CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

ANALYSIS OF SELECTED PARAMETERS AFFECTING THE QUALITY OF DRIED RABBIT MEAT

MASTER'S THESIS

Prague 2023

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Declaration

I hereby declare that I have completed this thesis entitled "**ANALYSIS OF SELECTED PARAMETERS AFFECTING THE QUALITY OF DRIED RABBIT MEAT**" independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague, April 2023

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Oyinkansola Damilola Folarin

Acknowledgements

I would like to express my heartfelt gratitude to God Almighty for his assurance and faithfulness in my academic journey (Phillipians 1:6) and to all those who have supported me throughout this process: To my very kind and brilliant supervisor, Dr Klara Urbanova, thank you for your unwavering support throughout this process, you cared so much about my work and was always available. I want to thank you for taking the time to explain things thoroughly, for answering any question I had, and for constructive criticism and ongoing guidance. Thank you, you really are a gem.

I want to thank the Czech Government for this opportunity, all my professors in the Department of Sustainable Technologies and the Department of Animal Science and Food Processing at FTA, for all the lectures and support I have received throughout my studies and the course of my research.

I must express my gratitude to my parents; Mr and Mrs Folarin for their constant support in all ramifications, my siblings; Lanre, Kofo and Jide, Mr Adedapo Orekoya, Mr Salufu Kola for their kindness and help with this work, and to all my friends and loved ones for their emotional support.

Abstract

In a bid to cater for the growing interests of consumers for nutritive, wholesome, and safe ready-to-eat rabbit meat products via meat drying techniques, a total of 20 rabbit legs were used to compare the physiochemical characteristics, volatile compounds, fatty acid profile and sensory quality of rabbit meats subjected to different drying temperatures. The meats were cut, dried at different temperatures (40, 60, 80, 100 and 120 °C, respectively) in a controlled drying chamber. Afterward, meats were allowed to cool and used for physiochemical analysis. Results showed the lowest meat pH and highest L* were observed in samples dried at 40 °C. However, highest a* and b* values were recorded in samples dried at 100 and 120 °C. Water activity was lowest in samples dried at 120 °C. Moreover, 25 volatile compounds were identified with Hexanal being the most abundant compound, most present in meat sample dried at 100 °C. Octadecenoic acid and Hexadecanoic acid were the first and second most abundant fatty acids identified were highest in meats dried at 60 and 40 °C, respectively. Sensorial descriptors such as pleasantness of smell and intensity of colour were highest in meat dried at 120 °C. However, excellent scores for juiciness and chewiness were recorded in samples dried at 40 °C. Therefore, rabbit meats dried at 40 °C have improved shelf life with less effect on the protein structures, are easy to chew, and juicy with less off-flavors along dried meat edges.

Key words: Rabbit meat; Fatty acids; Volatile compounds; SPME; GC-MS

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List of the abbreviations used in the thesis

ALA: α-linolenic acid

a_w: Water activity

DHA: Docosahexaenoic acid

DHE: Dynamic headspace extraction

EPA: Eicosapentaenoic acid

FAME: Fatty acid methyl ester

FAOSTAT: Food and Agriculture Organization Corporate Statistical Database

FFA: Free fatty acids

FTIR: Fourier transform infrared spectroscopy

GC-MS: Gas Chromatography - Mass Spectrometry

HPLC: High-performance liquid chromatography

HPLC–MS: High-performance liquid chromatography-mass spectrometry

HPSEC: High-performance size exclusion chromatography

HS-SPME: Headspace-solid phase microextraction

KI: Kovats Index

LCFA: Long-chain fatty acids

MCFA: Medium-chain fatty acids

MUFA: Monounsaturated fatty acids

MW: Molecular weight

NIR: near-infrared spectroscopy

NIST: National Institute of Standards and Technology

NMR: Nuclear magnetic resonance

P&T: Purge and trap

PUFA: Polyunsaturated fatty acids

RI: Retention index

RT: Retention time

SCFA: Short-chain fatty acids

SD: Strecker degradation

SDE: Simultaneous distillation and extraction

SFA: Saturated fatty acids

SPME: Solid-phase microextraction

TAG: Triacylglycerol

TBARS: Thiobarbituric acid reactive substance

UFA: Unsaturated fatty acids

USV: Ultrasound-assisted vacuum drying

VLCFA: Very-long-chain fatty acids

1. Introduction and Literature Review

1.1 Introduction

Rabbit meat has been an important delicacy for people in many European countries as well as the Mediterranean areas and other parts of the world. In the past 50 years, global rabbit production has increased 2.5 fold with China, Italy, Spain and France being the major rabbit meat producers (FAOSTAT, 2010). Rabbit meats are reared systematically on a large scale reaching about 1.8 million tonnes annually (FAOSTAT, 2012). From the nutritional point of view, rabbit meat contains high levels of protein and lower cholesterol content when compared with meats from other livestock (Dalle Zotte & Szendro 2011). Moreover, rabbit meat is often considered a functional food rich in docosahexaenoic acid (DHA), and low n-6/n-3 ratio (Dalle Zotte & Szendro 2011). This is the reason why rabbit meat is easily digested when compared with meats from other livestock (beef, lamb, or pork) (Enser et al. 1996) and is recommended for consumption, especially for persons with cardiovascular illnesses, type II diabetes and cancer (Hu & Willett 2002). Rabbit meat has a strong appeal across many ethno-religious groups and has become a common alternative to poultry in many new recipes. Thus, to cater for the growing interests of consumers for nutritive, wholesome, and safe ready-to-eat rabbit meat products, meat drying; an operation whereby heat penetrates into the product and causes moisture transfer from within the food product to its surface with subsequent evaporation to the air stream as vapour has been adopted over the years (Mewa et al. 2018).

Drying is one of extensively used techniques of food preservation that inhibits the deterioration of perishable foods and ensuring their availability all year round, especially during periods of scarcity. The use of different preservation methods and technologies usually results in certain physiochemical changes that modifies tissue structure in meat, thereby influencing sensory characteristics (such as appearance, aroma, color, taste, texture, and volume) of the final product, reduced surface moisture due to dehydration, enhanced functional properties of proteins and increases moisture and fat retention due to protein denaturation, (Beriain et al. 2011; Gómez et al. 2020). When thermal treatment is applied, chemical changes in meat occur due to molecular interactions such as denaturation, hydrolysis, and gelation suffered by proteins may be responsible for changes in nutritional value and consumer acceptance (Diéguez et al.

2010). Studies concerned with the rabbit meat quality have focused largely on parameters such as colour, pH, and water holding capacity (Pla et al. 1998; Hernández et al. 2000). Fatty acid profile of rabbit meat (Cambero et al. 1991; Ramírez et al. 2005), mineral composition (Hermida et al. 2006), as well as organoleptic properties, for example; appearance, flavour, texture, and overall acceptability are also available (Hernández et al. 2000; Dalle Zotte 2002; Polak et al. 2006; Ariño et al. 2007). However, dearth of information exists on studies concerning the impact of drying on quality attributes of rabbit meat. Based on these backgrounds, the comparison of physical and chemical qualities of rabbit meat under different drying temperatures was carried out in this study.

1.2. Literature review

1.2.1. Rabbit meat quality

Most rabbit meats are sold as a whole carcass, retail cuts, and ready-to-cook meat products, consequently, many elements of both carcass and meat qualities have to be considered. According to the safety and quality standards of the World Rabbit Science Association, the most commonly measured characteristics of body composition are dressing percentage, proportions of fore, intermediate and hind parts, carcass fatness estimated by dissectible fat weights relative to carcass weight, and muscle to bone ratio assessed in the hind leg (Blasco & Ouhayoun 1996). Meat quality is usually assessed via a mixture of physiochemical and histologically determined traits measured instrumentally in raw or cooked meat, as well as scores attributed by trained panelists. It encompasses the measurement of parameters such as water-holding capacity, colour assessed on fresh-cut surface, cooking loss, chilling loss and pH as a value that predicts technological and eating quality of the meat. According to report by Dalle-Zotte (2002), sensory properties majorly influence consumer's preferences in meat and meat products, especially regarding flavor and tenderness. Rabbit meat tenderness can be evaluated both instrumentally and sensory evaluation. In addition, the size, number, and type frequency of the muscle fibers could possible affect meat quality attributes. Correlations between fat content in rabbit meat and cooking losses and/or sensory descriptors have remain somewhat controversial according to researchers (Gondret et al. 1998; Hernandez et al. 1998; 2000), however, contents and composition of intramuscular fat of meat are important actors for eating quality.

1.2.2 Nutritional quality of rabbit meat

Amongst the copious factors determining quality and consumer acceptability of meat and meat products, the nutritive value of meat has growing importance. Meat serves as a major source of proteins, essential amino-acids, saturated fatty acids, for which a high consumption may be related to chronic non-deficiency diseases, e.g., obesity, cardiovascular diseases and type II diabetes. However, the nutritional value of rabbit meat has been reviewed (Combes 2004; Dalle Zotte 2004; Combes & Dalle Zotte 2005), showing that rabbit meat is rich in nutrients when compared with meats from other livestock. Rabbit meat contains 20–21 % of proteins, energy value of 427–849 kJ/100 g of fresh meat, unsaturated fatty acids (oleic and linoleic; 60 % of all fatty acids), minerals (magnesium, potassium, selenium, and phosphorus), low concentrations of fat, cholesterol, and sodium (Bielanski et al. 2000; Dalle Zotte 2002; Hermida et al. 2006). The fat content of rabbit meat varies depending on the part of the carcass studied (Pla et al. 2004) and different productive factors (Dalle Zotte 2002). Chemical composition of rabbit meat can also be influenced by genetics (Pla et al. 1998; Hernández et al. 1998), and age (Gondret et al. 1998; Hernández et al., 2004), but it is hardly affected by gender (Pla et al. 1996). In addition, nutrition plays a major role in the chemical composition of rabbit meat, particularly in its lipid composition (Dalle Zotte 2002). Finally, rabbit meat is tender as a result of its low elastine content (Ouhayoun & Lebas, 1987) and the high solubility of its collagen when compared with meats from other farm animals (Combes et al. 2003).

Rabbit meat fat contains mostly 36.90 % saturated fatty acids (SFAs), 34.60 % polyunsaturated fatty acids (PUFAs), and around 28.50 % Monounsaturated fatty acids (MUFAs) (Pla et al. 2004). The most abundant fatty acids are oleic (C18:1), palmitic (C16:0), and linoleic (C18:2) acids, showing percentages higher than 20% of total fatty acids. Among the polyunsaturated fatty acids (PUFAs), linoleic (C18:2) and linolenic (C18:3) are essential fatty acids since the animals are unable to synthesize them. Linoleic acid is the precursor of ω6 family of PUFA, while linolenic acid serves the same function for the ω3 family, especially for eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids. For those with cardiovascular health issues, 500 mg/day of combined EPA and DHA is recommended (ISSFAL 2004). The quantity of linoleic fatty acid in rabbit meat is around ten times greater than in beef and mutton and double the amount reported for pork (Enser et al. 1996). Linolenic acid is also abundant

in rabbit meat (3%) when compared with other meats; 1.37 % in mutton, 0.70 % in beef and 0.95 % in pork (Enser et al. 1996). Though rabbit meat has a very low quantity of DHA and EPA (Ramírez et al. 2005), Dal Bosco et al. (2004) confirmed the capability of rabbit tissues to produce these fatty acids from dietary linolenic precursor. In addition to fatty acids, cholesterol content of rabbit meat is around 59 mg/100 g of muscle (Combes 2004), and even lower values (45 and 50 mg/100g) were recorded for as *Longissimus dorsi* and *Psoas major* muscles, respectively (Alasnier et al. 1996). These values are much lower than those presented in meat from other farm animals; 61 mg in pork, 81 mg in chicken, and 70 mg in beef (Dalle Zotte 2004).

Moreover, rabbit meat is rich in phosphorus (230 and 222 mg/100g for the hind leg and loin, respectively) but has low concentrations of sodium (49 and 37 mg/100g for the hind leg and loin, respectively) and iron (1.3 and 1.1 mg/100g for hind leg and loin, respectively) (Combes 2004). Lombardi-Boccia et al. (2005) also found that rabbit meat has a low zinc concentration (0.55 mg/100g) and Skřivanová et al. (2002) observed copper concentrations of 0.048 and 0.035 mg/100 g in the hind leg and loin of rabbit, respectively. In general, rabbit meat showed a high retention of those minerals after cooking (Lombardi-Boccia et al. 2005). The selenium concentrations in rabbit meat vary between 9 µg/100g (Díaz-Alarcon et al. 1996) and 22 µg/100g (Wiesner et al. 1978). Furthermore, daily consumption of 100g of rabbit meat contributes 8 % of vitamin B2, 12 % of vitamin B5, 21 % of vitamin B6, 77 % of vitamin B3, and fulfillment of the daily vitamin B12 requirement (Combes 2004). In addition, rabbit meat contains trace amounts of vitamin A. Nevertheless, edible rabbit liver contains high amounts of this vitamin (Ismail et al. 1992). Dietary supplementation of 200 mg/kg vitamin E to improve oxidative stability resulted in an increase of almost 50 % of vitamin E in rabbit meat (Castellini et al. 2000).

1.2.3 Meat Drying

Although fresh meat is a rich source of protein and it is very fast in spoilage. Thus, meat processors from all over the world have over the years adopted meat drying as a sustainable technique towards preserving and enhancing the flavors of meat. Drying as demonstrated in Figure 1 is a unit operation whereby heat penetrates into the food product and causes transfer of moisture from within the product to its surface with

subsequent evaporation to the air stream (Tulek 2011). Heat drying is an ancient food preservation technique used to extend food shelf life. The removal of moisture from food consists of two basic phenomena; vaporization of moisture from the surface of the material and movement of moisture from the interior of the food to its surface as a result of diffusion, cell contraction and vapour pressure gradient. Drying removes the moisture from the food to inhibit the spoilage actions of microorganisms (bacteria, yeast and mould) and slows down enzymatic reactions, but does not inactivate them. It makes handling easier by reducing the size, weight, and risk of microbial contamination of foods as well as reduces transportation and storage costs (Akhtar and Pandey 2015).

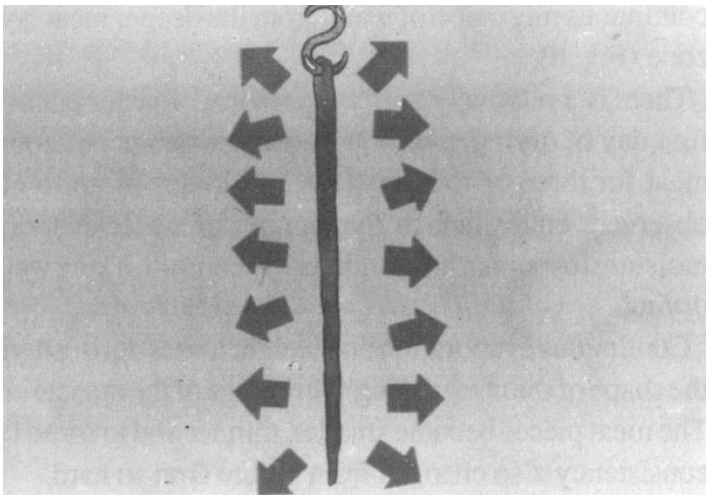


Figure 1: Dehydration process of a piece of meat suspended under drying conditions (schematic)

1.2.3.1 Benefits of meat drying

One of the health benefits of dried meat is that it maintains the sought-after protein from fresh meat thereby making it easier to store and usually in ready-to-eat packages for consumers. Carnosine and anserine are dipeptides that are commonly found in fresh meats are found in dried meat products as well (Zdanowska-Sasiadek et al. 2018). Carnosine is related to cardiac cell contractions and possesses great potential in heart failure treatment (Aristoy et al. 2018). Anserine is a derivative of carnosine that can be readily transformed back to carnosine through multiple enzymatic reactions (Aristoy et al. 2018). Dried meat products are also rich in α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Zdanowska-Sasiadek et al. 2018). These three fatty acids prevent cardiovascular diseases (Fleming & Kris-Etherton 2014), while

DHA aids brain development (Zdanowska-Sasiadek et al. 2018; Kris-Etherton et al. 2007; Jasinska & Kurek 2017). Since iron is compulsory for oxygen transfer in the human blood circulatory system, dried meat contains abundant minerals, especially iron, zinc, and magnesium (Zdanowska-Sasiadek et al. 2018). However, it was found that higher temperatures reduce the levels of available iron in dried meats; hence lower temperatures were recommended for the drying process. In addition, zinc and magnesium, which serve significant purposes in human health are high in dried meat products (Djinovic-Stojanovic et al. 2017). Zinc regulates antioxidant and anti-inflammatory pathways and supplementary doses of zinc are aid in treatment of liver disease, cancer, and heart disease (Santos et al. 2020). Therefore, the presence of zinc and magnesium in dried meat products is relieving consumers of the health benefits that come with the micronutrients. In general, dried meat products bring a multiplicity of health benefits with the level of beneficiaries differing amid the kind of meats used.

1.2.3.2 Technologies for drying meat and meat products

In orthodox drying processes, nutrients (vitamins and amino acids), color, and flavor, are lost as a result of thermal deterioration which reduces the drying rate and rehydration ratio (Doymaz et al. 2016). Drying influences the bulk densities and porosity structure of dried meats (Laopoolkit & Suwannaporn 2011), therefore loss of water and application of heat can lead to the formation of pores and shrinkage which in turn impact the taste and texture of the meats (Koc et al. 2008). Thus, advanced technologies such as vacuum drying, ultrasound-assisted vacuum drying (USV), and freeze-drying have been adopted for effective drying and production of higher quality dried meat products (Aksoy et al. 2019).

In vacuum-drying (Figure 2), discoloration and loss of nutritional components and flavor can be avoided due to the low temperature and absence of oxygen used to preserve heat-sensitive and rapidly oxidizable foods (Tekin & Baslar 2018). However, the vacuum-drying does not entirely prevent the quality loss in dried meat (Aksoy et al. 2019). On the other hand, the USV technology combines vacuum drying and ultrasonic treatment to produce consistent drying and allows the process to be accomplished in a shorter time. The mechanical waves created by ultrasonic treatment contribute to the movement of heat and water from within the meat to the surface. Additionally, the pressure and frequency of ultrasonic waves significantly influence the transfer rate

(Tekin & Baslar 2018). To ensure meat dries safely, air pressure should be kept very low with the boiling point of water less than 40 °C (Chibuzo et al. 2021). Another mechanism used during ultrasonic drying is cavitation, which speeds up water transport via removal of tightly bound water. Ultrasonic modifications in meat drying ensure a higher drying rate, prevents food quality loss, and decrease energy consumption and process cost (Tao & Sun 2015).

Freeze-drying is another drying method based on a three-state transition of water (Figure 3). The triple point temperature of the water is 0.0098 °C, and the pressure is 4.579 mmHg (Liu et al. 2022). In this process, water in food is first frozen at a low temperature and instantly sublimated from solid to gas in a vacuum. As a result, freeze-dried foods retain their nutrient composition and this drying technique does not denature protein or promote vitamin loss (Ma et al. 2018). It also preserves the original appearance, aroma, color, and flavor, of dried meat to the maximum degree possible while protecting its composition (Berk 2013). Freeze-dried products have low moisture content and can be reconstituted if need be with a high rehydration speed (Liu et al. 2022). Moreover, dried meat products with a porous structure and good rehydration capabilities can be achievable using freeze-drying methods (Sagar & Suresh 2010).

Furthermore, optimizing drying temperature is essential when choosing the proper drying techniques for meat and meat products. At high temperatures, drying periods should be reduced through rapid heat and moisture transfer. On the other hand, high temperature may increase in shrinkage rates, breakdown of proteins and vitamins, as well as decline color quality and rehydration capacity (Baslar et al. 2014; Xu et al. 2019). Although the drying period may be prolonged, low-temperature drying methods, such as freeze-drying, are recommended for meat drying as it prevents biochemical and microbiological degradation (Kilic 2009).

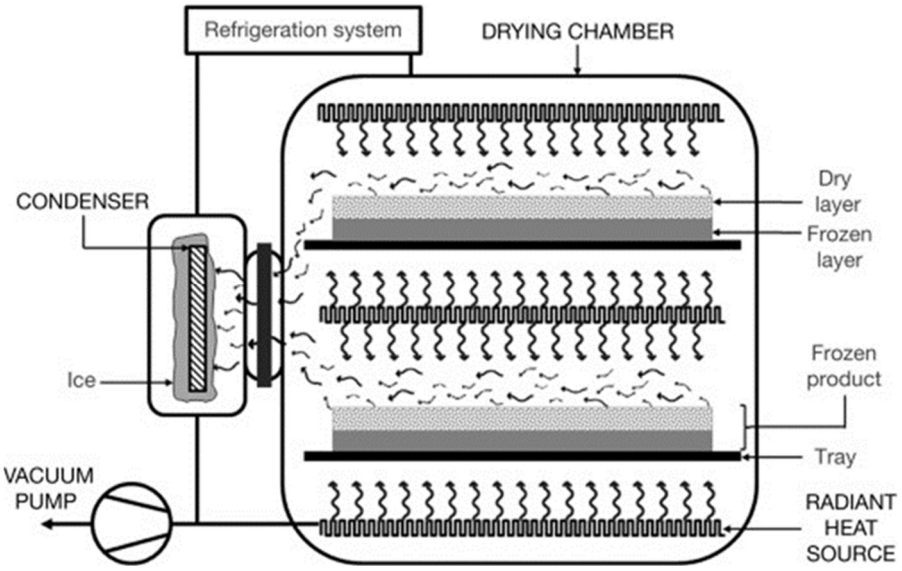


Figure 2: Vacuum dryer

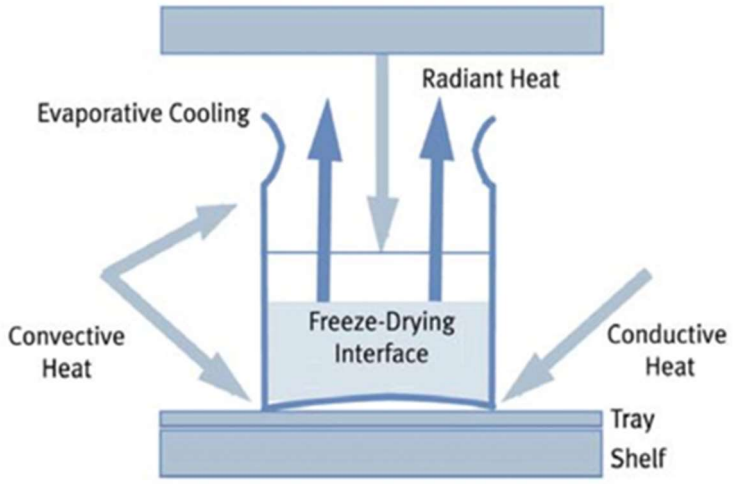


Figure 3: Heat transfer in a shelf freeze dryer

(Source: https://www.spscientific.com/freeze-drying-lyophilization-basics/)

1.3 Impact of drying on meat quality

Meat drying is a process that involves the removal of moisture from meat surfaces to obtain an ideal condition for dried meat product preservation. Drying conditions should be meticulously well thought-out as they influence meat quality attributes after going through some processes which may alter the condition of the meats. Fundamental factors for drying such as temperature, relative humidity, drying period, and water content are will affect the quality of the dried meats (Mishra et al. 2017). A lot of nutritional loss, which includes degradation of vitamins and amino acids, as well as flavor and color loss, occur due to thermal degradation that decreases the drying rate and rehydration ratio when using traditional thermal drying techniques (Doymaz et al. 2016). The benefit of adopting high temperature is that less drying period will be required because of more rapid heat and moisture transfer, however high temperature may deteriorate muscle proteins and vitamins (Aksoy et al. 2019). In contrast, low temperatures are recommended for drying as it will preserve heat-sensitive and easily oxidized foods (Aksoy et al. 2019), and reduce biochemical and microbial decomposition (Kilic 2009). However, it is time-consuming as the drying duration will increase. Therefore, it is best to choose a drying temperature and drying duration that do not compromise a product's physicochemical, nutritional, or sensory qualities.

1.3.1 Physicochemical characteristics

The physicochemical properties are essential indicators such as pH, color, water activity, lipid oxidation, proximate composition, and sensory characteristics used in assessing the role various drying techniques has on quality of dried meat.

1.3.1.1 Meat pH

Meat pH directly impacts the meat's functional attributes, eating and storage quality (Mishra et al. 2017). The pH of dried meats ranges between pH 5.4–5.8 (Lim et al. 2012). Low pH is essential to avoid protein denaturation of the meat. Different processes of dried meat revealed freeze-drying produced the lowest pH at 5.89 in contrast with air-drying method with high pH of 6.08. The variation in pH values was caused by the loss of the free acidic group as the result of different drying techniques (Mishra et al. 2017). In addition, a study by (Lim et al. 2012) discovered that hot air-dried jerky had a higher pH than sun-dried and shade-dried. The air-dried method

obtained a pH of 6.25, while both the sun-dried and shade-dried jerkies recorded pH of 6.03. The elevated pH values may be due to beef protein denaturation during drying (Lim et al. 2012).

1.3.1.2 Color

Color is one of the major attributes in food evaluation since consumers may instantly appraise it. Lightness (L), redness (a), and yellowness (b) which are sources of light scattering variations from meat surfaces have been revealed to differ during drying (Elmas et al. 2021). Depending on the drying methods used, the L value of dried meats was within the range of 20–50, a value was between 6 - 10, and b value was around 10–20 (Afifah et al. 2021). Furthermore, different drying techniques have an impact on the color of the products as well. Freeze-dried meat products had whiter color compared to meat products that were sun, air, or vacuum-dried. This color variation is primarily due to uniform light reflection from the surface of large pores (Afifah et al. 2021). Additionally, freeze-dried meats had higher L, a, and b values than other drying techniques, indicating that freeze-drying had less effect on the protein structures of meat samples when compared to sun-drying methods with darker brown color indicating browning effects (Mishra et al. 2017). Nevertheless, L and b values for air-dried meat were 27.74 and 11.50, respectively, which was generally higher than 25.91 and 7.95, respectively measured in sun-dried meats (Dinçer 2021).

1.3.1.3 Water activity

The water activity coefficient, a measure of the thermodynamics chemical potential of water in the system, expresses the condition of water in the food (Aksoy et al. 2019). The water activity (a_w) is defined as the ratio of the vapor pressure of water in food (p) to the vapor pressure of pure water (p0) at the same temperature (Mishra et al. 2017). In dried products, a_w is often less than 0.7 as it is responsible for microbiological stability to inhibit microbial growth (Taormina & Sofos 2014). In the study of beef jerky, air-dried and sun-dried samples achieved low a_w with values of less than 0.75 (Lim et al. 2012). Another study on dried biltong indicated low a_w ranging from 0.65 to 0.68 (Petit et al. 2014). The addition of additives such as sodium chloride (NaCl) in the meat prior to drying may also lower a_w levels (Petit et al. 2014). Moreover, the presence of active

water may be more important for food stability than the overall quantity of water in the food (Taormina & Sofos 2014).

1.3.1.4 Lipid oxidation

Lipid oxidation, also known as autoxidation or peroxidation is a process that can occur through auto-oxidation (production of free radicals in the dark at room temperature through the), photo-oxidation (occurs in the light) and by enzymatic action (such as lipase) thereby causing loss of nutritional value, flavour deterioration (rancidity), loss of colour, functional property changes, as well as formation of toxic compounds in meat and meat products (Saxby 1993; Hamilton 2003; Min and Ahn 2005). Lipid oxidation can modify color, aroma, texture, and nutrient composition of dried meat (Mishra et al. 2017). The oxidation of lipids may be related to antioxidant degradation, protein denaturation, and enzymatic reactions (Amaral et al. 2018). The level of ferrous ions in dried meat is substantially raised up after drying as a result of increase in non-heme iron and breakdown of heme pigments enabling auto-oxidation and resulting in rancidity (Mishra et al. 2017). The thiobarbituric acid reactive substance (TBARS) is mostly used to evaluate the degree of lipid oxidation in meat products (Lim et al. 2012). Dried meats have higher TBARS (8.33 mg/g) than fresh (0.11 mg/g) and smoked (0.21 mg/g) meats (Mishra et al. 2017). TBARS is higher in dried meat products because of processing activities such as mincing and mixing during drying which result in an extensive breakdown of cellular structures that allow the fusion of meat and pro-oxidants (Mishra et al. 2017). Sun-dried meats produced lower TBARS (0.77 mg/kg) than 0.68 mg/kg recorded in air-dried meat suggesting that the sun-dried meat is more susceptible to lipid oxidation (Lim et al. 2012). However, TBA progress can be delayed in dried meat products with low-fat content meat and under good storage environment (Mishra et al. 2017). Moreover, the peroxide dried meat products increased (83.3 mEq/kg) by using freeze-drying methods, whereas air-dried meat was lower (20.8 mEq/kg) (Dinçer 2021).

1.3.1.5 Sensory characteristics

Sensory qualities involve assessing descriptors such as taste, tenderness, color, juiciness, and overall attractiveness of meat to predetermine consumer preferences (Jalarama et al. 2013). When assessing dried meat, the lower the score in sensory evaluation the less preferable the method of drying is. Sensory attributes of air-dried

meat products decline as storage time increased when compared to sun-dried meat products. Lim et al. (2012) conducted a study on beef jerky using the air-drying at 80 °C for 4 h and sun-drying at 25–28 °C for 3.5 h. Although, there were no apparent differences in color and flavor, the authors concluded that air-dried beef jerky scored lower in consumer's overall acceptability than sun-dried meat. However, the sun-dried beef jerky exhibited greater tenderness and juiciness compared to the air-dried beef jerky (Lim et al. 2012). Flavor and texture scores of sun-dried meat products are comparatively higher when compared to meats from oven-drying and air-drying methods (Mishra et al. 2017). In air-dried meat, the high temperature from hot air makes the meat hard, more chewy and with off-flavors along the edges and corners of the meat.

1.3.2 Volatile compounds in meat

Post-mortem processes commences instantly after an animal is slaughtered, thus generating great amount of volatile compounds such as aliphatic aldehydes, alcohols, acids, ketones, and nitrogen- and sulfur-containing compounds as the lipid fraction of meat, particularly phospholipids, undergoes autoxidation phenomena (Mottram 1998). Although fresh meat is usually bloody, weakly-flavored, with faintly sweet aroma, it is still a rich source of compounds that can serve as precursors of volatile compounds (Soncin et al. 2007). The creation of volatile compounds has also been linked deterioration of meat especially during refrigeration (Vinauskiene et al. 2002), breed and farming system (Elmore et al. 1999; Cameron et al. 2000). However, lipids are conceivably the most significant precursor of volatile compounds in meat (Gray et al. 1996; Ahn et al. 1997). Heat treatment of lean meat startup chains of reactions such as lipid oxidation, maillard reactions, interactions between products of lipid oxidation and products of maillard reaction along with thiamine degradation that eventually impart the characteristic flavor of meat (MacLeod 1998; Warris 2000). The species-specific flavor of heat-treated meat products is caused by mixtures hundreds of volatile compounds, for example, in cooked beef, 880 volatiles were documented (Mottram, 1994). During thermal processing of meat, many volatiles belonging to a number of chemical classes such as aldehydes, alcohols, carboxylic acids, thiazoles, pyridines, hydrocarbons, pyrazines, thiophenes, lactones, oxazoles, ketones, thiazolines, esters, pyrroles, furans, pyrans, phenols, and other nitrogen or sulfuric compounds are produced (Kosowska et al. 2017). Thus, the impact of a single volatile compound in developing the

characteristic flavor may vary and a small part of these varieties of volatile compounds contributes towards flavor development.

Table 1: Main reactions of secondary aroma-active compound formation

Main classes of compounds produced	Reactions
Acids, alcohols	Hydrolysis
Aldehydes, ketones, hydrocarbons, lactones	Oxidation
Heterocycles, hydrocarbons	Pyrolysis
Amines, hydrocarbons, ketones	Decarboxylation
Esters, acids, acetals	Fermentation
Aldehydes, heterocycles	Strecker degradation
Heterocycles, aldehydes	Maillard reactions

Source: (Davidek et al. 1990)

Table 2: Main reactions leading to aldehyde formation

Precursor	Type of Reaction
Unsaturated lipids	Autoxidation and lipoxygenase-catalyzed oxidation
Amino acids	Strecker degradation, oxidative deamination
Saccharides	Non-enzymatic browning reactions, reverse aldolization
Phenolic substances	Enzymatic reactions
Primary alcohols, cyclitols	Free-radical or enzymatic oxidations
Acetals	Hydrolysis
Hydroxy acids	Decarboxylation

Source: (Davidek et al. 1990)

Table 3: Main reactions producing hydrocarbons

Precursor	Type of Reaction
Terpenes	Enzymatic reactions
Fatty acids, esters	Oxidation reactions
Various aromatic, alicyclic substances	Pyrolysis
Secondary alcohols, sterols	Dehydration
Carboxylic acids	Decarboxylation

Source: (Davidek et al. 1990)

Table 4: Formation of heterocyclic aroma compounds

Precursor	Type of Reaction	Products
Monosaccharides, oligosaccharides	Pyrolysis (caramelization, roasting) Maillard reactions	Furans and pyrans
Amino acids, peptides, proteins	Pyrolysis (frying, roasting) Strecker degradation Maillard reactions	Pyrroles, pyrazines, piperazines, imidazoles
Fatty acids, lipids	Oxidation	Furans and pyrans
Sulphur-containing amino acids	Pyrolysis (frying, roasting)	Thiophens, thiazoles, thiolans, trithians

Source: (Davidek et al. 1990)

1.3.2.1 Strecker degradation

Strecker degradation (SD) plays a number of roles in the production of flavour compounds in foods especially processed foods. It is the major predominant pathway for amino acids transformation into structurally related aldehydes of important flavour value. Correspondingly, SD make available a comparatively low energy path for mobilizing amino acids' nitrogen and sulfur to form ammonia, hydrogen sulfide and other important S/N/O-containing heterocyclic compounds with flavor characteristics. Furthermore, SD provides a reduction mechanism for conversion of dicarbonyls into acyloins thereby responsible for diverse flavour compounds formation (Rizzi, 1999).

1.3.2.2 Maillard reaction

Maillard reaction, also termed browning reaction is an important flavour-producing reaction in meat during cooking. The chemical mechanisms involve the condensation of the carbonyl group of the reducing sugar with the amino compound to give a glycosylamine. During thermal processing of meat products, reaction between reducing sugars in connective tissues and muscle proteins provide the basis for the colours and aromas which characterize cooked foods (Forrest et al. 1975; Mottram 2007). In a study by Estevez et al. (2003) involving the SPME-GC-MS analysis of volatiles in meat from pigs after refrigeration and heat-treated, methyl alcohols and ketones (such as 2-methyl-butan-1-ol, 2-ethyl-hexan-1-ol, 3-hydroxy-butan-2-one and 3-methyl-butan-1-ol) were the most representative in refrigerated meat due to the degradation of carbohydrates and proteins together with the Strecker degradation pathway while lipid-derived volatiles were the most abundant in heat-treated meat and refrigerated cooked meat. Another study by Olmo et al. (2014) on the effect of high-pressure-processing and modified-atmosphere-packaging on the volatile compounds and odour characteristics of sliced ready-to-eat cured-cooked pork meat product revealed vacuum-packaged meat products had high levels of esters, alcohols, acids and benzenic compounds until day 120 while ketones and sulphur compounds peaked on day 60 and declined afterwards. Kang et al. (2013) also detected off-flavour volatiles such as 9,12-octadecadienoic acid and octadecanoic acid in Korean native black goat meat under high pressure processing.

1.3.3 Extraction methods of volatile compounds

Certain methods have been investigated globally by researchers concerning the extraction methods of volatile compounds from meat, based on time, money, as well as type of sample and solvent. Thus, the choice of extraction technique is significant since its effectiveness could influence the chemical identification vividly (Lin 2014). Extraction methods such as simultaneous distillation and extraction (SDE), dynamic headspace extraction (DHE), purge and trap (P&T), and solid-phase microextraction (SPME) are well known developments in sample preparation (Xu et al. 2016). However, SPME has been demonstrated to be useful in the extraction of volatiles meat and meat products due to its high flexibility and one-step combination of sampling, isolation, concentration, and enrichment. SPME is a simple and swift solvent-free extraction method to analyze gaseous, liquid and solid samples which can be routinely used in combination with the GCMS system (Merkle et al. 2015; Vas and Vékey 2004). The

two major parameters that influence extraction efficiency are extraction temperature and time, as high temperature will speed up the equilibrium so as to reduce the required extraction time (Perestrelo et al. 2011).

Previous studies on the evolution of volatile compounds extraction methods such as the findings of Rivas-Canedo et al. (2011) compared DHE and SPME for analyzing volatile profile in cooked beef observed SPME with a 50/30 µm DVB/CAR/PDMS fibre was more effective in extracting compounds such as ethyl esters, 1-alcanols, and acids. Argyri et al. (2015) also reported that HS-SPME-GC/MS analysis provided valuable data about quite a number of volatile metabolic compounds detected during meat storage. In another study by Rivas-Canedo et al (2012), SPME was more efficient extraction method for a great amount of chemical compounds especially fatty acids in sliced cooked pork and low-acid fermented sausage. Acevedo et al. (2012) also found out 65 µm PDMS/DVB and 50/30 µm DVB/CAR/PDMS used GC/MS-SPME were the most suitable fibres for extracting volatile compounds in beef. Furthermore, Ma et al. (2013) heightened HS-SPME for GC/MS analysis of aroma compounds in cooked beef with the authors recommending HS-SPME should be carried out for 25 minutes at 40 °C with 10 minutes equilibrium time for optimal concentration.

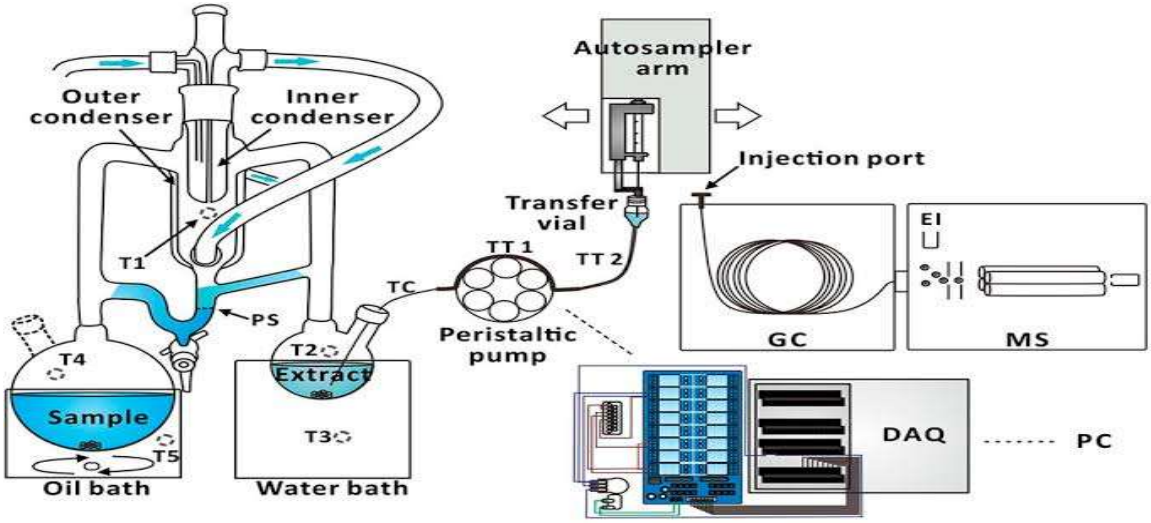


Figure 5: Schematic diagram of simultaneous distillation and extraction (SDE)

Source: Liao et al. (2020)

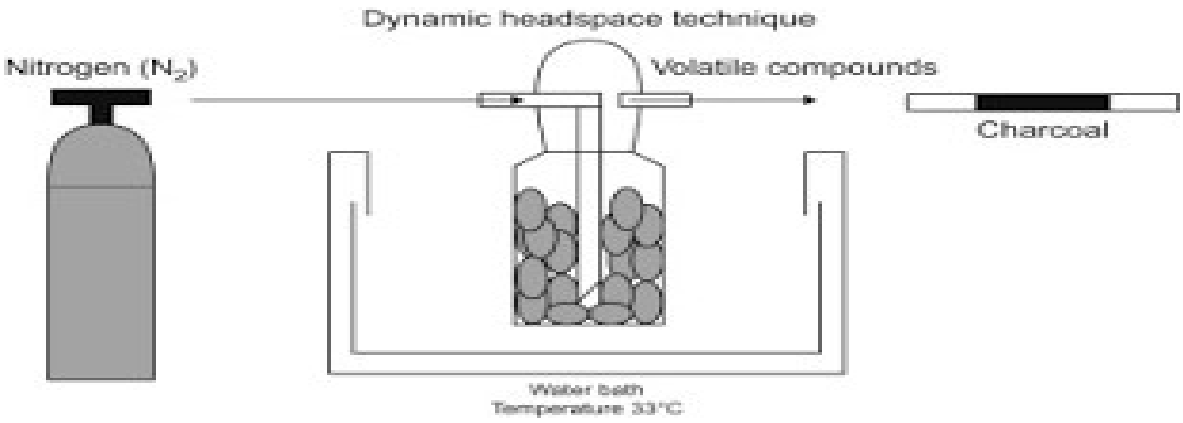


Figure 6: Schematic diagram of dynamic headspace extraction (DHE)

Source: Sabatini & Marsilio (2008)

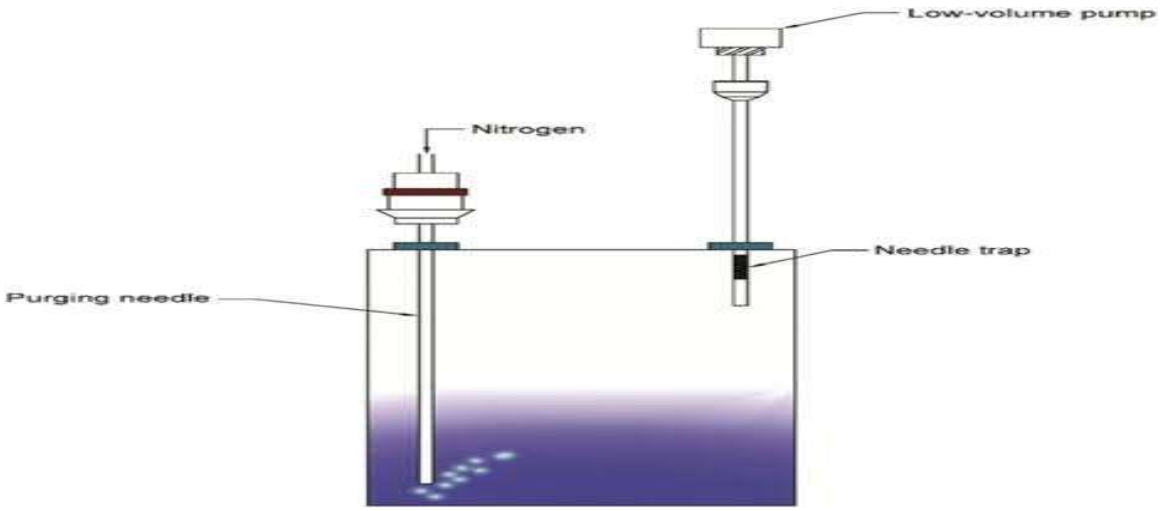


Figure 7: Schematic diagram of purge and trap (P&T)

Source: Barkhordari et al. (2017)

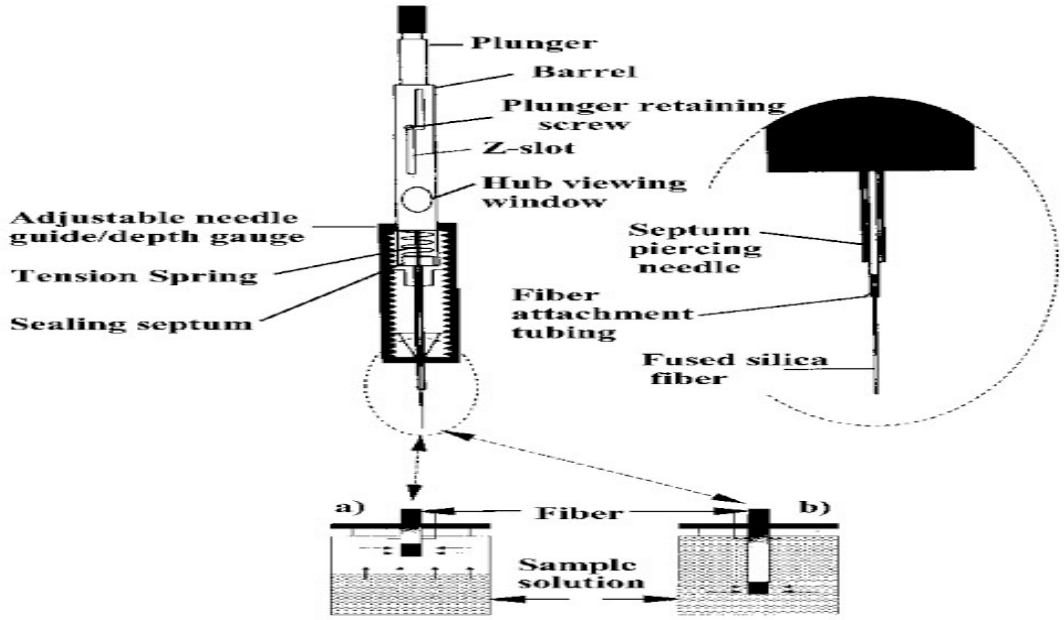


Figure 8: Schematic diagram of solid-phase microextraction (SPME)

Source: Wrobel et al. (2003)

1.4 Fatty acids

Fatty acids which was first isolated and named by French chemist Chevreul M. E. in the year 1818 are colourless solids or liquids that make up the major component of lipids and edible oils (Dijkstra 2009; Grofová 2010; Velíšek 2014; Moulodi et al. 2015). There are more than 100 fatty acids with most compounds having even number of carbons for the reason that their biosynthesis occurs by adding acetate with two carbons. The fatty acid compounds are complex monocarboxylic acids with at least two carbon atoms and they serve as an important source, storage and transporter of energy representing 30 – 35 % of total energy intake in many countries. Therefore, consumption of foods rich in fatty acids is highly beneficial especially for proper development of the central nervous system (Grofová 2010). Dietary sources of fatty acids include grain, vegetable oils, meat, dairy products, fish and fish oils (Rustan & Drevon 2005; IUPAC 2014). Fatty acids are present in food as esters in the form of fats and oils, and glycerophospholipids (Velíšek & Cejpek 2006), and are divided based on criteria such as chain length and/or saturation.

The chain length classification of fatty acids is usually based on the number of carbon atoms; for example short-chain fatty acids (SCFA) with less than 6 carbon atoms, medium-chain fatty acids (MCFA) with 6 – 12 carbon atoms, long-chain fatty acids

(LCFA) with 14 – 20 carbon atoms and very-long-chain fatty acids (VLCFA) with more than 20 carbon atoms. According to saturation criterion, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and unsaturated fatty acids (UFA) are typical examples. Many naturally occurring fatty acid compounds possess an un-branched chain with between 4 to 28 carbon atoms. In addition, the carbon atoms of some fatty acid compounds are linked by single bonds (-C-C-) for example SFA while double bonds (-C=C-) link others such as MUFA, PUFA and UFA). According to the configuration of double bonds, cis- and trans-fatty acids have been reported (Žák 2011; Velíšek 2014). However, the double bonds in certain fatty acid compounds can be converted to form single bonds under certain chemical reactions with hydrogen (Rustan & Drevon 2005; Belitz et al. 2009; IUPAC 2014). The two common methods for fatty acid determination are; Infrared spectrometry (IR) and gas chromatography (GC). Although the IR methodology is much easier to use nonetheless GC is a more accurate method (Moigradean et al. 2013). FAs are extracted with nonpolar solvents from solutions via lowering the pH to form the uncharged carboxyl group (Rustan & Drevon 2005).

1.4.1 Transesterification and GC-MS

Transesterification or alcoholysis is an organic reaction used to prepare fatty acid methyl ester (FAME) from triacylglycerol (TAG) to enable their chromatographic analysis. To accelerate the transesterification process, either a strong acid or a base catalyst will be used. The product of the transesterification is fatty acid alkyl esters and glycerol and the intermediates monoglycerides and diglycerides. For example, in the transesterification of vegetable oils, TAGs together with an alcohol reacts in the presence of a strong acid or base which creates a mixture of FAMEs and glycerol (Otera 1993; Schuchardt et al. 1998; Meher et al. 2006). Factors such as presence of FFAs, moisture, catalyst concentration, temperature, molar ratio of alcohol to oil, reaction time, type of alcohol, catalyst type, mixing intensity or using organic co-solvents can influence the transesterification process (Schuchardt et al. 1998; Hammond 2003; Meher et al. 2006; Prošková et al. 2009; Wang et al. 2014). Analytical methods for the identification of FAMEs in oils include; gas chromatography (GC), gas chromatography-mass spectrometry (GC–MS), high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), high-performance liquid chromatography-mass spectrometry (HPLC–MS), high-performance size exclusion

chromatography (HPSEC), Fourier transform infrared spectroscopy (FTIR) or near-infrared spectroscopy (NIR) and Raman spectroscopy. However, for the determination of individual profiles of FAs in oils and fats is the most widely used is the GC-MS technique.

1.4.1.1 Acid-Catalysed Transesterification

In this method, the transesterification process makes use of acid catalysts thereby resulting in high yields in alkyl esters. However, this reaction takes more than 3 hours and requires temperature above 100 °C for a complete conversion making it much slower than base-catalysed reactions. This process is particularly appropriate for oils with high water content and free fatty acids (FFAs) content greater than 3 % and. (Meher et al. 2006; Bhatti et al. 2008; Ejikeme et al. 2010). In acid-catalysed transesterification reactions, protonation of the carbonyl group of the ester leads to carbonation and creates a tetrahedral intermediate in the process after a nucleophilic attack of the alcohol. The intermediate eradicates glycerol to form a different ester as well as regenerates the catalyst.

1.4.1.2 Base-Catalyzed Transesterification

This is a rapid and less corrosive method that produces FAME from glycerol esters and not FFA components via the use of an alkali catalyst for the transesterification process. Potassium hydroxide, sodium hydroxide and sodium methoxide are the mostly used alkali catalysts (Metcalffe 1981). In the first step these transesterification processes, the attack of the alkoxide ion to the carbonyl carbon of the TAG molecule results in the development of a tetrahedral intermediate. Secondly, tetrahedral intermediate reacts with alcohol to produces alkoxide ion. Finally, intermediate rearrangement produces an ester and a diglyceride. This method is appropriate for fats and oils with FFA content lower than 3 % (Meher et al. 2006; Bhatti et al. 2008; Ejikeme et al. 2010).

1.5 Gas chromatography-mass spectrometry

Gas chromatography/mass spectrometry (GC/MS) has been broadly used to analyze biochemical and organic mixtures (Skoog et al., 2007) in many fields of science such as forensics, environmental science, medical and biological research, flavour and fragrances industry, food science and technology and many others (Penton et al., 2011). The GC/MS system is a combination of two analytical procedures; the gas chromatography separates the components of a mixture in time while mass spectrometer

provides information that aids the structural identification of each component (McLafferty and Turecek 1993; Larsen et al. 1996; Hübschmann 2008; McMaster 2008; Sparkman and Penton, 2011). During the analysis of complex biochemical compounds with the GC–MS system, poor results may be observed due to leaks. Therefore, the septum at the injection port should be replaced at around 100 injections, while a properly maintained Merlin Microseal can be used for a lot of injections. The interface between the gas chromatograph and the mass spectrometer is another source of leak that should be remedied to optimize results. In addition, different sequences of temperature programs may loosen the nut connecting the column to the mass spectrometer, thereby causing huge amount of air background in the chromatogram (Elmore, 2014).

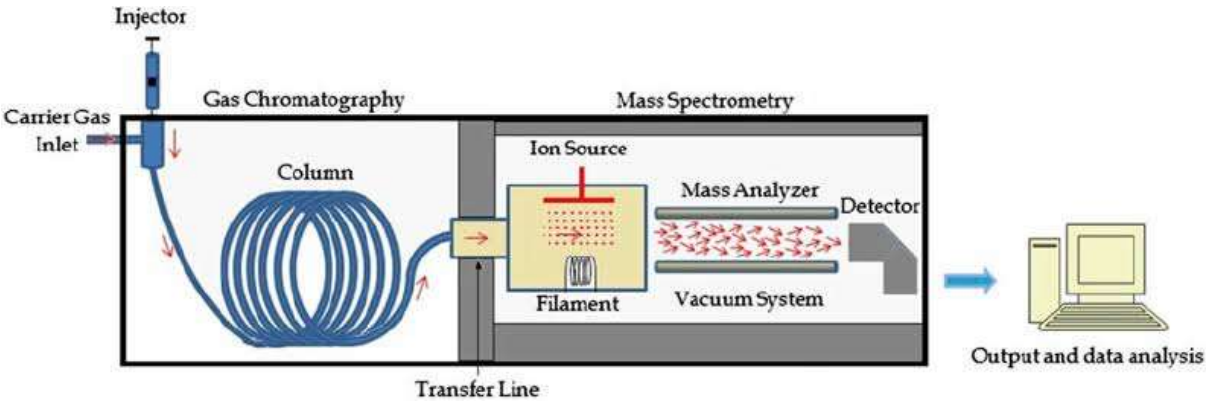


Figure 4: Schematic diagram of a GC-MS system

Source: Emwas et al. (2015)

One of the most important characteristics of GC is its ability to separate components with very similar distribution constants between the two phases (Stashenko and Martinez, 2014). In gas chromatography, distribution or partitioning of complex compounds between a mobile phase and a stationary phase takes place. The mobile phase of the GC is a carrier gas, usually comprising of argon, helium, hydrogen or nitrogen, while the stationary phase is immobile comprising of liquid with high molecular weight which is chemically bonded to the inner walls of the chromatographic column. Samples are swept through the column by a stream of carrier gas while components within the samples are separated based on the amount of time consumed to pass through the column which greatly depends on their chemical structure; this is termed retention time (Hussain and Maqbool, 2014). On the other hand, mass

spectrometry is used to acquire the fingerprint of molecules in samples; as soon as molecules move into the source chamber of the mass spectrometer, they are bombarded by electrons via ionization and fragmentation which causes energy transfer during this process. Ions will be identified and separated according to mass-to-charge ratio (m/z) by the electron multiplier after drifting across the analyzer section. The mass spectrum will be attained via plotting abundance of ions identified versus their mass-to-charge ratio (Ministry of Environment 1991). This technique is suitable for as Vasta et al. (2007), with the aid of mass spectrometry analysis, distinguished feeding type of the animals with the identification of 33 volatiles among the 204 compounds identified in meat samples. Fischer et al. (2012) also identified skatole in meat juice by using stable isotope dilution analysis – direct immersion-SPME-GC/MS.

2. Aims of the Thesis

This thesis aimed to compare the physiochemical characteristics, volatile compounds, fatty acid profile and sensory quality of rabbit meats subjected to different drying temperatures. The objectives were:

- To evaluate the effect of different drying temperatures on the physicochemical characteristics of rabbit meat.
- To analyze the volatile compounds present in rabbit meats dried at different temperatures.
- To determine the effect of different drying temperatures on the fatty acid composition of rabbit meat
- To compare the effects of drying temperatures on the sensory attributes of rabbit meats assessed by a trained panel.

2.1 Research questions

- Does drying temperatures influence the quality attributes of rabbit meat?
- How do the drying temperatures affect the physical quality, chemical quality and consumer acceptability of dried rabbit meat?

2.2 Hypotheses

H_0: Drying temperatures did not influence the quality attributes of rabbit meat.

H_1: Drying temperatures influenced the quality attributes of rabbit meat.

H_0: Drying temperatures did not affect the physical quality, chemical quality and consumer acceptability of dried rabbit meat.

H_1: Drying temperatures affected the physical, chemical and consumer acceptability of dried rabbit meat.

3. Materials and Methods

3.1. Location of Experiment

This study was conducted at the Laboratory of Food Processing Technologies and Laboratory of Ethnobotany and Ethnopharmacology of the Faculty of Tropical Agrisciences, Czech University of Life Sciences, Prague.

3.2. Sample collection and processing

A total of 20 skinned rabbit legs were purchased from a reputable store in Prague, Czech Republic and immediately transported to the freezer (-20 °C) in the laboratory of Food Processing Technologies for the purpose of the experiment. Preparatory operations such as thawing and cutting were carried out on the frozen rabbit meat. The meat was thawed at 4 °C for 24 hours before use. The meats were cut parallel to the fibres, approximately 20 mm x 20 mm thick and 260 mm long, and were subsequently divided into 5 groups for drying at different temperatures. Hundred grams (100g) of meat were dried at temperatures 40, 60, 80, 100 and 120 °C, respectively in a controlled drying chamber or oven (Memmert SF30, Schwabach, Germany). The meat was dried to a constant weight of the sample. The samples intended for sensory analysis were dried to a value of aw 0.5, which ensured the microbial safety of the tested samples. The samples were weighed after drying, placed in separate Petri dishes and allowed to cool before further analyses.

3.3 Physicochemical characteristics

The dried meat samples were pulverized using a Grindomix GM 100 knife mill (Retsch, Haan, Germany), pH and color of the dried samples were recorded. The pH values of dried rabbit meat samples were obtained using a calibrated pH and temperature probe (Thermo Scientific, Orion Star A211, Orion 8302BNUMB ROSS ultra pH/ATC Triode, USA). 10 g of dried meat was blended with 100 ml of water, the pH meter probe was inserted into the mixture and readings were recorded and repeated for all samples. The probe was cleaned between samples to prevent cross-contamination. Colour measurements L* (lightness), a* (redness), b* (yellowness) at random points on dried meat samples were measured with a CIE Lab surface colour with a portable spectrophotometer (CM-600d, Konica Minolta Optics inc., Japan; aperture diameter: 8 mm; illuminant: D65; observer angle: 10° and specular component: 0% UV). The water activity of dried meats samples per treatment was measured by a water activity meter of

Novasina (Lab touch water activity). Moisture content of samples was determined by expressing weight loss as a percentage of initial weight before drying while amount of fat extracted after drying was measured with a digital sensitive weighing balance. All monitored measurements were repeated five times, and average values were calculated.

3.4 Volatile Compounds Analysis GC-MS/SPME

3.4.1 Sample preparation and extraction

1 g of the dried samples was collected in a 2 ml vial with a sealed cap which was refrigerated until the analysis. The crushed samples were subject to heat at 50 °C for 15 mins, after which the vial was then subjected to Headspace-solid phase microextraction (HS-SPME). The SPME device, a silica fibre coated with 100 µm thick polydimethylsiloxane (PDMS) film was placed inside the headspace of the sample for 15 minutes. The fibre was inserted into the injection system of a gas chromatograph where the substances desorbed at 25 °C. The fibre was left in the injection system until further extraction. Before analysis, the SPME fibre was reconditioned at 250 °C to eliminate contamination from any previous usage. This blank measurement was performed for a total of 1 hour. One fibre was used throughout the entire extraction process.

3.4.2 GC-MS Analysis

The GC-MS analysis was processed on the Agilent 7890B/5977a GC/MSD System (Agilent Technologies, Santa Clara, California, USA) equipped with an autosampler Agilent 7693, a HP-5 column (30 m x 0.25 mm, film thickness 0.25 µm, Agilent19091s-433). The carrier gas helium had a flow rate of 1 ml/min. The injecting temperature was set at 250 °C and maintained during the whole chromatography run, which was set to 37 minutes. The optimised GC oven temperature program was 45 °C (5 min) to 180 °C at 5 °C/min, then to 280 °C at 20 °C/min. The GC injector port operated in splitless mode with a 0.75 mm i.d. liner. MassHunter Workstation Software Qualitative Analysis Version B.07.00 was utilized to analyze the mass spectra. The software program was then set to obtain the peak areas by integration. The identification of compounds was based on a comparison of the mass spectra with the National Institute of Standards and Technology library version 2.2 (NIST, USA). The confirmation of the identification of components was based on the comparison of Retention Index (RI) values, which were

calculated by using the retention times of n-Alkanes series ranging from C7 to C40 (Sigma-Aldrich). The area under each identified peak was calculated using electronic integration by MassHunter software. Substances that were not possible to be confirmed by comparison of RI were due to their unavailable retention time data.

3.5 Fatty acid Composition

3.5.1 Extraction of fat and transesterification of fatty acids

After the drying process, 10 g each of the three samples per treatment underwent fat extraction using the Soxhlet fat extraction technique (Velp Scientifica SER 158 Series Automatic Solvent Extractor, Italy). Fatty acids in the extracted fat were converted to fatty acid methyl esters (FAME) by transesterification process according to Hammond (2003). Fat was collected from the extractor cups and 100 mg was weighed and put in a vial for each sample, 1.0 ml of toluene was added using a 500 µl Hamilton gastight syringe to dilute the fat. Then, 4 ml of Sodium Methoxide was added using a 5 ml measuring cylinder, the solution was covered and mixed gently. At this point, the FAs began to detach from triacylglycerols to form glycerin, soap, and FAMEs, it was safe to say the transesterification reaction began to occur. After about 15 minutes, the solutions were uncapped and 5 ml of 5 % Acetic Acid was added using the 5ml measuring cylinder to stop the transesterification reaction. 5 ml of n-hexane was added to dilute the solution, capped and shaken slightly. In few minutes, three layers were noticed in each sample, the lower layer being glycerol, the middle layer formed by soap and the upper layer formed by dissolved FAMEs in n-hexane. 500 µl of the top layer of each sample was collected using the 500 µl Hamilton gastight syringe into a 2 ml vial, and the vial was capped. The samples were labelled accordingly and placed into the autosampler of the GC for the gas chromatography-mass spectrometry (GC-MS) analysis.

3.5.2 GC-MS analysis of fatty acids

The subsequent GC-MS analysis was performed on an Agilent 7890B/5977A GC/MSD System (Agilent Technologies, USA) equipped with an HP-5 column (5 %-phenyl)-methylpolysiloxane with diameters 30 m length, 250 µm internal diameter, 0.25 µm film thickness. For the analysis of substances, Agilent Technologies 5977A mass spectrometer was used. Helium with a flow rate of 1 ml/min was used as the carrier gas. The optimised GC oven temperature program was 70 °C (2 min) to 280 °C at 10 °C/min, final temperature held for 10 min. The chromatography run was set to 33

minutes. The MSD transfer line temperature was maintained at 250 °C with the electron energy of 70 eV. Mass spectra were acquired in the mass range from m/z 30 to 550, using a scan time of 1 s. Data were elaborated through MassHunter Workstation Software, Qualitative Analysis Version B.07.00, service pack 2, build 7.0.7024.29. This software generated integration peaks. Identification of FAME was based on comparing of mass spectra of detected substances against mass spectra covered by the NIST/EPA/NIH library version 2.2. For accuracy, detected fatty acid was compared with standard FAME Mix 37.

3.6 Sensory evaluation

The sensory evaluation was performed by 12 panelists who were instructed to sit in individual booths. The panelists were provided with 2cm-thick dried rabbit meats from each treatment that were placed in containers with random codes to avoid biases, water and bread to neutralize their palate between samples as well as an evaluation form to score descriptors. The panelists received the samples in a randomized fashion. Panelists were asked to open their container and take the time to evaluate appearance, pleasantness of smell, intensity of smell, likeableness of colour, intensity of colour, pleasantness of texture, juiciness, chewiness, pleasantness of taste and intensity of taste of each meat sample which was assessed on a continuous scale from 0 to 100 as demonstrated in Table 5.

3.7 Statistical Analysis

Data collected were subjected to one-way analysis of variance using general linear model as contained in the Minitab® software version 19.1.0. Significant ($p < 0.05$) differences among treatment means were separated using Tukey test of the same software. Due to the sub-sample size of meat samples used for the volatile compounds and fatty acid analyses, no statistics were performed on the data.

Table 5. Description and scale of the sensory attributes used to evaluate dried rabbit meats

Descriptor	Attribute	Scale
Appearance	Before eating sample	0 = very low 100 = very high
Pleasantness of smell	Before eating sample	0 = very low 100 = very high
Intensity of smell	Before eating sample	0 = very low 100 = very high
Likeableness of colour	Before eating sample	0 = very low 100 = very high
Intensity of colour	Before eating sample	0 = very low 100 = very high
Pleasantness of texture	Before eating sample	0 = very low 100 = very high
Juiciness	After first five or ten chews	0 = very low 100 = very high
Chewiness	After at least fifteen chews	0 = difficult to chew 100 = easily chewable
Pleasantness of taste	After first five or ten chews	0 = very low 100 = very high
Intensity of taste	After first five or ten chews	0 = very low 100 = very high

4. Results

4.1. Physicochemical characteristics

The results for physicochemical characteristics of dried rabbit meat after drying at different temperatures are presented in Table 6. All parameters measured except moisture loss were significantly ($p<0.05$) different across treatments. The pH of dried rabbit meat samples differed significantly ($P < 0.001$) across treatments with the pH ranging from 5.89 in samples dried at 40 °C to 6.28 in samples dried at 120 °C. The lightness of colour of the dried rabbit meat also differed ($P < 0.001$), L* decreased from 79.43 with drying at 40 °C to 66.04 in samples dried at 120 °C. The redness of colour of the dried meats differed across treatment means ($P < 0.001$), with statistically similar highest values (11.53 and 11.07) recorded at drying at 100 and 120 °C, respectively and lowest (3.72) at drying at 40 °C. Similarly, significantly ($P < 0.001$) highest values statistically (27.81 and 28.26) for yellowness colour of meat samples were recorded at drying at 100 and 120 °C, respectively and lowest (17.92) at drying at 40 °C. Water activity differed ($P < 0.001$) across treatment means with values ranging from 0.22 in samples dried at 120 °C to 0.45 in samples dried at 60 °C. Moisture loss of dried meats was not influenced ($P=0.706$) by the effect of drying temperature. However, fat extracted after drying differed ($P<0.002$) with values ranging from 0.45 g in meat dried at 120 °C to 1.30 g in meats dried at 80 °C.

Table 6: Physicochemical characteristics of dried rabbit meat after drying at different temperatures

Parameter	Drying temperature (°C)					SEM	P-value
	40	60	80	100	120		
pH	5.89[b]	6.19[a]	6.27[a]	6.20[a]	6.28[a]	0.04	<0.001
L* (Lightness)	79.43[a]	75.33[b]	72.56[b]	66.26[c]	66.04[c]	0.91	<0.001
a* (Redness)	3.72[d]	6.97[c]	8.97[b]	11.53[a]	11.07[a]	0.27	<0.001
b* (Yellowness)	17.92[d]	22.86[c]	25.19[b]	27.81[a]	28.26[a]	0.37	<0.001
Water activity	0.41[a]	0.45[a]	0.42[a]	0.28[b]	0.22[c]	0.01	<0.001
Moisture loss (%)	77.23	73.83	77.68	76.54	78.43	2.39	0.706
Extracted Fat (g)	0.60[b]	0.93[ab]	1.30[a]	0.56[b]	0.45[b]	0.114	0.002

[a,b,c, d] Means with different superscripts within the same row differ significantly ($P < 0.05$)

4.2 GC-MS Analysis of Volatile Compounds

In Table 7, the GC-MS analysis of volatile compounds in dried rabbit meat after drying at different temperatures is presented. A total of 25 compounds of different classes (aldehydes, alkanes, esters, furans, acids, ketones, and alcohols) were identified with Hexanal being the primary compounds identified was highest (516.15) in meat samples dried at 100 °C. Similarly, the highest values for Hydroxyurea (9.04), 3-Amino-2-oxazolidinone (50.60), 3-methyl-butanal (38.21), Heptan-2-one (18.74), Octanal (20.33), 3,5-Octadien-2-ol (8.37) were recorded in samples dried at 100 °C. On the other hand, the highest values for compounds such as n-Hexane (63.53), Pentan-1-ol (23.31), O-Isobutylhydroxylamine (6.81), 2,5-dimethyl-pyrazine (18.37), Heptanal (20.69), Heptan-1-ol (11.53), 2-Ethyl-1,4-dimethyl-benzene (9.68), 1-Octen-3-ol (49.16), 2-Pentyl-furan (32.92), 3-Octen-2-one (40.37), 3,5-Octadien-2-one (10.49) and Nonanal (12.09) were observed in rabbit meat samples dried at 120 °C. Moreover, meat samples dried at 40 °C had the abundance of 2-Methyl-butanal (8.59), 1-Methylethyl-benzene (8.90), Mesitylene (7.29), while o-Xylene (8.13) and D-Limonene (12.97) were prominent in dried meat samples at 80 °C.

Table 7: Volatile compounds identified using gas chromatography-mass spectrometry in dried rabbit meat after drying at different temperatures.

Compound	MW	RI		Area (mean area values x 10^4)				
		Obs a	Pubs b	40°C	60°C	80°C	100°C	120°C
2-Aziridinylethyl amine	86	e	d	37.64	46.97	17.64	31.40	42.69
Hydroxyurea	76	e	d	7.55	7.66	4.68	9.04	7.67
3-Amino-2-oxazolidinone	102	e	d	45.70	12.25	31.13	50.60	34.78
n-Hexane	86	e	d	10.07	29.32	35.21	0	63.53
2-Methyl-butanal	86	e	d	8.59	4.32	4.81	3.34	4.98
3-Methyl-butanal	86	e	d	18.90	25.18	3.14	38.21	4.32
Pentan-1-ol	88	756	758	10.68	16.92	c	17.80	23.31
O-Isobutyl hydroxylamine	89	e	d	c	4.24	c	1.19	6.81
Hexanal	100	795	796	374.36	388.08	43.32	516.15	316.87
o-Xylene	106	872	888	c	1.89	8.13	0.86	c
Heptan-2-one	114	889	889	3.91	3.57	c	18.74	8.32
Heptanal	114	900	902	10.39	3.66	1.30	11.90	20.69
2,5-Dimethyl-pyrazine	108	913	927	4.07	0.90	c	6.07	18.37
1-Methylethyl-benzene	120	941	930	8.90	1.00	0.88	c	c
Heptan-1-ol	116	975	970	c	c	c	0. 47	11.53
1-Octen-3-ol	128	986	978	11.34	16.67	0.17	12.03	49.16
2-Pentyl-furan	138	993	993	7.40	3.57	c	27.92	32.92
Mesitylene	120	996	996	7.29	2.97	4.20	c	c
Octanal	128	1005	1006	4.16	2.30	0. 47	20.33	5.85
D-Limonene	136	1031	1036	10.20	11.08	12.97	1.27	9.32
3-Octen-2-one	126	1043	1036	5.88	c	c	0.48	40.37
2-Ethyl-1,4-dimethyl-benzene	134	1068	1072	3.08	1.67	2.23	1.12	9.68
3,5-Octadien-2-one	124	1077	1098	5.03	c	c	c	10.49
Nonanal	142	1107	1104	7.94	7.68	c	6.99	12.09
3,5-Octadien-2-ol	126	1143	1039	1.98	c	c	8.37	2.33

MW: Molecular weight; RT: retention time; RI: retention index; Area - 10^4

a Retention index is calculated from retention times and based on C7-C40 alkanes

b Data taken from NIST Database, National Institute of Standards and Technology

c Compound not detected in the sample

d Literature data not available/found

e Retention index is out of calibration

4.3 Fatty acid profile

The Fatty acid profile of the dried rabbit meat after drying at different temperatures was determined after transesterification to fatty acid methyl esters (FAME) and analysis by GC-MS. 17 fatty acids were detected across the five different temperatures as shown in Table 8. Octadecenoic acid was the most abundant compound in the dried meat samples with the highest peak area (14300.32) being derived from dried meat samples at 60 °C. The second most abundant compound, Hexadecanoic acid was the highest (6075.27) in meats dried at 40 °C. Tetradecanoic acid (1438.00) and 12-Methyltridecanoic acid (377.72) were also highest in meats dried at 40 °C. Meats dried at 120 °C had the highest area for Octanoic acid (37.25), Dodecanoic acid (184.47), Pentadecanoic acid (280.96), Heptadecanoic acid (432.13), Nonadecanoic acid (69.18), and Eicosenoic acid (376.18). Other compounds such as Decanoic acid (177.86), Hexadecenoic acid (1780.33), Eicosatetraenoic acid (191.98), and Eicosanoic acid (89.54) were highest in samples dried at 100 °C while 14-Methylpentadecanoic acid (55.09), and Octadecanoic acid (1647.46) were highest in rabbit meats dried at 80 °C. In addition, chromatographs showing the samples at the 5 different temperatures with the compounds at their peaks, in order 40, 60, 80, 100 and 120 °C were presented in Figure 9.

Table 8: Fatty acid profile of dried rabbit meat after drying at different temperatures

Corresponding Fatty Acid	MW	RI		Area (mean area values x 10^4)				
		Obs a	Pubs b	40°C	60°C	80°C	100°C	120°C
Octanoic acid	144	1127	1128	9.02	9.60	7.23	17.60	37.25
Decanoic acid	172	1327	1328	88.45	86.91	86.70	177.86	159.13
Dodecanoic acid	200	1527	1527	102.65	41.91	113.22	148.54	184.47
Tetradecanoic acid	228	1718	1719	1438.00	12.81	26.19	8.19	11.58
12-Methyltridecanoic acid	228	1731	d	377.72	303.26	15.14	20.98	24.62
Pentadecanoic acid	242	1820	1826	155.49	193.44	153.99	244.57	280.96
14-Methylpentadecanoic acid	256	1893	1884	48.54	11.47	55.09	34.81	28.48
Hexadec-9-enoic acid	254	1915	1898	981.71	1366.88	1012.04	1780.33	913.75
Hexadecanoic acid	256	1945	1933	6075.27	5030.88	4579.44	4533.23	1392.08
Heptadecenoic acid	270	2011	2028	96.60	27.88	127.69	153.27	111.85
Heptadecanoic acid	270	2033	2030	229.01	279.66	127.69	329.60	432.13
Octadec-9-enoic acid	282	2130	2141	3386.24	14300.32	11831.17	2231.96	10669.63
Octadecanoic acid	284	2143	2139	590.93	835.40	1647.46	1160.91	1215.87
Nonadecanoic acid	298	2233	2228	31.13	25.36	52.56	37.31	69.18
Eicosatetraenoic acid	304	2276	2274	72.69	86.28	112.02	191.98	153.99
Eicosenoic acid	310	2311	2300	54.17	156.62	112.02	320.41	376.18
Eicosanoic acid	312	2333	2332	17.59	26.12	68.41	89.54	78.02

MW: Molecular weight; RT: retention time; RI: retention index; Area - 10^4

a Retention index is calculated from retention times and based on C7-C40 alkanes

b Data taken from NIST Database, National Institute of Standards and Technology

d Literature data not available

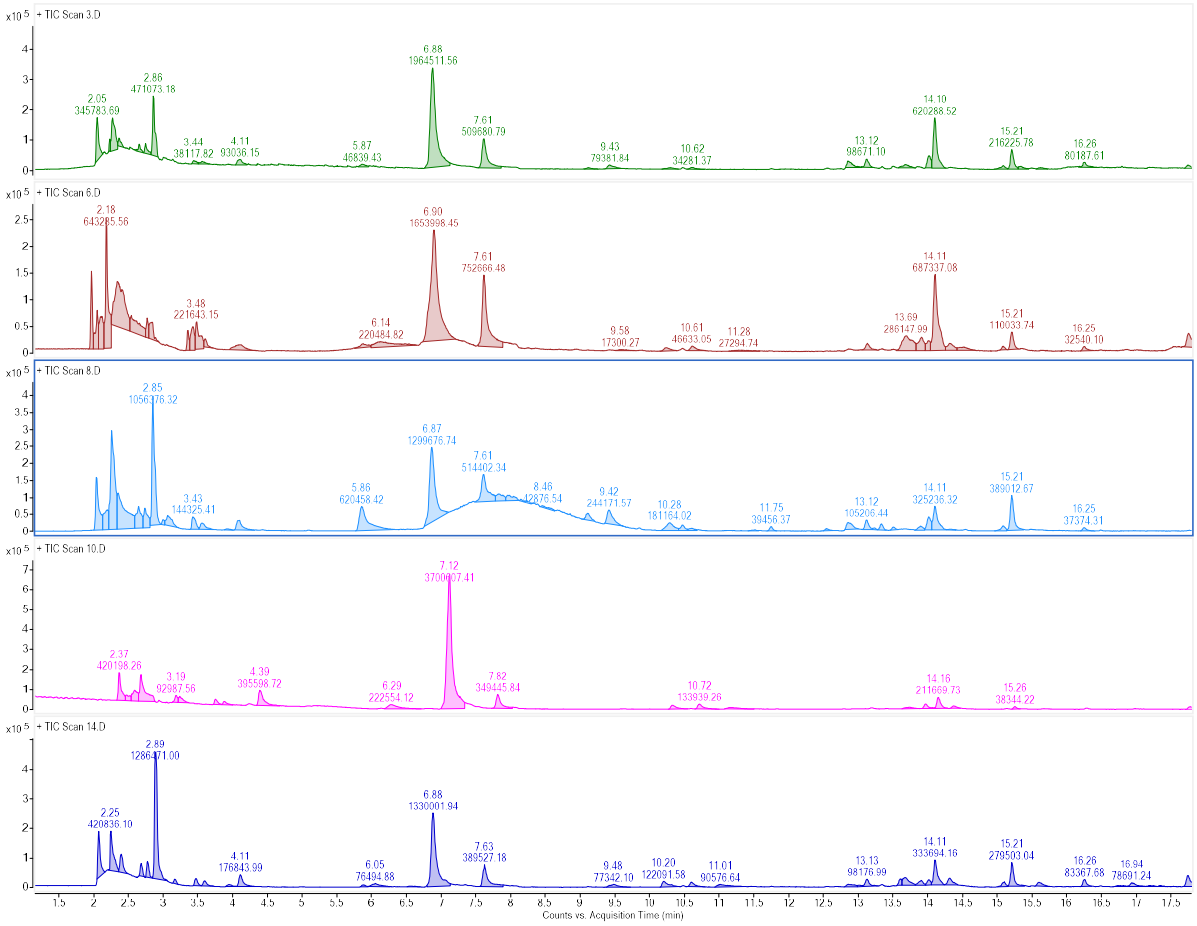


Figure 9: A chromatograph showing the samples at the 5 different temperatures with the compounds at their peaks, in order 40, 60, 80, 100 and 120 °C

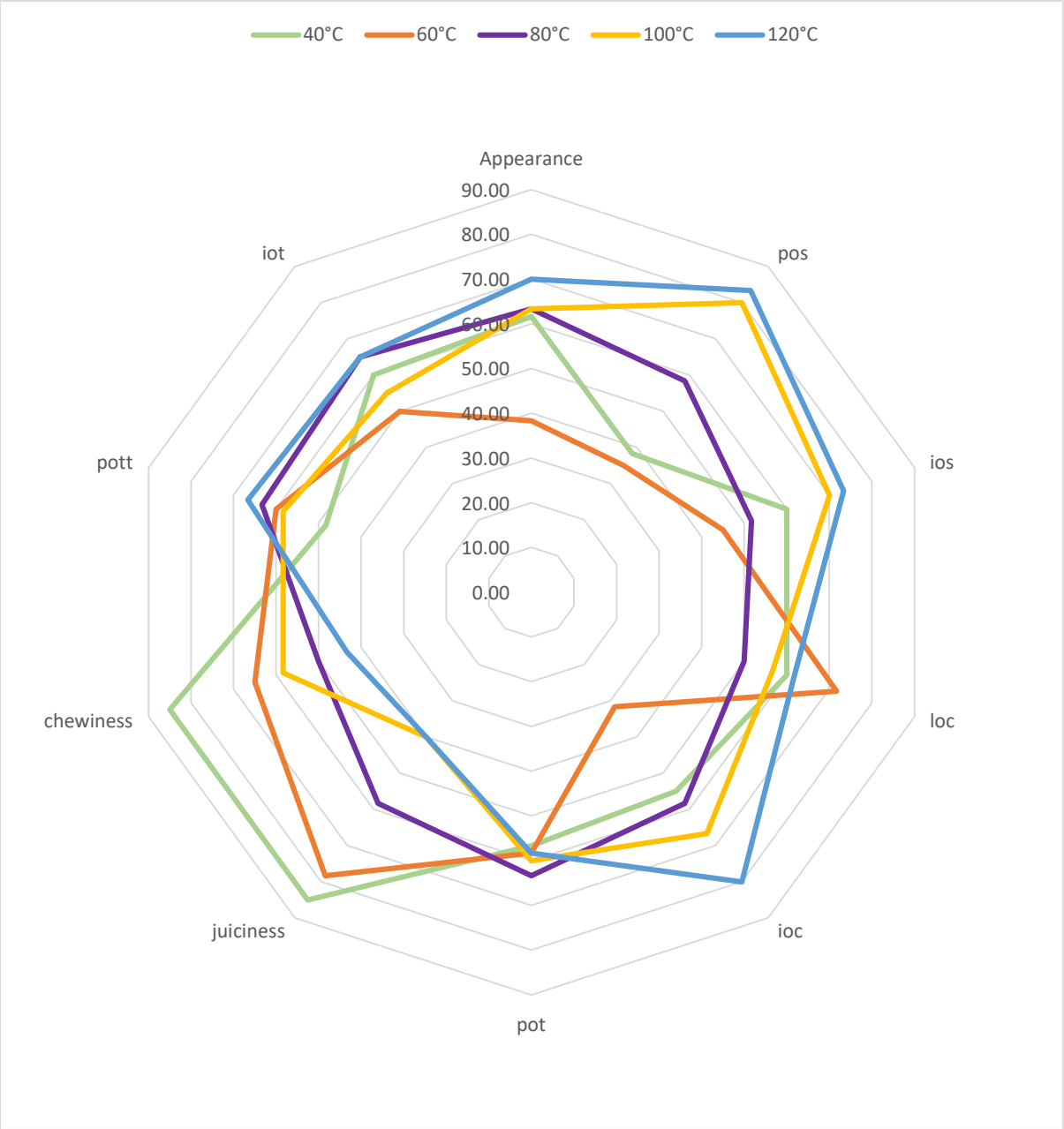
4.4 Sensory Evaluation

The effect of drying temperature on sensory characteristics of dried rabbit meat was depicted in Table 9. Sensory parameters such as appearance (P= 0.066), intensity of smell (P= 0.071), likeableness of colour (P= 0.461), pleasantness of texture (P= 0.987), pleasantness of taste (P= 0.623), and intensity of taste (P= 0.667) were no significantly different across treatments. However, pleasantness of smell of dried rabbit meats differed significantly (P= <0.001) with values ranging from 35.00 in meats dried at 60 °C to 83.33 in meat dried at 120 °C. Intensity of colour also differed (P= <0.001) with values ranging from 31.67 in meat dried at 60 °C to 80.00 in dried meat at 120 °C. In addition, the range of 40.00 to 85.00 for juiciness (P= <0.001), and 43.33 to 85.00 for chewiness (P= 0.002), differed significantly across varying drying temperature treatments. In addition, radar chart illustrated the effect of drying temperature on sensory characteristics of dried rabbit meat which was presented in Figure 10.

Table 9: Effect of drying temperature on sensory characteristics of dried rabbit meat

Descriptor	Drying temperature (°C)					SEM	P-value
	40	60	80	100	120		
Appearance	61.67	38.33	63.33	63.33	70.00	7.95	0.066
Pleasantness of smell	38.33^{c}	35.00^{c}	58.33^{bc}	80.00^{ab}	83.33^{a}	6.12	<0.001
Intensity of smell	60.00	45.00	51.67	70.00	73.33	7.89	0.071
Likeableness of colour	60.00	71.67	50.00	56.67	61.67	8.26	0.461
Intensity of colour	55.00^{ab}	31.67^{b}	58.33^{ab}	66.67^{a}	80.00^{a}	7.00	<0.001
Pleasantness of texture	56.67	58.33	63.33	60.00	58.33	8.64	0.987
Juiciness	85.00^{a}	78.33^{ab}	58.33^{bc}	40.00^{c}	40.00^{c}	6.52	<0.001
Chewiness	85.00^{a}	65.00^{ab}	50.00^{b}	58.33^{ab}	43.33^{b}	7.22	0.002
Pleasantness of taste	48.33	60.00	63.33	58.33	66.67	8.53	0.623
Intensity of taste	60.00	50.00	65.00	55.00	65.00	8.45	0.667

^{a,b,c} Means with different superscripts within the same row differ significantly ($P < 0.05$)



N.B: Appearance; POS – Pleasantness of smell; Intensity of smell; LOC - Likeability of color; IOC – Intensity of color; POT – Pleasantness of texture; POTT - Pleasantness of taste; IOT – Intensity of taste

Figure 10: Radar chart illustrating the effect of drying temperature on sensory characteristics of dried rabbit meat

5. Discussion

Dried meat quality is measured by a variety of indicators which include pH, colour water activity, lipid oxidation, proximate composition, and sensory characteristics (Mediani et al. 2022). The pH of meat is one of the important indices of meat quality as it has direct effects on the meat's functional attributes, eating and storage quality (Mishra et al. 2017). In this study, the drying temperature had a significant ($P<0.05$) influence on meat pH with results showing the lowest pH in rabbit meats dried at 40 °C when compared with meat samples from other drying temperatures with the pH above 6. This result was consistent with earlier report by Mediani et al. (2022) that the pH of dried meat products is significantly affected by drying procedures. The pH of dried meat samples according to (Lim et al. 2012) and Rahman et al. (2005) ranged between pH 5.4–5.8 and 5.89 - 6.08, respectively. Thus, drying rabbit meat at 40 °C could potentially result in a longer shelf-life as low pH values are important to avoid protein denaturation in the meat (Mishra et al. 2017). The observed differences in pH might be due to the loss of the free acidic groups as a result of different drying temperatures. According to previous reports (Hamm 1960; Lawrie 1998; Daszkiewicz & Gugołek 2020), the degree of losing free acidic groups explains the variation in meat pH, isoelectric point of and glycolytic potential of muscle.

Colour is another important quality attributes that influences consumer acceptance of many meat and meat products. The colour stability of meat plays a vital role in consumer's decision in meat selection (Needling et al. 2016). The differences ($p<0.05$) in colour values of dried rabbit meats in the current study revealed L* of meat samples reduced with increase in drying temperature while a*, and b* increased with increase in drying temperature. This is in line with reports by Elmas et al. (2021), who revealed lightness (L), redness (a), and yellowness (b) which are sources of variation in light scattering from the surface of the meat that represents the degree of browning were significantly influenced by drying. Results may indicate that drying rabbit meat at 40 °C had less effect on the protein structures of meat samples as compared to other drying temperatures that indicated browning effects and/or darker brown colour of meat samples. According to Rahman et al. (2002), L* value point toward the extent of browning of dried samples as a higher L* value shows less brown colouration of the product. Also, a decrease in L* values of dried meat revealed drying at a higher temperature caused significant protein structural changes in meat samples (Lawrie

1998). This could be linked to the Maillard browning reactions resulting from the reaction between reducing sugars in connective tissues and muscle proteins during heat processing of meat products (Forrest et al. 1975).

In addition, the water activity of meat samples ranged from 0.22 – 0.45 with meats dried at 40, 60 and 80 °C showing high water activity than meat samples dried at 100 and 120 °C. This was consistent with previous findings by Taormina and Sofos (2014) that the a_w contents of dried meat products are often less than 0.7 as it is responsible for the prevention of microbial growth. The non-significance in moisture loss of dried meat samples in this study was in contrast with the findings of Chimel et al. (2017) who revealed drying decreased water content in the case of poultry-pork kabanosy. In addition, the moisture content of the beef lung samples in a study by Reshan Jayawardena et al. (2022) reduced as drying temperature increased from 50 to 100 °C. The variations may be as a result of drying samples to achieve constant weight irrespective of drying time in this present study when compared with previous works. Furthermore, the quantity of extracted fat after drying which was lowest in meat dried at 120 °C may imply high drying temperature ensured less fat melted to the surface of the meat resulting from shorter drying time. This was in line with various investigations (Serrano et al., 2007; Braeckman et al., 2009; Sanghoon, 2011), that reported longer cooking time resulted in greater fat loss.

During the thermal processing of meat, many volatile compounds are generated that belong to different chemical classes: hydrocarbons, alcohols, aldehydes, pyrans, ketones, carboxylic acids, esters, furans, pyrroles, lactones, pyrazines, phenols, thiazolines, pyridines, thiazoles, oxazoles, thiophenes, and other nitrogen or sulfuric compounds (Kosowska et al., 2017). A total of 25 volatile compounds were identified and quantified in dried rabbit meats used in this study. Hexanal being aldehydes was the most abundant compound identified with the highest being recorded in meat samples dried at 100 °C. This result was in agreement with reports by Xie et al. (2016) that aldehydes were the highest in amount among the volatile compounds present in rabbit meat. In addition, different studies found that hexanal was the predominant aldehyde in dry-cured meat products (Domínguez et al. 2019) and cured meat products (Armenteros et al. 2012; Lorenzo et al. 2014; Domínguez et al. 2016). Though aldehyde groups are common in several meat species, especially when they are heat-treated, aldehydes are

not the main odorants in most meat species, as other volatile compounds have sturdier characteristic odours (Kang et al., 2013; Duan et al., 2015).

Similarly, Hydroxyurea, 3-Amino-2-oxazolidinone, 3-methyl-butanal, Heptan-2-one, Octanal, 3,5-Octadien-2-ol were highest in samples dried at 100 °C while compounds such as n-Hexane, Pentan-1-ol, O-Isobutylhydroxylamine, 2,5-dimethyl-pyrazine, Heptanal, Heptan-1-ol, 2-Ethyl-1,4-dimethyl-benzene, 1-Octen-3-ol, 2-Pentyl-furan, 3-Octen-2-one, 3,5-Octadien-2-one and Nonanal were highest in rabbit meat samples dried at 120 °C. This confirms reports by Kosowska et al. (2017), who stated that although the formation of volatile compounds is a multi-directional process that occurs due to the transformations associated with lipid oxidation, Maillard reaction, interactions between lipid oxidation products and Maillard reaction products, and thiamine degradation. However, temperature, duration and type of the heat treatment applied may result in a number of chemical reactions leading to the formation of many volatiles responsible for the species-specific meat flavour. In addition, Many volatiles were also identified in heat-treated chicken meat as high temperatures induce the formation of vast amounts of heterocyclic compounds (Shi & Ho 1994).

Fatty acids in meat and meat products are important not only for nutritional value, but also for organoleptic properties and shelf-life of meat products. The fat composition of meat, especially the fatty acid content when combined with a specific cooking methodology, mostly influences the final quality of meat products (Badiani et al. 2002; Serrano et al. 2007). In this study, 17 compounds comprising saturated and unsaturated fatty acids were identified in dried rabbit meat at different temperatures. Octadecenoic acid and Hexadecanoic acid, the first and second most abundant compound in dried meat samples, were highest in dried meat samples at 60 °C and 40 °C, respectively. This may imply that drying temperature affected the fatty acid constituents of rabbit meat. Despite the various studies (Badiani et al. 2004; Maranesi et al. 2005; Sarriés et al. 2009), focusing on the effect of heat treatment on the fatty acid composition of meat, a dearth of information still exist on the impact of drying temperature on the fatty acid composition of rabbit meat. However, previous studies (Keller & Kinsella 1973; Pearson et al. 1977) showed lipid oxidation of meat increased when heating temperature reached 70 °C. Variations in the fatty acid composition of raw and heat-treated meats differed significantly between chicken and beef patties in another study by Echarte et al.

(2003). Moreover, Scheeder et al. (2001) observed changes in fatty acid composition during grilling of beef patties. In addition, variations were observed in other fatty acid compounds as influenced by different drying temperatures. One of such Hexadecanoic acid was lowest in meat samples dried at 120 °C. This result was in agreement with the study of Abdel-Naeem et al. (2021), where lower Palmitic acid (C16:0) concentration were recorded in rabbit meat samples cooked by different heat treatments.

Furthermore, sensory descriptors such as pleasantness of smell, the intensity of colour, juiciness and chewiness differed significantly across different drying temperatures. Pleasantness of smell which was significantly highest in meat samples dried at 120 °C may be linked to the presence of volatile compounds with stronger characteristic odours such as n-Hexane, Pentan-1-ol, O-Isobutylhydroxylamine, 2,5-dimethyl-pyrazine, Heptanal, Heptan-1-ol, 2-Ethyl-1,4-dimethyl-benzene, 1-Octen-3-ol, 2-Pentyl-furan, 3-Octen-2-one, 3,5-Octadien-2-one and Nonanal. According to Zhao et al. (2020), 2,5-dimethyl-pyrazine is an aromatic compound that contribute to the taste and aroma of foods. Also, Heptan-2-one is a natural product that serves as a flavoring agent (Hall and Andersson, 1983). The intensity of colour, which was scored highest in samples dried at 120 °C can be attributed to the lower L* and higher a* and b*, which reflected in the appearance and colour of these dried rabbit meat samples. However, the low scores for juiciness and chewiness for samples dried at 120 °C is in line with reports by Mediani et al. (2022), who stated that high temperature from hot air leads to more hardness, and formation of off-flavors along the edges and corners of the meat.

6. Conclusion

According to results, it is proven that different drying temperatures influence some of the quality attributes of rabbit meat such as the pleasantness of the smell, intensity of the meat color as well as the juiciness and chewiness of the meat amongst others that have been researched in this work. Meat dried at 120 °C resulted in improved smell and color attributes, this could be due to the presence of volatile compounds such as 2-Ethyl-1,4-dimethyl-benzene, Hexanal and Heptan-2-one which are natural compounds that contribute to the strong characteristic odours in food. Also, the dark brown colour observed in meat dried at 120 °C is a desirable color change in dried meat and could be attributed to Maillard reaction. However, there was a great preference by sensory panellists for rabbit meats dried at 40 °C as they are easy to chew, juicy and with less off-flavors along dried meat edges. Furthermore, in order to achieve optimal improvement in the shelf life of rabbit meat while minimizing the impact on protein structures, drying at 40 °C is recommended. This drying temperature is ideal to achieve dried rabbit meats with a pH below 6, lower scores for darker brown coloration, and a reasonable amount of bound water that falls within the safe threshold for preventing microbial growth. The most abundant volatile compound, Hexanal, was identified, with the highest concentration recorded in dried meat samples dried at 100 °C. However, the first and second most abundant fatty acids in dried meat samples, Octadecenoic acid and Hexadecanoic acid, respectively, were highest when rabbit meats were dried at 60 °C and 40 °C, respectively.

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