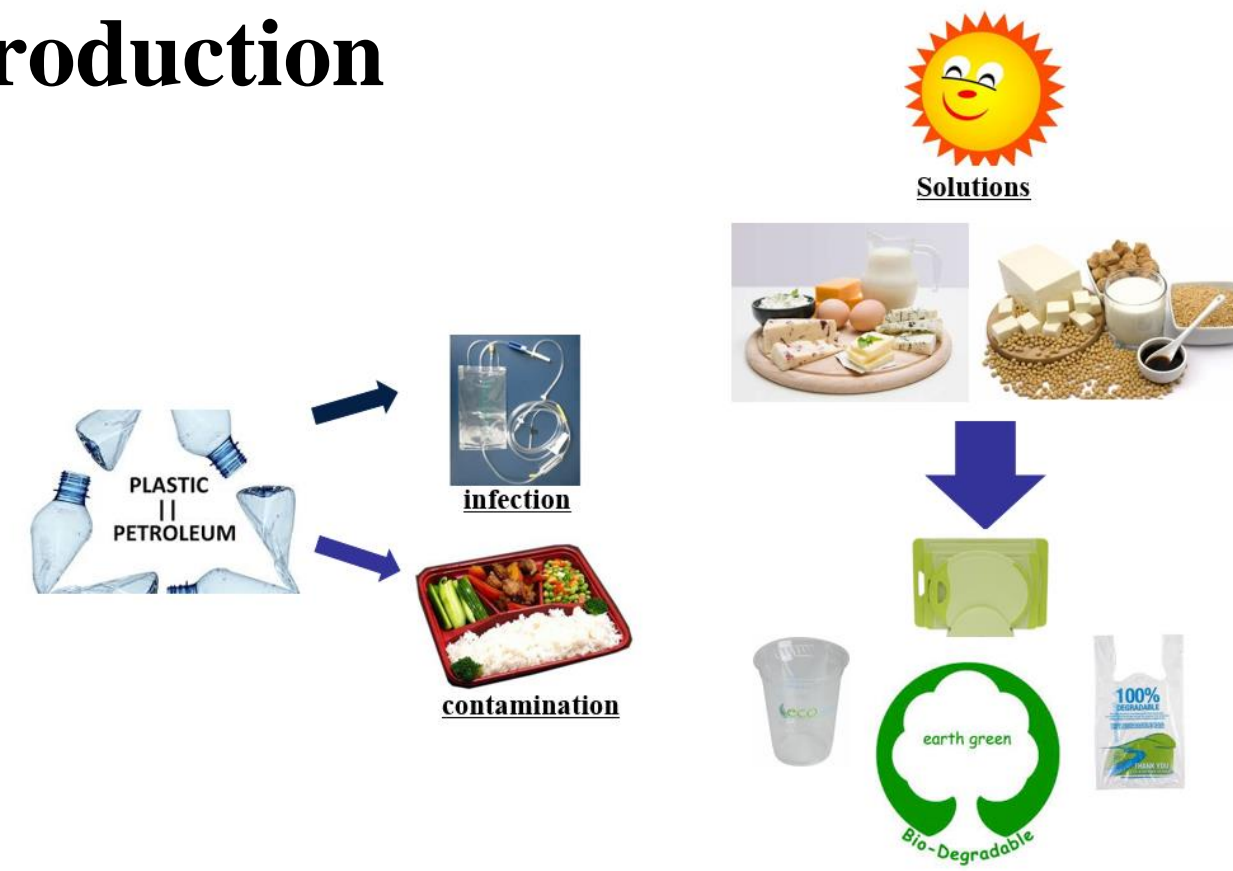# Protein based bio-plastics and their antibacterial potential

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Introduction

Objectives

- To evaluate antibacterial properties of protein-based bioplastics through the use of different plasticizers
- To obtain the best protein-plasticizer combination that has least bacterial count for bioplastic production

Experimental & Treatment Design

- **Experimental design:** completely randomized design
- **Treatment design:** bacterial counts with negative binomial
- **Treatment factors:**
  - Proteins
    - albumin (A), soy (B), zein (C), whey (D)
  - Plasticizes
    - water (w), glycerol (g), natural rubber latex (n)
- **Experimental unit:** petri dishes
- **Response variable:** bact. count after 24hrs
  - *Escherichia coli* as Gram (-) species

Model & Assumptions

- **Model:**

  Linear predictor is: $\eta_{ij} = \eta + \tau_i + \alpha_j + (\tau\alpha)_{ij}$

  Link function: $\eta_{ij} = log(\lambda_i)$

- **Assumptions:**

  Distribution of the observations: $y_{ij} \sim$ Negative Binomial(λ_i, ϕ),

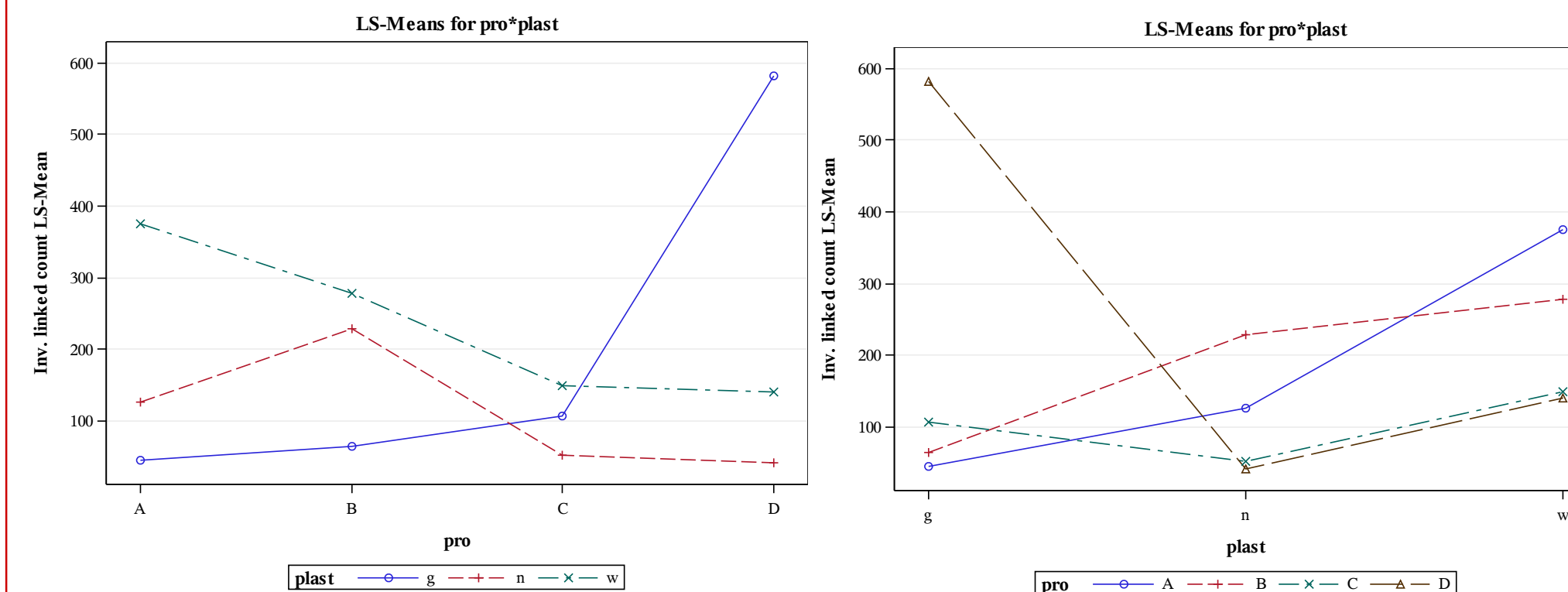 where $E(y_{ij}) = \lambda$ and $V(y_{ij}) = \lambda + \lambda^2\phi$

  $\tau_i = $ ith treatment, plasticizer (water, glycerol, natural rubber) with fixed effect

  $\alpha_j = $ jth treatment, protein (albumin, soy, zein, and whey) with fixed effect

  $(\tau\alpha)_{ij} = $ combination of ith treatment and jth treatment with fixed effect

Statistical Analysis

- **There appears to be an interaction between treatment combinations.**

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
pro	3	36	0.74	0.5368
plast	2	36	3.53	0.0398
pro*plast	6	36	4.16	0.0028

- There is a significant interaction between proteins and plasticizers.

  F = 4.16

  P-value = 0.0028

- **Simple effects**

Simple Effect Comparisons of pro*plast Least Squares Means By pro

Simple Effect Level	plast	_plast	Estimate	Standard Error	DF	t Value	Pr > |t|
pro C	n	w	-1.0671	0.6939	36	-1.54	0.1328
pro D	g	n	2.6594	0.6939	36	3.83	0.0005
pro D	g	w	1.4217	0.6907	36	2.06	0.0469
pro D	n	w	-1.2378	0.6949	36	-1.78	0.0833

- The most significant difference is between whey + glycerol (Dg) and whey + natural rubber (Dn).

Simple Effect Comparisons of pro*plast Least Squares Means By plast

Simple Effect Level	pro	_pro	Estimate	Standard Error	DF	t Value	Pr > |t|
plast g	A	B	-0.3902	0.6960	36	-0.56	0.5785
plast g	A	C	-0.8816	0.6949	36	-1.27	0.2127
plast g	A	D	-2.5827	0.6936	36	-3.72	0.0007
plast g	B	C	-0.4914	0.6936	36	-0.71	0.4832

- The most significant difference is between glycerol + soy (gA) and glycerol + whey (gD).

Convert logit into meaningful ratio

- Convert the logit estimate into bacterial count to find the ratio of treatment combinations:
  - glycerol + soy vs. glycerol + whey:

    $$e^{log(Ag)-log(Dg)} = e^{log\left(\frac{Ag}{Dg}\right)} = e^{-2.5827} = 0.0755$$

  - whey + glycerol vs. whey + natural rubber:

    $$e^{log(Dn)-log(Dg)} = e^{log\left(\frac{Dn}{Dg}\right)} = e^{-2.6594} = 0.0699$$

- The count ratio of whey + glycerol vs. whey + natural rubber ($\frac{Dn}{Dg}$) is smaller, thus whey + glycerol (Dn) and whey + natural rubber (Dg) have the most different bacteria counts.

The winner is …

pro*plast Least Squares Means

pro	plast	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper	Mean	Standard Error Mean	Lower Mean	Upper Mean
D	g	6.3669	0.4877	36	13.05	<.0001	0.05	5.3777	7.3561	582.25	283.98	216.53	1565.68
D	n	3.7075	0.4935	36	7.51	<.0001	0.05	2.7065	4.7084	40.7500	20.1121	14.9766	110.886
D	w	4.9452	0.4891	36	10.11	<.0001	0.05	3.9532	5.9372	140.50	68.721	52.1035	378.87

- Since whey + natural rubber (Dn) is the numerator, this treatment combination has the smallest mean bacteria count.

  $$\text{whey + natural rubber } (Dn) = e^{log(Dn)} = e^{log(3.7075)} = 40.7500$$

- Least Square Means also provides:
  - Standard error mean = 20.1121
  - 95% CI (14.9766, 110.88)

Conclusion

- Whey protein-natural rubber latex (Dn) combination has been proved to produce plastic that shows best antibacterial potential.