How properties of cast films made with Cottonseed Protein concentrate can be influenced ?

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Summary:

- Physical and microbiological stability of COTPROT
- Characteristics of film forming solutions
- Characteristics of films

- Physical and microbiological stability of COTPROT solutions

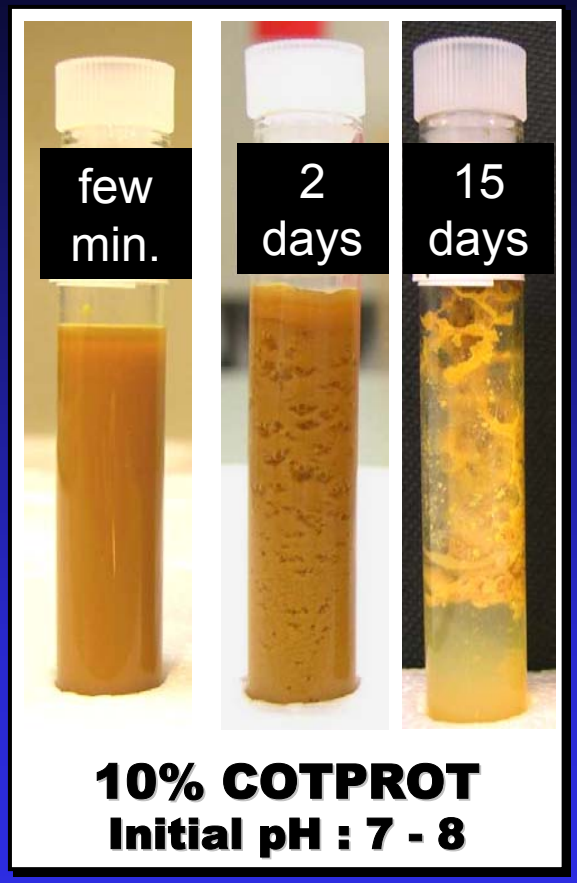
The preparation of COTPROT-based solutions in non aseptic conditions…

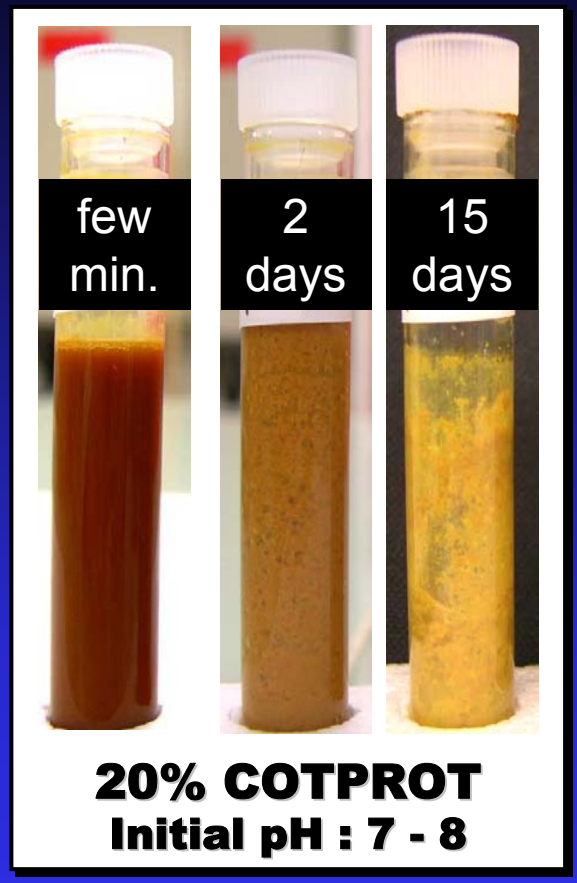
- From cotton-seed raw materials (flours, cakes),
- From freeze-dried COTPROT (obtained from the previous)
- Mild temperature and pH

…favour micro-organisms development

- Rich nutritive medium (proteins, sugars, minerals…)
- with high water content (High a_w values)

Evidence of biological spoilage (anaerobic)



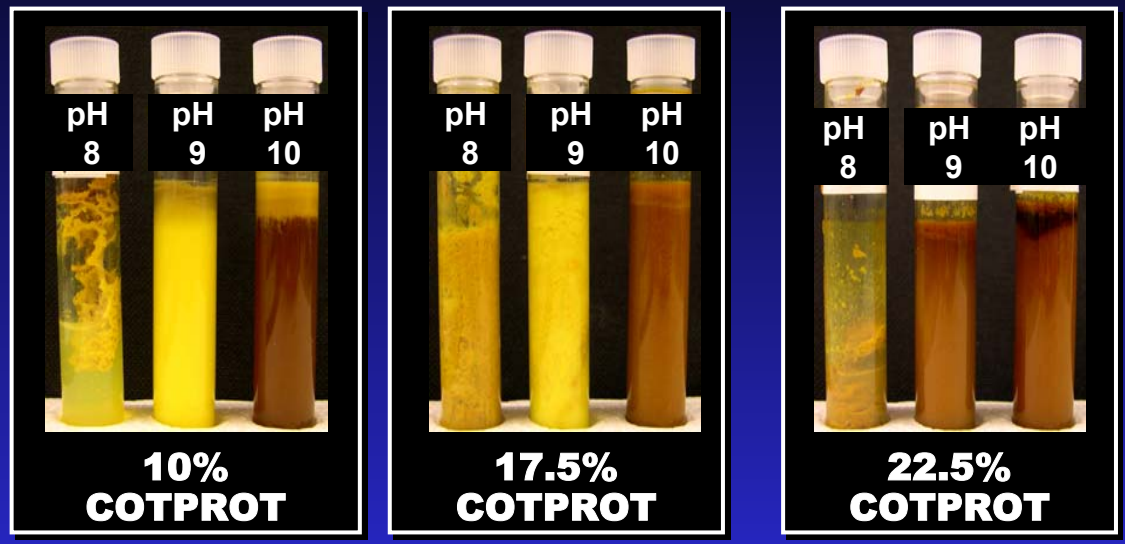


- ☑ Gas production and acidification of the mediun (pH ≅ 4.5)
- ☑ Proteins coagulation and « cheese » like odor
- ☑ Color changes (quinone reduction)

Reduction of micro-organisms growth

☑ Combination of high pH and high COTPROT content

Evolution of COTPROT Solutions after 3 weeks/ T_{amb}

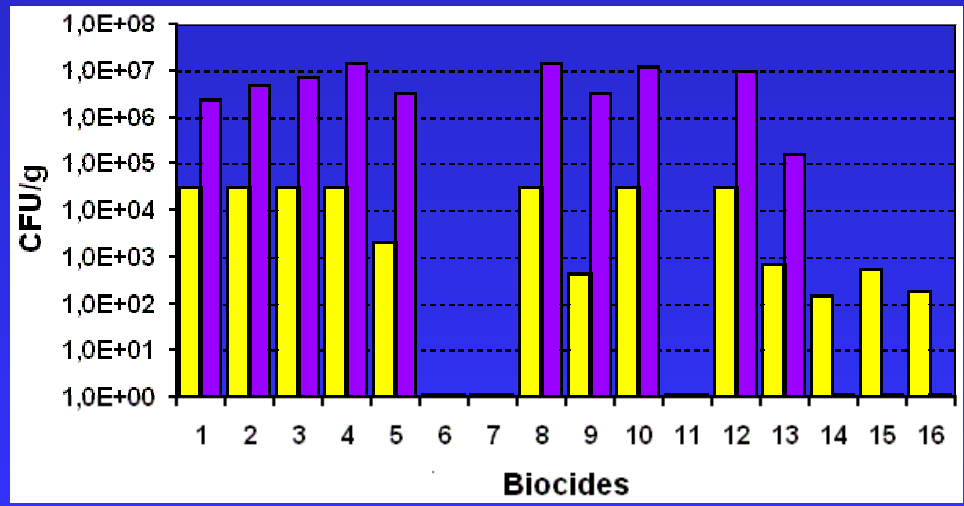


☑ Biocide use (or high temperature solvation)

Bacterial growth
No Yeast / Fungi

Best biocides :
A/ Zincpyrithione
B/ CIT/MIT or BIT/MIT

(INCOTEC Data)



Physical stabilization of COTPROT solutions

COTPROT solutions tends to settle with time

- ☑ Very slight or no effect of high pH
- ☑ Reduction of water content through freeze-drying
- ☑ Effect of additives such as paraben

COTPROT STABILITY

COTPROT extraction
pH = 9.0
T = 57ºC
t = 30 min

COTPROT solution
Control

COTPROT solution
+
Methylparabene
(0.06g of stabilizer/100g of COTPROT)

COTPROT solution
+
Propylparabene
(0.06g of stabilizer/100g of COTPROT)

Incubation
T = 25ºC
(TECNAL, BOD – TE-390)

Characterization
Total microorganisms counting (TMC)

EFFECT OF STABILIZING AGENTS AND OF THE STORAGE TIME ON THE NUMBER OF COLONY FORMING UNITS (CFU)

Storage time (days)	CFU/ml		
	COTPROT Control	COTPROT + methylparabene	COTPROT + propylparabene
10	67 ± 15	65 ± 7	80 ± 14
21	63 ± 12	57 ± 21	53 ± 6
31	40 ± 26	53 ± 15	63 ± 21
40	53 ± 12	57 ± 6	110 ± 10
60	300 ± 113	30 ± 14	85 ± 35

- Characteristics of film forming solutions

A result of factor combinations…

☑ Extrinsic Factors such as temperature

☑ Intrinsic factors related to solution composition :

- nature and concentration of constitutive elements,
- pH, ionic strength…,
- Reaction and interactions between molecules

…with various influences on :

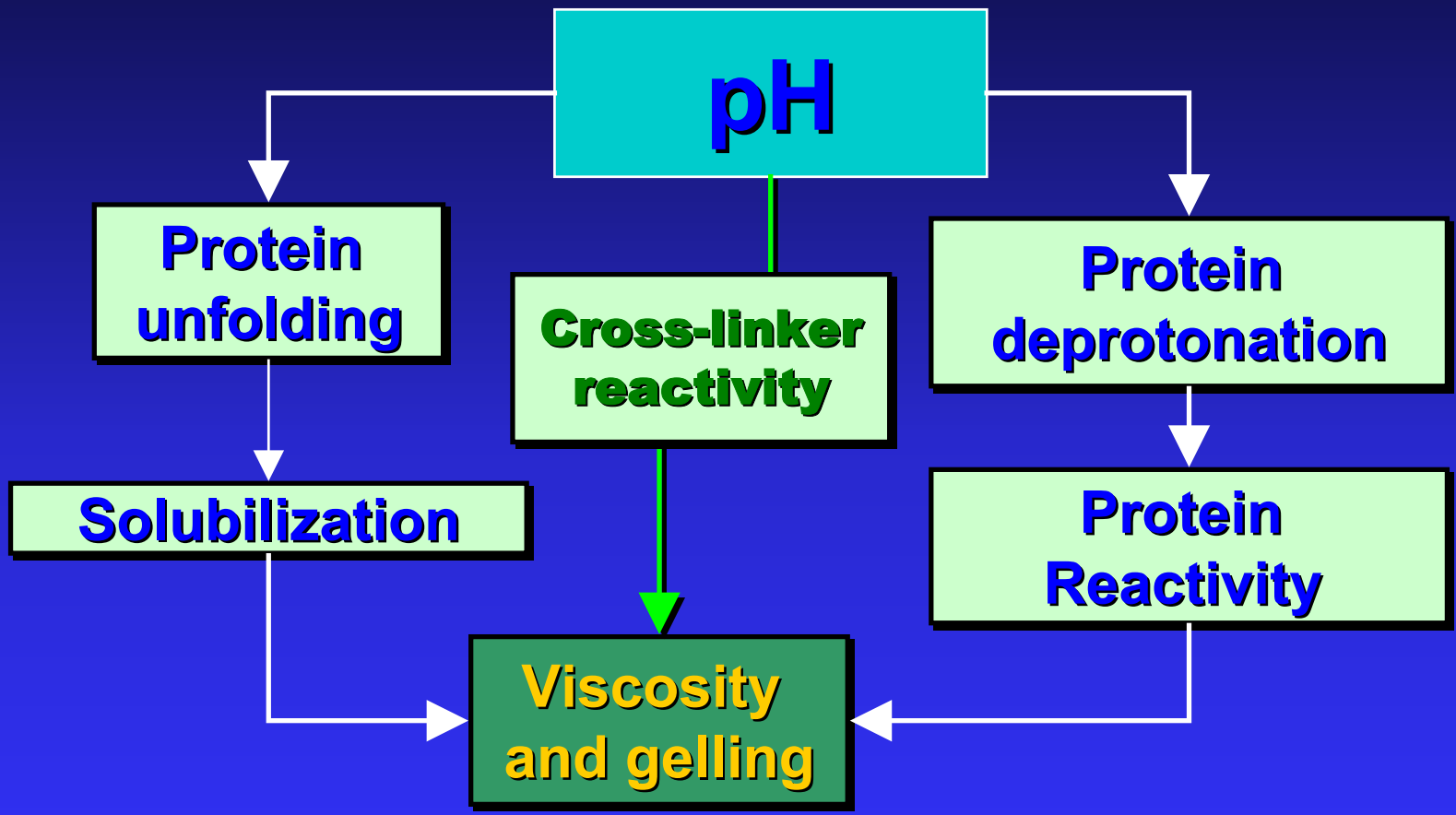
☑ Solubility

☑ Viscosity

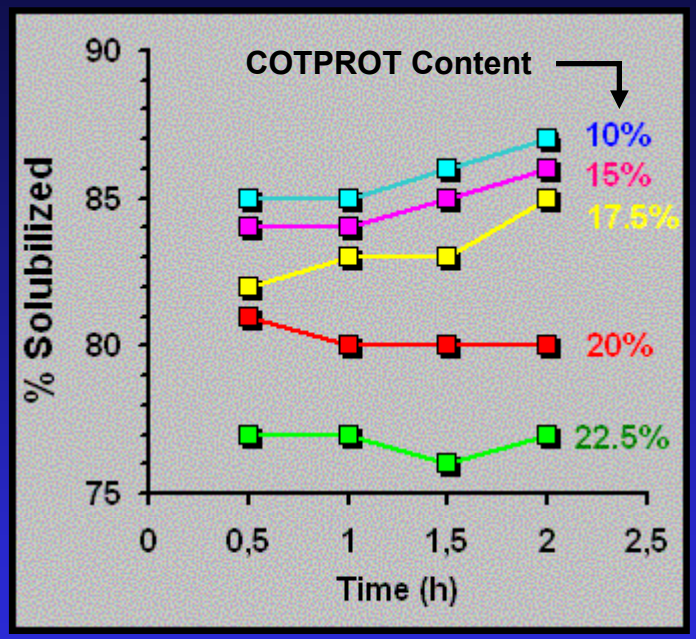
☑ Proteins and cross-linkers reactivity

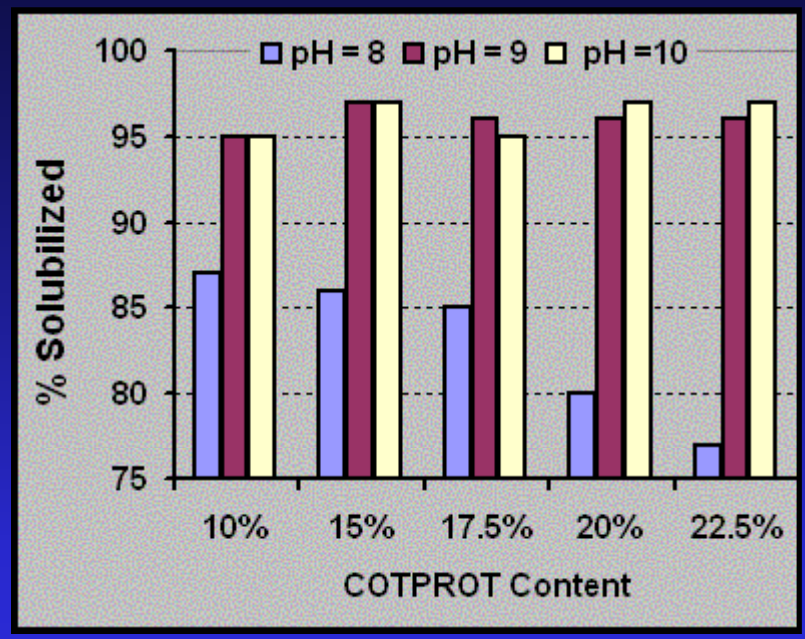
☑ Gelling

Role of pH and temperature, in film forming solution preparation



Effect of pH on freeze-dried COTPROT Solubilization

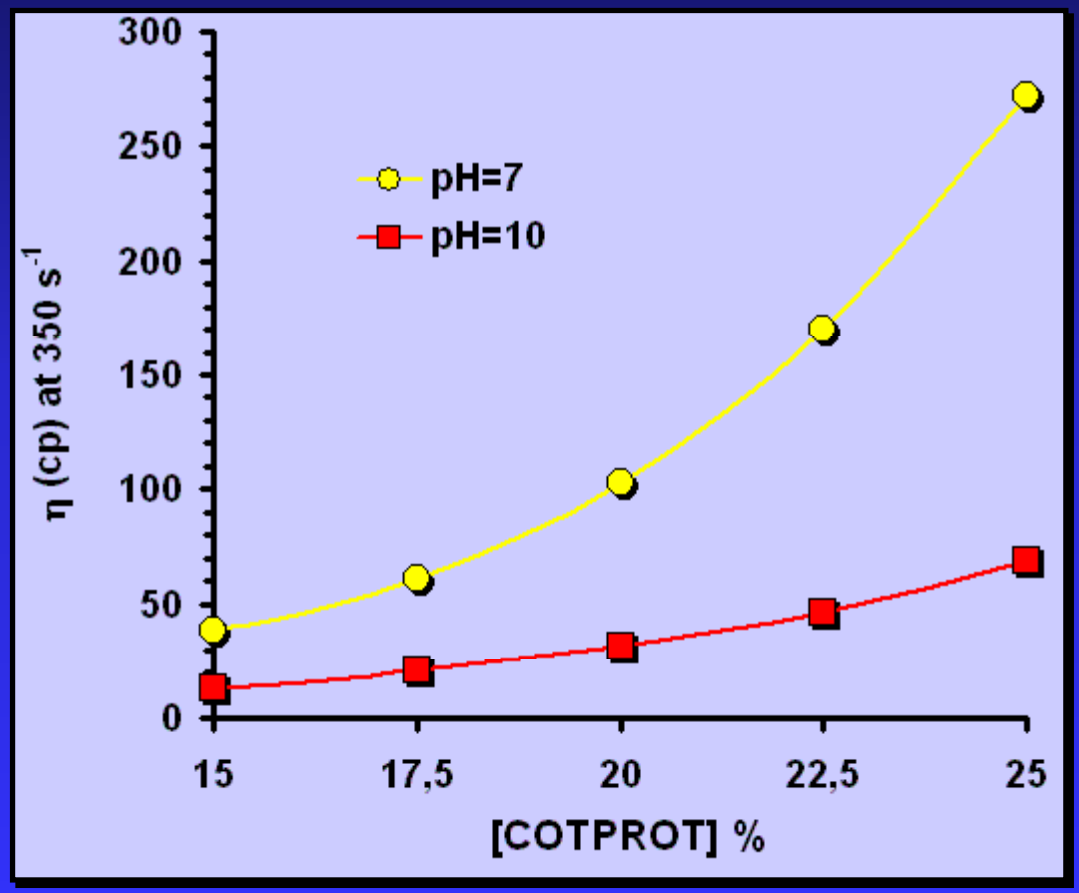




- ☑ increase of COTPROT concentration reduce solubility at neutral pH
- ☑ increase of pH leads to solubility > 95% , whatever the concentration

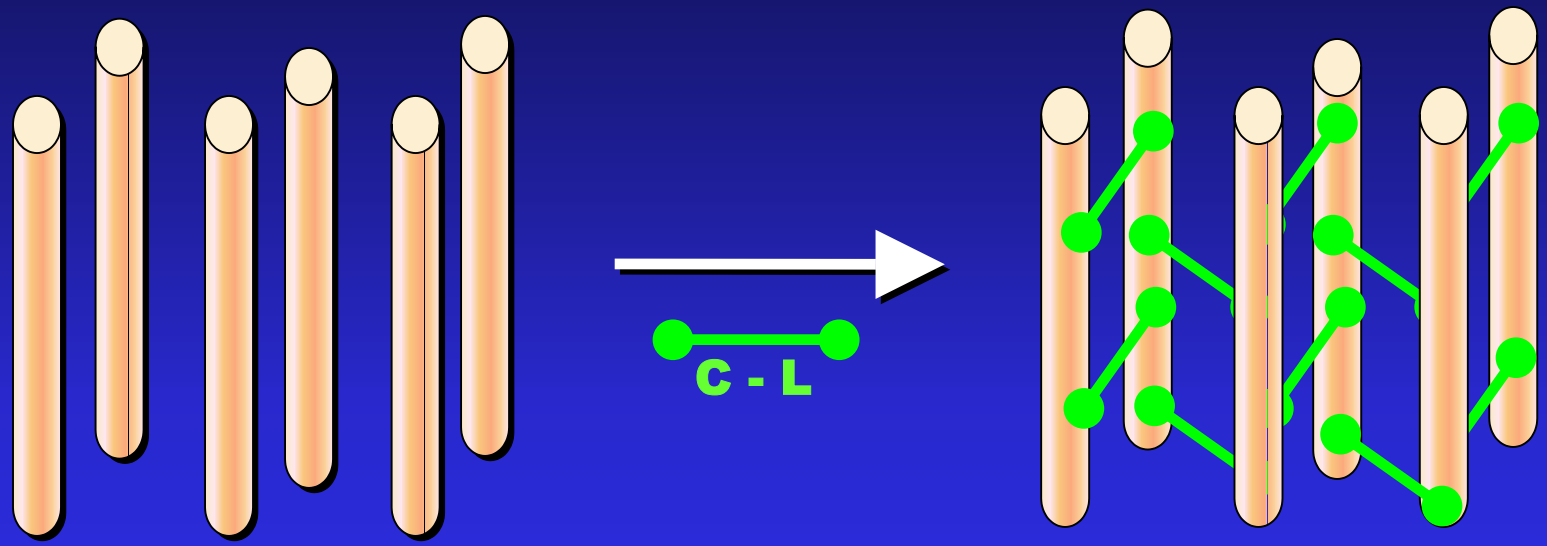
Effect of pH and COTPROT concentration on viscosity

☑ **Starting solutions (Uncross-linked, unplasticized)**



Influence of cross-linking on viscosity and gelling

☑ Principle : Formation of covalent bonds between proteins



☑ Results :

- Formation of 3D cohesive network
- ↗ molecular weight
- ↗ viscosity [Mark Houwink law $\eta = a.(M_w)^b$]

Contrasted effects of cross-linkers (1)

☑ Cross-linker reactivity : a matter of pH

	REACTIVITY	
	Low pH	High pH
Glutaraldehyde	+++++	++
Glyoxal	+	++++

☑ Polymerisation of glutaraldehyde at high pH

- formation of high Mw species
- ↘ Mobility and/or ↘ number of reactive site
- BUT SELECTIVE REACTIVITY towards reactive lysine

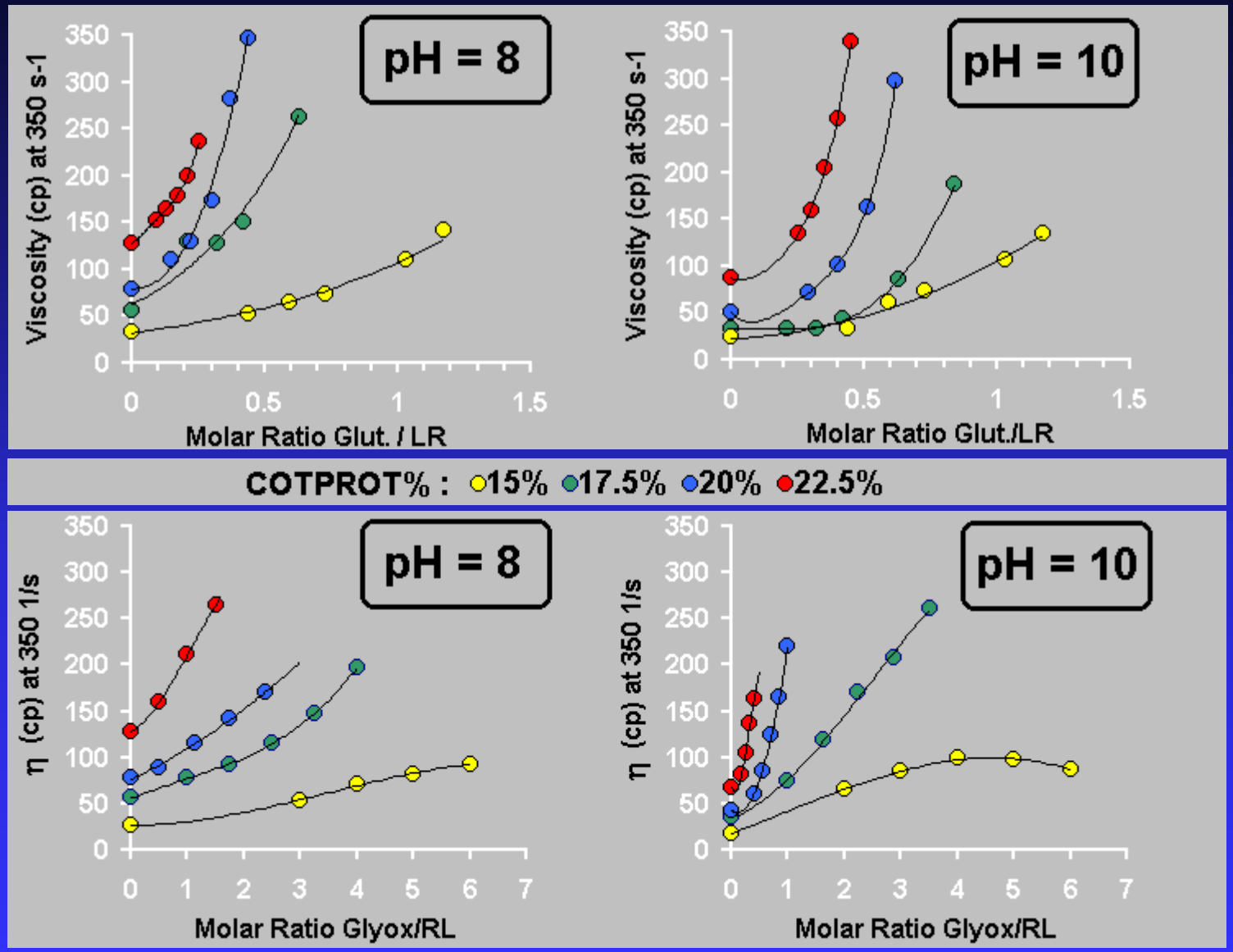
☑ Non selective action of glyoxal

- existence of 3 reactive species (Mono-, di- and trimer)
- REACTION with several amino-acids and formation of cyclic and linear products BUT few cross-links

Contrasted effects of cross-linkers (2)

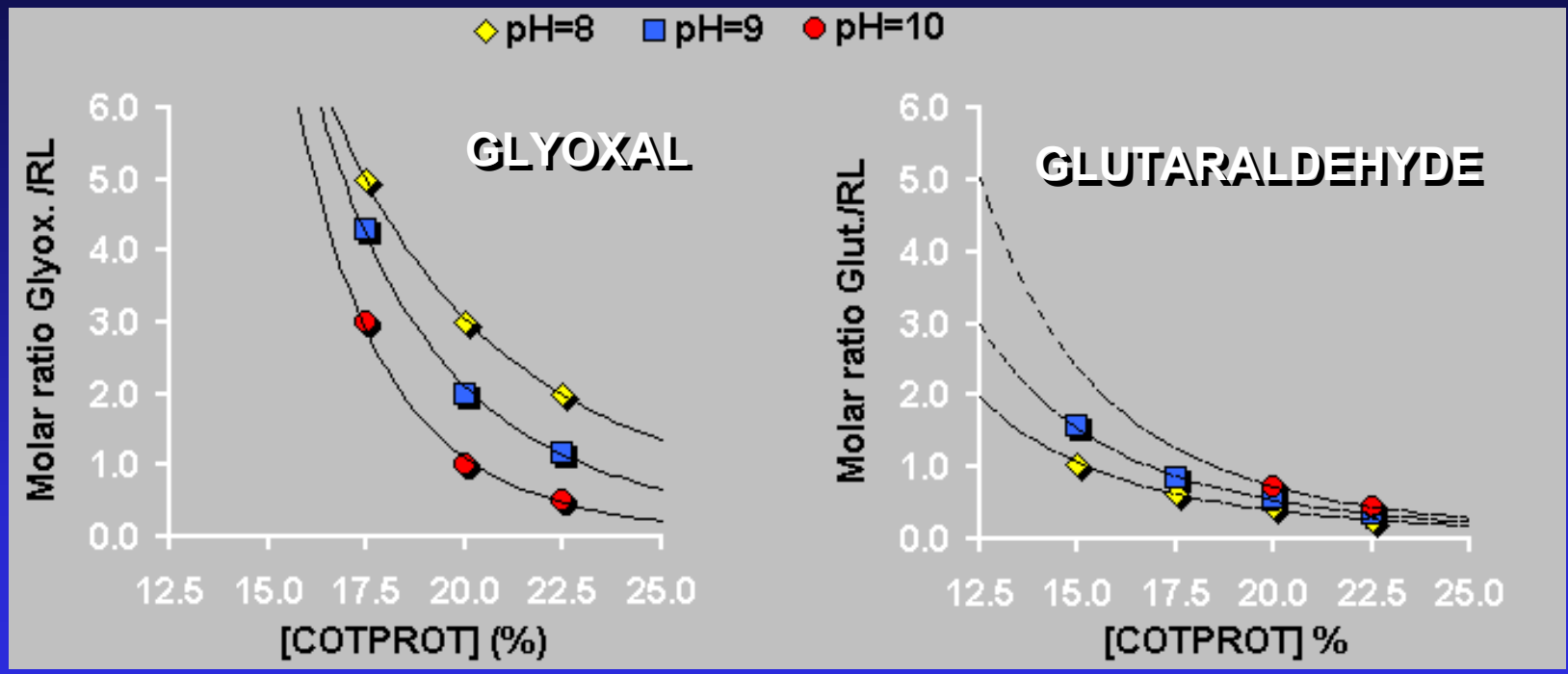
GLUTARALDEHYDE

GLYOXAL



Contrasted effects of cross-linkers (3)

☑ **Gelling limits of film forming solutions**

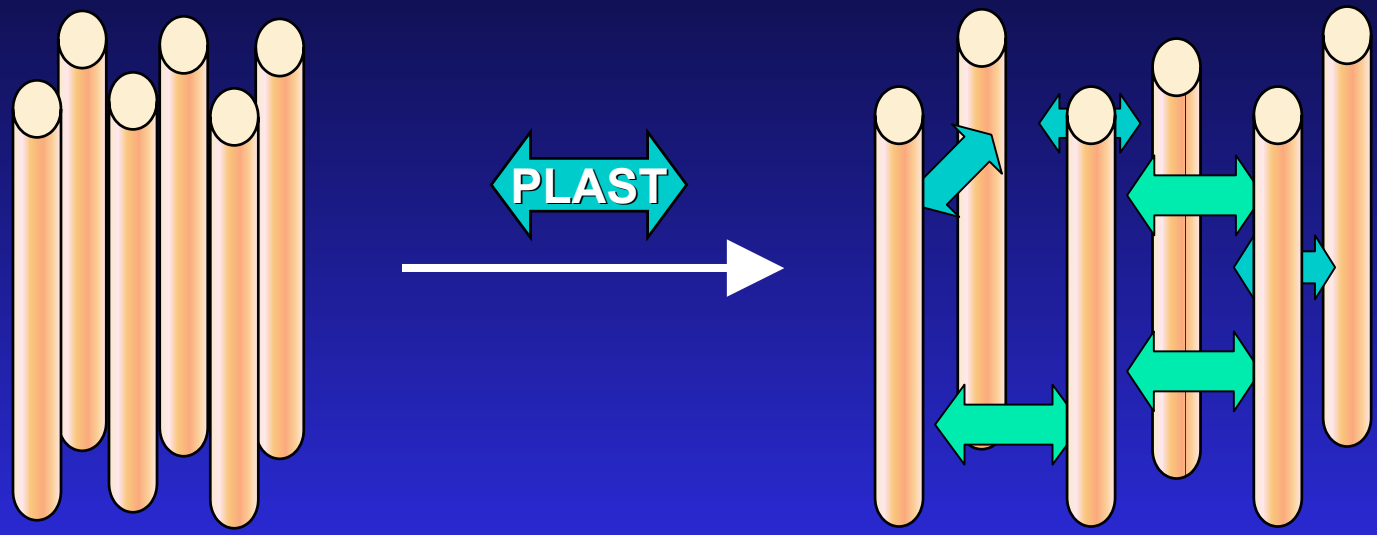


Whatever the cross-linker, gel formation appeared at ratio much lower than the theoretical maximum (complete consumption of RL) of :

4 for glutaraldehyde **10 for glyoxal**

Role of plasticization

☑ Principle : insertion of plasticizer between proteins chains

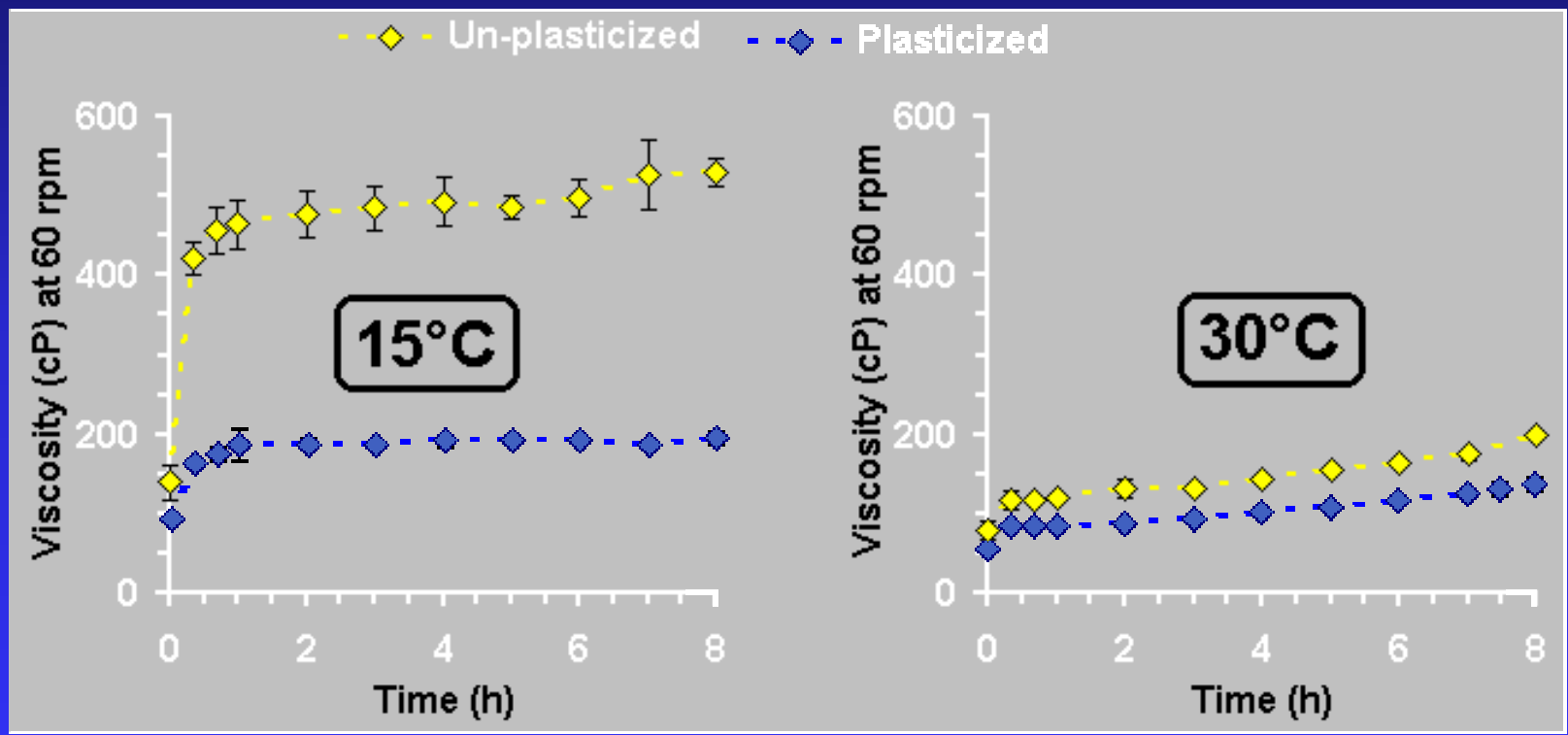


☑ Effects :

- disruption of protein-protein H- Bonds,
- ↗ flexibility/elasticity of the network and ↘ viscosity

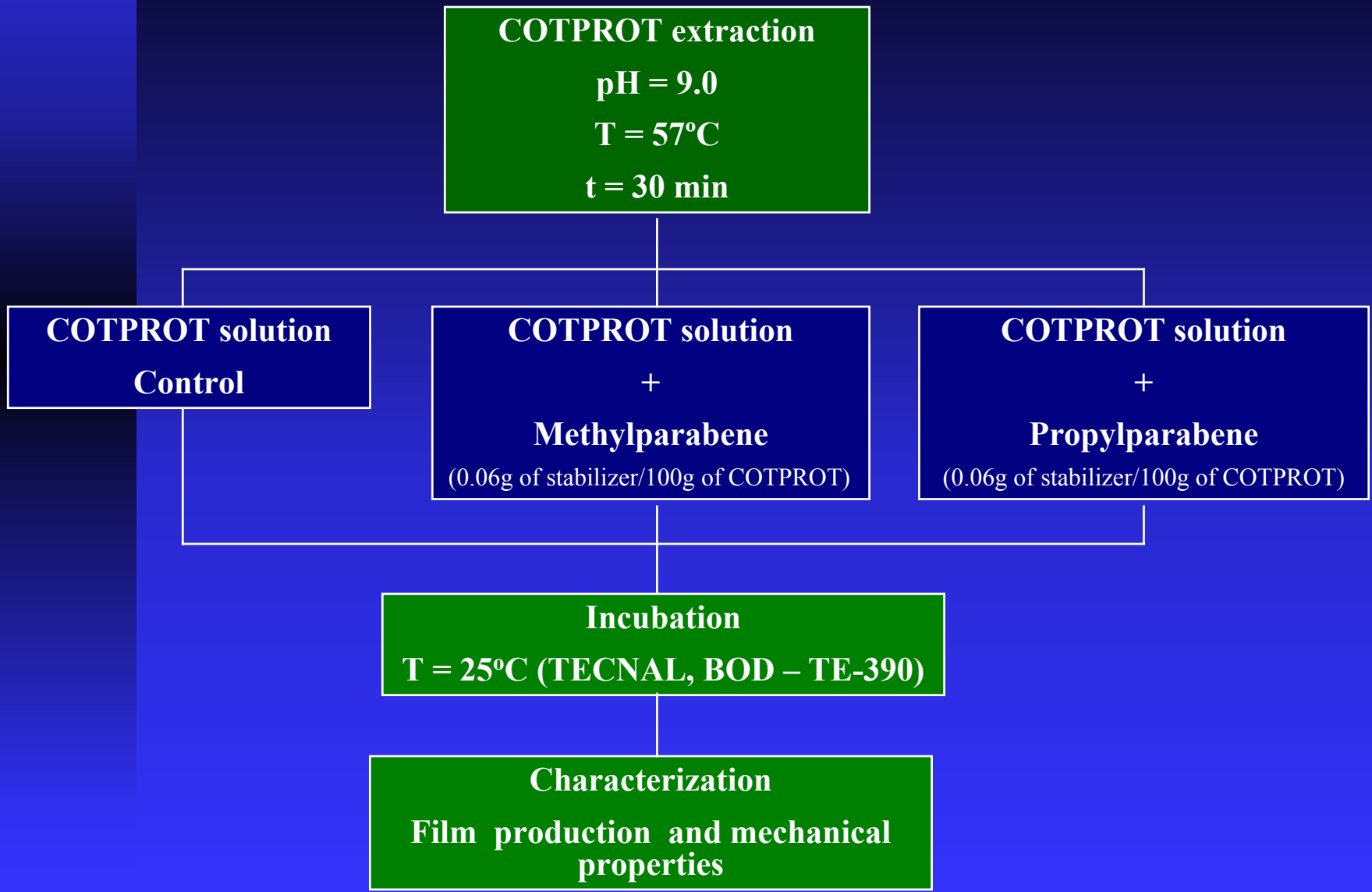
Effect of Temperature and plasticization on viscosity

☑ Plasticization before cross-linking



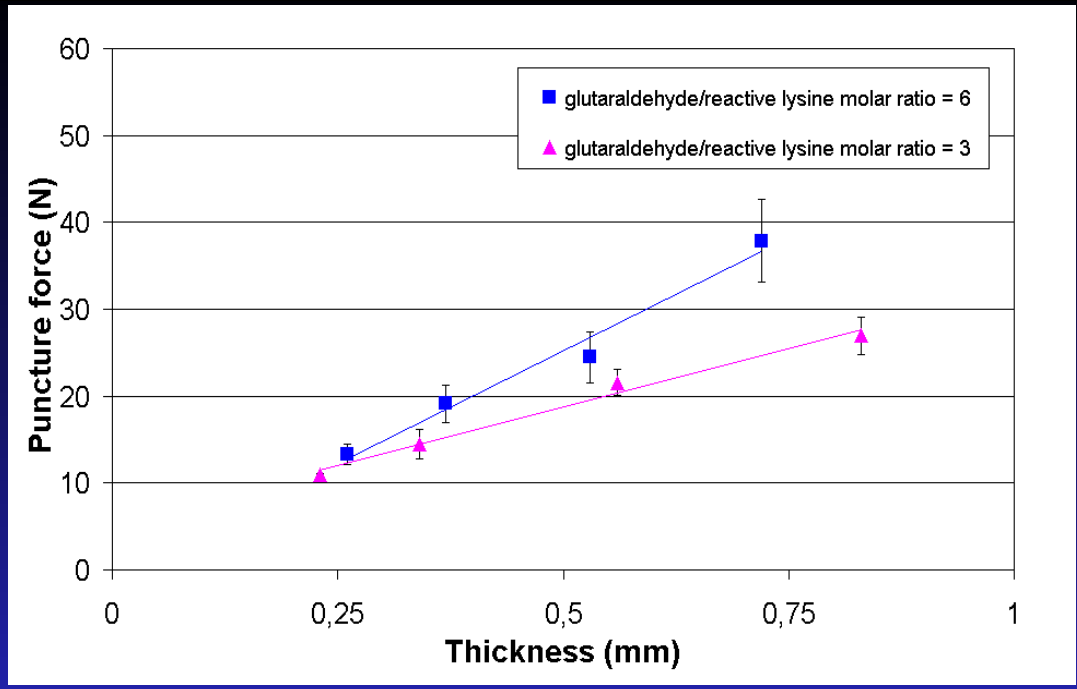
- Characteristics of films

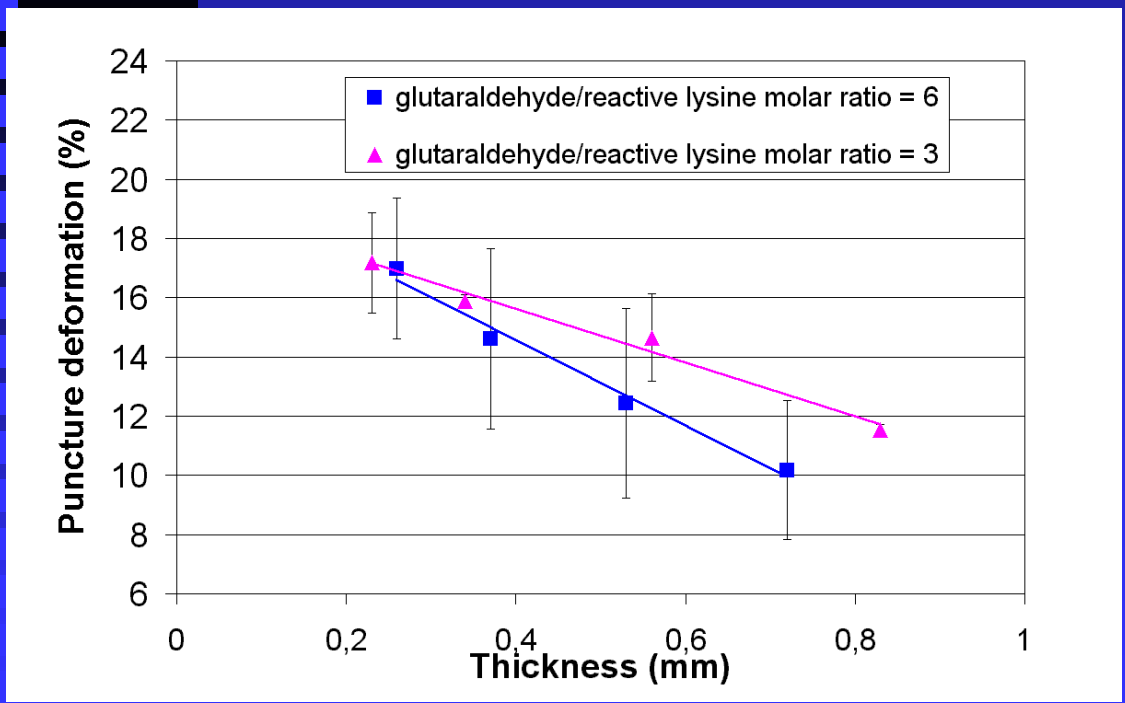
FILM PRODUCTION USING STABILIZER AGENT



RESULTS

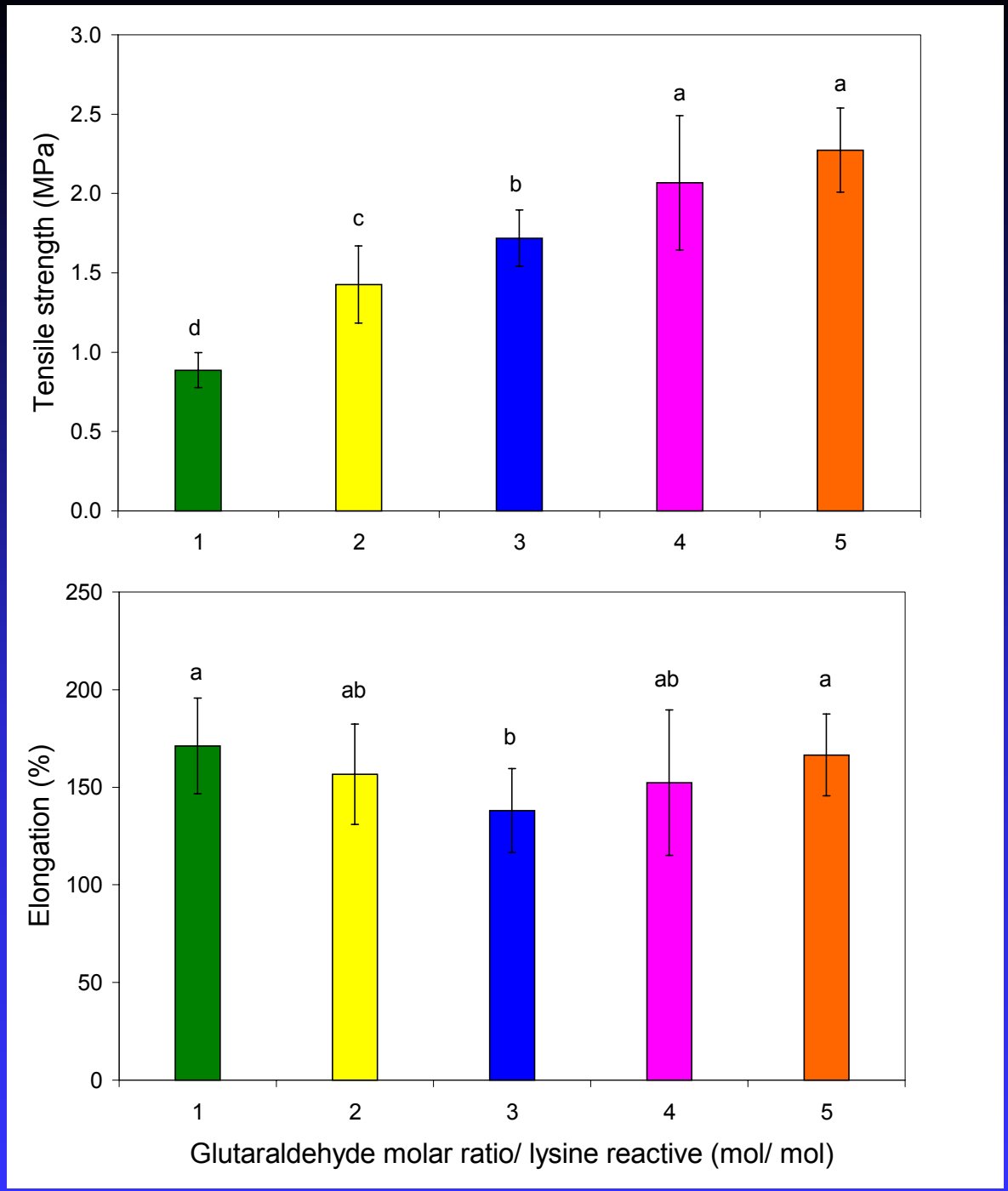
Properties	Without stabilizers	With Methylparabene	With Propylparabene
Puncture force (N)	13.8 ± 1.3	12.6 ± 1.8	13.6 ± 1.7
Puncture deformation (%)	14.7 ± 1.1	18.6 ± 3.5	16.0 ± 1.6
Tensile strength (MPa)	1.8 ± 0.2	1.4 ± 0.2	1.1 ± 0.4
Deformation at break (%)	138.1 ± 21.5	134.0 ± 26.0	90.1 ± 29.5
Elastic modulus (MPa)	0.104 ± 0.005	0.081 ± 0.03	0.089 ± 0.026
Viscosity of COTPROT (cP)	6.8 ± 0.6	6.8 ± 0.4	5.0 ± 0.9
Viscosity of filmogenic solution (cP)	140.6 ± 5.8	12.3 ± 4.3	5.1 ± 0.5





Film Thickness

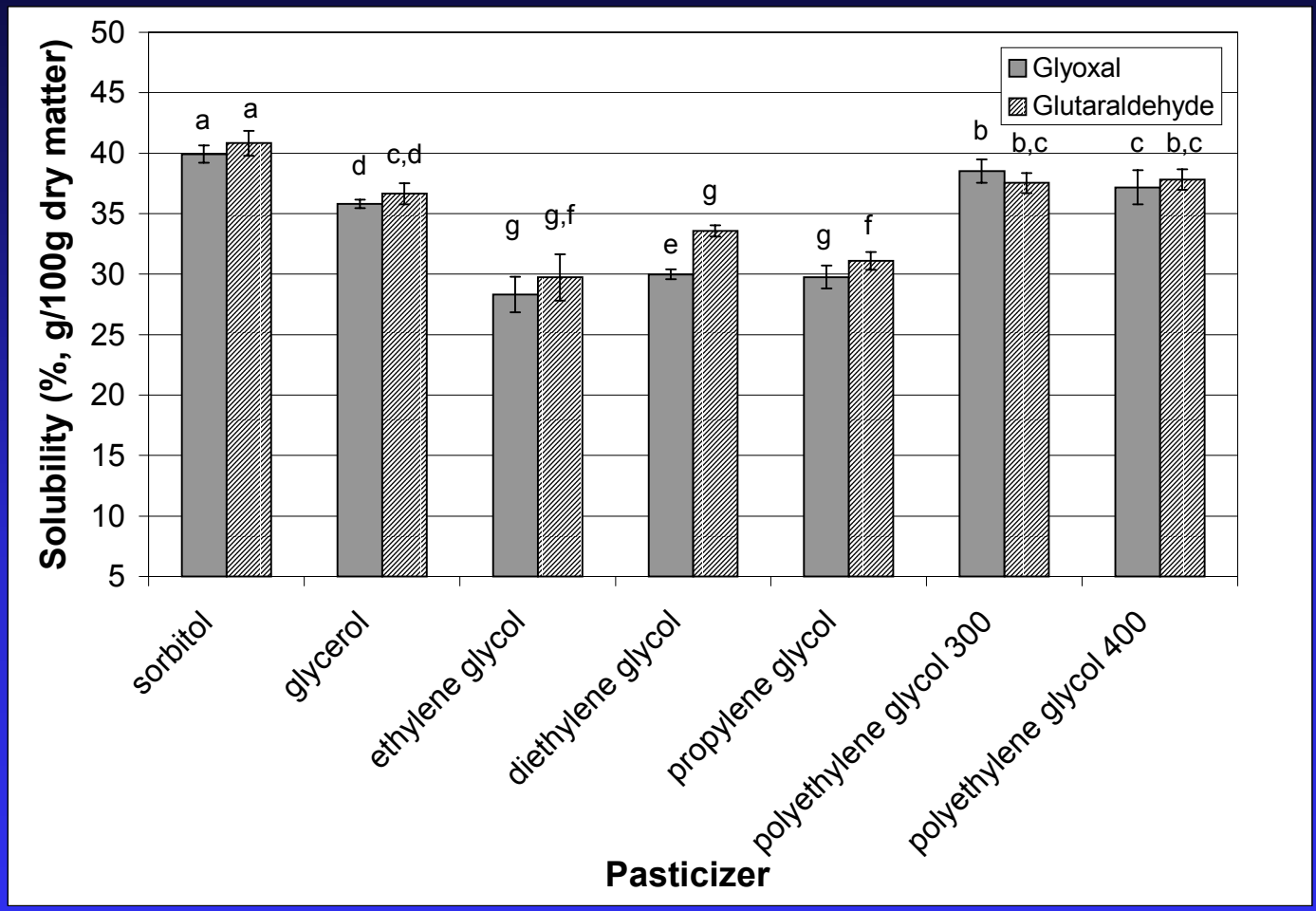
Concentration of crosslinking agent

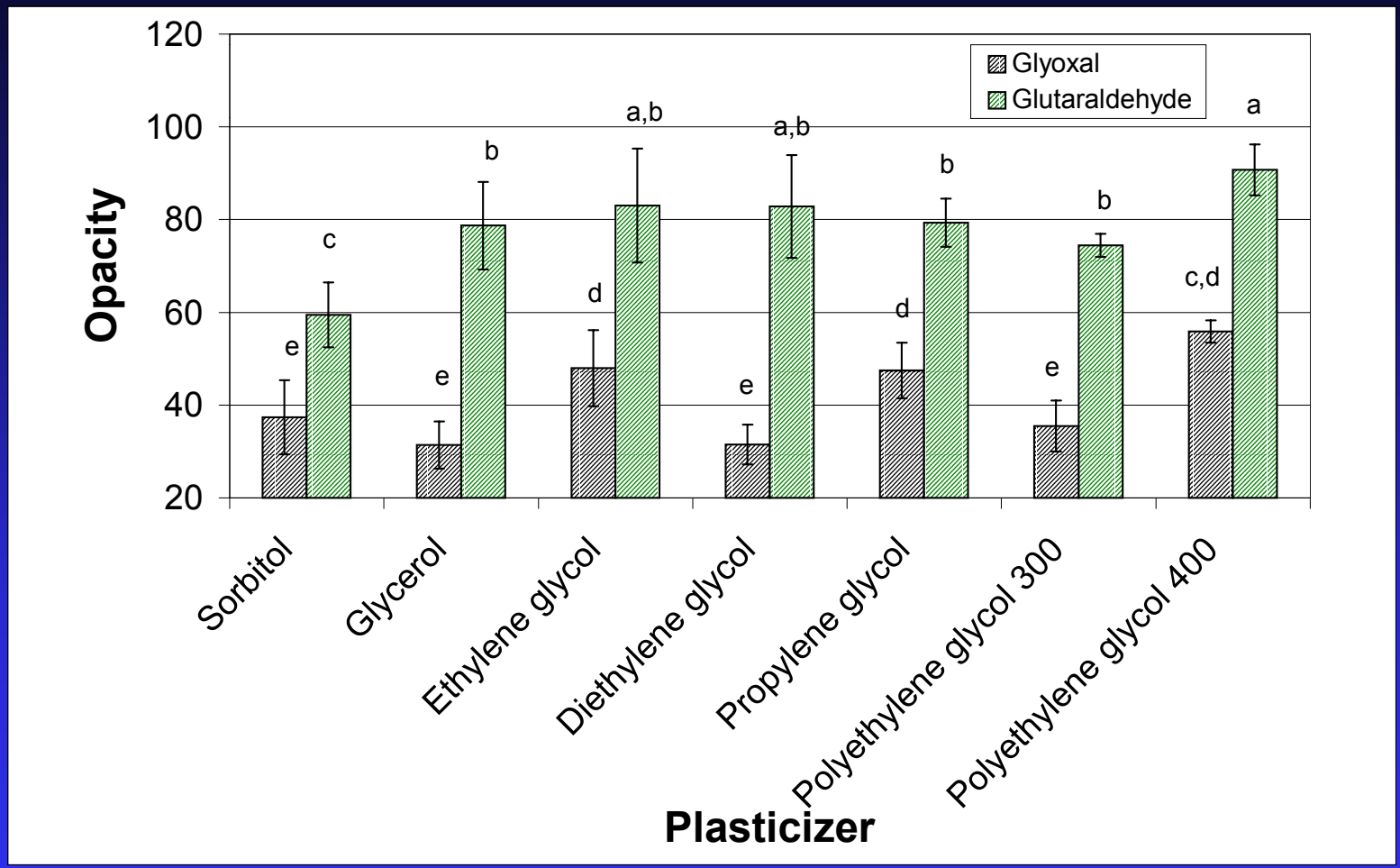


Plasticizer type

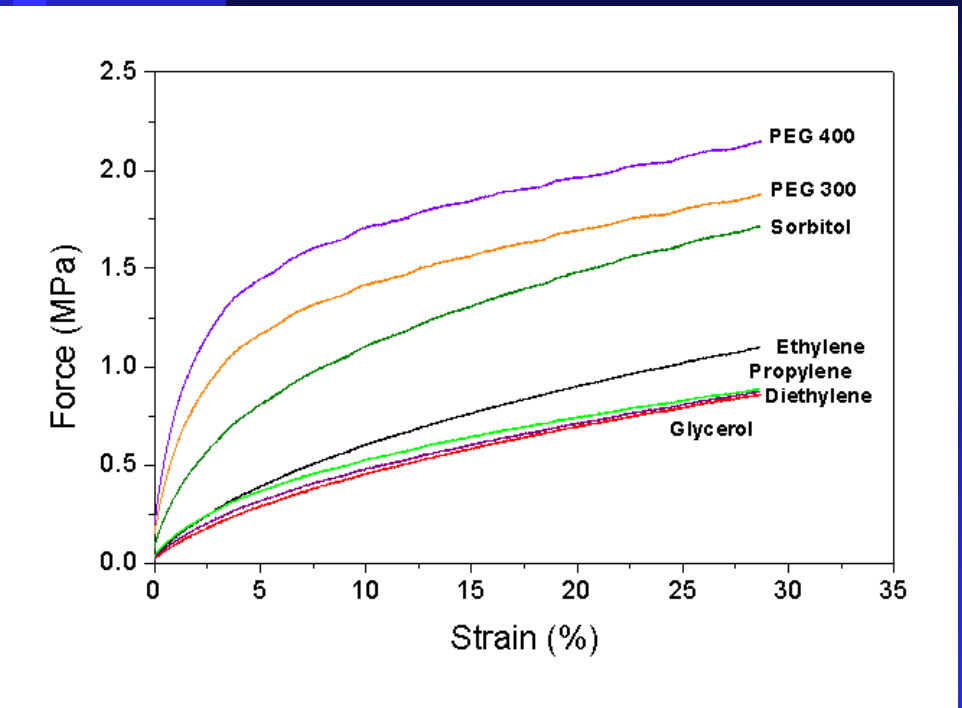
Plasticizer type	Formula	MW	mol plasticizer / 100g dry matter	g Oxygen / 100g dry matte	mol Oxygen in plasticizer / 100g dry matter
Ethylene glycol	$C_2H_6O_2$	62	0.32	10.32	0.166
Prolpylene glycol	$C_3H_8O_2$	76	0.26	8.42	0.111
Glycerol	$C_3H_8O_3$	92	0.22	10.43	0.113
Diethylene glycol	$C_4H_{10}O_3$	106	0.19	9.06	0.085
Sorbitol	$C_6H_{14}O_6$	182	0.11	10.55	0.058
PEG 300	$H(OCH_2 - CH_2)_6 OH$	300	0.07	7.47	0.025
PEG 400	$H(OCH_2 - CH_2)_8 OH$	400	0.05	7.20	0.018

Plasticizers and Crosslinking agents types

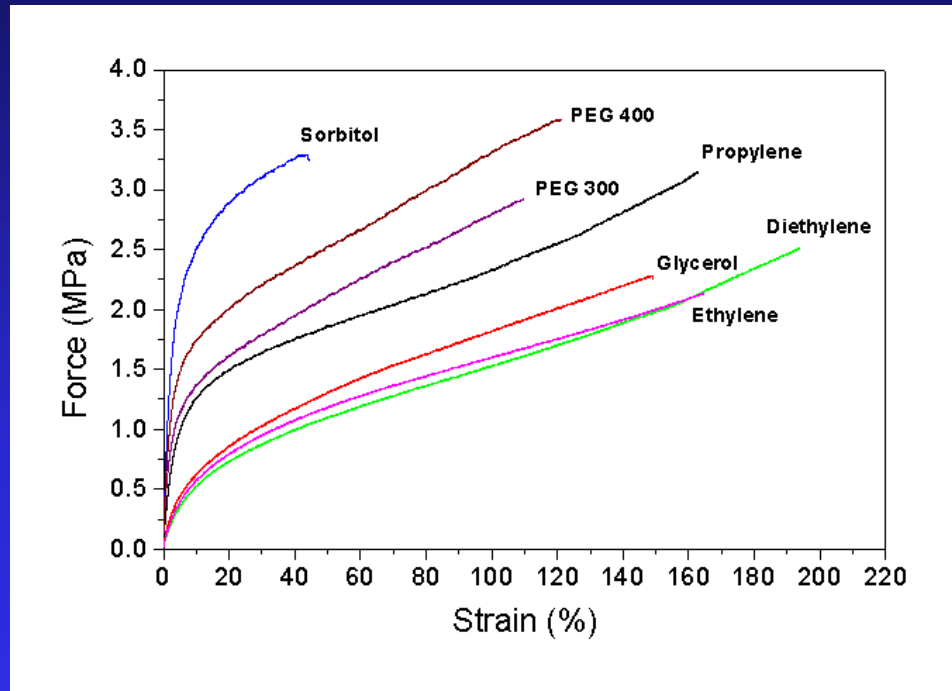



120
100
80
60
40
20
Opacity
Glyoxal
Glutaraldehyde
e
c
e
b
d
a,b
e
a,b
d
b
e
b
c,d
a
Sorbitol
Glycerol
Ethylene glycol
Diethylene glycol
Propylene glycol
Polyethylene glycol 300
Polyethylene glycol 400
Plasticizer

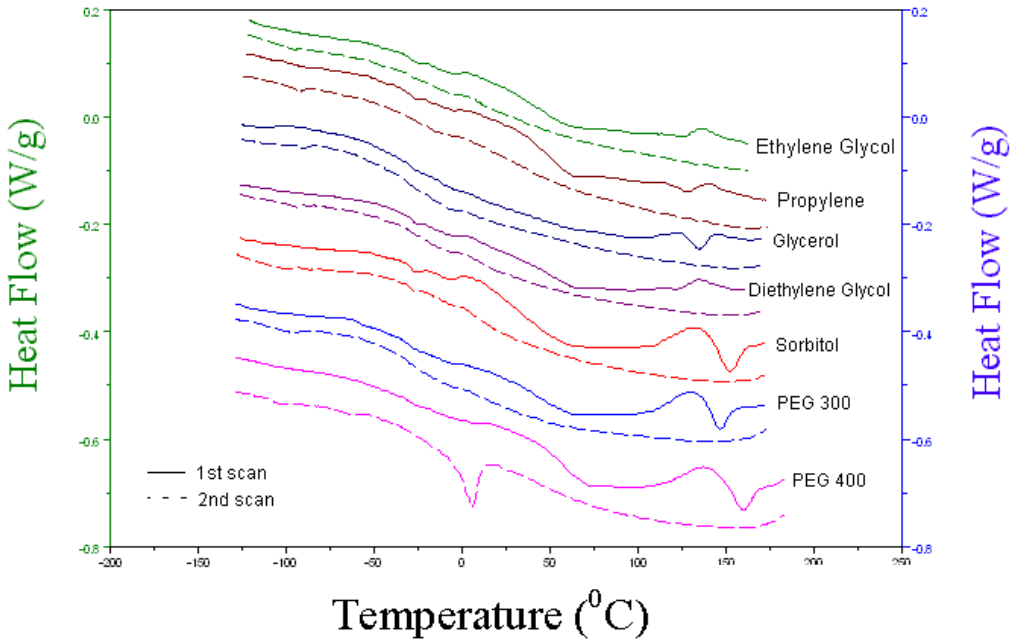
Glyoxal



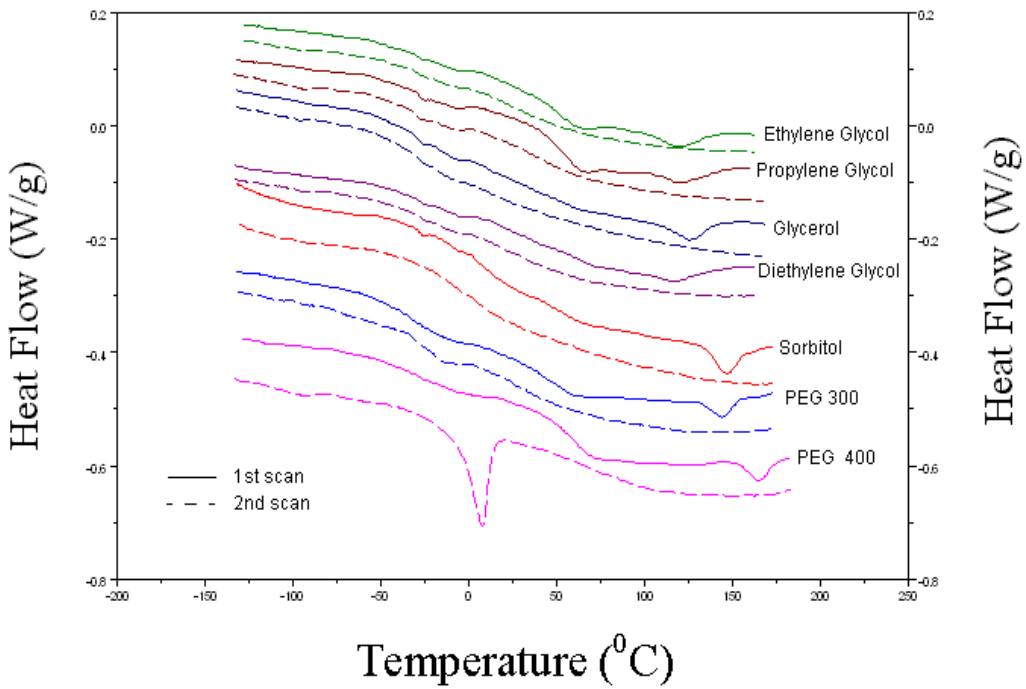
Glutaraldehyde



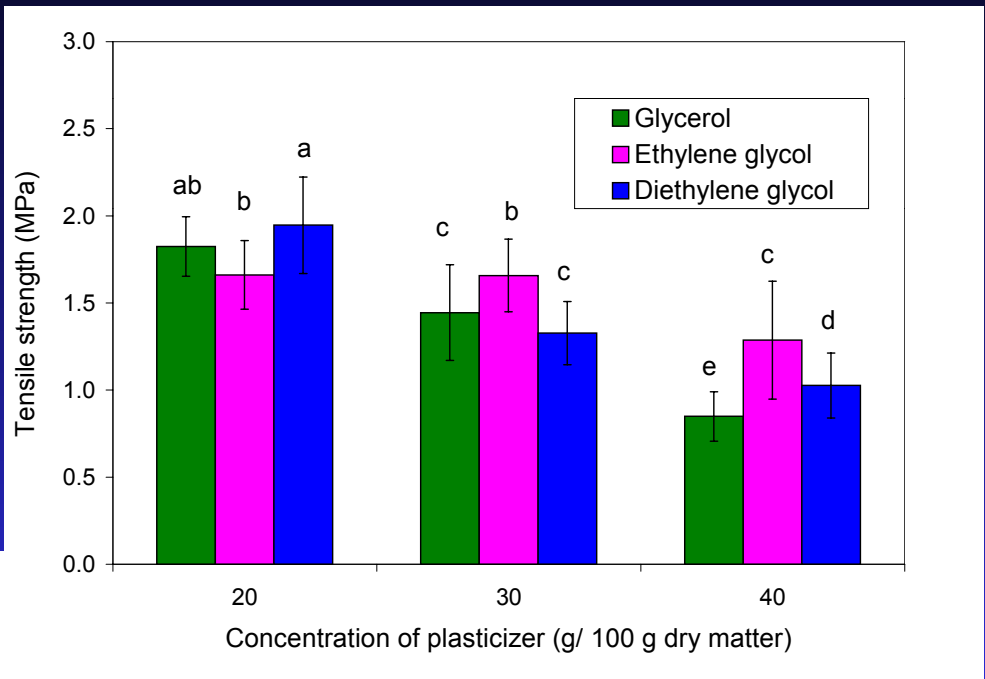
Glyoxal

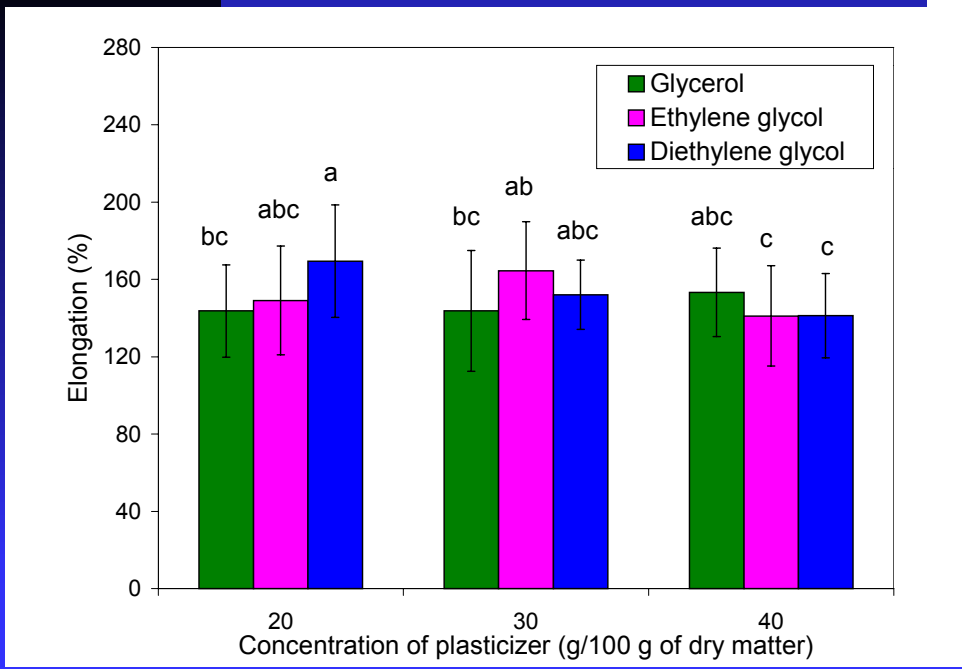


Glutaraldehyde



Plasticizers types and concentration





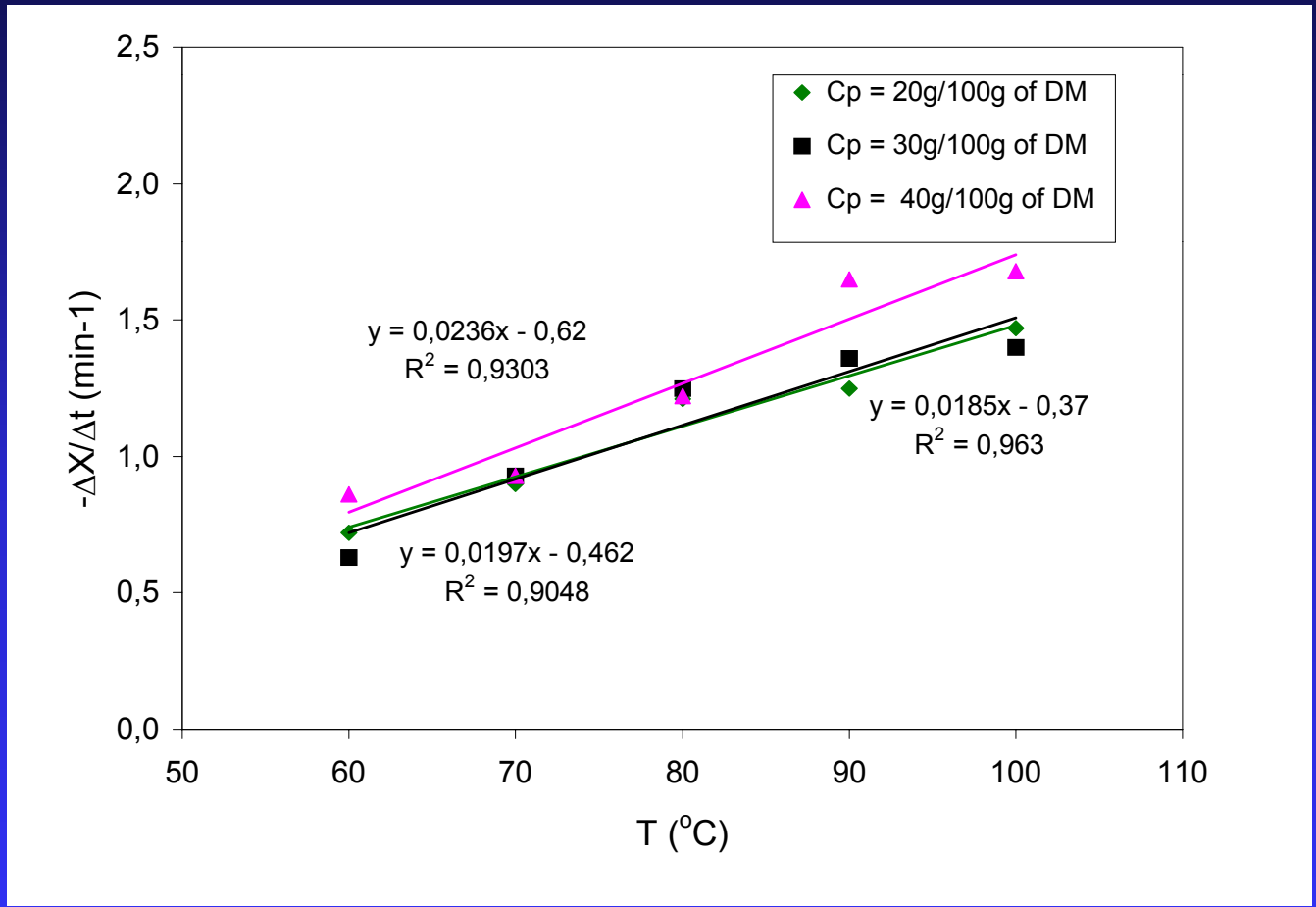
Glutaraldehyde crosslinked films produced by spreading

Casting or spreading

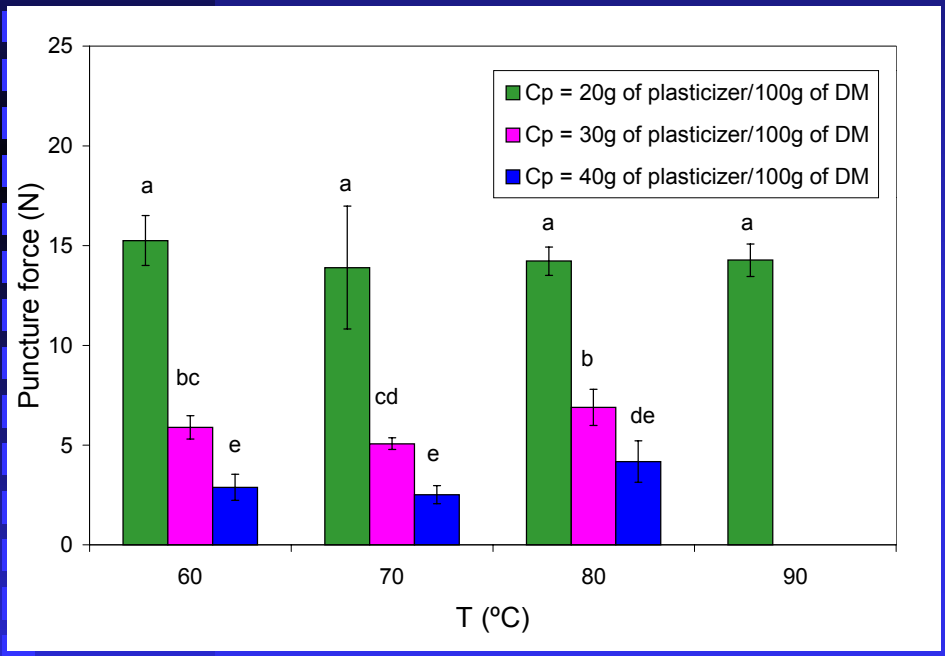
Properties	Ethylene glycol		Glycerol		Diethylene glycol	
	Casting	Spreading	Casting	Spreading	Casting	Spreading
PF (N)	18.1 ± 2.4^{a}	12.5 ± 1.8^{b}	16.3 ± 1.5^{a}	13.3 ± 1.2^{b}	15.1 ± 1.9^{a}	11.8 ± 1.9^{b}
PD (%)	14.5 ± 2.8^{a}	15.0 ± 2.2^{a}	16.2 ± 0.8^{a}	15.1 ± 2.0^{a}	18.7 ± 1.1^{a}	20.3 ± 3.2^{a}
TS (MPa)	2.6 ± 0.4^{a}	1.7 ± 0.2^{b}	2.5 ± 0.3^{a}	1.8 ± 0.2^{b}	2.5 ± 0.3^{a}	1.9 ± 0.3^{b}
E (%)	176.8 ± 16.1^{a}	149.1 ± 28.1^{b}	147.3 ± 16.1^{a}	143.7 ± 23.9^{a}	201.1 ± 8.7^{a}	169.5 ± 29.1^{b}
EM (MPa)	0.061 ± 0.019^{a}	0.076 ± 0.009^{a}	0.131 ± 0.037^{a}	0.080 ± 0.016^{b}	0.040 ± 0.007^{a}	0.068 ± 0.007^{b}

Different letters, same line and plasticizer type, represent significant differences ($p>0.05$) between averages obtained through the Duncan test.

Drying temperature



Puncture force



Puncture deformation