Characterization of the Cottonseed Protein Concentrate (CSPC) made from cottonseed raw materials

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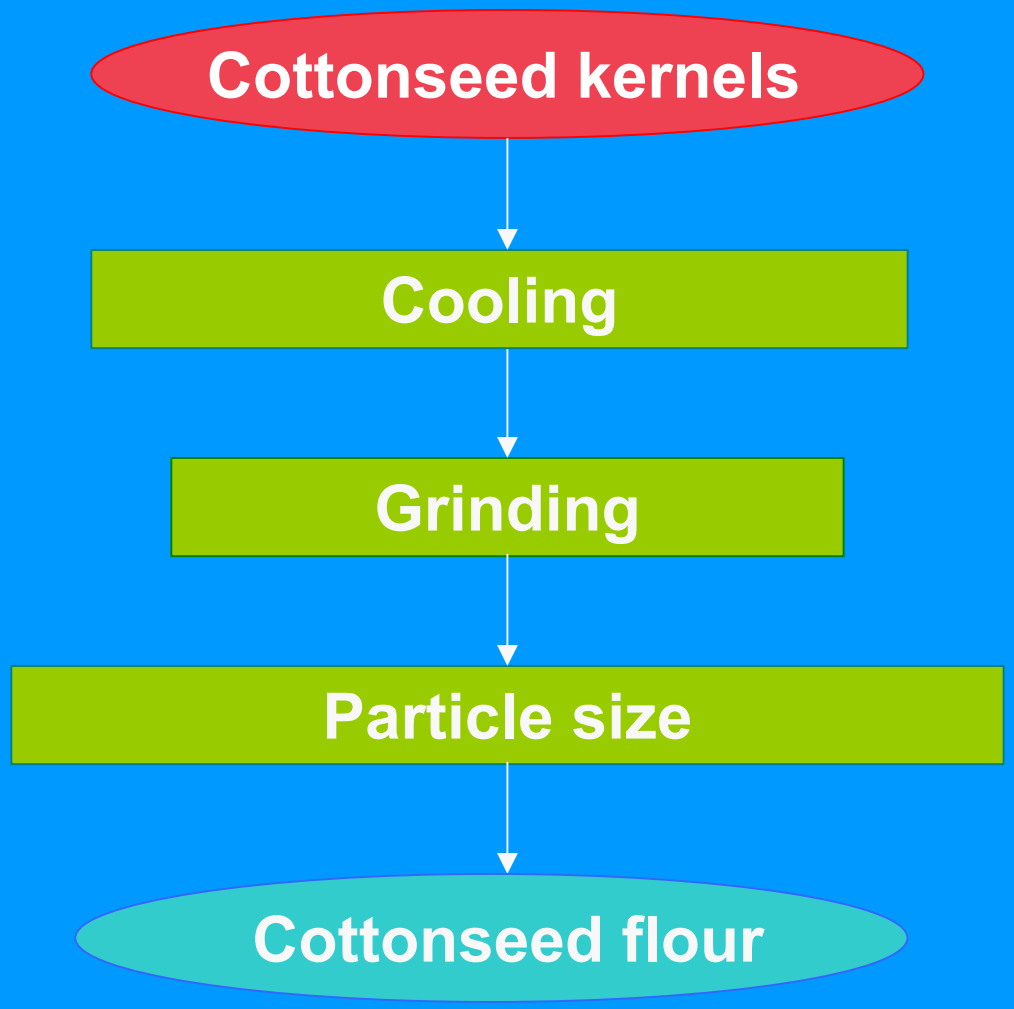
1st RAW MATERIAL: COTTONSEED KERNELS

KERNELS GRINDING



2nd RAW MATERIAL: COTTONSEED FLOUR

Chemical Composition

Lipid content	28.41 (g/100g dry matter)
Protein	27.98
Total lysine	1.25
Reactive lysine	0.85
Total gossypol	0.63
Free gossypol	0.55
Combined gossypol	0.08
Water content	7.73

PROTEINS IN THIS RAW MATERIAL

Molar mass - protein content

111.0 kD – 6.0%
94.4 kD – 5.5 %
50.3 kD – 16.5 %
49.0 kD – 14.5 %
29.6 kD – 9.0 %
20.0 kD – 10.5 %
18.0 kD – 8.5 %
15.3 kD – 8.5 %
13.9 kD – 11.0 %

Aminogram

ASX	2.85	MET	0.49
GLX	6.18	ILE	0.87
SER	1.30	LEU	1.67
THR	0.95	TRP	-
GLY	1.22	PHE	1.58
ALA	1.28	CYS-S	0.47
ARG	3.49	LYS	1.25
PRO	1.16	HIS	0.97
VAL	1.16	TYR	0.97

(g/100g dry matter)

COTPROT PROCESS STEPS

Flour dispersion

↓

pH adjust

↓

Extraction

↓

Centrifugation

↓

Filtration

Flour dispersion & pH adjust

Before alkali

After alkali

Centrifugation

Screening:
Saturated Plackett & Burman planning

Factors

- Flour/water ratio
- pH
- Particle size
- Centrifugation time

Temperature

Alkaline agent

Dispersion time

Centrifugation force

Experimental essays

Exp.	Flour/Water (%)	pH	Temperature (ºC)	Duration (min)	Part. Size (mm)	Alcaline agent	Centrifugal Force	Cent. time
1	40	10	25	60	0,42-0,6	NH_4OH	500	5
2	40	8	60	60	0,42-0,6	TEA	500	5
3	20	10	60	60	<0,3	TEA	500	15
4	40	10	60	30	<0,3	TEA	1500	5
5	40	10	25	30	<0,3	NH_4OH	500	15
6	40	8	25	30	0,42-0,6	TEA	1500	15
7	20	8	25	60	<0,3	NH_4OH	1500	5
8	20	8	60	30	0,42-0,6	NH_4OH	500	5
9	20	10	25	60	0,42-0,6	TEA	1500	15
10	40	8	60	60	<0,3	NH_4OH	1500	15
11	20	10	60	30	0,42-0,6	NH_4OH	1500	5
12	20	8	25	30	<0,3	TEA	500	5

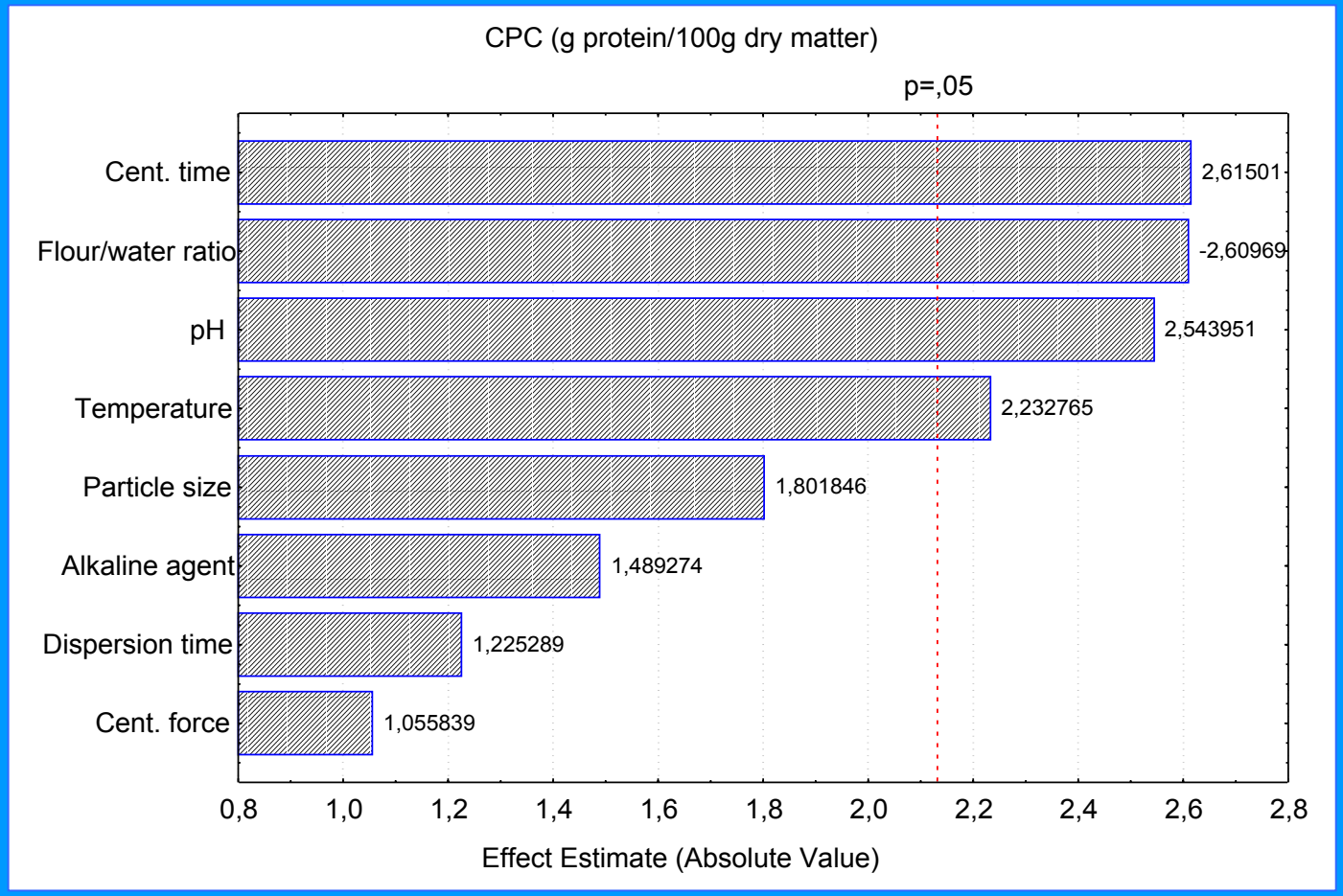
COTPROT Parameters

Exp.	CPC	Viscosity (cP)	L*	a*	b*
1	39,71	-	54,57	1,40	47,19
2	37,28	16,15	57,86	3,35	49,36
3	47,35	3,04	30,70	11,14	43,87
4	40,22	-	-	-	-
5	39,57	33,75	54,83	4,99	55,45
6	37,86	4,72	50,58	-0,43	32,13
7	41,89	4,28	40,2	1,25	23,17
8	48,33	0,93	41,79	4,71	36,90
9	47,38	0,71	51,01	6,24	54,61
10	39,51	52,75	48,30	2,37	39,72
11	45,43	0,93	42,57	11,87	51,85
12	29,60	7,01	42,055	1,69	29,79

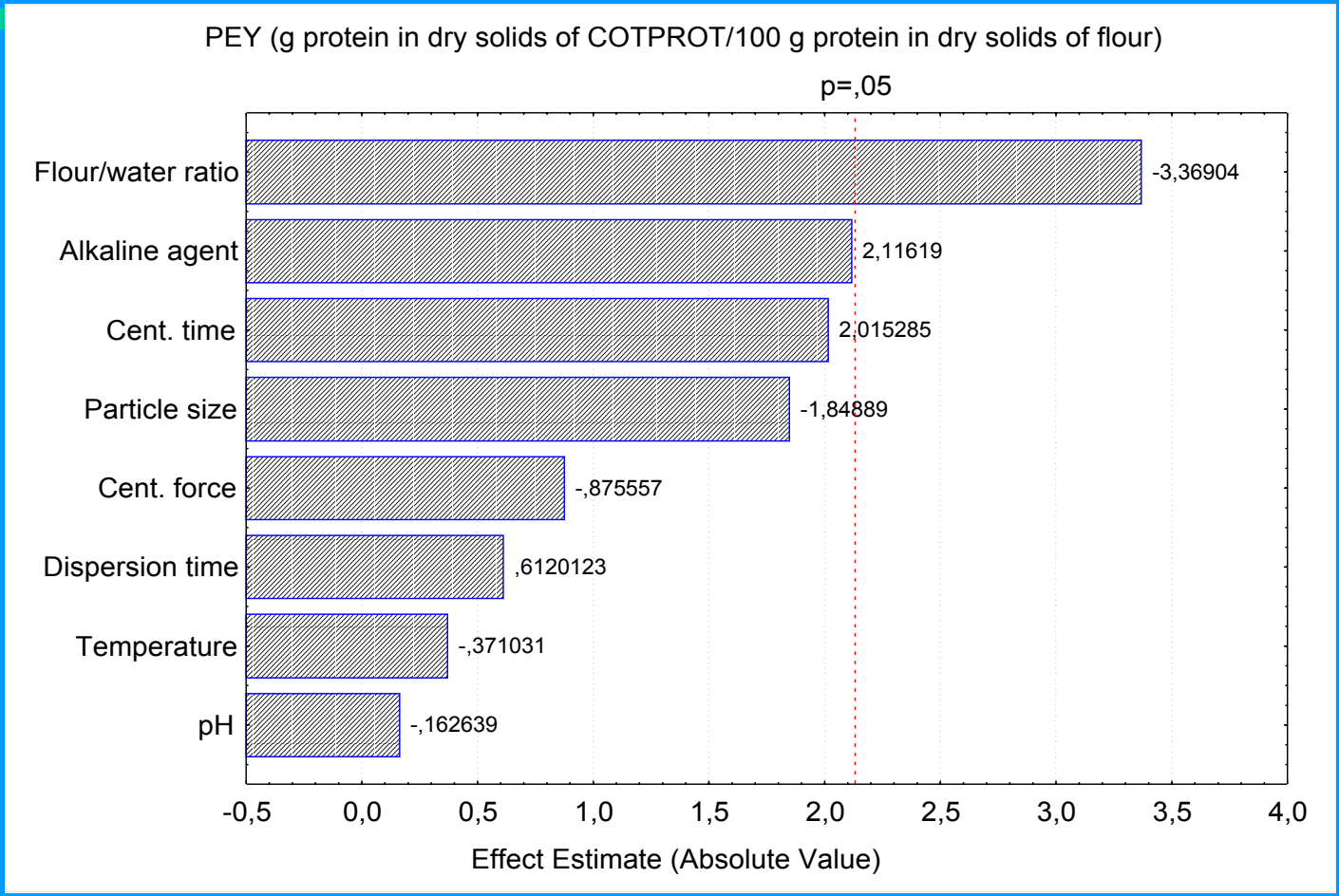
COTPROT Yields

Essay	DMY	PEY	DR
1	16,25	21,73	74,82
2	20,72	26,01	72,74
3	43,03	63,48	44,93
4	4,62	5,88	80,30
5	44,44	55,67	47,46
6	24,08	30,65	68,94
7	43,60	57,66	48,62
8	25,50	41,46	60,88
9	31,10	49,59	62,42
10	32,91	41,20	56,22
11	33,39	50,95	59,97
12	43,26	40,52	49,75

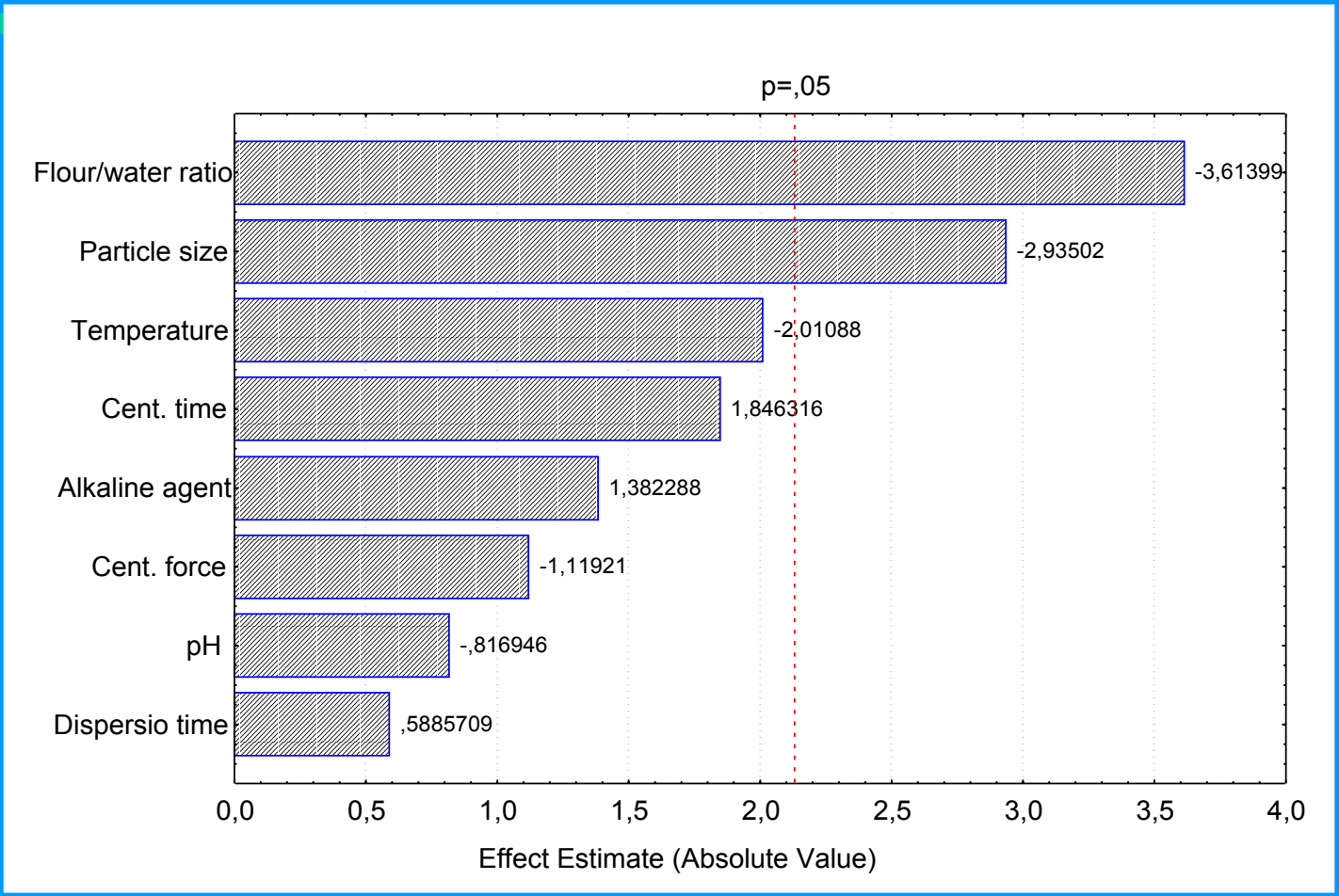
Pareto charts of standardized effects



COTPROT protein concentration (CPC): g protein/100 g COTPROT dry solids.



Protein Extraction Yield (PEY): g protein in COTPROT dry solids/100 g protein in cottonseed kernel flour dry solids.



Dry Matter Yield (DMY): g COTPROT dry solids/100 g cottonseed kernel flour dry solids.

Parameters chosen to optimize the COTPROT extraction process

- pH, temperature and flour/water ratio.

- Fixed Parameters:
  - Particle size
  - Alkaline agent
  - Time of agitation
  - Centrifugation conditions.

Planning Matrix

	Independent variables[1]					
	Flour/water (g/100ml)		PH		Temperature (ºC)	
Exp	X_1		X_2		X_3	
1	22	(-1)	8.4	(-1)	33	(-1)
2	28	(+1)	8.4	(-1)	33	(-1)
3	22	(-1)	9.6	(+1)	33	(-1)
4	28	(+1)	9.6	(+1)	33	(-1)
5	22	(-1)	8.4	(-1)	57	(+1)
6	28	(+1)	8.4	(-1)	57	(+1)
7	22	(-1)	9.6	(+1)	57	(+1)
8	28	(+1)	9.6	(+1)	57	(+1)
9	20	(-1.68)	9	(0)	45	(0)
10	30	(1.68)	9	(0)	45	(0)
11	25	(0)	8	(-1.68)	45	(0)
12	25	(0)	10	(1.68)	45	(0)
13	25	(0)	9	(0)	25	(-1.68)
14	25	(0)	9	(0)	65	(1.68)
15	25	(0)	9	(0)	45	(0)
16	25	(0)	9	(0)	45	(0)
17	25	(0)	9	(0)	45	(0)
18	25	(0)	9	(0)	45	(0)
19	25	(0)	9	(0)	45	(0)

Dependent Variables

	PEY (%)	DMY (%)	CPC (%)	CV (cP)	L*	a*	b*	DRY (%)
Exp	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8
1	69.03	39.25	4.37	0.96	48.26	3.32	45.21	44.21
2	58.09	33.30	5.68	1.86	46.90	5.10	46.97	52.26
3	73.17	40.95	4.77	0.86	48.59	7.00	53.08	46.17
4	57.27	37.12	5.24	1.35	43.12	8.26	51.74	44.55
5	68.35	39.01	4.56	1.01	37.52	7.61	43.73	43.04
6	58.05	31.67	6.06	1.61	29.04	8.51	39.05	61.15
7	87.32	49.14	5.87	1.11	43.88	9.72	54.65	37.50
8	65.22	37.69	6.58	1.76	33.67	9.10	46.35	24.23
9	76.65	42.52	4.27	0.94	45.68	6.37	49.41	42.85
10	67.38	36.50	6.65	1.48	31.39	8.82	41.91	50.03
11	60.49	35.64	5.03	1.46	49.70	5.47	50.00	60.79
12	77.85	44.99	5.78	1.01	41.73	10.83	54.87	38.79
13	60.30	37.31	4.90	1.07	49.49	5.13	52.46	53.74
14	71.91	41.24	5.88	1.33	37.21	8.10	46.00	51.49
15	70.59	38.27	5.59	1.01	39.62	7.92	47.47	53.80
16	70.12	39.16	5.64	1.07	39.78	7.96	47.82	60.76
17	60.11	36.81	4.77	1.01	37.99	8.34	46.89	57.70
18	66.16	36.54	5.23	1.29	37.20	7.94	46.03	46.01
19	68.84	37.95	5.46	1.09	37.33	8.03	45.01	44.99

Statistical Study

- Variance analysis with Statistica 5.0 for Windows (StatSoft Inc.)
- Quadratic model: explained variability percent >84.06 % for all the responses
- $Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$

Regression coefficients

	PEY	DMY	DRY	CPC	CV	L	b	a
Coefficients	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8
b_0	67.25	37.80	52.86	5.35	1.09	38.44	46.71	8.04
Linear								
b_1	-5.48***	-2.83***	1.71	0.70***	0.26***	-3.63***	-1.84**	0.54***
b_2	4.30	2.74	-6.24	0.34**	-0.08	-0.43	2.86***	1.36***
b_3	3.00**	0.99*	-1.84	0.23**	0.07	-4.65***	-1.76**	1.19***
Interactions								
b_{12}	-2.10	-0.25	-5.13*	-0.40	-0.05**	-0.73	-0.84	-0.26
b_{13}	-0.69	-1.13	-0.20	0.25	-0.02	-1.48	-1.68**	-0.35
b_{23}	2.85	1.33	-4.59	0.43	0.11**	1.81	0.70	-0.52**
Quadratic								
b_{11}	1.26	0.34	-3.33	-0.01	0.06	-0.25	-0.71	-0.18
b_{22}	-0.84	0.26	-1.14	-0.03	0.07	2.29**	1.70*	0.02
b_{33}	0.25	0.63	-2.14	-0.03	0.06	1.46	0.56	-0.52***
% explained variability (R^2)	84.06	90.87	87.37	91.48	85.10	90.81	96.50	89.15

*Probability level between 5 to 10%; **Probability level between 1 to 5%; ***Probability level less than 1%.

Optimization results

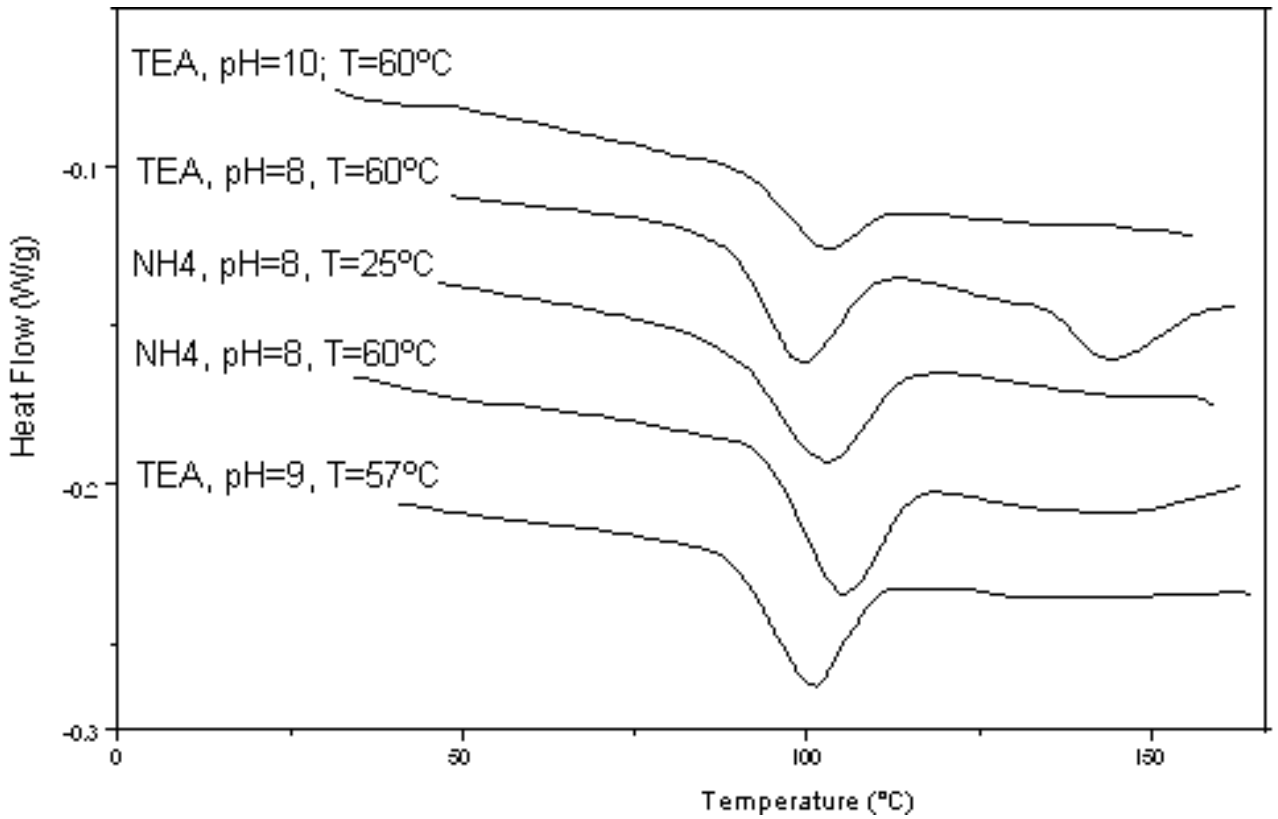
- The surface-response planning allowed to verify optimal conditions only in the case of L* (minimum) and DRY (maximum).

- Also, considering DMY and PEY such the most important parameters, it can be suggested that there was an optimal condition located at high temperature, low FWR and high pH domain.

Chemical Composition of Optimized COTPROT (freeze-dried)

Lipid content	5.6	(g/100g dry matter)
Protein	57.9	
Reactive lysine	1.73	
Total gossypol	1.5	
Free gossypol	<0.04	
Moisture content	7.1	

DSC studies


TEA, pH=10; T=60°C
TEA, pH=8, T=60°C
NH4, pH=8, T=25°C
NH4, pH=8, T=60°C
TEA, pH=9, T=57°C
Heat Flow (W/g)
-0.1
-0.2
-0.3
0
50
100
150
Temperature (°C)

Chemical Analysis Of COTPROT Residue

Analyses	S2* (g/100gdry matter)	S3 (g/100gdry matter)
Lipids	29.99	30.13
Protein content (N x 5.3)	25.49	25.48
Raw celulose	13.67	11.73
Hemicellulose	9.36	7.6
Lignin	7.72	11.49
Total fiber	11.82	9.71
FDA	21.37	19.14
FDN	30.73	26.74
Minerals	6.86	4.86

* Results were presented in the first year report of the project.

Aminoacid Composition Of COTPROT Residue

Amino acids	Content (%)	Amino acids	Content (%)
- Aspartic acid	2.41 ± 0.15	- Valine	1.29 ± 0.09
- Glutamic acid	5.91 ± 0.21	- Methionine	0.24 ± 0.01
- Serine	1.23 ± 0.06	- Isoleucine	0.84 ± 0.04
- Threonone	0.91 ± 0.04	- Leucine	1.62 ± 0.004
- Glycine	1.44 ± 0.25	- Phenilanine	1.35 ± 0.08
- Alanine	1.12 ± 0.06	- Cisteine	0.31 ± 0.02
- Arginine	2.94 ± 0.19	- Lysine	1.36 ± 0.15
- Proline	1.13 ± 0.08	- Histidine	0.71 ± 0.04

Mineral Composition Of COTPROT Residue

Mineral contents (dry basis)	S2* (CR3)	S3 (CR3)
Ca (%)	74.6 ± 1.1	0.16 ± 0.04
P(%)	1.46 ± 0.01	0.97 ± 0.04
K (%)	3.5 ± 0.1	3.16 ± 0.03
Mg (%)	0.75 ± 0.02	0.78 ± 0.04
Na (ppm)	68.9 ± 4.3	12.9 ± 0.4
Mn (ppm)	16.4 ± 0.9	16.8 ± 0.2
Fe (ppm)	111.8 ± 5.9	111.1 ± 0.1
Zn (ppm)	90.1 ± 3.9	110.6 ± 11.5
Cu (ppm)	4.2 ± 0.2	3.3 ± 0.1

* Results were presented in the first year report of the project.