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Glycation : de l'inflammation au vieillissement



# Characterization of a bovine serum albumin model system for in vitro fortification of N $\epsilon$ -carboxymethyl-lysine

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# Summary

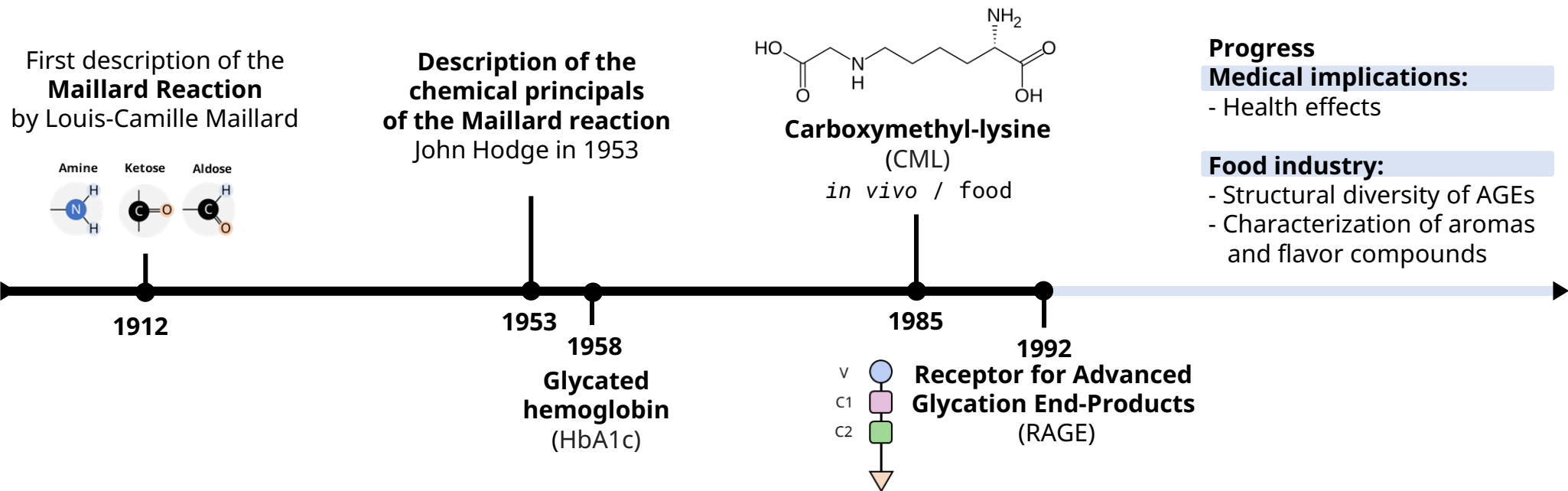
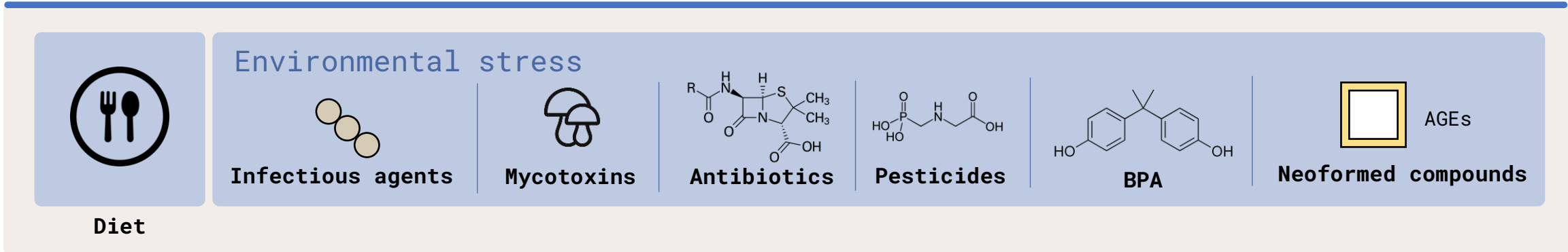
1 | **Introduction**  
Why building a new model protein?

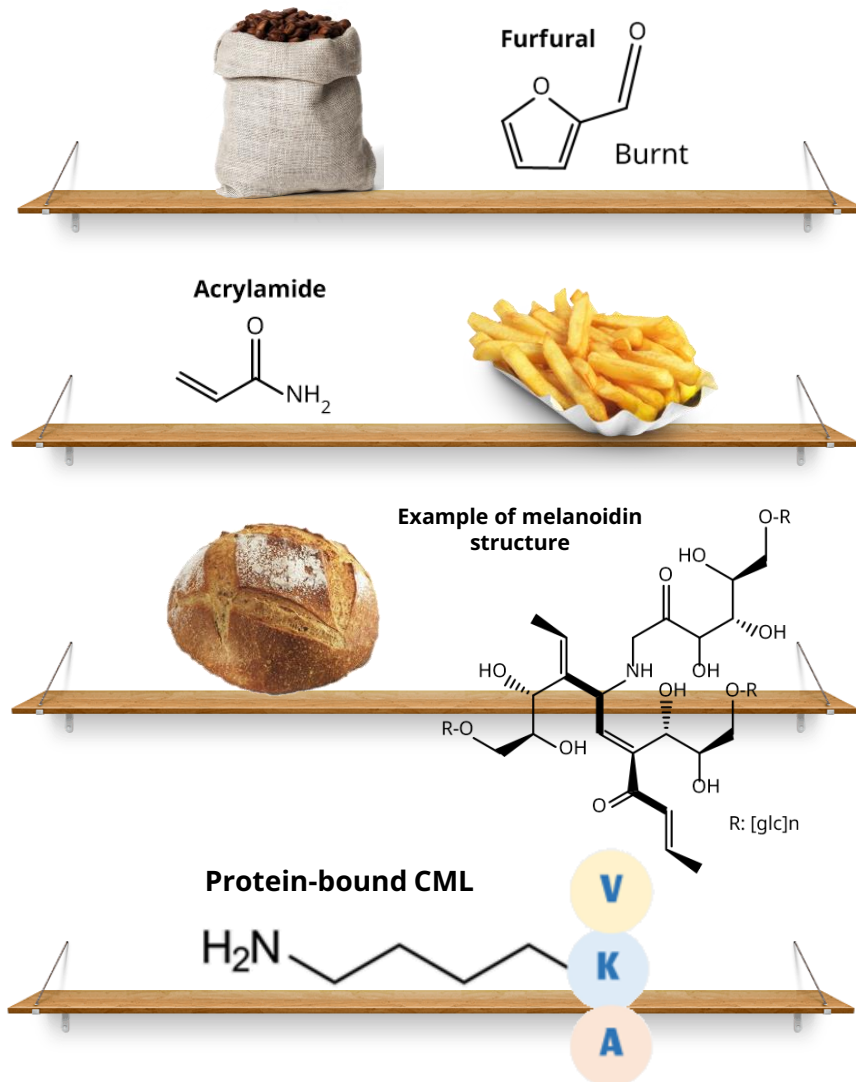
2 | **PROS and CONS**  
Different dietary protocols

3 | **Results and discussion**

4 | **Key findings**







## Exogenous AGEs (Neoformed compounds)

High heat



### Example Infant formula

Up to 40.5 mg CML/100 g prot.  
(TUD - AGE Database)

## Tissue accumulation/dysfunction

Tessier et al., 2015

### Gut



Microbial community shift  
Metabolic endotoxemia

van Dongen et al., 2021

### Aorta



Arterial stiffness

Grossin et al., 2015

### Kidneys



Glomerulosclerosis

Teissier et al., 2019

# Investigating the effects of dAGEs

## *Dietary protocols*



<b>Pros</b>	Depict the effect/kinetics of a target compound	Closer to homemade/ industrial processing	Investigation of a target compound/conservation of vitamins
<b>Cons</b>	Not representative of a complex food matrix	Vitamin loss/ uncontrolled formation of AGEs	Less representative of the reactions on food matrix

## Goals EXOAGEING

Comparative characterization of **classical** and **alternative** methods of converting lysine into CML, investigating other glycation hotspots such as arginine residues as well as more complex forms of glycation products such as crosslinks.

Method to prevent nutritional loss associated with heat treatment.

Extensive use of quantitative and qualitative analytical methods (LC-MS/MS) and proteomics.

Evaluate the digestibility of modified protein in vivo.

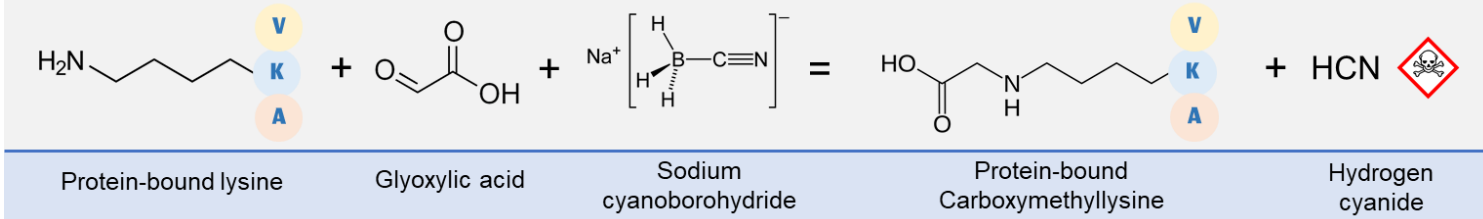


Prepare a dCML enriched mice food

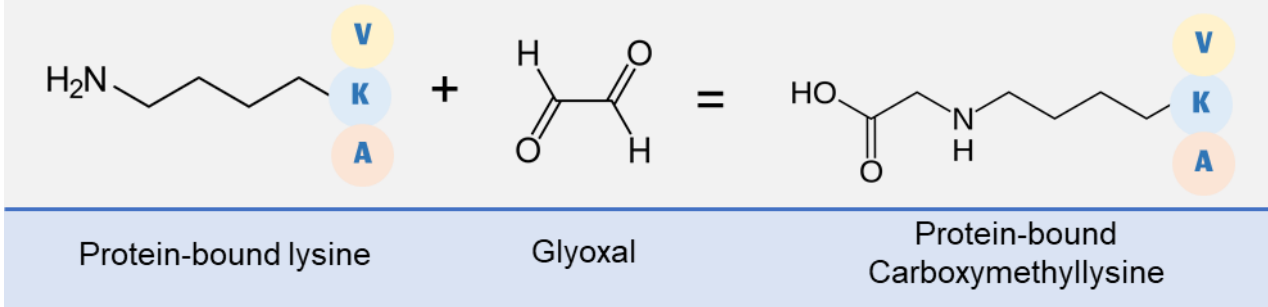


**200 µg CML/g food**  
(Grossin et al., 2015)

**Classic**

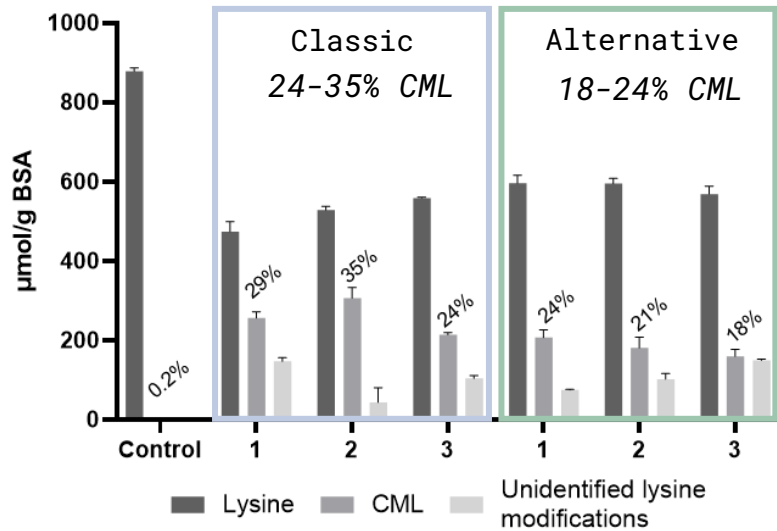


**Alternative**



# > CML formation ... and other AGEs

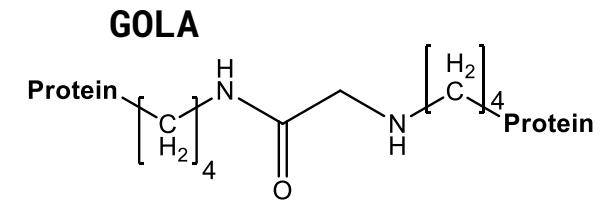
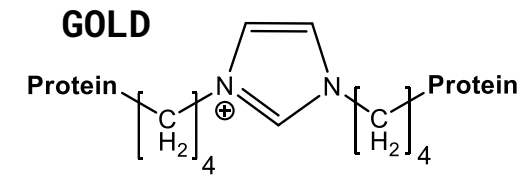
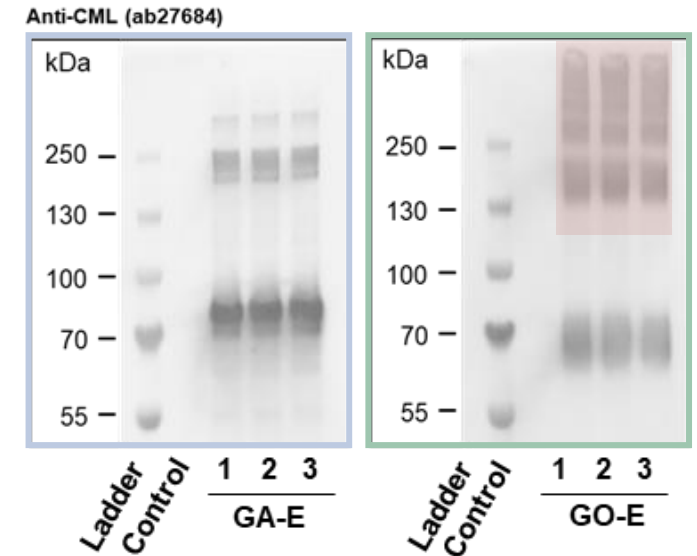
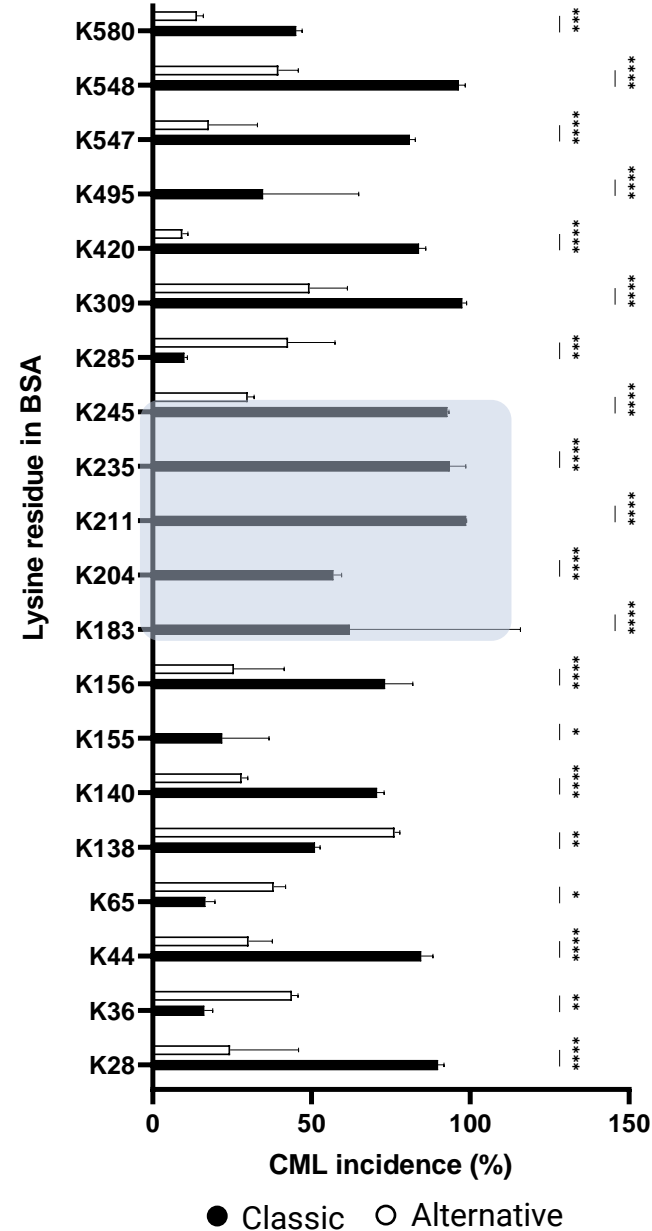
## Lysine modifications



**Classic method:**  
Higher CML formation

Able to glycate lysine residues not occurring in alternative methods

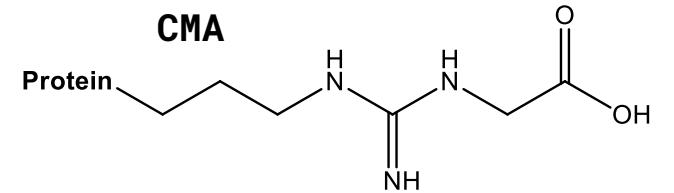
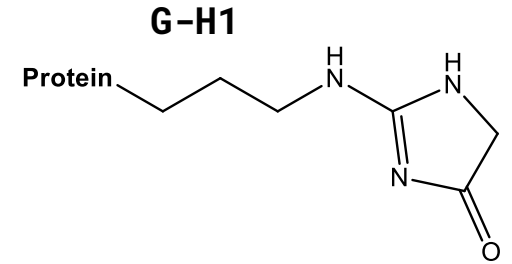
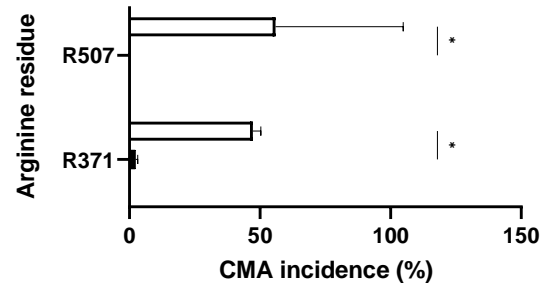
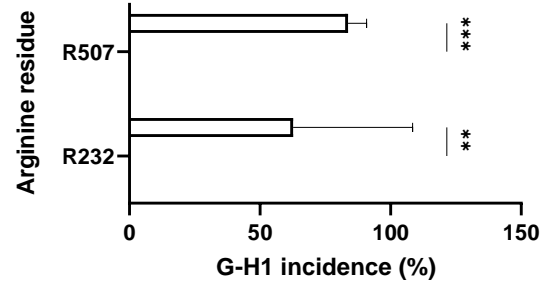
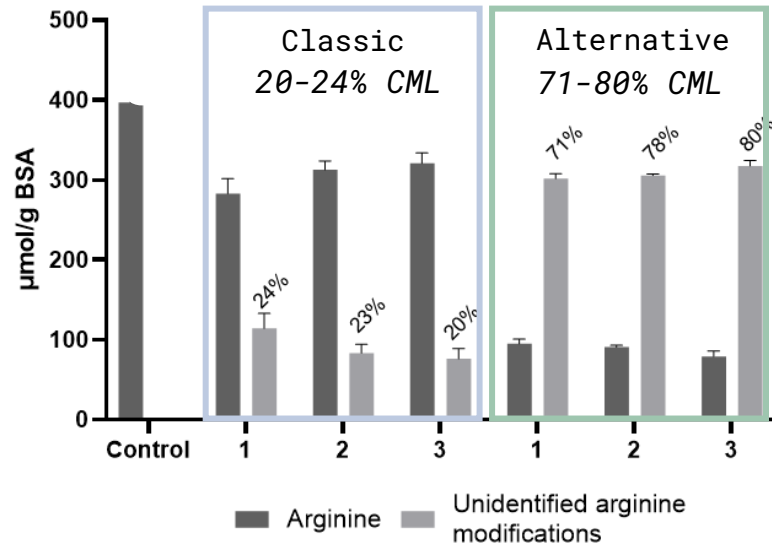
**Alternative method:**  
Greater formation of high Molecular weight products  
Including glycation crosslinks  
GOLD-GOLA





> Not only lysines were modified...

Arginine modifications



Argines are preferential targets of glycation

G-H1 was the main arginine product formed mainly withing glyoxal alternative method



26  $\mu\text{g}$  CML/g food

X



270  $\mu\text{g}$  CML/g food

## Feces



About 1/3 of ingested dCML was eliminated in feces



26% dCML

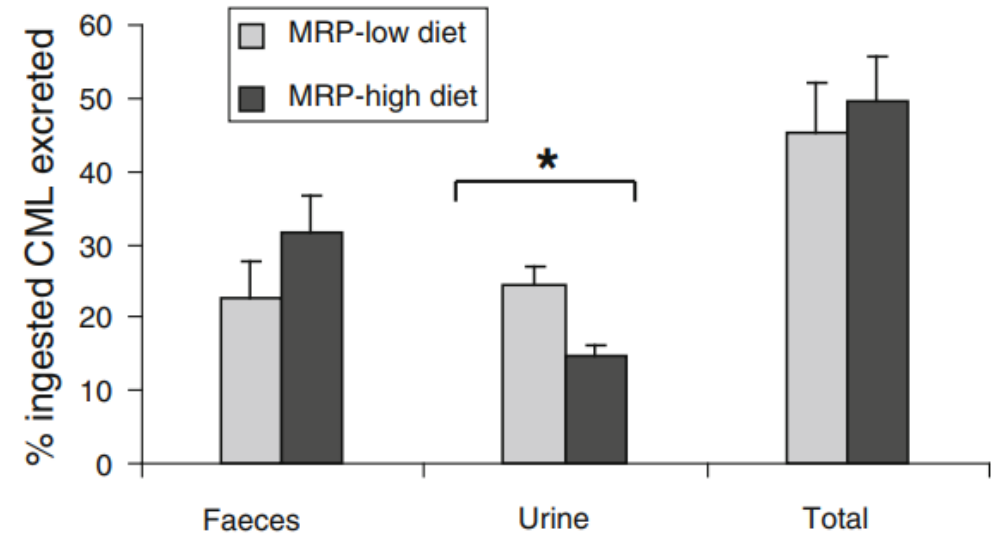


33% dCML

### Remaining CML:

- Metabolized
- Accumulated into tissues
- Urine

## Sample: Healthy male adolescents

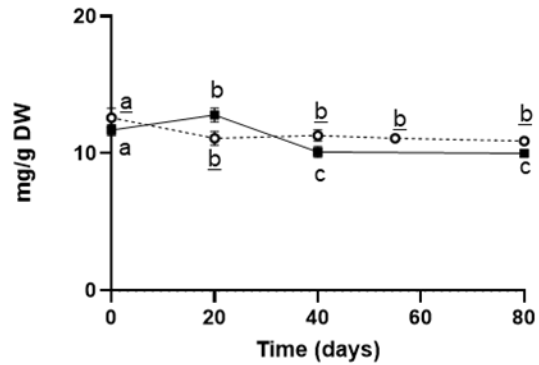


(Delgado-Andrade et al., 2012)

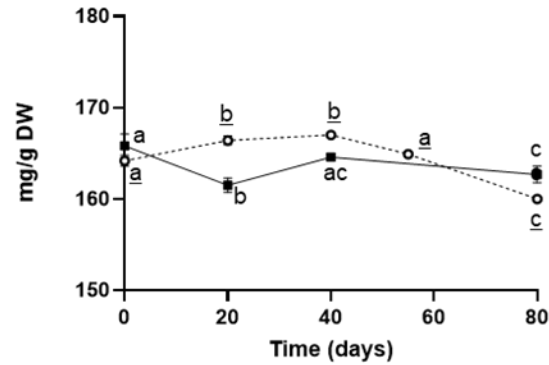


# Storage stability

## Lysine



## Total protein



## Furosine:

Time dependente increase/20°C

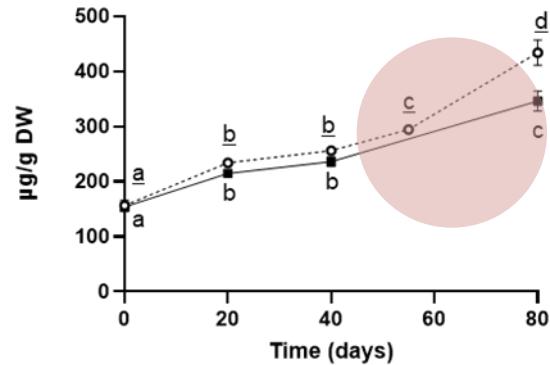
## CML:

Not a stable product

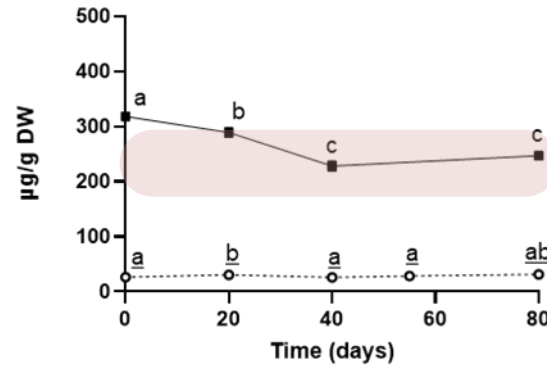
## CMFL

(N-ε-carboxymethyl-N-ε-(1-deoxyfructosyl)-l-lysine)  
(Hellwig et al., 2022)

## Furosine



## CML



○ STD ■ dCML



## Glycated mice diet

Building and characterizing mice diet enriched in glycated bovine serum albumin

## Key findings

The use of GA + cyanoborohydride remained the most effective way in terms of CML yield and the lower incidence of another glycation adducts

The use of glyoxal was demonstrated to be a reproducible method for CML production without the formation of HCN as side product

The extensive modification of BSA by glyoxal did not affect the fecal excretion of CML

No method is still known to the exclusive synthesis of CML.

# Acknowledgments



## Advisors

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