P-06-16 5:30 PM

Optimization of the fluorogenic assay conditions to determine caspase 3 activity in meat extracts (#263)

Claudia Fuente-Garcia[1, 2], Noelia Aldai[2], Enrique Sentandreu[1], Eva Botella[1], Miguel Angel Sentandreu[1]

[1] Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Paterna (Valencia), Spain; [2] Lactiker Research Group, University of the Basque Country (UPV/EHU), Department of Pharmacy and Food Sciences, Vitoria-Gasteiz, Spain

Introduction

Caspases are cysteine-dependent proteases involved in apoptosis and inflammation processes by selectively cleaving C-terminal aspartic acid residues of peptides/proteins.Researchers put their efforts mainly on the study of caspases in skeletal muscle since they are activated in early metabolic responses associated with hypoxia/ischemia [1,2]. Current approaches addressing the determination of caspase activity in meat are performed with general caspase assay kits which are not properly optimized for muscle tissue. Then, this research presents a tailored fluorogenic assay, as an alternative to the general commercial kits, to measure the activity of caspase 3 in meat extracts.

Methods

Sarcoplasmic extracts were obtained from 0.5g of beef (*Longissimus thoracis* muscle) homogenized in 2mL of extraction buffer containing HEPES buffer, pH 7.5, 10% sucrose and 1mM EDTA. The homogenate was centrifuged at 20000*g* for 20min at 4°C, and the supernatant was filtered and stored at -80°C until analyzed. Different concentrations of cysteine preservative DTT and Ac-DEVD-AMC substrate were tested in order to optimize the fluorogenic assay until reaching final experimental conditions: 50µL of sarcoplasmic extracts were added to each of the microplate wells followed by 20µL of 24mM DTT solution. Microplate was pre-incubated for 30min at 37°C and then the enzyme reaction was initiated by adding 50µL of 0.1mM of Ac-AMC-DEVD dissolved in HEPES-CHAPS buffer (pH 7.5). Fluorescence intensity was then measured for 40min at 37°C every 2min interval. Moreover, different volume ratios (R-) of extract/substrate (1:1 and 3:5) were tested.

Results

The fluorescence assay proposed is based on the hydrolysis of the fluorogenic substrate Ac-DEVD-AMC by caspase 3 that releases fluorescent AMC group with a maximum emission at 460nm wavelength [3]. Figure 1 shows the enzyme activity at different substrate concentrations (mM) and extract/substrate ratios during the experimental optimization process finding the highest caspase activity at 0.083 mM (R-1:1) after 46min of incubation at 37°C. However, such a substrate concentration was discarded to avoid saturation of the fluorescence detection of samples with extremely high enzymatic activity. The effect of extract/substrate ratio had a great influence in muscle caspase 3 activity: R-1:1 showed higher fluorescence values than R-3:5. Finally, 0.042mM, R-1:1 and 40min conditions were selected as definitive substrate concentration, extract/substrate ratio and reaction time, respectively.

Figure 2 illustrates the effect of DTT concentration on enzyme activity of 1:2 and 1:4 diluted extracts studied under aforementioned optimized conditions. Hydrolysis of Ac-DEVD-AMC did not seem to be affected by DTT concentration, although caspase 3 activity was negatively affected in absence of DTT in samples with low enzymatic presence (data not shown). Therefore, 4mM DTT concentration was finally selected as final optimized conditions to determine caspase 3 activity in meat extracts (▪ in Figure 2 - undiluted sample).

Conclusion

After an appropriate optimization, results demonstrated that the methodology proposed can accurately and with high sensitivity determine caspase 3 activity in meat extracts. The straightforward microtiter plate format allowed the simultaneous analysis of a high number of samples and can be a better alternative to general caspase assay kits.

Acknowledgements

Department of Economic Development and Infrastructures of the Basque Government for the doctoral fellowship of C.F.G.

References

1. Ouali, A., Herrera-Mendez, C.H., Coulis, G., Becila, S., Boudjellal, A., Aubry, L., Sentandreu, M.A. (2006). Revisiting the conversion of muscle into meat and the underlying mechanisms. Meat Science, 74, 44-58.

2. Kemp, C.M., Bardsley, R.G., Parr, T. (2006). Changes in caspase activity during the postmortem conditioning period and its relationship to shear force in porcine longissimus muscle. Journal of Animal Science, 84, 2841-2846.

3. Stennicke, H.R., Salvesen, G.S. (1999). Caspases: preparation and characterization. Methods, 17, 313-319.

Notes

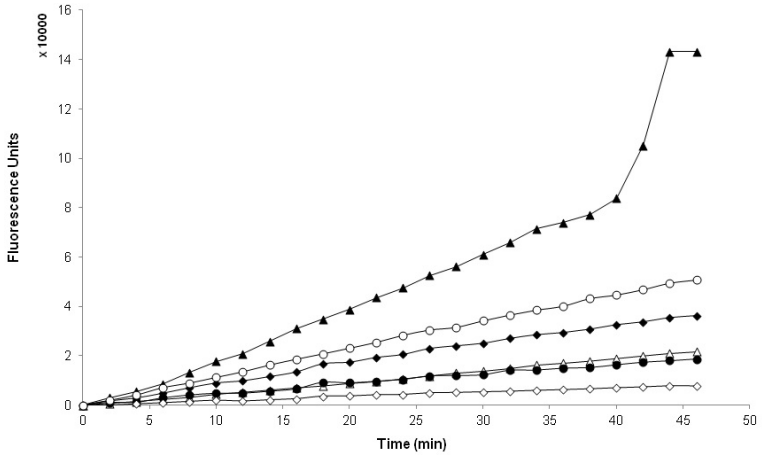

Figure 1. Effect of substrate concentration (mM) and extract/substrate ratio (R-) on caspase 3 activity: (Δ) 0.021 mM (R-1:1); (▲) 0.083 mM (R-1:1); (◊) 0.025 mM (R-3:5); (●) 0.05 mM (R-3:5); (♦) 0.1 mM (R-3:5) and (○) optimized conditions (0.042 mM and R-1:1).

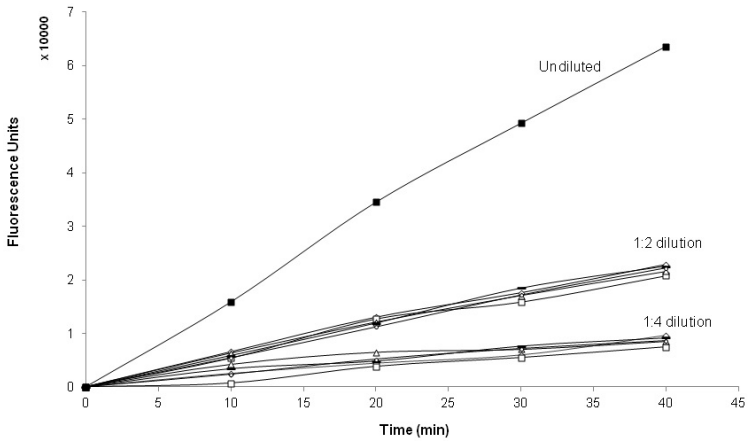

Figure 2. Effect of DTT concentration on caspase 3 activity in 1:2 and 1:4 extract dilutions: (◊) 16 mM; (□) 8 mM; (Δ) 4 mM; (━) 1 mM and (○) 0 mM (R-1:1). Undiluted sample assayed under experimental optimized conditions (■).

Notes