USE OF MODIFIED STARCH FOR COLORLESS GLOBIN FROM BLOOD CELLS.

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SUMMARY: Working conditions for the preparation of colorless globin using modified maize starch (MMS) were stablished using soluble carboximethyl cellulose (CMC) as reference. Response was calculated using two parameters of heme content and protein recovery. Three levels of temperature (30, 50 and 80 °C), final pH (2.25, 2.70 and 3.00) and phosphatation grade (1, 3.1 and 5.2 g H3PO4/100 g starch) were tested as well as two levels of polymers concentration (0.3 and 0.5 %). Results suggest that thermal denaturation of hemoglobin could help to achieve a more efficient decolorization and that MMS is at least as efficient for decolorization as CMC is.
Better results are obtained when final pH is 2.25, polymer concentration is 0.3% and phosphatation grade is between 1 and 3.1 %.

INTRODUCTION: Although blood cells are a valuable source of edible protein, its utilization is limited at present mainly because its dark reddish color is not desirable.
Decolorization can be achieved through the separation of hemoglobin into heme and globin fractions using differents methods. Sato et al (1981) developed a simple method for the preparation of a colorless globin using carboxymethil cellulose (CMC) column chromatography. Autio et al (1984) showed that soluble CMC also can be used for the same purposes, and Hayakawa et al (1986) determined that optimum conditions were: initial pH 1.5, CMC concentration 0.72%, final pH 2.25 and heating temperature 77 °C.
The aim of this paper is to achieve the conditions for the preparation of a colorless globin, using modified maize starch (MMS) in order to coprecipitate the separated heme.

MATERIALS AND METHODS: Bovine blood cells concentrate was obtained from the slaughterhouse and kept frozen (-20 C). 50 mL of blood cells concentrate were hemolyzed by adding it on 250 mL of water, or polymer (CMC or MMS) acid solution (0.3 or 0.5 %). Three levels of phosphatation (1, 1.3 and 5.2g H3PO4/100g MMS) were tested.
The pH of the suspentions was adjusted to 1 by HCl and then blood was added. After 30 min mixing, pH was adjusted to 1.8 and after 30 min mixing more, pH was adjusted to final value (2.25, 2.7 or 3) by NaOH. Sample were heated to 30, 50 or 80 °C and mixed 30 min more.
When heated sampled got room temperature the co-precipitated heme was centrifuged off. The co-precipitated was washed out and was centrifuged off again. Both supernatans were poored into a 100 mL volumetric flask and volume was adjusted. Protein content was determined by the method of Lowry et al and Absorbance at 380 nm was also determined. All the treatments were tested three times.
Response (RE) wasdefined as RE=(HC/PR^2) * 10000 using protein recovery (RP) and residual heme content (HC) defined by Hayakawa

et al (1986).
Analysis of variance were performed

RESULTS AND DISCUSSION: Lower HC values are found when polymer concentration is 0.3% (table 1). In such conditions, better results are obtained when samples are heated and phosphatation grade is 3.1. Final pH value didn't affect the HC values at the lower polymer concetration, but when polymer concentration is 0.5%, the lowest the final ph value the better the decolorization is.

Table 1.- Mean value of heme content.

Concentration	Polymer	Mean	Temp.	Mean	pH	Mean
0.3%	MMS-1	0.530 b	30	0.617 b	2.25	0.559 a
	MMS-3.1	0.458 a	50	0.537 a	2.70	0.553 a
	MMS-5.2	0.601 c	80	0.519 a	3.00	0.560 a
	CMC	0.634 c				
0.5%	MMS-1	0.714 a	30	0.820 b	2.25	0.722 a
	MMS-3.1	0.820 b	50	0.730 a	2.70	0.762 b
	MMS-5.2	0.785 b	80	0.750 a	3.00	0.816 c
	CMC	0.747 a				

Values in the same column and concentration not showing a common letter are significantly different ($p < 0.05$)

As is well known the protein-heme and polymer-heme interaction are competitive at low pH, being the former relatively stronger than the later in the presence of salt wich is present in the solution because of the pH adjustment. Therefore, lower HC values can be expected if the heme-protein interaction is reduced by thermal denaturation of hemoglobin. In fact, in the unheated samples, heme group could be retained in the hydrofobic pocket of the protein even after the linkage between the iron and imidazole groupes has been broken. The thermically induced unfolding of the protein molecule allowed the heme group to coprecipitate with the polymer, but interactions between thermal treatment, pH value and polymers concentration are present as Hayakawa et al (1986) has pointed out.
Table2 shows the protein recovery results. CMC and MMS-3.1 at 0.3% concentration yield the better values specially when final pH is 2.25. Increasing the phosphatation grade or polymer concentration, yield values are not improved. Some kind of interaction between protein and polymers which is dependent of the ionic strength seems to be present. This could explain the effect of the phosphatation grade and the polymer concentration. The effect of the pH could partially be explained because the lower the pH the farest the isolectric point.

Table 2.- Mean value of protein recovery

Concentration	Polymer	Mean	Temp.	Mean	pH	Mean
0.3%	MMS-1	0.769 a	30	0.805 b	2.25	0.852 b
	MMS-3.1	0.814 b	50	0.784 a	2.70	0.746 a
	MMS-5.2	0.767 a	80	0.784 a	3.00	0.768 a
	CMC	0.813 b				
0.5%	MMS-1	0.645 a	30	0.707 a	2.25	0.725 a
	MMS-3.1	0.680 b	50	0.738 b	2.70	0.711 a
	MMS-5.2	0.702 b	80	0.725 b	3.00	0.735 a
	CMC	0.850 c				

Values in the same column and concentration not showing a common letter are significantly different ($p < 0.05$)

Table 3 shows the response (RE) values,being clear that MMS-1 and MMS-3 could sucessfully substitute CMC for globin production. Better valuesarefound when final pH is 2.25 and polymer concentration is 0.3%, the better conditions for decolorization. As thermal denaturation didn't help the protein recovery and PR has a big effect on the RE values is clear why RE is not improved raising the temperature.

Table 3.- Response (RE) values.

Conc.(%)	Temp. (°C)	pH	MMS-1	MMS-3.1	MMS-5.2	CMC
0.3%	30	2.25	66.90	80.11	97.82	87.21
		2.70	107.60	104.60	100.90	106.80
		3.00	104.30	67.75	129.80	102.69
	50	2.25	68.50	79.57	84.10	95.97
		2.70	99.37	57.59	114.90	108.37
		3.00	121.60	94.49	126.50	140.86
	80	2.20	69.72	67.80	74.15	68.70
		2.70	102.20	84.88	103.87	99.31
		3.00	119.70	91.90	101.85	73.78
0.5%	30	2.25	161.60	141.73	158.80	183.95
		2.70	145.10	200.00	156.10	148.97
		3.00	221.50	152.63	172.80	137.49
	50	2.25	173.80	154.45	127.19	69.22
		2.70	185.36	195.99	184.98	59.52
		3.00	297.20	211.49	174.28	71.65
	80	2.25	85.61	271.00	142.22	121.36
		2.70	222.50	183.97	149.33	105.16
		3.00	164.80	131.58	153.05	106.93

Note:Each value is the mean of 9 replications.

CONCLUSIONS: Modified maize starch can be used to co-precipitate the heme group after the acid hydrolisis, as well as carboxy methil cellulose is. The better results are get when final pH 1s 2.25, polymer concentration is 0.3 % and phosphatation grade is between 1 and 3.1 %

REFERENCES :

- Autio, K., Kiesvaara, M., Malkki, Y. and Kanko, S. (1984) Journal of Food Science 49:859.
- Hayakawa, S., Matsuura, Y., Nakamura, R. and Sato, Y. (1086) Journal of Food Science 51:786.
- Sato, Y., Hayakawa, S. and Hayakawa, M. (1981) Journal of Food Technology 16:81