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ANTIOXIDANT PROTEIN FRACTION HYDROLYSATES DERIVED FROM ANTIDORCAS MARSUPIALIS AND THEIR FUNCTIONAL ACTIVITIES

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I. INTRODUCTION

Oxidation is a significant challenge for the food industry, driving concerns over spoilage, nutrient degradation, and the formation of potentially harmful compounds. Among these, lipid oxidation stands out for its impact on meat quality, resulting in rancidity, flavour alterations, and texture deterioration (1). Historically, the food industry has depended on synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) to prevent the oxidation of lipids (2). However, these synthetic solutions caused concern among consumers due to their potential health risks and adverse environmental impact. This growing unease has fuelled a shift toward natural, sustainable alternatives, sparking interest in plant (2) and animal (3) derived antioxidant sources. The search for novel sources is vital given the diverse antioxidant potencies and efficacies.

The study of dietary protein hydrolysates derived from animal muscles is interesting due to their potential as natural antioxidants and functional ingredients in food systems. Antioxidant protein hydrolysates can neutralise free radicals, thereby reducing oxidative damage linked to chronic diseases and extending the shelf life of food products (4).
This research examined the antioxidant characteristics of protein hydrolysates from the *longissimus dorsi* muscle of springbok (*Antidorcas marsupialis*), generated via enzymatic hydrolysis using Alcalase and Papain. The unique characteristics of springbok, including its lean muscle composition and high-quality protein content, suggest that it could yield antioxidant hydrolysates with promising functional applications (5). The study analysed hydrolysates from whole protein, sarcoplasmic, and myofibrillar fractions for antioxidant activ ity.

II. MATERIALS AND METHODS

Longissimus dorsi muscle meat from springbok (*Antidorcas marsupialis*) was obtained from Lamerina, Johannesburg, South Africa. After collection, the meat was stored at −18°C for preservation until further processing. Essential reagents, including 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrous sulphate, and hydrogen peroxide, as well as molecular weight cut-off membranes (10-kDa, 5-kDa, and 3-kDa), were sourced from Sigma-Aldrich (Tallaght, Ireland). All other reagents used were of analytical grade. The experiments were repeated three times.

Sample preparation
Meat samples were thawed overnight at 4°C and blended using a Waring blender. Freshly prepared meat was then utilised for protein extraction.

DPPH Radical Scavenging Activity Assay
The DPPH radical scavenging activity was evaluated using a method by Najafian and Babji (2015). For each sample, 1.5 mL was mixed with 1.5 mL of 0.15 mM DPPH in 95% ethanol. The reaction mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm, and scavenging activity was calculated as follows:
Scavenging Effect (%) = [(B – A) / B] × 100,
where A is the sample absorbance and B is the control absorbance.

TBARS
This assay measured lipid peroxidation by mixing 0.5 g of sample with 2.5 mL of TBA reagent (0.375% TBA, 15% TCA, and 0.25 N HCl), heating at 100°C for 10 minutes, and centrifuging. Absorbance of the supernatant at 532 nm provided the TBARS value, calculated using:
TBA Value (mg malonaldehyde equivalents/kg sample) = Absorbance at 532 nm × 2.77

III. RESULTS AND DISCUSSION

The findings revealed that hydrolysates treated with Alcalase exhibited significantly higher DH and antioxidant activity, particularly in the whole protein and sarcoplasmic fractions (Table 1). Alcalase hydrolysates contained smaller peptides (<3 kDa), which exhibited enhanced radical scavenging abilities in DPPH and hydroxyl radical assays, while Papain hydrolysates, generating larger peptides, had lower antioxidant potential. These results

indicate that the choice of enzyme and protein fraction type affects the bioactive properties of protein hydrolysates, highlighting their potential as natural antioxidants in food products.
The TBARS results, shown in Figure 1, revealed that lipid oxidation levels varied significantly among hydrolysate samples prepared from different protein fractions (whole protein, sarcoplasmic protein, and myofibrillar protein) and treated with two proteases (papain and alcalase). The whole protein hydrolysate treated with alcalase showed the highest TBARS value (~0.5 mg malonaldehyde/kg), indicating the greatest lipid oxidation, likely due to increased exposure of lipid substrates. In contrast, sarcoplasmic and myofibrillar hydrolysates treated with alcalase had lower TBARS values (~0.2 mg malonaldehyde/kg), suggesting that these fractions are less susceptible to oxidation when hydrolysed with alcalase.

Table 1: %Radical scavenging activity of alcalase and papain hydrolysates at different molecular weights

Molecular weight cut-off	%RSA HYDROLYSATE SAMPLES (DPPH ASSAY)					
	ALCALASE HYDROLYSATES			PAPAIN HYDROLYSATES		
	WP	SP	MP	WP	SP	MP
>30kda	20.17	16.81	11.61	12.26	14.53	36.98
>10kDa	32.10	29.28	20.39	21.51	26.97	40.74
>3kDa	47.11	38.39	31.67	33.80	35.39	40.56
<3kDa	56.51	45.59	41.03	36.70	45.26	44.22

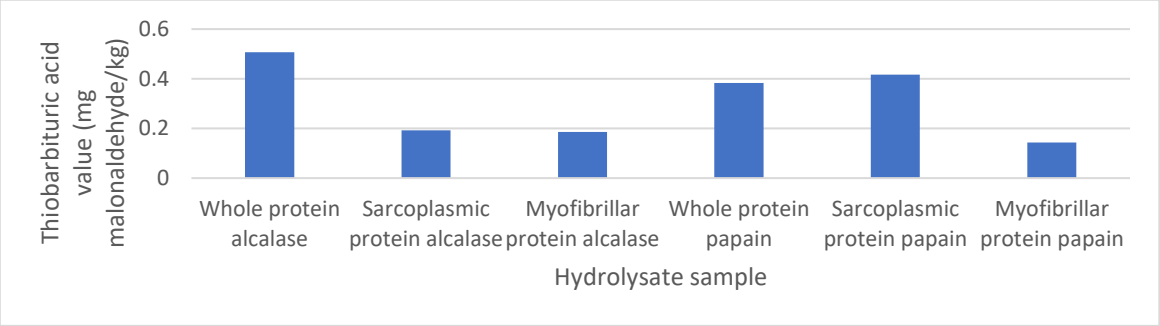


Figure 1: Thiobarbituric acid value of alcalase and papain hydrolysed protein fractions

IV. CONCLUSION

This study demonstrates the efficacy of enzymatically derived springbok protein hydrolysates as promising natural antioxidant ingredients. The findings support that Alcalase-derived hydrolysates, particularly from whole and sarcoplasmic protein fractions, offer enhanced antioxidant activity due to their smaller peptide sizes, favouring interactions with free radicals.

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