jerky type meat product.

Part VI

Summary

Jerky is made by drying lean buffalo meat strips with humectants combination. This product is of most concern as it does not require refrigeration during the marketing and further preparation before consumption. As, Indigenous moisture meat of Nepal, *sukutii*, are still based on traditional drying technology and hot smoking has adverse effects on appearance, odor, texture, and taste. Also, these products are more susceptible to lipid oxidation and microbial spoilage in meat products which reduces shelf-life. To overcome such problems, a mixture of humectants was incorporated to optimize buffalo jerky and study its storage stability.

To optimize different humectants (salt, sucrose, brown sugar, maltodextrin, and citric acid) for the preparation of buffalo meat jerky through D E®. The water activity of the final product was the response to the experiment. Sensory, physiochemical, and microbiological qualities of the buffalo jerky were also studied. The overall experiment was divided into two parts. At first pretreatment solution of humectants was optimized and the best formulation in terms of sensory was selected. The optimized samples were compared with the control sample (untreated) concerning physicochemical properties, lipid oxidation, and yeast and mold count. Water activity (a_w) was found to be affected by the pretreatments of humectants and effects can be predicted by the model equation with high precision. An increase in salt concentration was the main factor in decreasing water activity in buffalo jerky. From graphical and numerical optimization, the optimized combination obtained was 4 g salt, 2 g sucrose, 2 g brown sugar, 0.5 g maltodextrin, and 0.5 g citric acid and superior sensory quality.

Crude protein content and pH had no significant difference ($P>0.05$) between the optimized sample and control sample whereas moisture content, fat, ash content, and water activity had a significant difference ($P<0.05$) between the optimized sample and the control sample. There was a significant difference between ($P<0.05$) optimized and control in terms of storage stability study except on the 5^{th} day of moisture content of the jerky, there was no significantly different ($P>0.05$) between both samples. The optimized jerky sample was

stable for 30 days without any decrease in sensory attributes and microbiological spoilage to the control jerky sample which lasted for only 20 days.

References

Adhikari, B. M. (2006). Study on hygenic quality of buffalo meat markated in Dharan. B. Tech Food Technology Dissertation. Tribhuvan Univ., Nepal.

Akthar, J. and Pandey, R. K. (2015). Meat drying technology and drying characteristics of meat and meat products. *Int. J. Appl. Pure Sci. Agric.* **1** (8), 21-25.

Albright, S. N., Kendall, P. A., Avens, J. S. and Sofos, J. N. (2002). Effect of marinade and drying temperature on inactivation of *Escherichia coli* O157:H7 on inoculated home dried beef jerky. . *J. Food Safety*. **22** (3), 155-167. [doi./10.1111/j.1745-4565.2002.tb00338.x].

Allen, K., Cornforth, D., Whittier, D., Vasavada, M. and Nummer, B. (2007). Evaluation of high humidity and wet marinade methods for pasturization of jerky. *J. Food Sci.* **72** (7), 351-356. [doi: 10.1111/j.1750-3841.2007.00458.x].

Anon. (2007). Paw kit standard operating procedure [Brochure]. pp. 1-13. Retrieved from www.Decagondevices.inc.com/standard-operating-procedure/. [Accessed 23 November, 2019].

Anon. (2021). The best homemade beef jerky recipe An Elite Cafemedia Food Publisher. Retrieved from https://www.onceuponachef.com/recipes/the-best-homemade-beef-jerky-recipe.html. (Last update 11 October, 2019). [Accessed 12 January, 2021].

AOAC. (2005). "Official Methods of Analysis" (18th ed.). Vol. 4th. Association of official Analytical Chemists. Washington DC.

Belitz, H.-D., Grosch, W. and Schieberle, P. (2009). "Food Chemistry" (4th ed.). Springer. Berlin, Germany. [ISBN 978-3-540-69933-0].

Buck, J., Sant, L. and Robertson, A. (2020). "Making Jerky at Home Safely". A Pacific Northwest Extension Publication. USA.

Burfoot, D., Everis, L., Mulvey, L., Wood, A. and Betts, R. (2010). Literature Review on Microbiological Hazards Associated with Biltong and Similar Dried Meat Products [Report]. 41. Vol. 41. Food Standard Agency. London, UK. [Accessed 8 March, 2022].

Chakrabarty, M. M. (2003). "Chemistry and Technology of Oils and Fats" (1st ed.). Allied Publishers Pvt. Ltd. India. [ISBN 81-7764-495-5].

Chen, W. S., Liu, D. C. and Chen, M. T. (2002). Effects of high level of sucrose on the moisture content, water activity, protein denaturation and sensory properties in

chinese-style pork jerky. *Asian-Aus. J. Animal Sci.* **15** (4), 585-590. [doi.org/10.5713/ajas.2002.585]

Chen, W. S., Liu, D. C., Chen, M. T. and Ockerman, H. W. (2000). Improving texture and storage stability of chinese-style pork jerky by the addition of humectants. *Asian-Aus. J. Anim. Sci.* . **13** (10), 1455-1460.

Choi, J.-H., Jeong, J.-Y., Han, D.-J., Choi, Y.-S., Kim, H.-Y., Lee, M.-A., Lee, E.-S., Paik, H.-D. and Kim, C.-J. (2008). Effects of pork/beef levels and various casings on quality properties of semi-dried jerky. *Meat Sci.* **80** (88), 278-286.

Chukwu, O. and Imodiboh, L. I. (2009). Influence of storage conditions on shelf-life of dried beef product (kilishi). *World J. Agric. Sci.* . **5** (1), 34-39.

Das, A. K., Anjaneyulu, A. S. R., Verma, A. K. and Kondaiah, N. (2006). Physicochemical, textural, sensory characteristics and storage stability of goat meat patties extended with full-fat soy paste and soy granules. *Int. J. Food Sci. Technol.* **43** (22), 383-392.

Das, H. and Jayaraman, S. (2003). A study on quality and stability of convenience dehydrated chicken pulav. *J. Food Sci. Technol.* . **40** (1), 97-101.

DFTQC. (2010). "Khadya Prasodhan Prabidhi". Department of Food Technology and Quality Control, Babarmahal, Kathamandu. Kathmandu.

FAO. (2007). Composition of meat Food and Agriculture Organization Retrieved from http://www.fao.org/ag/againfo/themes/en/meat/backgr_composition.html. (Last update 5 March, 2015). [Accessed 12 October, 2021].

FAO. (2019). Meat & Meat Products. Food and Agriculture Organization. Retrieved from http://www.fao.org/ag/againfo/themes/en/meat/home.html. (Last update 15 March, 2019). [Accessed 13 May 2019].

Fennema, O. (1996). Water and ice. *In:* "Food Chemistry" (1st ed.). (O. Fennema, Ed.). pp. 17-94. New york Marcel Dekker.

Frazier, W. and Westhoff, D. (2003). "Food Microbiology" (6th ed.). Tata McGrew hill Publishing, New Dehli. India.

FritoLay. (2020). Ingredients. Frito-Lay North America, Inc. a Dividion of PepsiCo. Retrieved from https://www.fritolay.com/products/matador-original-jerky. (Last update 2018-05-23). [Accessed 2022-01-25].

Gailani, M. B. and Fung, D. Y. C. (1989). Microbiology and water activity relationship in the processing and storage of Sudanese dry meat (sharmoot). *J. Food Protect.* . **52** (1), 13-20.

Giordano, G., Guarini, P., Ferrari, P., Biondi-Zoccai, G., Schiavone, B. and Giordano, A. (2010). Beneficial impact on cardiovascular risk profile of water buffalo meat consumption. *Eur. J. Clin. Nutr.* **64** (9), 1000-1006. [doi:10.1038/ejcn.2010.108].

Gu, Z., Liu, S., Duan, Z., Kang, R., Zhao, M., Xia, G. and Shen, X. (2021). Effect of citric acid on physicochemical properties and protein structure of low-salt restructured tilapia (*Oreochromis mossambicus*) meat products. *J. Sci. Food Agricult.* **101** (4), 1636-1645. [doi:/10.1002/jsfa.10784].

Huang, T. and Nip, W. (2001). Intermediate moisture meat and dehydrated meat. . *In:* "Meat Science and Applications" (1st ed.). (Y. H. Hui, W. Nip and R. W. Rogers, Eds.). pp. 337-425. USA. Marcel Dekker, Inc.

Jamhari, E. S. and Lakshmiwati, D. A. (2018). Effect of sugarcane levels and drying methods on chemical and physical qualities of ground beef "dedeng". *Bulletin Animal Sci.* **42** (1), 67-71.

Jang, S., Kim, H., Hwang, K., Song, D., Kim, Y., Ham, Y., Lim, Y., Jeong, T., Kim, S. and Kim, C. (2014). Effects of replacing sucrose with various sugar alcohols on quality properties of semi-dried jerky. *Korean J. Food Sci.* **35** (5), 622-629.

Kandeepan, B. and Rajkumar, A. (2009). Buffalo as a potential food animal. *J. Livestock Product.* . **1** (1), 45-56.

Karthikeyan, J., Kumar, S., Anjaneyulu, A. S. R. and Rao, K. H. (1999). Application of hurdle technology for the development of caprine keema and its stability aat ambient temperature *Elsevier*. **54** (39), 9-15.

KC, J. B. and Rai, B. K. (2019). "Basic Food Analysis Handbook" (3rd ed.). Pragati Printers, Anamnagar, Kathmandu, Nepal. Kathmandu.

Kharb, R. and Ahlawat, S. S. (2010). Effect of pre cooking and spices on quality characteristics of dehydrated spent hen meat mince. *Indian J. Poultry Sci.* . **45** (1), 100-102.

Kharel, G. P., Acharya, P. P. and Rai, B. K. (2010). "Indigenous Foods of Nepal" (1st ed.). Highland Publication Kathmandu. [ISBN 978-9937-2-2013-2].

Kong, J., Perkins, B., Dougherty, M. P. and Camire, M. E. (2011). Control of lipid oxidation in extruded salmon jerky snacks. *J. Food Sci.* **76** (1), 8-12. [doi:10.1111/j.1750-3841.2010.01897.x].

Kumar, P., Verma, A. K., Kumar, D., Umaraw, P., Mehta, M. and Malav, O. V. (2019). Meat snacks: A novel technological perspective. *In:* "Innovations in Traditional Foods" (3rd ed.). (C. M. Galanakis, Ed.). pp. 293-321. India. Woodhead publishing

Lapitan, R. M., Del Barrio, A. N., Katsube, O., BAN-TOKUDA, T., Orden, E. A., Robles, A. Y., Fujihara, T., Cruz, L. C., Homma, H. and Kanai, Y. (2007). Comparison of carcass and meat characteristics of Brahman grade cattle (*Bos indicus*) and crossbred water buffalo (*Bubalus bubalis*). *Animal Sci. J.* **78** (6), 596-604. [doi:10.1111/j.1740-0929.2007.00480.x].

Lee, S. W. and Kang, C. S. (2003). Effects of moisture content and drying temperature on the physicochemical properties of ostrich jerky. *J. Meat Sci.* **47** (5), 330-333.

Leistner, L. (1992). Food preservation by combined methods. *Int. J. Food Res.* **25**, 151-158.

Lim, D.-G., Lee, S.-S., Seo, K.-S. and Nam, K.-C. (2012). Effects of Different Drying Methods on Quality Traits of Hanwoo Beef Jerky from Low-Valued Cuts during Storage. *Korean J. Food Sci.* . **32** (4), 531-539. [doi:10.5851/kosfa.2012.32.5.531].

Lima, D. M., Rangel, A., Urbano, S., Mitzi, G. and Moreno, G. M. (2013). Oxidação lipídica da carne ovina. *J. Acta Veterinaria Brasilica.* **7** (1), 14-29.

McCabe, S. (2019). Brown sugar vs white sugar: what is the difference? Healthline online magzine. Retrieved from https://www.healthline.com/nutrition/brown-sugar-vs-white-sugar. (Last update 23 January 2020). [Accessed 23 May 2020].

Min, B. and Ahn, U. (2005). Mechanism of lipid peroxidation in meat and meat products- a review. *J. Food Sci. Biotechnol.* **14** (1), 152-163.

Mishra, B., Mishra, J., Pati, P. and Rath, P. (2017). Dehydrated meat products- A review. *Int. J. Livest. Res.* . **7** (22), 10-22. [doi:10.5455/ijlr.20170812035616].

MOALD. (2021). "Statistical Information on Nepalese Agriculture". Planning & Development Cooperation Coordination Division, Statistics And Analysis Section. Singhdurbar, Kathamndu, Nepal

Msagati, T. A. M. (2013). "The Chemistry of Food Additives and Preservatives" (3rd ed.). Wiley Online Library. London.

Nam, D.-G., Jeong, B.-G. and Chun, J. (2017). Physicochemical properties and oxidative stabilities of chicken breast jerky treated various sweeting agents. . *Korean J. Food Preserv.* . **24** (1), 84-92.

Naveena, B. M. and Kirant, M. (2014). Buffalo meat quality, composition, and processing characteristics; contribution to the global economy and nutritional security. *J. Animal Frontiers*. **4** (4), 18-25. [doi:10.2527/af.2014-0029].

Nummer, B., Harrison, J. A., Harrison, M. A., Kendall, P., Sofos, J. N. and Andress, E. L. (2004). Effects of prepration methods on the microbiological safety of home dried meat jerky. *J. Food Protect.* **67** (10), 2337-2341. [doi:10.4315/0362-028X-67.10.2337].

Obanu, Z. (2001). Preservation of meat in Africa by control of the internal aqueous environment in relation to product quality and stability. *J. Afric. Meat Sci,* . **48** (2), 34-47.

Ogunsola, O. O. and Omojola, A. B. (2008). Qualititative evaluation of Kilishi prepared from beef and pork *Afr. J. Biotechnol.* . **7** (11), 1753-1758.

Park, Y. W. (2009). Moisture and water activity *In:* "Processed Meat and Poultry " (2nd ed.). (L. M. L. Nollet and F. Toldra, Eds.). pp. 35-69. New york CRC Press.

Pimental, T. C., Gomes da Cruz, A. and Deliza, A. (2016). Sensory evaluation: Sensory rating and scoring. . *In:* "The Encyclopedia of Food and Health" (12th ed., Vol. 4). (F. P. Caballero and F. Toldra, Eds.). pp. 744-749. England Oxford acedemic Press.

Potter, N. N. and Hotchkiss, J. H. (1995). "Food Science" (5th ed.). Vol. 1. Chapman and Hall Publisher. New York, USA.

Prabhakar, K. (1999). "Teaching Manual " (1st ed.). *Self-archived* Teaching manual. Department of Meat Science and Technology. India. [Accessed 7, november, 2020].

Rao, K. V. and Kowale, B. N. (1991). Changes in phospolipids of buffalo meat during processing and storage. *J. Meat Sci.* . **30** (5), 115-129.

Reyes-Canoa, R., Dorantes-Alvareza, L., Hernandez-Sancheza, H. and GutierrezLopez, G. (1994). A traditional intermediate moisture meat: Beef cecina. *J. Meat Sci.* . **47** (3), 121-127.

Sahoo, J. and Anjaneyulu, A. S. R. (1997). Effect of natural antioxidants and vacuum packaging on the quality of buffalo meat nuggets during refrigerated storage. *J. Meat Sci.* **47** (3), 223-230.

Shaefer, A. (2018). Is maltodextrin bad for me? *Healthline*. Wellness newsletter. Retrieved from https://www.healthline.com/health/food-nutrition/is-maltodextrin-bad-for-me#maltodextrin-and-diabetes. [Accessed 12 September, 2018].

Shi, S., Kong, B., Wang, Y., Liu, Q. and Xia, X. (2020). Comparison of the quality of beef jerky processed by traditional and modern drying methods from different districts in Inner Mongolia. *Meat Science*.

Shumaker, K. and Kendle, C. (2017). Food Preservation: Making Jerky [Report]. Ohio State Univ. Extension. USA, USA. Retrieved from ohioline.osu.edu/factsheet/hyg-5362. [Accessed 2 january, 2022].

Skandamis, P. N. and Goundaki, A. S. (2009). Dried meats, poultry and related products. *In:* "Microbiology Handbook of Meat Products" (1st ed.). (R. Fernendes, Ed.). pp. 83-95. London. Leatherhead Publishing

Sorapukdee, S., Uesakulrungrueng, C. and Pilasombut, K. (2016). Effects of humectants and roasting on physiological and sensory properties of jerky made from spent hen meat. . *Korean J. Food Sci.* . **36** (3), 326-334.

Srinivas, V. (1996). A study on the prepration of Intermediate Moisture Meat using certain humectants. M.V.Sc Livestock Product Technology Thesis. Sri Venkateswara Veterinary Univ. , India.

Sritongtae, B., Mahawanich, T. and K., D. (2011). Drying of osmosed cantaloupe: Effect of polyols ondrying ans water mobility. *Int. J. Chem.* **4**(5), 53-62.

Subba, D. (2010). Analysis of meat and meat products. *In:* "Textbook of Meat and Poultry Technology " (2nd ed.). (D. Subba, Ed.). pp. 57-73. Kathmandu. National College of Food Science and Technology

Subba, D. (2018). "Handbook of Meat Technology-II" (1st ed. ed.). *Self-archived* Teaching manual Central Department of Food Technology Nepal.

Sureshkumar, S., Venkataramanujan, V., Dushyantham, K. and Kalaikannan, A. (2006). Effect of water activity on the physio-chemical, microbiological and sensory qualities of meat sausage stored under ambient temperature. *Am. J. Food Sci.* **1** (2), 166-172.

Tamburrano, A., Tavazzi, B., Calla, C. A., Amorini, A. M., Lazzarino, G., Vincenti, S., Zottola, T., Campagna, M. C., Moscato, U. and Laurenti, P. (2019). Biochemical and nurtitional characteristics of buffalo meat and potential implications on human health for a personalized nutrition. *Italian J. Food Safety* **8**(3), 45-52.

Taoukis, P. S. and Richardson, M. (2020). Principles of Intermediate-Moisture Foods *In:* "Water Activity in Foods: Fundamentals and Applications" (7th ed.). (G. V. Barbosa-

Canovas, A. J. Fontana JR, S. J. Schmidt and T. P. Labuza, Eds.). pp. 385-424. Wiley Online Library.

Tapia, M. S., Alzamore, S. M. and Chirife, J. (2007). Effects of water activity aw on microbial stability: as a hurdle in food preservation *In:* "Water Activity in Foods" (1st ed.). (G. V. Barbosa-Canovas, A. J. Fontana JR, S. J. Schmidt and T. P. Labuza, Eds.). pp. 239-273. UK. Blackwell Publishing. [ISBN 978-0-8138-2408-6/2007].

Thomas, R., Anjaneyulu, A. S. R., Mendiratta, S. K. and Kondaiah, N. (2008). Technologies for value added buffalo meat product: a review. *J. Food Technol.* **2**, 104-114.

Thornton, P. K. (2010). Livestock production: recent trends, future prospects. *Philosophical Transactions of the Royal Society: Biol. Sci.* . **365** (1554), 2853-2867.

Toldrá, F. (2017). "Lawrie's Meat Science" (16th ed.). Woodhead Publishing. London. [ISBN 0081006977].

USDA. (2019). Jerky and Food Safety [Press release]. Food Safety and Inspection Service Retrieved from https://www.fsis.usda.gov/. [Accessed 12 November, 2019].

Williams, P. (2007). Nutritional composition of red meat. *J Nutr. Dietetic.* **64** (22), 23-31.

Wongwiwat, P. and Wattanachant, S. (2015). Quality changes of chicken meat jerky with different sweeteners during storage. *J. Food Sci. Technol.* **52** (12), 8329-8335. [doi:10.1007/s13197-015-1884-2].

Yang, H.-S., Kang, S.-W., Joo, S.-T. and Choi, S.-G. (2012). Effects of salt concentration and drying time on the quality characteristics of pork jerky during dehydration. *Korean J. Food Sci. Ani. Resour.* . **32** (3), 285-292. [doi:10.5851/kosfa.2012.32.3.285].

Ziegler, G. R., Rizvi, S. S. H. and Acton, J. C. (1987). Relationship of water content to textural characteristics, water activity, and thermal conductivity of some commercial sausages. *J. Food Sci.* **52** (4), 901-905. [doi:10.1111/j.1365-2621.1987.tb14238.x].

Appendix

Appendix A

A.1 Chemical used:

- Salt, sucrose, brown sugar, maltodextrin & citric acid
- Soy sauce and other masala & spices
- Food grade vinegar
- MSG
- Nitrite curing salt
- Ascorbic acid
- Catalyst mixture, Potassium permanent, silver nitrate
- Potassium Iodide
- Sodium thiosulphate
- Potato dextrose agar
- Other basic laboratory chemicals

A.2 Apparatus used:

- Water bath and incubator
- Colony counter
- Weighing balance
- Thermometer,
- pH meter
- Cabinet drier
- Soxhlet apparatus
- Kjeldahl digestion and distillation set
- Muffle furnace
- Glassware
- Water activity meter
- Refrigerator

Appendix B

Table B. 1 Combinations of factors and responses for optimization of humectants mixture

Run	A: Salt g	B: Sucrose g	C: Brown sugar g	D: Fructose g	Citric acid g	Water activity (a_w)
1	1.80	2.74	1.29	3	1.12	
2	5.11	0	1.59	1.51	1.80	
3	6	0	0	3	1	
4	4	0	3	0	3	
5	6	1.	3	0	0	
6	2.13	2.49	3	1.02	1.36	
7	2.57	0	2.06	2.97	2.40	
8	5.67	2.48	0	0	1.87	
9	2.74	1.99	0.05	2.23	3	
10	0.55	6	0	2.10	1.35	
11	3.39	2.37	1.41	0	2.84	
12	0	1	3	3	3	
13	3.11	4.33	0	2.56	0	
14	2.13	2.49	3	1.02	1.36	
15	0	5.25	2.66	2.09	0	
16	5.11	0	1.59	1.51	1.79	
17	2.60	6	1.21	0	0.2	
18	0	5.15	1.66	0.31	2.88	
19	5.17	2.55	0.64	1.64	0	
20	4	0	3	3	0	

Appendix C

Sensory evaluation card

Date:

Product: Buffalo jerky

Name of Panelist:

Please taste the jerky samples provided to you and give points for your evaluation as given below for each sensory quality parameter.

Perceptions	**Points**
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike slightly	3
Dislike very much	2
Dislike extremely	1

Sensory Parameters					
Samples	Color	Taste	Texture	Juiciness	Overall Acceptability
A					
B					
C					
D					
E					

Comments (If any):

……

Signature

Appendix D

Sensory analysis of the jerky samples

Table D1 ANOVA table for sensory parameters of the jerky samples

		Sum of Squares	df	Mean Square	F	Sig.
Color	Between Groups	33.050	9	3.672	8.325	.000
	Within Groups	39.700	90	.441		
	Total	72.750	99			
Taste	Between Groups	37.360	9	4.151	8.302	.000
	Within Groups	45.000	90	.500		
	Total	82.360	99			
Texture	Between Groups	26.360	9	2.929	6.102	.000
	Within Groups	43.200	90	.480		
	Total	69.560	99			
Juiciness	Between Groups	27.760	9	3.084	6.196	.000
	Within Groups	44.800	90	.498		
	Total	72.560	99			
OA	Between Groups	28.450	9	3.161	6.422	.000
	Within Groups	44.300	90	.492		
	Total	72.750	99			

Table D2: Tukey HSD for color

Tukey HSD[a]					
Formulation of the product	N	Subset for alpha = 0.05			
		1	2	3	4
C	10	5.50			
G	10	6.00	6.00		
A	10	6.10	6.10		
D	10		6.50	6.50	
b	10		6.60	6.60	
e	10		6.60	6.60	
h	10		6.60	6.60	
f	10		6.80	6.80	6.80
i	10			7.10	7.10
j	10				7.70
Sig.		.588	.192	.588	.088

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Table D3 Tukey HSD for taste

Tukey HSD[a]				
Formulation of the product	N	Subset for alpha = 0.05		
		1	2	3
c	10	5.50		
d	10	6.00	6.00	
b	10	6.10	6.10	
h	10	6.20	6.20	
A	10	6.30	6.30	
F	10	6.30	6.30	
E	10	6.40	6.40	
G	10	6.50	6.50	
I	10		7.00	7.00
J	10			7.90
Sig.		.062	.062	.137

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Table D4: Tukey HSD For Texture

Tukey HSD[a]				
Formulation of the product	N	Subset for alpha = 0.05		
		1	2	3
b	10	5.50		
d	10	5.90	5.90	
e	10	6.10	6.10	
g	10	6.10	6.10	
a	10	6.40	6.40	
c	10	6.40	6.40	
f	10	6.50	6.50	6.50
h	10		6.70	6.70
i	10		6.70	6.70
J	10			7.50
Sig.		.052	.242	.052

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Table D5: Tukey HSD for Juiciness

Tukey HSD[a]				
Formulation of the product	N	Subset for alpha = 0.05		
		1	2	3
d	10	5.40		
h	10	5.50		
b	10	5.60		
c	10	5.70	5.70	
a	10	6.30	6.30	6.30
e	10	6.30	6.30	6.30
f	10	6.30	6.30	6.30
i	10	6.30	6.30	6.30
g	10		6.70	6.70
j	10			7.10
Sig.		.135	.061	.264

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Table D6: Tukey HSD for Overall acceptability

Tukey HSD[a]			
Formulation of the product	N	Subset for alpha = 0.05	
		1	2
d	10	6.10	
b	10	6.30	
c	10	6.40	
a	10	6.50	
h	10	6.60	
e	10	6.70	
g	10	6.70	
f	10	7.00	
i	10	7.10	7.10
j	10		8.10
Sig.		.058	.058

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Appendix E

Table E1: Independent sample t-test for the physicochemical parameter of the jerky

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
MC	Equal variances assumed	.050	.834	-7.130	4	.002	-4.62667	.64886	-6.428	-2.82514
	Equal variances not assumed			-7.130	3.993	.002	-4.62667	.64886	-6.430	-2.82388
Fat	Equal variances assumed	.798	.422	-3.371	4	.028	-2.13000	.63189	-3.884	-.37558
	Equal variances not assumed			-3.371	3.421	.036	-2.13000	.63189	-4.008	-.25193
Protein	Equal variances assumed	.127	.739	-39.91	4	.000	-22.24333	.55731	-23.790	-20.69601
	Equal variances not assumed			-39.91	3.782	.000	-22.24333	.55731	-23.826	-20.66019
Ash	Equal variances assumed	.026	.879	12.309	4	.000	9.06000	.73603	7.017	11.10353
	Equal variances not assumed			12.309	3.983	.000	9.06000	.73603	7.013	11.10688
pH	Equal variances assumed	5.241	.084	-1.972	4	.120	-.90333	.45802	-2.175	.36832

	Equal variances not assumed			-1.972	2.312	.170	-.90333	.45802	-2.64014	.83348
	Equal variances assumed	.400	.561	-11.500	4	.000	-.07667	.00667	-.09518	-.05816
aw	Equal variances not assumed			-11.500	3.200	.001	-.07667	.00667	-.09715	-.05618

Appendix F

Homogenous subsets of physio-chemical and microbiological quality of optimized jerky during storage

Table F1: Tukey HSD for change in moisture content

Tukey HSD[a]		
days	N	Subset for alpha = 0.05
		1
0 day	3	14.1400
5 day	3	14.2700
10 day	3	14.4900
15 day	3	14.7367
20 day	3	14.8867
25 day	3	15.0800
30 day	3	15.1167
Sig.		.599

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table F2: Tukey HSD for change in water activity

Tukey HSD[a]				
days	N	Subset for alpha = 0.05		
		1	2	3
0 day	3	.6267		
5 day	3	.6267		
10 day	3	.6333		
15 day	3		.6600	
20 day	3		.6733	.6733
25 day	3		.6800	.6800
30 day	3			.6967
Sig.		.965	.163	.076

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table F3: Tukey HSD for change in peroxide value

Tukey HSD[a]			
days	N	Subset for alpha = 0.05	
		1	2
0 day	3	.2367	
5 day	3	.2767	.2767
15 day	3	.2867	.2867
10 day	3	.2900	.2900
20 day	3		.3100
25 day	3		.3133
30 day	3		.3300
Sig.		.054	.054

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table F4: Turkey HSD for change in yeast and mold count

Tukey HSD[a]			
days	N	Subset for alpha = 0.05	
		1	2
0 day	3	.2367	
5 day	3	.2767	.2767
15 day	3	.2867	.2867
10 day	3	.2900	.2900
20 day	3		.3100
25 day	3		.3133
30 day	3		.3300
Sig.		.054	.054

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix G

Homogenous subsets of physio-chemical and microbiological quality of control sample during storage

Table G1: Tukey HSD for Moisture content of control sample during storage

Tukey HSD[a]

Days	N	Subset for alpha = 0.05				
		1	2	3	4	5
0 day	3	10.7800				
5 day	3		13.2233			
10 day	3			17.7300		
15 day	3				22.6267	
20 day	3					25.7300
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table G2: Tukey HSD for water activity of control sample during storage

Tukey HSD[a]						
Days	N	Subset for alpha = 0.05				
		1	2	3	4	5
0 day	3	.6133				
5 day	3		.6600			
10 day	3			.7233		
15 day	3				.7700	
20 day	3					.8167
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table G3: Tukey HSD for PV of control sample during storage

Tukey HSD[a]						
Days	N	Subset for alpha = 0.05				
		1	2	3	4	5
0 day	3	.2633				
5 day	3		.4467			
10 day	3			.6433		
15 day	3				.8233	
20 day	3					1.0700
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Table G3: Tukey HSD for yeast and mold count of control sample during storage

Tukey HSD[a]

Days	N	Subset for alpha = 0.05			
		1	2	3	4
0 day	3	.2633			
5 day	3		.4467		
10 day	3		.4467		
15 day	3			.8267	
20 day	3				1.1300
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix H

Paired Sample T-Test of Optimized and Control samples during Storage

Table H1: Paired Samples Test for moisture content of Optimized and Control samples

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	OS0 - CS0	3.38000	.12767	.07371	3.06285	3.69715	45.855	2	.000
Pair 2	OS5 - CS5	1.06000	.44643	.25775	-.04899	2.16899	4.113	2	.054
Pair 3	OS10 - CS10	-3.38000	.16093	.09292	-3.77978	-2.98022	-36.377	2	.001
Pair 4	OS15 - CS15	-7.97333	.68369	.39473	-9.67172	-6.27495	-20.200	2	.002
Pair 5	OS20 - CS20	-10.89333	.53519	.30899	-12.22283	-9.56384	-35.254	2	.001

Table H2: Paired Samples Test for water activity of Optimized and Control samples

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	OS0 - CS0	.01333	.01155	.00667	-.01535	.04202	2.00	2	.184
Pair 2	OS5 - CS5	-.03333	.01528	.00882	-.07128	.00461	-3.78	2	.063
Pair 3	OS10 - CS10	-.09000	.01000	.00577	-.11484	-.06516	-15.6	2	.004
Pair 5	OS20 - CS20	-.14333	.03055	.01764	-.21922	-.06744	-8.13	2	.015

Table H3: Paired Samples Test for PV of Optimized and Control samples

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	OS0 - CS0	-.0266	.03055	.01764	-.10256	.04922	-1.512	2	.270
Pair 2	OS5 - CS5	-.170	.03606	.02082	-.25957	-.08043	-8.167	2	.015
Pair 3	OS10 - CS10	-.35	.01528	.00882	-.39128	-.31539	-40.06	2	.001
Pair 4	OS15 - CS15	-.4833	.01528	.00882	-.52128	-.44539	-54.81	2	.000
Pair 5	OS20 - CS20	-.710	.17578	.10149	-1.14667	-.27333	-7.00	2	.020

Table H4: Paired samples test for yeast and mold count in optimized and control samples.

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	OS0 - CS0	-.02667	.03055	.01764	-.10256	.04922	-1.512	2	.270
Pair 2	OS5 - CS5	-.17000	.03606	.02082	-.25957	-.08043	-8.167	2	.015
Pair 3	OS10 - CS10	-.35333	.01528	.00882	-.39128	-.31539	-40.064	2	.001
Pair 4	OS15 - CS15	-.54000	.04583	.02646	-.65384	-.42616	-20.410	2	.002
Pair 5	OS20 - CS20	-.71000	.17578	.10149	-1.14667	-.27333	-6.996	2	.020

Appendix I

Table I ANOVA table for the model and water activity (a_w) as the response variable

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**P-value**	
Model	0.0031	14	0.0002	4.73	0.0480	significant
*Linear Mixture	0.0005	4	0.0001	2.80	0.1444	
AB	0.0018	1	0.0018	38.99	0.0015	
AC	2.249E-06	1	2.249E-06	0.0485	0.8343	
AD	0.0004	1	0.0004	8.20	0.0353	
AE	0.0002	1	0.0002	3.39	0.1249	
BC	8.887E-06	1	8.887E-06	0.1918	0.6797	
BD	0.0006	1	0.0006	12.84	0.0158	
BE	0.0003	1	0.0003	5.51	0.0658	
CD	0.0001	1	0.0001	1.65	0.2558	
CE	0.0000	1	0.0000	0.2350	0.6484	
DE	0.0003	1	0.0003	6.97	0.0459	
Residual	0.0002	5	0.0000			
Lack of Fit	0.0000	3	0.0000	0.1054	0.9495	not significant
Pure Error	0.0002	2	0.0001			
Cor Total	0.0033	19				
R^2	0.9298					
Adjusted R^2	0.7333					
Predicted R^2	-0.6690					
Adeq. Precision	8.5179					

*Interference for linear mixtures uses type I sums of squares.

Appendix J

Shelf-life study of the optimized sample (O. S.) and control sample (C. S.)

Table J1: change in moisture content, water activity, and peroxide value

Days	Moisture content		Water activity		Peroxide value	
	O. S.	C. S.	O. S.	C. S.	O. S.	C. S.
0	14.14 ± 0.52	10.78 ± 0.62	0.63 ± 0.01	0.61 ± 0.02	0.23 ± 0.03	0.26 ± 0.01
5	14.27 ± 0.58	13.22 ± 0.65	0.63 ± 0.01	0.66 ± 0.01	0.28 ± 0.01	0.45 ± 0.03
10	14.49 ± 0.71	17.73 ± 0.69	0.63 ± 0.01	0.72± 0.02	0.29 ± 0.02	0.64 ± 0.02
15	14.74 ± 0.72	22.63 ± 0.71	0.66 ± 0.01	0.77 ± 0.01	0.29 ± 0.02	0.83 ± 0.07
20	14.89 ± 0.87	25.73 ± 0.73	0.67 ± 0.02	0.82 ± 0.02	0.31 ± 0.01	1.02 ± 0.05
25	15.08 ± 0.70		0.68 ± 0.01		0.31 ± 0.02	
30	15.12 ± 0.60		0.70 ± 0.01		0.33 ± 0.02	

Table J2: Change in YMC during storage

Days	YMC (log cfu/ml)	
	O. S.	C. S.
0	0.24 ± 0.03	0.26 ± 0.01
5	0.28 ± 0.01	0.45 ± 0.02
10	0.29 ± 0.02	0.64 ± 0.02
15	0.29 ± 0.02	0.83 ± 0.06
20	0.31 ± 0.01	1.13 ± 0.07
25	0.31 ± 0.02	
30	0.33 ± 0.02	

Appendix K

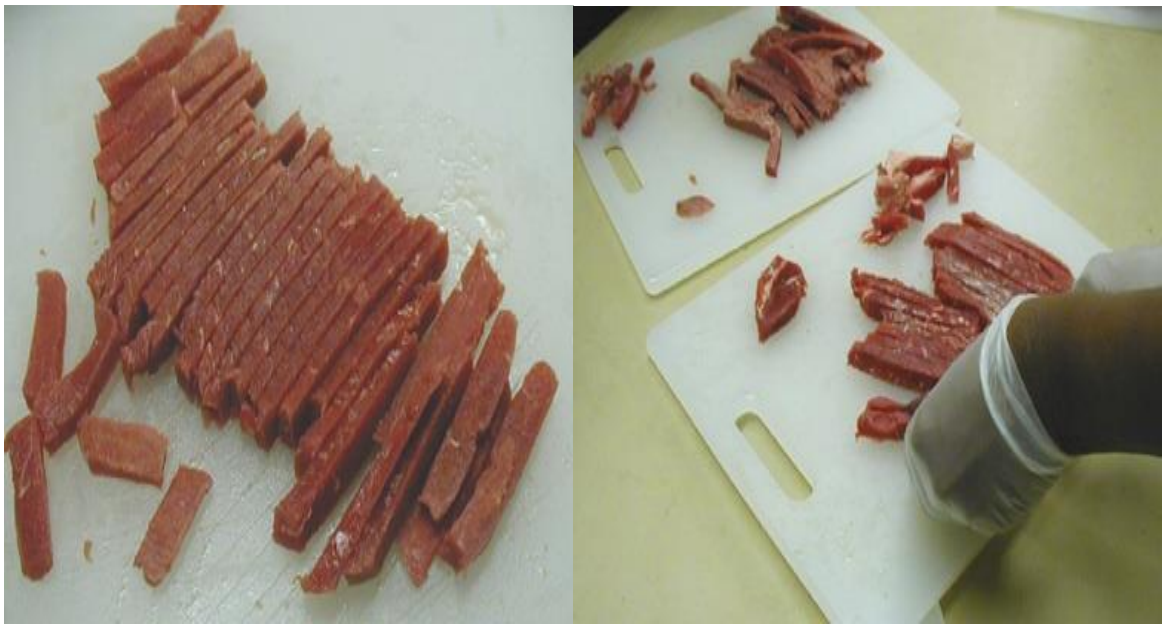

Plate 1 Meat strips preparation

Plate 2 Drying of jerky after marination with humectants mixture

Plate 3 Humectants infusion along with other jerky manufacturing food ingredients

Plate 4 Dried buffalo jerky type intermediate moisture meat

Plate 5 Storage of buffalo jerky

Plate 6 Water activity measurement of the samples

Plate 7 Sensory analysis of buffalo jerky

Plate 8 Crude protein determination by Kjeldahl method

Plate 9 Storage stability study

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