RESEARCH ARTICLE AGROBIOLOGICAL RECORDS
ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

INHIBITION OF CREATINE USING DIFFERENT CONCENTRATIONS OF ALOE VERA EXTRACT IN CHICKEN NUGGETS

Usama Mubarik [1§*], Usama Asif [2§] and Mehwish [3,4*]

[1]Faculty of Allied Health Sciences, Superior University, Lahore, Pakistan
[2]National Institute of Food Science and Technology (NIFSAT), Faculty of Food, Nutrition and Home Sciences, University of Agriculture, Faisalabad-38040, Pakistan
[3]Department of Biochemistry, Faculty of Life Sciences, Government College University, Faisalabad, Pakistan
[4]Lyallpur Institute of Management and Sciences, Faisalabad, Pakistan

[§]These authors have equal contribution to this endeavor.

*Corresponding author: umbhatti@outlook.com (UM), mehwishali35423542@gmail.com (Mehwish)

ABSTRACT

Meat is widely regarded as a valuable source of protein in the human diet. However, cooking, frying, and grilling meat can trigger chemical changes that have the potential to be carcinogenic. This is due to the reaction of creatine, which produces heterocyclic aromatic amines (HAAs) when exposed to high temperatures. The conversion of creatine to creatinine during cooking is a key factor in the production of HAAs. Frying meat is particularly risky because it typically involves the use of minimal spices, which could potentially inhibit the formation of HAAs. To address this issue, a recent study sought to explore the use of plant-based extracts to inhibit the development of HAAs in meat products. Specifically, the researchers used aloe vera gel to coat chicken nuggets in three different concentrations: 40, 60, and 80%. Aloe vera gel was chosen for its high content of bioactive compounds and antioxidants, as well as its cellulose content, which could potentially act as a barrier to HAA formation. The results of the study were promising, with aloe gel-coated samples showing up to 44% inhibition in DPPH analysis and a reduction in creatine levels from 1.12 to 0.52mg/mL. The color of the nuggets was also observed, with lower levels of redness and yellowness. Overall, the study concluded that aloe gel-coated nuggets were able to significantly reduce creatine levels during frying, which in turn resulted in a reduction in HAA production. These findings suggest that plant-based extracts may be a valuable tool in the effort to mitigate the potential carcinogenic effects of cooking meat.

***Keywords*:** Creatine, HAAs, DPPH, Chicken Nuggets, Frying

Article History (23-115) || Received: 19 Mar 2023 || Revised: 20 Apr 2023 || Accepted: 02 May 2023 || Published Online: 15 May 2023

1. INTRODUCTION

Meat is a valuable source of protein that plays a crucial role in building and strengthening the human body. It is also essential for the growth and development of various organ systems (Gibis and Weiss 2017; Haque et al. 2021). However, it is important to handle and cook protein carefully to ensure its effective absorption in the human digestive system. Cooking red meat at temperatures above 150°C has been found to produce carcinogenic compounds known as heterocyclic aromatic amines (HAAs) (Gibis 2016; Zamora and Hidalgo 2020; Nadeem et al. 2021). Traditional cooking methods such as frying, grilling, and barbecuing are typically conducted within a temperature range of 150-300°C, making it highly likely that HAAs will be produced through these methods (ur Rahman et al. 2014).

HAAs are formed when amino acids, sugars, and creatine from meat products react at elevated temperatures (>150°C). These compounds are reported to be carcinogenic due to the presence of hydrocarbon rings containing nitrogen. Glucose and creatine are the precursors in the reaction that produce HAAs in meat. Inhibiting creatine can help prevent the production of cancer-causing amines (Gibis and Weiss 2017; Chen et al. 2022). HAAs are known to increase the risk of cancer by mutating genes and disrupting or changing cell growth, leading to abnormal growth. Overconsumption of overcooked meat in a normal lifestyle can increase the risk of various types of cancer (Sugimura 1997; Bert et al. 2020; Rawat and Saxena 2021). Every year, approximately 13% or 7.5million of the world's deaths are attributed to cancer. The International Agency for Research on Cancer (IARC) reported approximately 173,000 cases in Pakistan in 2018, including five types of cancers, of which prostate cancer is related

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

to red meat consumption (dietary factors) (Tufail and Wu 2023). Smoking is the leading cause of cancer, followed by dietary factors as the second leading cause of cancer, especially for prostate and gastrointestinal cancer. In the north and south regions of Pakistan, colorectal cancer is reported in high ratios due to the consumption of smoke, grilled, and fried meat (Tufail and Wu 2023).

To minimize the production of carcinogenic HAAs in cooked meat, researchers have explored the use of spices and condiments to inhibit their formation. These compounds contain antioxidants that can prevent oxidation reactions and control HAAs production (ur Rahman et al. 2014). Additionally, fruits and their extracts have been studied for their potential to inhibit HAAs due to their phytochemical content and anti-cancer properties. For example, tomatoes have shown promise in reducing HAAs formation in beef (ur Rahman et al. 2014).

Both natural and synthetic compounds have been used to mitigate the production of HAAs in cooked meat. Synthetic compounds such as butylated hydroxy-anisole (BHA) and butylated hydroxyl-toluene (BHT) have been found to influence HAAs formation (Gibis 2016). It's worth noting that cooking time, temperature, and the type of meat can significantly affect the production of heterocyclic amines (Omojola et al. 2015). Imidazoquinolines (IQ) is a polar HAAs and have mutagenicity in a red meat cooked at high temperature, so it is necessary to remove or restrain this polar amine (Gibis 2016). Carboxymethyl cellulose (CMC) and microcrystalline cellulose is another appropriate way to reduce HAAs in grilled or fried meat. During heating process, it reduces the level of mass transfer of precursor to the surface. Synthetic MCC and CMC are applied to the meat to reduce the production of HAAs and also has ability as a source of fat replacer (Gibis and Weiss 2017). These objectives of the study were:

1. Assessing the effect of aloe vera gel for inhibition of HAAs in meat.
2. Formulation of standard concentration of Aloe Vera Gel to inhibit HAAs.
3. To evaluate the combined effect of aloe vera extract and antioxidants compounds.

2. MATERIALS AND METHODS

2.1. Chemicals and Materials

The research utilized several chemicals and materials in the experiments. Creatine monohydrate was obtained from UNI-CHEM, New York, while 2,2-Diphenyl-1-picrylhydrazl (DPPH) was purchased from Alfa Aesar USA. Other chemicals used were methanol, picric acid, sodium hydroxide, and trichloro-acidic acid, which were obtained from Merck KGa, Germany and used in their original form. All other chemicals used were of analytical grade, and distilled water was used to prepare solutions. For the preparation of chicken nuggets, standard ingredients such as egg, meat, refined flour, and spices were purchased from the local market in Lahore. Aloe vera plant spikes were also purchased and processed to obtain refined gel for coating the nuggets.

2.2. Preparation of Aloe Vera Extract

To prepare the aloe vera extract, 10 leaves of aloe vera were selected and washed with distilled water to remove dirt and any contaminants. The leaves were then dried with a clean cloth and boiled in water for approximately 15-20min. This treatment helped to soften the leaves for easier extraction of sol gel material. Different concentrations of sol gel were then formulated using distilled water, and three concentrations (40, 60, and 80% w/w) were selected for further experimentation.

2.3. Meat Sample Preparation

For the meat sample preparation, boneless chicken meat was obtained from the local market and ground into minced meat using an electrical blender. 100g of minced meat was then used to formulate the nuggets with refined flour, egg, and spices under optimal conditions. The formulated nuggets were coated with different concentrations of aloe gel and frozen for 1hr to achieve a uniform texture. Finally, the nuggets were fried at 180˚C for 5min.

2.4. Color Assessment

To assess color changes, a digital colorimeter (Konica Minolta CR-20) was used to measure the lightness (L*), redness (a*), and yellowness (b*) values of the samples at different concentrations. The assessment was carried out after the nuggets were freshly fried and the surface oil layer was removed. The sensor was placed vertically on the nuggets, with a distance of 1 mm to obtain accurate results.

2.5. Creatine Assay

For creatine assay, the association of creatine to total protein in chicken was estimated using spectrophotometric studies. The α-naphthol-diacetyl reaction was used to determine creatine with slight modifications following the method of (Dvořák 1981). In brief, 10g of sample was homogenized with 200mL of 3% trichloroacetic acid to completely hydrolyze creatine phosphate. After 1 hour of homogenization, the mixture was

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

filtered using Whatman no. 3 filter paper, and creatine monohydrate standard was used for further estimation. The quantification was done at 525nm using standard curve estimation with a coefficient of reliability of R2=0.9906.

2.6. Antioxidant Assay

To determine antioxidant activity, the DPPH assay was used following the methodology of Duh (1998). In brief, 20 g of sample was diluted in 60mL of distilled water and homogenized at 7000 rpm for 3 min. The grounded solution was centrifuged at 4000rpm for 5min and filtered using Whatman filter paper no. 3. The filtered supernatant was mixed with 400µL and 1600µL water, respectively, and the spectrophotometric measurements were done at 517nm.

2.7. Research Design

The present study utilized the research design shown in Fig. 1. Meat samples were treated with various concentrations of aloe vera gel to assess its effectiveness in inhibiting the production of heterocyclic amines. To obtain accurate and reliable results, three replicates were prepared for each test with each concentration. The mean values and standard deviations of the experimentations were calculated to ensure the consistency of the findings.

2.8. Statistical Analysis

The overall results were statistically analyzed using SPSS software package. The significant difference was estimated using one-way analysis of variance and ranking of means were obtained using Tukey's test.

3. RESULTS AND DISCUSSION

The present study was conducted with the aim to reduce heterocyclic amines in chicken nuggets.

3.1. Creatine Assay

The association of creatine to total protein in chicken was calculated according to the method of (Dvořák 1981). Fig. 2 showed the standard curve of creatine at 517 nm, that shows complete linearity with coefficient of determination (R^2) at 0.9906. Moreover, this curve was used to measure creatine in chicken nuggets.

Fig. 3 illustrates the research findings on the effect of different concentrations of aloe vera gel on the creatine content of meat samples. Three replicates were prepared for each concentration, and the mean values and standard deviations were calculated. The results showed a mixed response in reducing creatine content across the treatments, but a strong and significant difference ($P<0.05$, $P=0.001$) was observed between the control (To) and T3, which contained 80% (w/w) aloe vera gel solution. The reduction in creatine content in the T3 treatment may be attributed to the presence of various bioactive compounds that are bound within the cellulosic framework of the aloe vera gel. Additionally, cellulose may have created a protective coating that reduced the conversion of creatine into creatinine.

Osman et al. (2020) observed a persistent ratio of creatine to net protein in the muscles of animals with an extensive collagen network, and they further noted that the persistent ratio of creatine to total protein in chicken muscles could be a practical indicator of meat quality. This indicator could also aid in determining the levels of creatinine in urine, which is considered an index of the mass of lean body. Matarneh et al. (2021) also reported that the excretion of creatinine is lower among infants compared to adults.

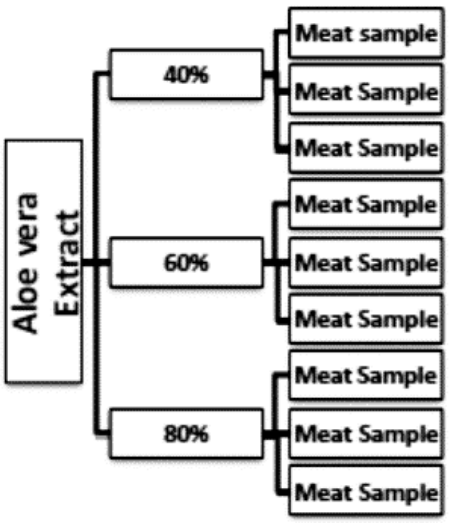

Fig. 1: Experimental design used for estimating effectiveness of aloe vera gel.

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

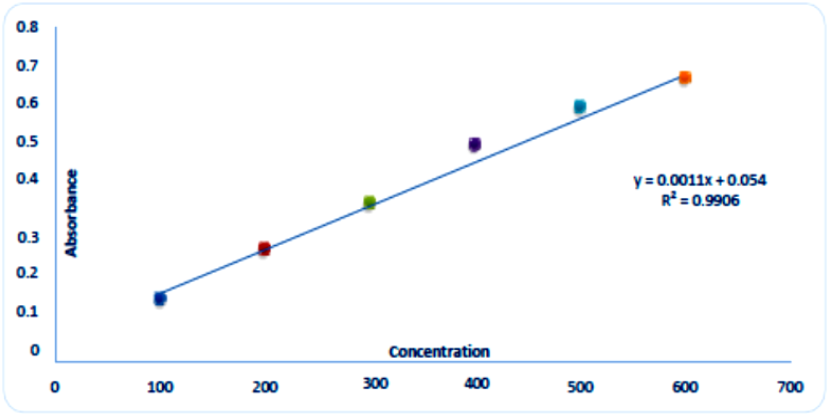

Fig. 2: The standard curve of creatine used for quantification of creatine in chicken nuggets. The absorbance was measured at 517nm.

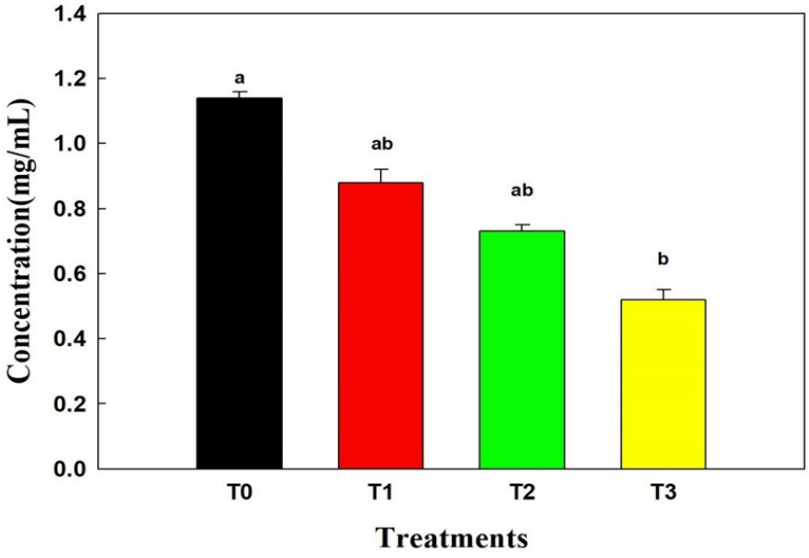

Fig. 3: The effect of different concentrations of aloe vera gel solutions in reducing the creatine contents in chicken nuggets.

3.2. Antioxidant Assay

Fig. 4 presents the results of the antioxidant activity of aloe vera-coated chicken nuggets, as measured by the inhibition of HAAs. The findings showed significant differences in percentage inhibition across all samples. The highest percentage inhibition was observed in T3, which contained 80% (w/w) aloe vera gel solution. Additionally, appreciable percentage inhibition was also observed in T2, which contained 29% (w/w) aloe vera gel, compared to the control. This strong inhibition may be attributed to the presence of various functional and nutraceutical components in the aloe vera gel.

According to Reza Nazifi et al. (2019), aloe vera extract exhibits significant antioxidant activity and scavenging activity due to the presence of antioxidant compounds in the extract. Fig. 4 demonstrates the antioxidant activity of aloe vera gel due to its functional nature and the presence of antioxidant components. The scavenging activity refers to the extract's ability to act as an antioxidant or to scavenge DPPH radicals (Lucini et al. 2015).

Furthermore, the phenolic compounds in aloe vera gel extract play an important role in preventing certain bacterial growth that can cause infections in animals and humans (Sánchez et al. 2020). The results of the present study support the findings of Radha and Laxmipriya (2015) that higher content of aloe vera gel extracts provide higher scavenging activity and protection against harmful bacterial infections.

3.3. Assay of Color

Color identification is a part of sensory evaluation and development of product needs to be identified from every aspect, streaks of color assessment graph determined changes in appearance of product. In present study has used colorimetric technique to carry out color assay of chicken meat by dividing them into four different groups, including a control group, a sample of three chicken meat pieces treated with 40, 60 and 80% respectively (Fig. 5). Barbin et al. (2016) investigated that colorimetric assays are most commonly useful to detect the levels of protein and glucose. The change in colors is generally indicated in three different axes, including L-axis, B-axis and A-axis. The L-axis indicates the lightness, the A-value shows the shift towards red or green while, the B-value depicts the

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

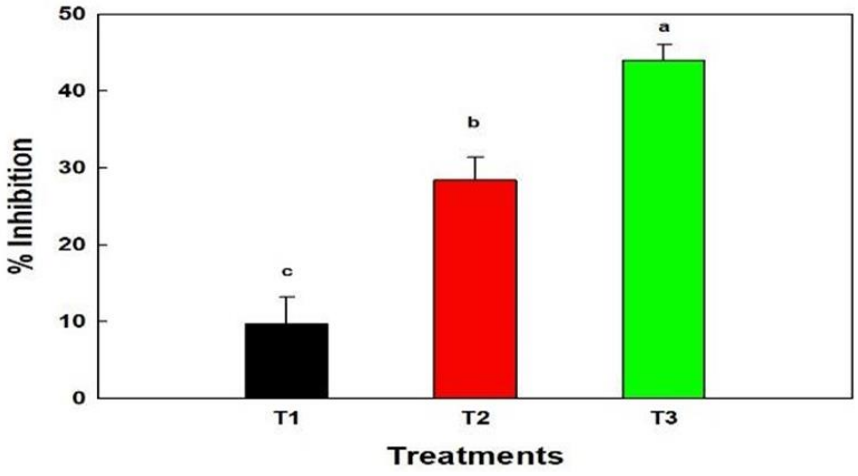

Fig. 4: % Inhibition of heterocyclic amines from aloe vera coated chicken nuggets.

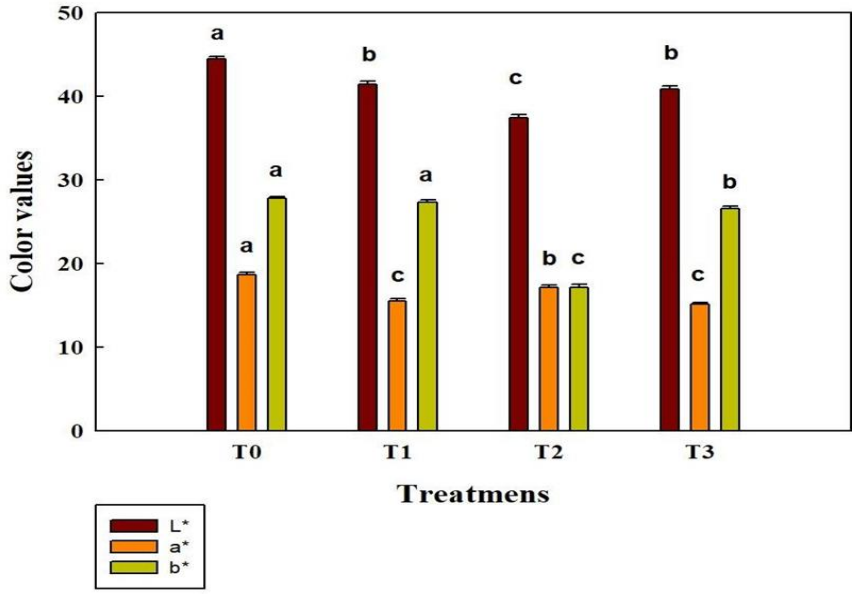

Fig. 5: Color values comparison with control and treated sample (L* a* b*)

shift towards blue or yellow color. Sohaib et al. (2017) observed that the color assay is mainly carried out with food items or additives to detect their quality based on their texture and primarily nutritiveproperties. On the other side, the color assay contributes to measuring the pH value of different food items thus, illustrating their acidic or basic nature and the potential impact of wide variety of food items on the physical and physiological health of individuals with or without any illness.

In this study, findings obtained were different from control treatment as L* lightness of T1, T2 and T3 decreased as aloe gel has cellulosic layer which can be barrier for light. Mixed results included non-significant results between T1 and T3 but have significant difference in T2, it might be due to greater absorbance of aloe gel before frying. Redness turning to blueish color a* have significant difference in T2 and decreased in other treatments as compounds in aloe vera mask redness of meat, but thick layer might not be properly absorbed due to which T3 shows non-significant results. Yellowness b* produced during frying but in nuggets yellow to brown color appears due to crumbscoating so there is no significant change in treatments except T2.

Conclusion: The results of the research study demonstrate that using aloe vera as a coating before frying meat can inhibit the formation of HAA's. The DPPH assay protocol performed showed that a 80% concentration of aloe vera gel solution inhibited 44% of HAA's, while the 60% concentration inhibited 29%, and the 40% concentration inhibited only 10%. These findings indicate that using aloe vera can effectively inhibit HAA's and improve the quality of meat. Additionally, the study found that aloe vera extract has significant scavenging activity due to its high content of phenolic compositions, which act as antioxidants. This scavenging activity is important as it indicates the extract's ability to act as an antioxidant or DPPH radical scavenger. Furthermore, the phenolic

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

composition of aloe vera gel extract plays an essential role in preventing certain bacterial growth that can cause infections in humans and animals. The study's results also demonstrate that higher concentrations of aloe vera gel extract provide greater scavenging activity and protection against harmful bacterial infections. The intensity of color depicted in the graphs and table relied on the concentration of aloe vera extract, as well as the axis at which the color was observed. Therefore, aloe vera extract can improve the quality of meat by optimizing the parameters of biochemical reactions within the meat. In conclusion, aloe vera extract is recognized as playing a significant role in improving the quality of meat and has the potential to treat both humans and animals with various conditions. Future researchers should investigate the antioxidants in aloe vera and their benefits for health, as well as the benefits of aloe vera in treating multiple disorders. Furthermore, research into how the cellulosic gel inhibits reactions of creatine during meat frying should also be conducted. By separating the antioxidants in aloe vera and converting them into a formula for drug preparation, aloe vera's benefits can potentially help treat various conditions.

Author's Contribution

Usama Mubarik and Mehwish: Conceptualization, investigation, and writing—original draft. Usama Asif: Methodology, writing—review and editing. Usama Mubarik: Validation, writing—review and editing. All authors approved the final version of the manuscript.

ORCID

Usama Mubarik https://orcid.org/0009-0007-6362-0900

REFERENCES

Barbin, D. F., Mastelini, S. M., Barbon Jr, S., Campos, G. F., Barbon, A. P. A., and Shimokomaki, M. (2016). Digital image analyses as an alternative tool for chicken quality assessment. *Biosystems Engineering*, *144*, 85-93.

Bert, F., Scaioli, G., Tolomeo, M., Lo Moro, G., Gualano, M. R., & Siliquini, R. (2020). Knowledge, attitudes and eating habits red and processed meat among gym users: a cross-sectional survey. *Perspectives in Public Health*, *140*(4), 203-213.

Chen, C., Yu, L. T., Cheng, B. R., Xu, J. L., Cai, Y., Jin, J. L., ... & Wan, J. (2022). Promising therapeutic candidate for myocardial ischemia/reperfusion injury: what are the possible mechanisms and roles of phytochemicals?. *Frontiers in Cardiovascular Medicine*, *8*, 2257.

Duh, P. D. (1998). Antioxidant activity of burdock (Arctium lappa Linne): its scavenging effect on free-radical and active oxygen. *Journal of the American Oil Chemists' Society*, *75*(4), 455-461.

Dvořák, Z. (1981). Creatine as an indicator of net muscle proteins. *Journal of the Science of Food and Agriculture*, *32*(10), 1033-1036.

Gibis, M. (2016). Heterocyclic aromatic amines in cooked meat products: causes, formation, occurrence, and risk assessment. *Comprehensive Reviews in Food Science and Food Safety*, *15*(2), 269-302.

Gibis, M., and Weiss, J. (2017). Inhibitory effect of cellulose fibers on the formation of heterocyclic aromatic amines in grilled beef patties. *Food Chemistry*, *229*, 828-836.

Haque, M. H., Sarker, S., Islam, M. S., Islam, M. A., Karim, M. R., Kayesh, M. E. H., ... & Anwer, M. S. (2020). Sustainable antibiotic-free broiler meat production: Current trends, challenges, and possibilities in a developing country perspective. *Biology*, *9*(11), 411.

Lucini, L., Pellizzoni, M., Pellegrino, R., Molinari, G. P., and Colla, G. (2015). Phytochemical constituents and in vitro radical scavenging activity of different Aloe species. *Food chemistry*, *170*, 501-507.

Matarneh, S. K., Silva, S. L., and Gerrard, D. E. (2021). New insights in muscle biology that alter meat quality. *Annual review of animal biosciences*, *9*, 355-377.

Nadeem, H. R., Akhtar, S., Ismail, T., Sestili, P., Lorenzo, J. M., Ranjha, M. M. A. N., ... & Aadil, R. M. (2021). Heterocyclic aromatic amines in meat: Formation, isolation, risk assessment, and inhibitory effect of plant extracts. *Foods*, *10*(7), 1466.

Omojola, A. B., Ahmed, S. A., Attoh-Kotoku, V., and Wogar, G. I. (2015). Effect of cooking methods on cholesterol, mineral composition and formation of total heterocyclic aromatic amines in Muscovy drake meat. *Journal of the Science of Food and Agriculture*, *95*(1), 98-102.

Osman, A., Bin-Jumah, M., Abd El-Hack, M. E., Elaraby, G., Swelum, A. A., Taha, A. E., ... and Ashour, E. A. (2020). Dietary supplementation of soybean glycinin can alter the growth, carcass traits, blood biochemical indices, and meat quality of broilers. *Poultry science*, *99*(2), 820-828.

Radha, M. H., and Laxmipriya, N. P. (2015). Evaluation of biological properties and clinical effectiveness of Aloe vera: A systematic review. *Journal of traditional and complementary medicine*, *5*(1), 21-26.

Rawat, G., & Saxena, C. (2021). A review on ameliorative role of food supplements in cancer. *ASIAN JOURNAL OF MULTIDIMENSIONAL RESEARCH*, *10*(4), 383-390.

Reza Nazifi, S. M., Asgharshamsi, M. H., Dehkordi, M. M., and Zborowski, K. K. (2019). Antioxidant properties of Aloe vera components: a DFT theoretical evaluation. *Free Radical Research*, *53*(8), 922-931.

Sánchez, M., González-Burgos, E., Iglesias, I., and Gómez-Serranillos, M. P. (2020). Pharmacological update properties of Aloe vera and its major active constituents. *Molecules*, *25*(6), 1324.

Sohaib, M., Anjum, F. M., Arshad, M. S., Imran, M., Imran, A., and Hussain, S. (2017). Oxidative stability and lipid oxidation

ISSN: 2708-7182 (Print); ISSN: 2708-7190 (Online)
Open Access Journal

flavoring volatiles in antioxidants treated chicken meat patties during storage. *Lipids in Health and Disease*, *16*(1), 1-10.

Sugimura, T. (1997). Overview of carcinogenic heterocyclic amines. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *376*(1-2), 211-219.

Tufail, M., and Wu, C. (2023). Exploring the Burden of Cancer in Pakistan: An Analysis of 2019 Data. *Journal of Epidemiology and Global Health*, 1-11.

ur Rahman, U., Sahar, A., Khan, M. I., and Nadeem, M. (2014). Production of heterocyclic aromatic amines in meat: Chemistry, health risks and inhibition. A review. *LWT-Food Science and Technology*, *59*(1), 229-233.

Zamora, R., & Hidalgo, F. J. (2020). Formation of heterocyclic aromatic amines with the structure of aminoimidazoazarenes in food products. *Food chemistry*, *313*, 126128.