Quantitative Determination of Casein in Heated Meat Products by Polyacrilamide-SDS Gel Electrophoresis.

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Introduction.

In this work we used the discontinous gel electrophoretic technique of Laemmli (1970) to study quantitatively casein in canned meat products treated by heat.
Casein determination by immunologic techniques have been reported by Huescar Fernandez et al.(1982).
We used the SDS-electrophoresis technique because the electrophoretic pattern of casein is make up of 3 bands (34 K , 30 K , 26 K). In this zone meat bands are weak. So that we thought that electrophoretic resolution with sodium dodecyl sulfate could be satisfactory.
Furthermore it seems that actin band is more stable (Lacourt et al. 1977) . So that we have used casein peack/ actin peack index to calculate casein concentration.

Material and Methods.

Sample manufacture.
Sample were canned meat products supplied by a local industry. The mixture was a " pâte " and the composition was the same for all samples.They varied only in casein concentration and heat treatment. The base composition of the mixture was: Lean pig meat (65 %) , head lean pig meat (20 %) , ice (15 %) , salt (20 gr/Kg) spice (4 gr/ kg) , phosphate (3 gr/kg),sugar (1gr/kg) , preservatives (1gr/ kg) , sodium ascorbate (0.5 gr/ Kg) and sodium nitrite (0.2 gr/kg) .
The mixture was prepared with a cutter (Vall-35). The way out temperature was 7ºC. The added protein was Sodium Caseinat EM-HV (DMW). Final casein concentration in meat products were 0 , 0.5 , 1 , 1.5 , 2 % .
The weighed base mixture was put into the cutter and then the casein was added. After a homogenization the way out temperature was 14 to 15ºC.
The products were prepared to reach inner temperatures of 60 - 70 - 80 - 90 - 100 - 110 ºC. A open boiler was used for preparing samples at 60 - 70 - 80 ºC and the others were prepared with a pressure cooker.
Sample treatment.
Centrifugation for 20 min at 6000 rpm with acetone was used in removing fat from samples. The acetone extraction

treatment was made for three times. Subsequently the residue was dried at 60ºC overnight.
Sample solubilization and extraction was made using Hofmann's method (1977) modified by Calsina et al.(1982).
Electrophoretic technique.
The discontinous gel electrophoretic technique of Laemmli (1970) combined with SDS-electrophoretic was used.
The Vernon PHI-3 densitometer was used at 470 nm wave lenght.

Results.
Electrophoretograms of samples at all temperatures described before were studied. The electrophoretic pattern of casein is make up of 3 bands (34 K , 30 K , 26 K). Firstly, it was studied the best electrophoretogram zone to establish casein peack/ actin peack index to casein concentration ratio in each sample.
For choosing the best zone we take account the following points :
a) zone where the correlations were higher.
b) zone where current (mA) changes influence was low.
c) zone where temperature changes influence was low.
d) zone where linnear regression slopes were higher.
The 32 - 26 K zone was selected from 40 - 26 K, 40 - 32 K and 32 -26 K zones.
Table 1 shows the obtained values of casein peack / actin peack index at different cook temperatures and casein concentrations. Coefficients of determination (R) of the linnear regressions (casein peack/actin peack index to casein concentration) were between 88 - 99 % .
At the same casein concentration the index values were not constant and they varied in function to temperature. As temperature increase from 60 to 80ºC the index value decrease, and from 80 to 110ºC this value increase. These variations with temperature in the analized zone in samples of 1.5% casein concentration can be observed in Figure 1 .
The electrophoretograms of the 70 ºC sample at each casein concentrations are presented in Figure 2a, where it can be observed visually the casein band increase with concentration. Figure 2b shows sample variations with temperature.
Densitograms of Figure 3a are presented in Figure 3a. Figure 3b shows densitograms of 2% casein concentration at all temperatures studied.

Discusion.

Provided that high correlation were found for casein peack / actin peack index to casein concentration for all temperature studied we can establish that casein concentration could be quantitatively determined at any of this temperatures.

Howewer because the values of casein peack / actin peack index were different within the same casein concentration at different temperatures studied, we can say that casein is not thermostable in the temperature range analized. For each cook temperature the regression lines were different at regular 10ºC intervals.

Proportionally casein destruction is higher than actin destruction at 60º C to 80º C temperature range. Actin can be virtually considered thermostable up to 80º C.

It seems that actin is thermolabile at 80º C to 110º C temperature range and its destruction is higher in relation to casein because the index values increase from 80º C. Our experiments to demostrate actin thermolability at these temperature ranges have not been completly successeful up to now. This subject will be studied in further works.

From this paper we conclude that quantitative casein determinations in cooked meat products are possible at any cook temperature if this temperature is known aproximately. From Hofmann's (1977) point of view this could be possible if the actin/miosin index is known.

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TABLE 1.- Values of casein peack/actin peack index at different cook temperatures and casein concentrations.

Cook temperatures (ºC)	Casein concentration (%)				
	0	0.5	1.0	1.5	2.0
60	0,38	0.44	0.57	0.65	0.85
70	0.35	0.39	0.49	0.53	0.57
80	0.25	0.30	0.37	0.45	0.60
90	0.31	0.34	0.40	0.57	0.62
100	0.36	0.56	-	0.64	0.70
110	0.52	0.64	-	0.72	0.81

FIGURE 1.- Casein peack/actin peack (C/A) variations with cook temperatures in samples of 1.5 % casein concentration.

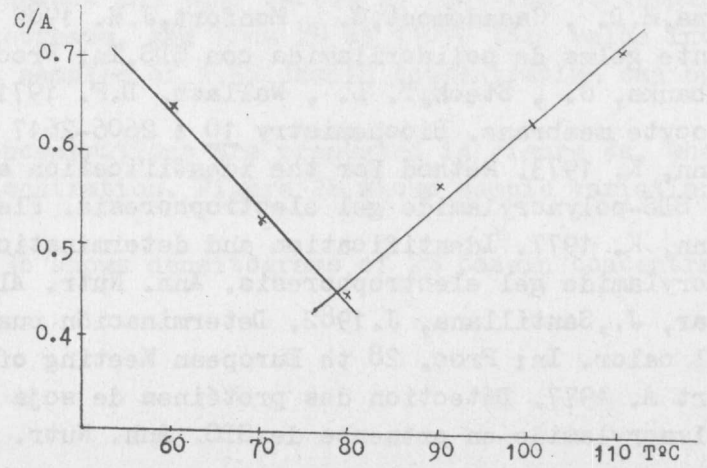
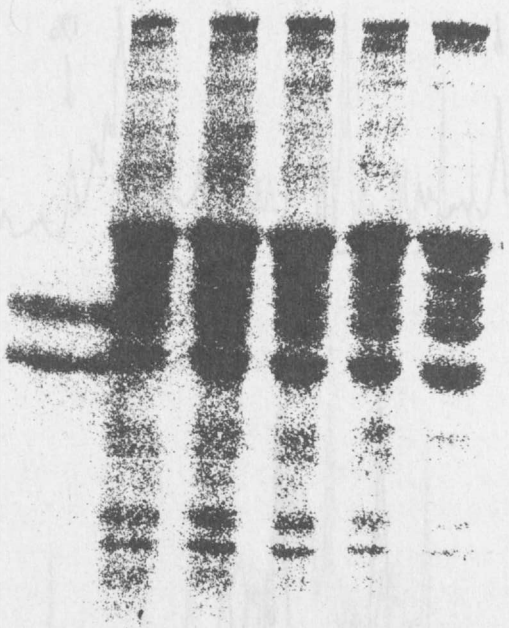


FIGURE 2 .- a) Electrophoretograms of 70ºC samples : 1) casein , 2) meat product with 0% casein , 3) meat product with 0.5% casein , 4) meat product with 1.0% casein , 5) meat product with 1.5% casein 6) meat product with 2.0 % casein

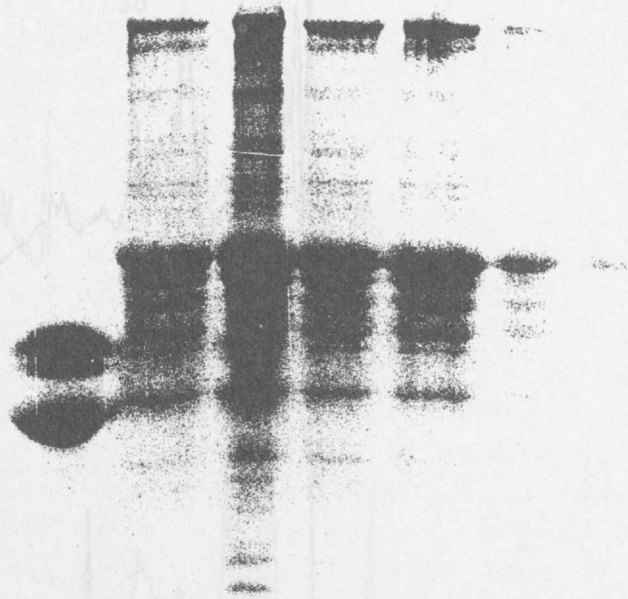
b) Electrophoretograms of samples variation with temperature : 1) casein , 2) meat product without casein at 60ºC , 3) meat product without casein at 70ºC , 4) meat product without casein at 80ºC 5) meat product without casein at 90ºC , 6) meat product without casein at 100ºC , 7) meat product without casein at 110ºC .

a)



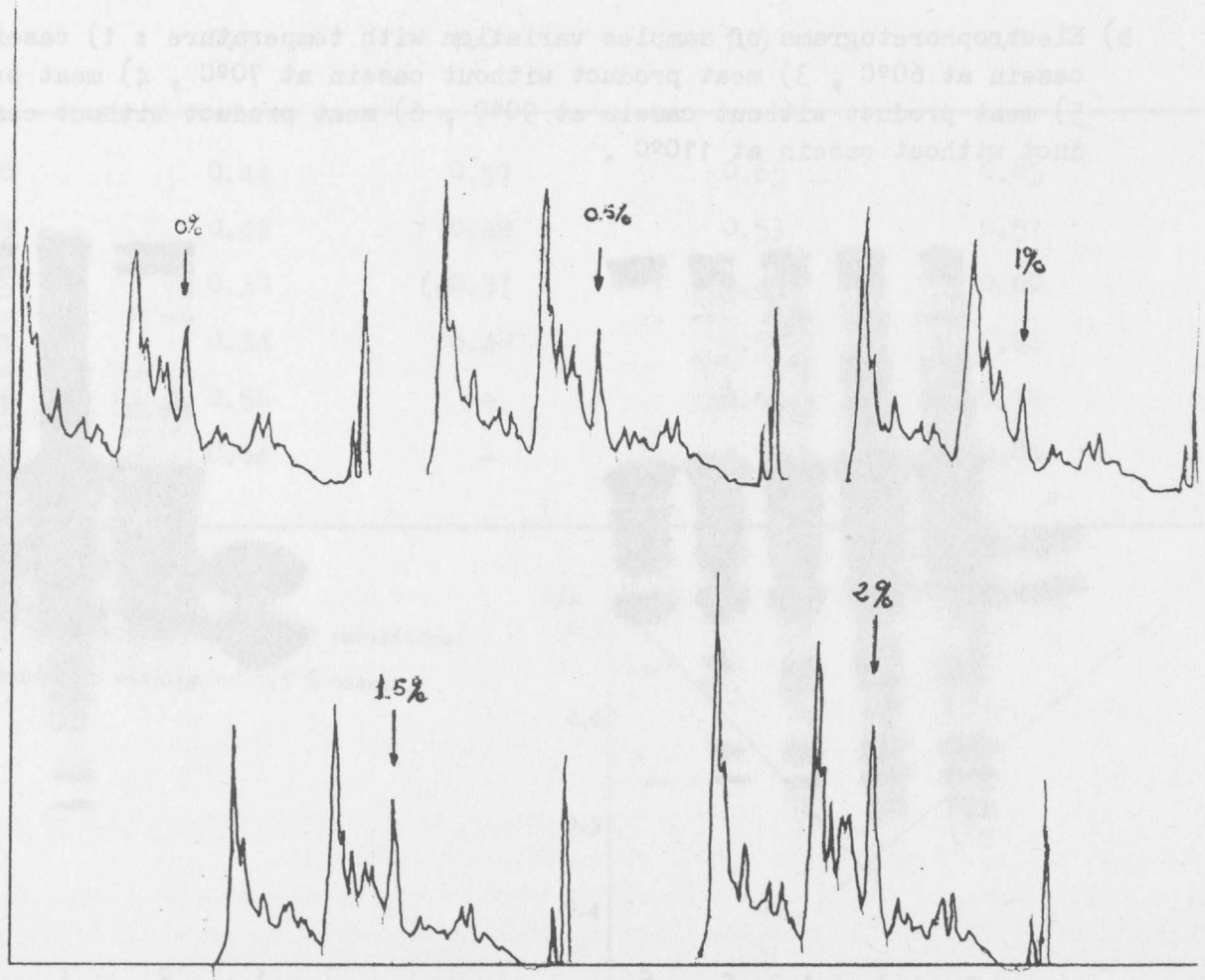
1 2 3 4 5 6

b)



1 2 3 4 5 6 7

FIGURE 3 .- a) Densitograms of 70º C at different casein concentration.



b) *Densitograms of 2 % casein concentration samples at different temperatures.*