

Department of Food Science



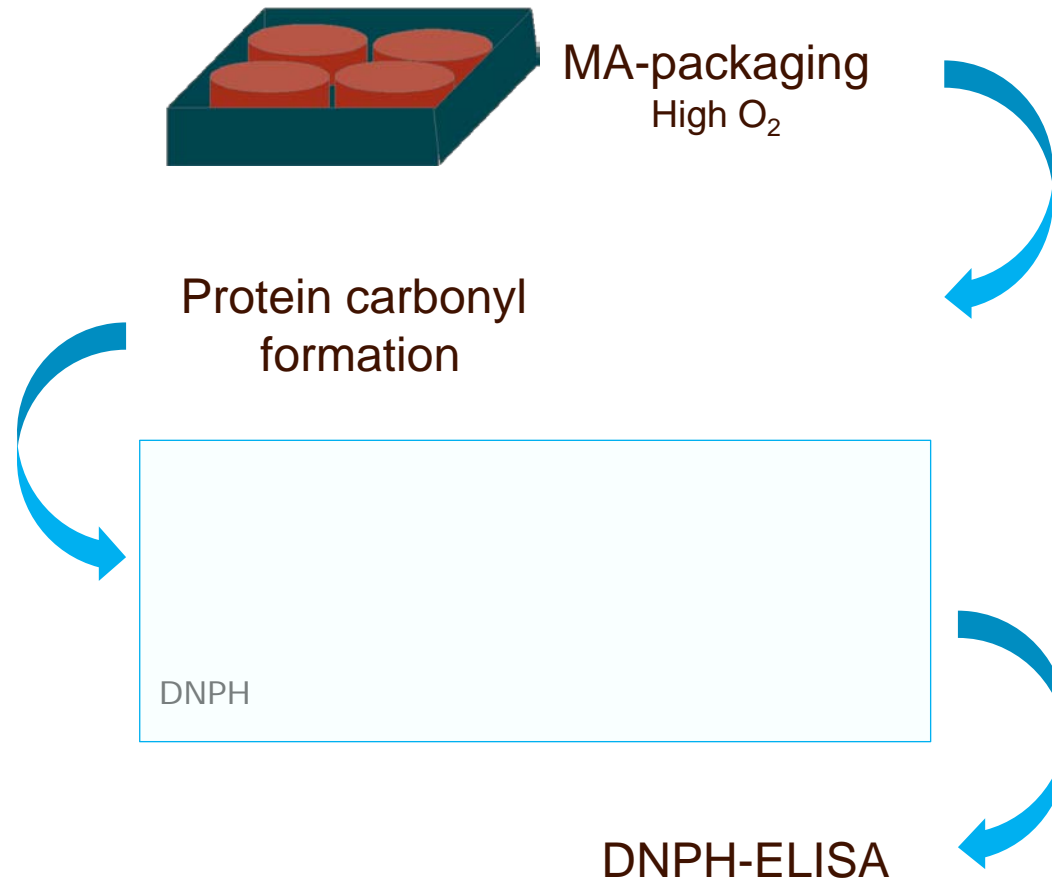
Quantification of protein carbonyls in meat by DNPH-ELISA

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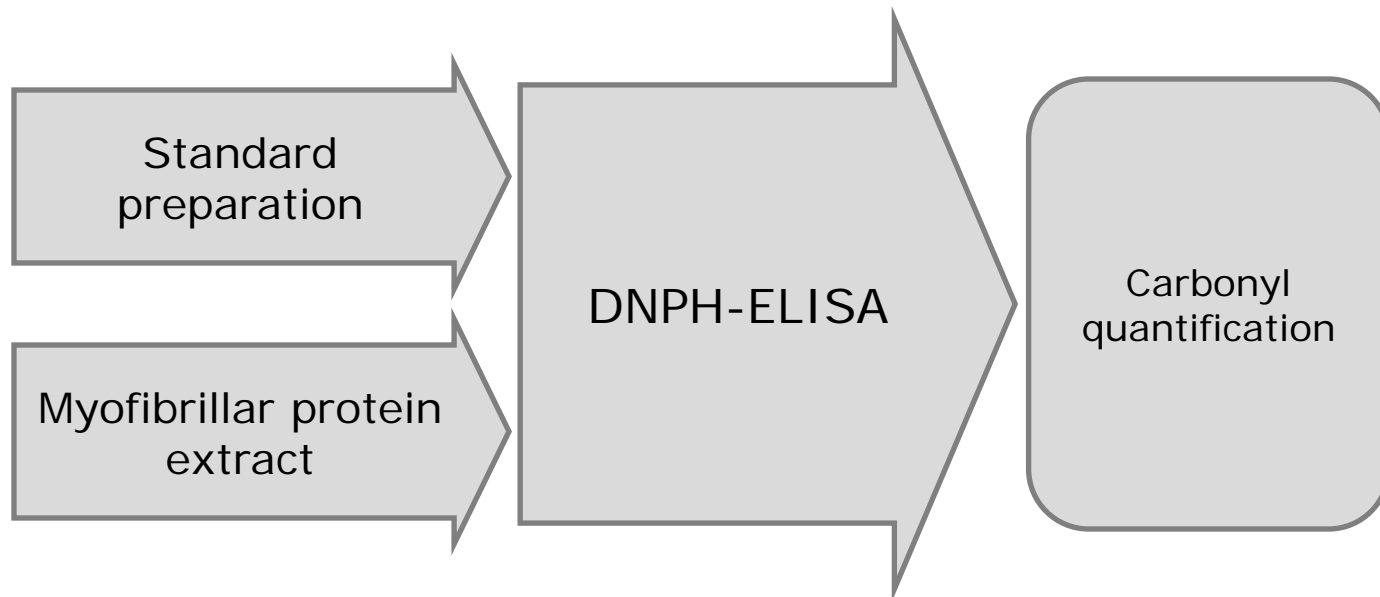
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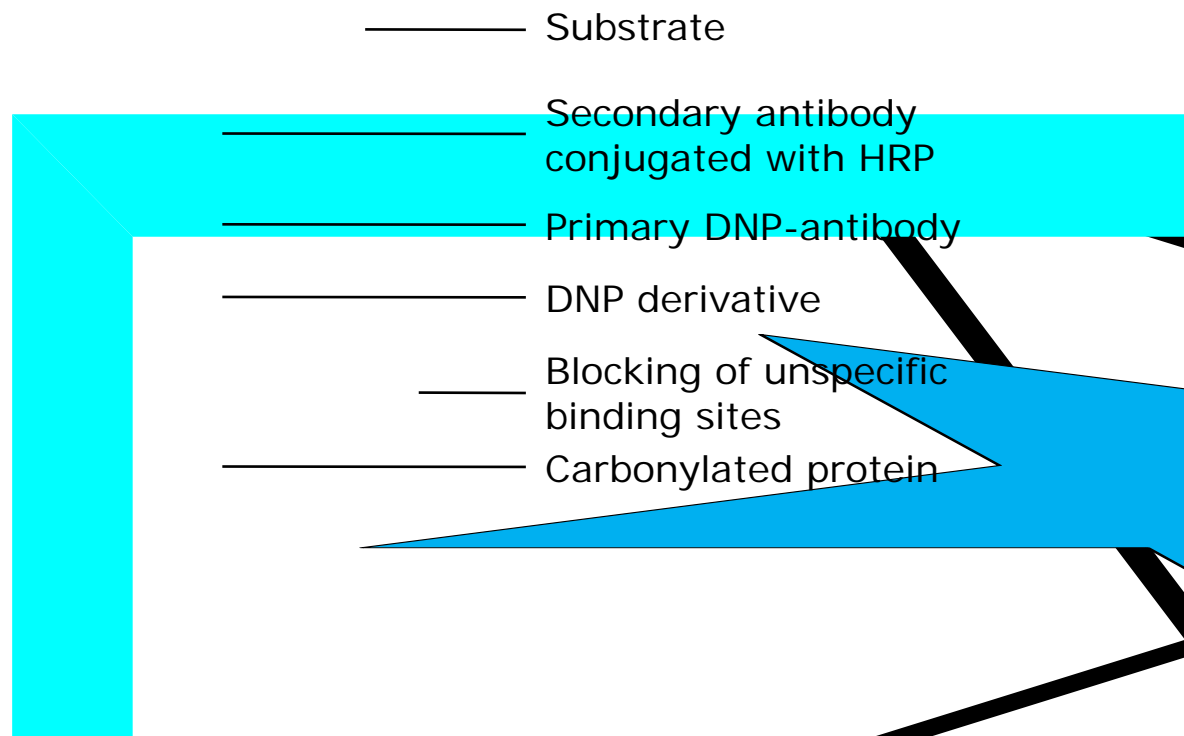
Protein carbonyl formation in meat products



Carbonyl quantification by DNPH-ELISA



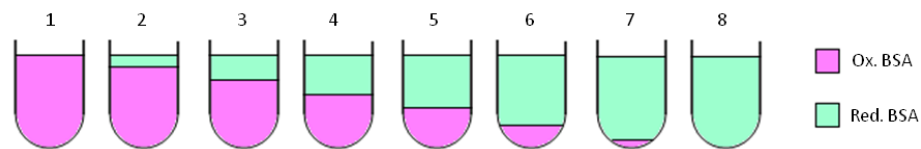
Principle of DNPH-ELISA



Carbonyl standard preparation

Protein concentration is maintained constant: 10 $\mu\text{g/ml}$ or 1 $\mu\text{g/well}$

Carbonyl concentration is varied: 0.7-10 nmol carbonyl/mg protein



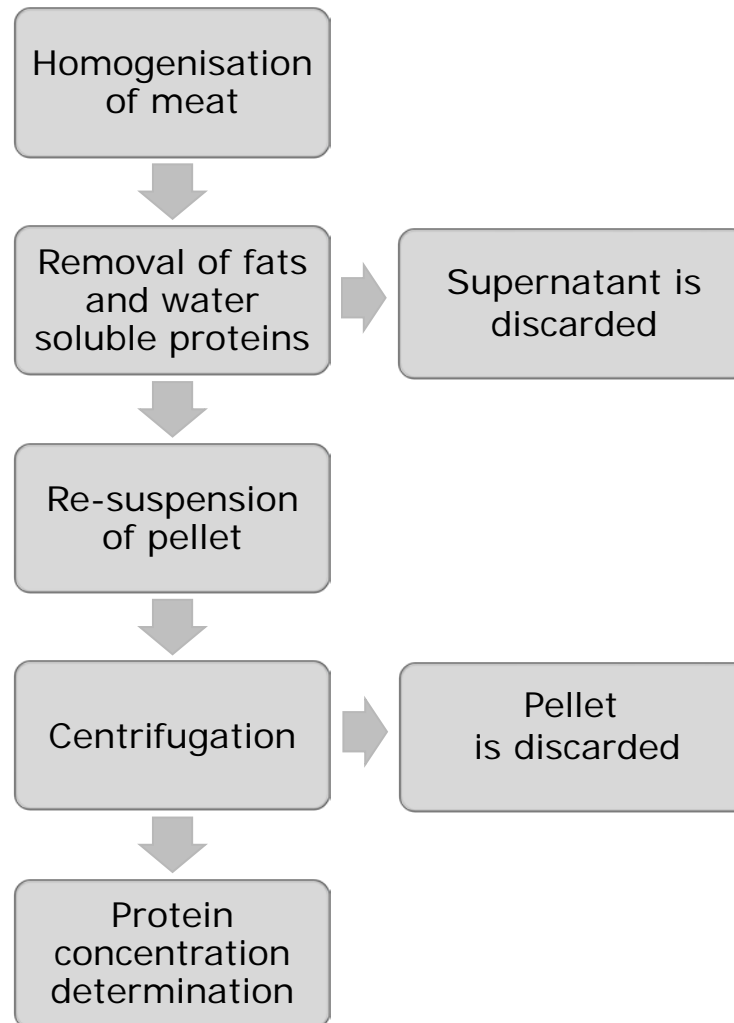
Carbonyl standard	Preparation ^a	Carbonyls ^b [nmol/mg protein]
Ox.BSA	Ferrous iron/EDTA	18
Red.BSA	NaBH ₄	0.7

^a Alamdari et al. (2005) Free Radical Biology and Medicine, 39, 1362-1367.

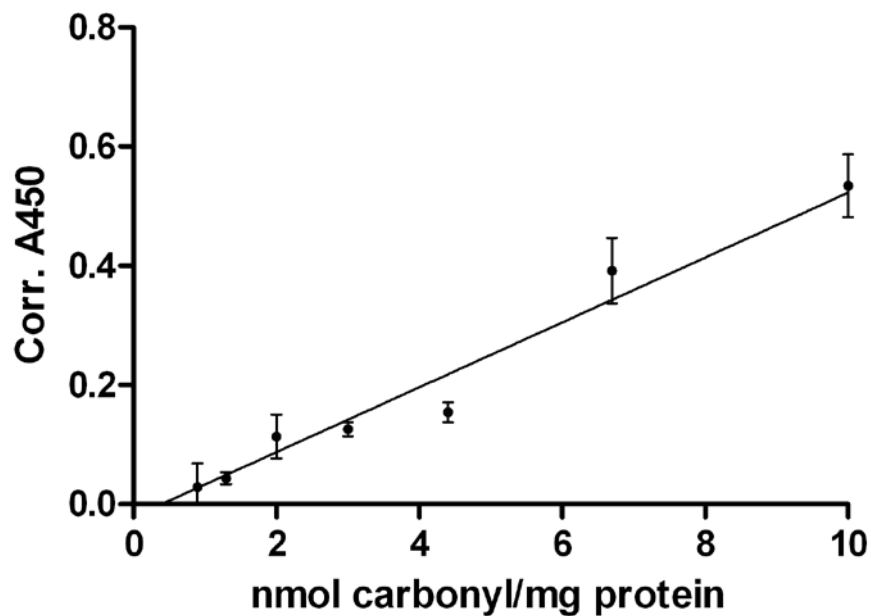
^b Levine et al. (1990) Methods in Enzymology, 186, 464-478.



Myofibrillar protein extraction from beef



Dose-response of the carbonyl standard

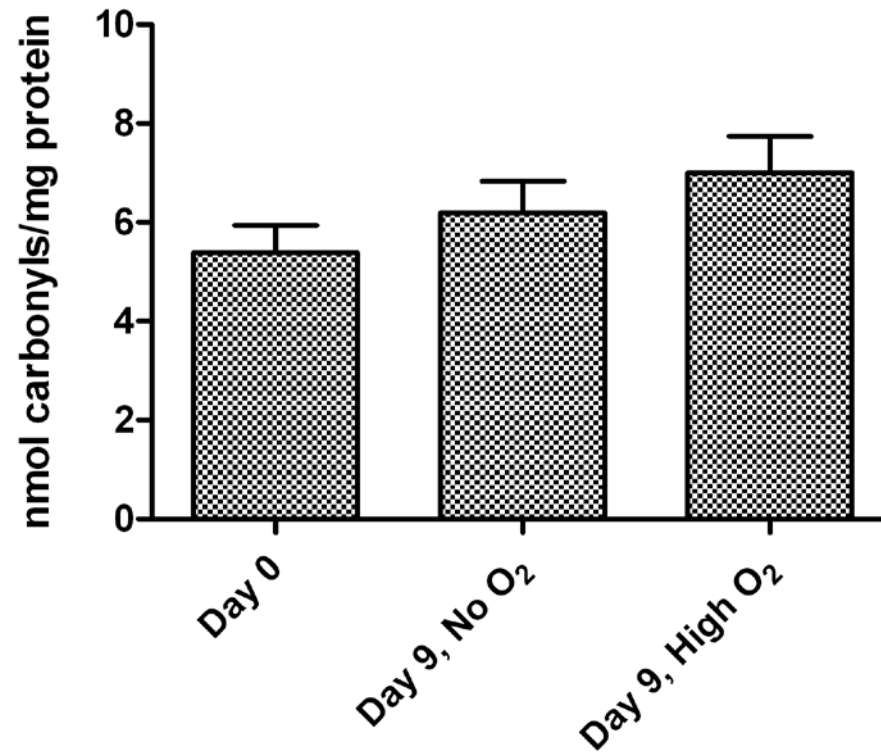


Dose-response of carbonyl standard (mean \pm SEM) for the quantification of carbonyl compounds in meat.

$$y = 0.05441x - 0.02139, R^2 = 0.8387$$



Protein carbonyl formation in beef



The carbonyl concentration in the myofibrillar extract of MA-packed minced beef stored for 9 days with or without O₂. The error-bars indicate a 95% confidence interval.



Comparison to previously found levels in beef

Protein extract	Experimental conditions	Storage time	Carbonyl analysis	Max. carbonyl (nmol/mg protein)	Reference
Myofibrillar	Not given	10 days	Colorimetric carbonyl analysis	5.1	<i>Martinaud et al, 1997*</i>
Myofibrillar	Not given	10 days	Colorimetric carbonyl analysis	6.9	<i>Martinaud et al, 1997*</i>
Myofibrillar	High oxygen, 4 °C, dark	9 days	Colorimetric carbonyl analysis	6.9	<i>Unpublished data</i>
Myofibrillar	High oxygen, 4 °C, dark	9 days	DNPH-ELISA	7.7	<i>Unpublished data</i>

* Martinaud et al. (1997) Journal of Agricultural and Food Chemistry, 45, 2481-2487.



Summary

- The protein carbonyl concentration in myofibrillar proteins from meat can be quantified by DNPH-ELISA
- Carbonyl quantification by DNPH-ELISA is comparable to:
 - Quantification by the colorimetric carbonyl analysis
 - Previously found levels of carbonyls in beef
- Improvement and validation are needed!



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