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Screening of *Lactococcus lactis* starters with Absorbance Reduction Activity Method (ARAM)

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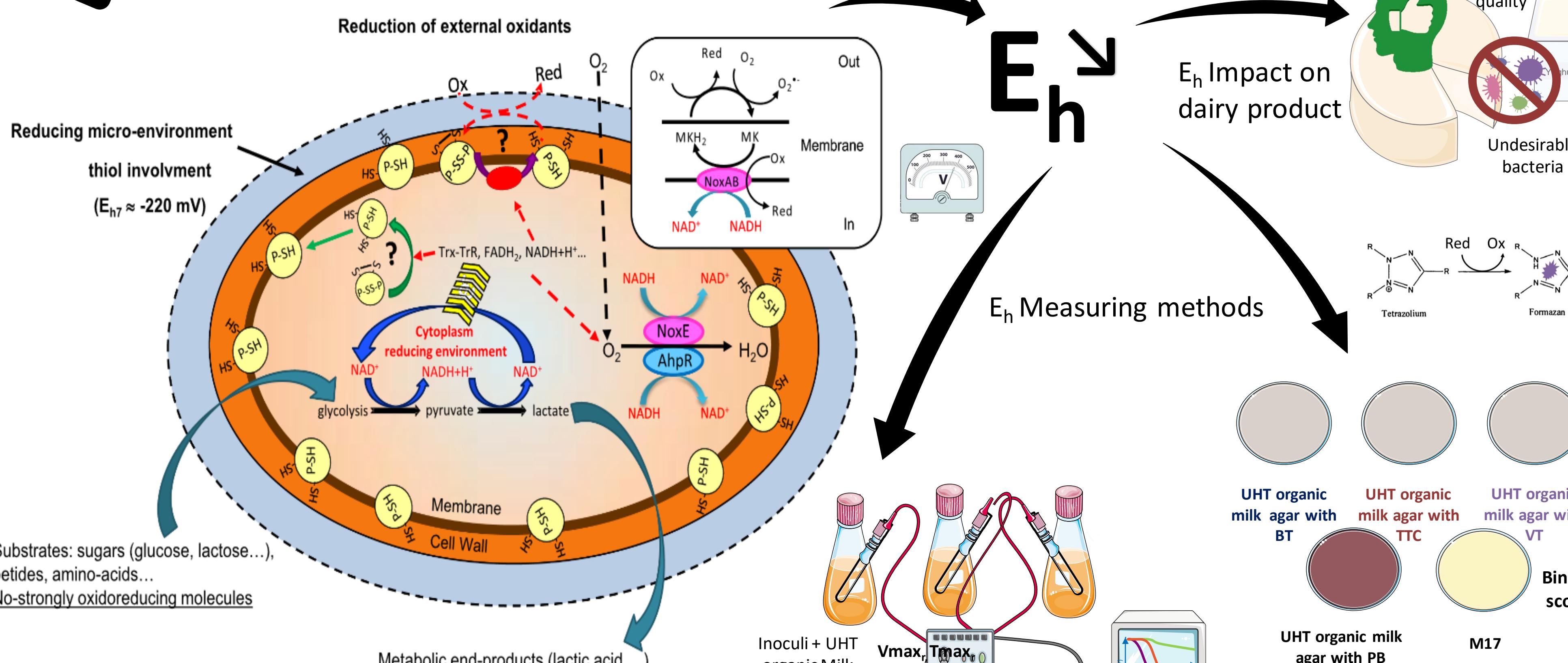
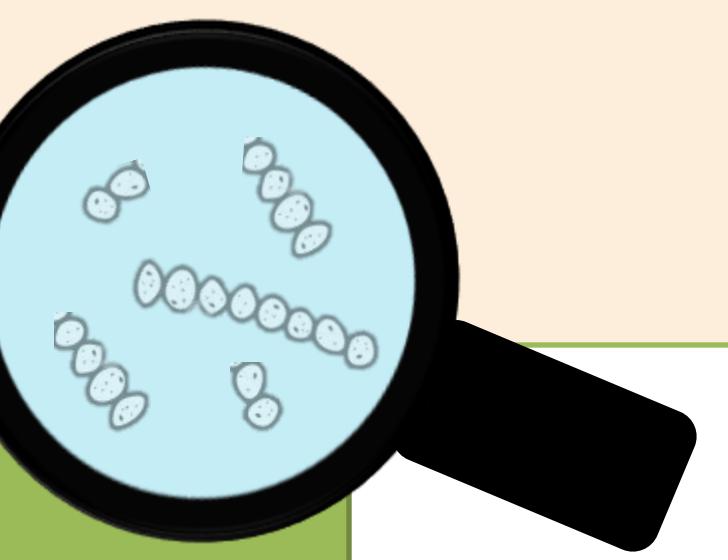


Fig 1: Cell functions involved in the reducing activity of *Lactococcus lactis* subsp. *lactis*^[1]

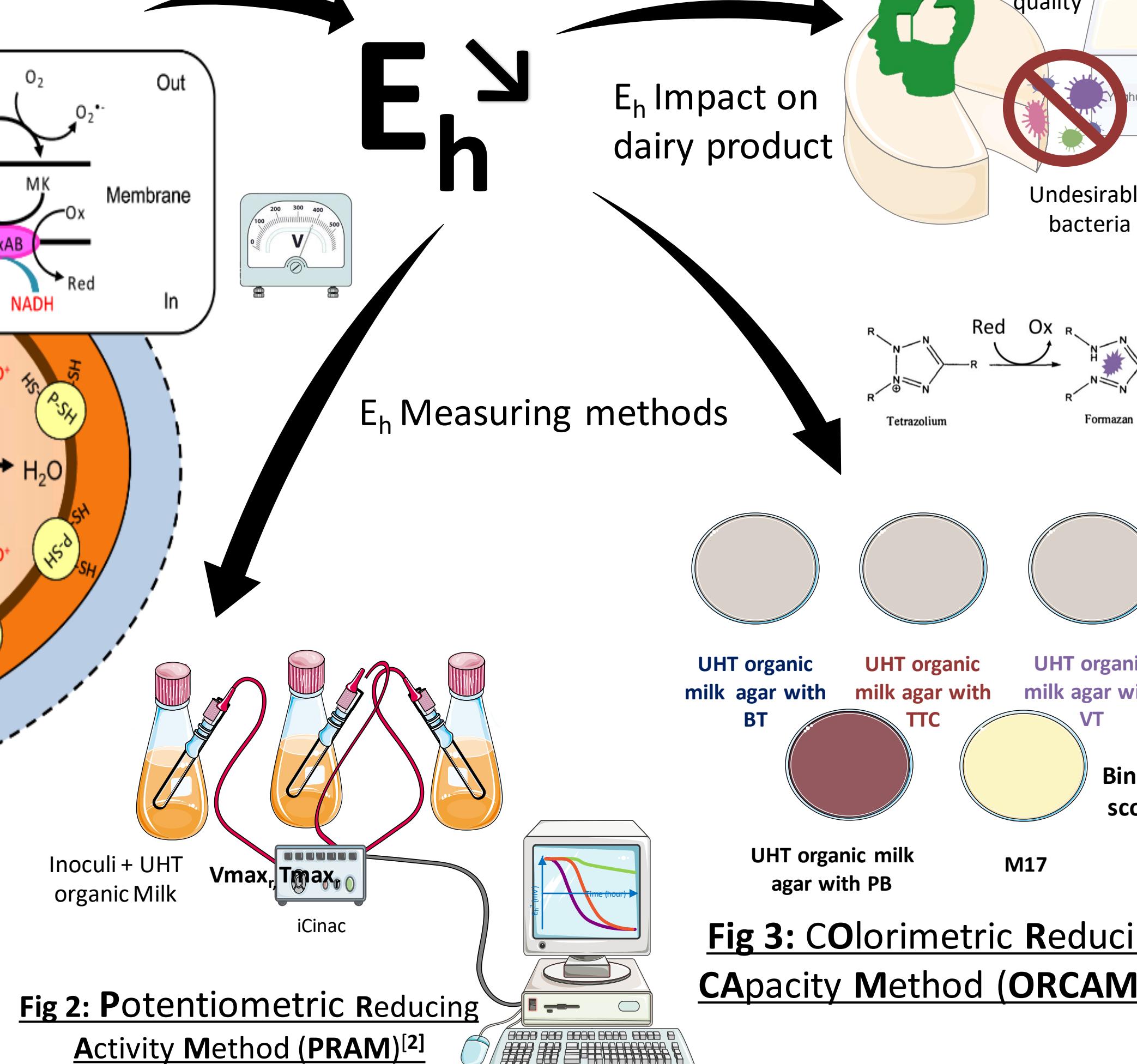


Fig 2: Potentiometric Reducing Activity Method (PRAM)^[2]

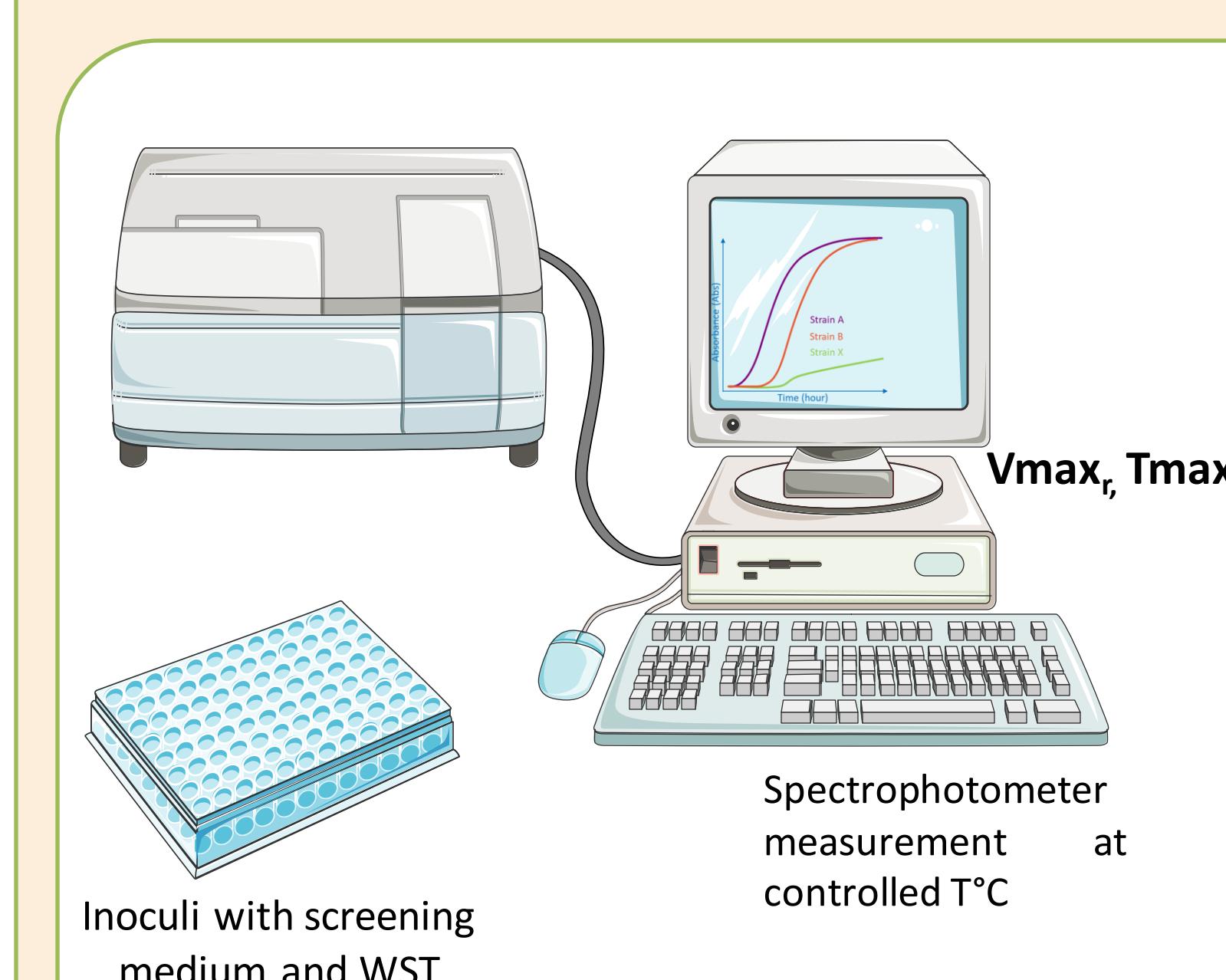


Fig 4: Absorbance Reducing Activity Method (ARAM)

Tab 1: Comparative table of the three methods according to acquisition, measurement and their characteristics

Method name Sources	PRAM ^[2]	ORCAM ^[3]	ARAM This work
Method	Potentiometric	Colorimetric	Spectrometric (Absorbance)
Time	15 to 24 hours	24 to 48 hours	16 hours
Material	Interface + probes + bottles	Petri dish + anaerobic chamber + gaz	Microplate + spectrometer
Cost	+ ++	++	-
Implementation	Long and thorough preparation	Fast	Fast
Definition	Reducing activity and acidification	Reducing capacity and acidification	Reducing activity
Type	Kinetic	Binary score	Kinetic
Parameters	pH & Eh	pH & Eh	Eh
pH Values	Raw data + Vmax _r + Tmax _r	Raw data + Vmax _r + Tmax _r	∅
Eh	Raw data + Vmax _r + Tmax _r	Visual observation	Visual observation
Fiability	Repeatable	Repeatable	Repeatable
Differenciation	Inter & Intra-species	Inter-species	Intra-species
Characteristics	Yield	1 probe per strain	8 Petri dishes per strain
	Repeatability	-	Analyst-dependent
	Constraints	Probe calibration	Gaz & results interpretation
	Risk	Glass probe	Gaz
			Small volumes

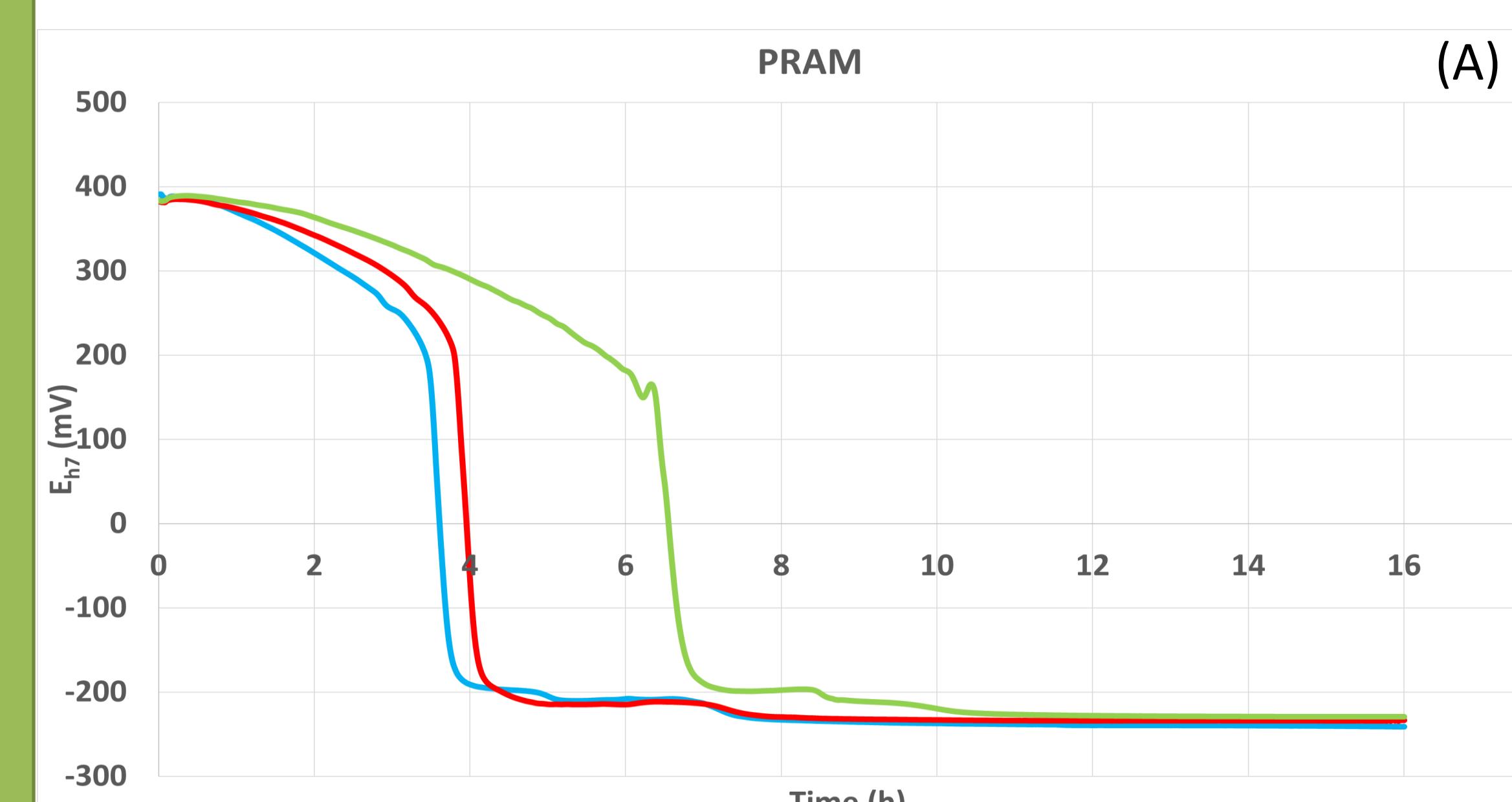


Fig 5: Typical curves of commercial cheese starters with PRAM (A) and ARAM (B). Vmax_r: Maximal reducing rate; Abs: Absorbance

	Starter 1	Starter 2	Starter 3
ARAM Vmax _r (Abs.h ⁻¹)	0,0410 (a)	0,0392 (a)	0,0286 (b)
PRAM Vmax _r (mV.h ⁻¹)	-1596 (a)	-1526 (a)	-1049 (b)
ORCAM Score	31	31	31

Thus ARAM:

- allows intra-species starter comparison
- compares until 30 strains simultaneously
- is applicable from frozen or freeze-dried bacteria
- combined with ORCAM, allow to compare larger quantities of starters before using PRAM to get more accurate results on reducing activity
- discriminates strains on their reduction kinetics

References:

[1] Roussel et al. 2022. Food Res Int 156,1111-54

[2] Cachon et al., 2002. Lait 82,281-8

[3] Michelon et al. 2013. J Biosci Bioeng 115,229-32