The effect of sample/water -ratio and added salt on the buffering capacity of meat

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SUMMARY

Sample/water -ratio used when titrating a meat sample affects the shape of the titration curve. The buffering capacity (BC) maximum between pH 6.5-7.0 moves towards lower pH-values when the amount of water used for diluting the sample diminishes. On the other hand, there is no systematic change in the net consumption of acid in pH range 5.5-7.0.

Also salt causes the BC maximum between pH 6.3-7.0 to move to lower pH values. The effect is clear when using sample/water -ratio 10/100 and salt concentrations from 0% to 5%.

INTRODUCTION

pH-value is the most important independent variable in meat. Several other properties of meat, eg. waterholding capacity, tenderness, colour and keepability depends on pH-value. Changes in pH-value, on the other hand, are affected by the buffering capacity (BC) of meat; that is, its ability to resist the change in pH-value when acid or base is added to the system or is formed in it during the post mortem metabolism.

BC determinations are generally carried out by homogenizing the sample with water, adjusting pH to a starting value and observing the changes in pH as acid or base is added in small amounts. BC is then calculated by dividing the amount of added acid with the corresponding change in pH. (see e.g. Bendall et al. 1962, Sayre et al. 1963, Connell et al. 1964, Honikel et al. 1974, Castellini et al.1981, Rao et al. 1989).

MATERIALS AND METHODS

1. Samples of beef trimmed free of visible fat and connective tissue were used. The sample was homogenized and two 10 g aliquots were weighted out and separately homogenized with distilled water. Sample/water -ratios used were 10/100 and 10/10. The homogenates were titrated using 0.1 N HCl and 0.1 N NaOH. The titration curve for pH range 4-9 was obtained by combining data from these two titrations.

Buffering capasity was calculated for each increment of acid and base as described by Hill et al. (1985).

$BC_1 = (T_1 - T_2) / (pH_1 - pH_2)$

where BC_1 is the average buffering capacity for the range between observations 1 and 2. BC values were plotted against the midpoint of each respective pair of pH values. Curves were fitted using the spline smoothing procedure (SAS/GRAPH 'GPLOT'-subrutine). The pH and BC values for the minimum and maximum points were read from the curve.

2. When studying the effect of salt the samples were first homogenized with salt, left to equilibrate for 30 min and then mixed with salt solution. The concentrations of salt used were 1%, 2%, 3%, 4% and 5%. The salt percentage is calculated including both sample and diluent.

RESULTS

Fig 1. shows two typical titration curves which were obtained using two different sample/water -ratios.

The BC-curves shown in Fig 2. were derived from data shown in Fig 1. When different sample/water -ratios are used the change in the shape of the BC-curve is evident.

Table 1. gives the coordinates (pH- and BC-values) for the minimum and maximum points of BC-curves. Table 2. also shows values for area A (see Fig 2.) which indicate the change in the shape of the BC-curve. In column 10 are listed the consumptions of acid/base (mmol H^+/pH-unit) calculated from the pH range minimum-maximum and in column 11 the consumptions calculated from the pH range 5.5-7.0.

The 'hump' in the titration curve got smaller and the curve straightened when the amount of water used in titration diminished. The changes in titration curve are small and difficult to detect, while in the BC-curve which is the reciprocal of

the derivative of the titration curve, the changes are easily seen. In the BC-curve the difference between both pH and BC values for the maximum and minimum points got smaller and the curve straightened.
When the sample/water -ratio changes from 10/100 to 10/10 the pH value for the BC-maximum in pH range 6.5-7.0 decreases by 0.1 pH units and the BC-minimum in pH range 5.6-5.9 increases by 0.1 pH units.
Due to small number of replicates and high standard deviation the differences are not statistically significant, but the trends can be seen especially if one looks at the results of titrations carried out consecutively (consecutive codenumbers, Table 1.).

The consumption of acid/base when read for the pH range BC minimum-BC maximum from the titration curve (column 9, Table 1.) diminished when the amount of water used in titrating decreased. This is obvious because the pH range also diminished. On the other hand, the consumption calculated per pH-unit (column 10) changed hardly at all. Neither did the comsumption when read for the pH range 5.5-7.0 -the typical range for both the postmortem reaction sequence and technological processes.
Fig 3. and 4. show some titration- and BC-curves from tests in which the effect of salt on the BC was studied and Table 2. shows the results of these tests. Increasing the NaCl concentration caused the maximum to move to lower pH values (0% NaCl, maximum at pH 6.93, 1% NaCl, maximum at pH 6.64, 4% NaCl maximum at pH 6.32) and the minimum around pH 5.5 to disappear (see Fig 4.) With sample/water -ratio 10/100 the change is clear and systematic. With ratio 10/10 no such change in the curves can be detected.

DISCUSSION

Several compounds in meat can act as as proton donors or acceptors and thus affect the BC of meat. The compounds that most affect the buffer capacity in the pH range 5.5-7.0 are:
1) dipeptides carnosine ja anserine, the histidylimidazole residues of which have pK-values 6.9 (carnosine) and 7.0 (anserine) (Honikel et al. 1974) 2) histidylimidazole residues of myofibrillar proteins, pK=6.4-6.8 (Bendall 1973) 3) several phosphatecompounds having pK values between 6.1-7.1 (Honikel et al. 1974).

Together these compounds cause the BC-maximum between pH values 6.5-7.0. The contribution of different compounds to the maximum cannot be identified.
Apparently there are changes in the way in which each compound contributes to buffering capacity as the sample/water -ratio changes. This can be detected as a slight change in the pH-value of the BC-maximum. Conformational changes and aggregation between protein molecules might change the number of titratable charged groups some groups becoming more some less accessible to the solvent (Connell et al. 1964).
Post mortem glycolysis is in fact comparable to a titration of muscle tissue with lactic acid without added water. It is often stated that there is a linear correlation between the pH-value and the amount of lactic acid formed.
Hamm (1977) has, however, pointed out that it is by no means self-evident that the relationship between the linear magnitude of lactate concentration and the logarithmic magnitude of pH is linear. Actually, the pH-lactate curve between pH 7 and 5.5 represents the almost linear medium part of the S-shaped titration curve of muscle tissue. Shape and position of this curve are determined by the buffering capacity of the tissue.
Likewise, the results presented in this paper seem to suggest that the less added water is used when diluting the sample the smaller is the 'hump' in the titration curve and the more linear the correlation between the amount of acid and pH-value in pH range 5.5-7.0.

Salt affects the proteins of meat by 1) increasing their solubility (at pH values higher than the isoelectric point) 2) through binding of Cl^--ions which increases the space between filaments. The increase in WBC caused by increase of NaCl

concentration from 0% to 5% is based on these effects. The same effects probably cause the change in the shape of the BC-curve. The change in the shape of the BC-curve is clear when using sample/water -ratio 10/100 although there is no systematic trend in the net consumption of base.

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TABLE 1. Data from titrations with different sample/water-ratios

1	2	3	4	5	6	7	8	9	10	11
341 F	10/100	6.14	5.73	32.8	6.93	50.9	10.9	52	43	64
277 F	10/100	6.11	5.80	39.1	6.81	58.0	9.5	48	48	70
343 F	10/10	6.01	5.75	44.9	6.78	58.5	7.0	46	45	68
278 F	10/10	5.95	5.90	39.5	6.78	48.5	3.9	40	45	66
276 T	10/100	6.80	5.65	44.2	6.81	63.8	11.4	64	55	83
279 T	10/10	6.76	5.73	48.5	6.65	55.8	3.4	49	53	78
403 G	10/100	5.63	5.65	36.0	6.83	56.0	11.8	57	48	72
421 G	10/100	5.65	5.65	33.5	7.03	52.4	13.0	61	44	66
404 G	10/10	5.60	5.72	44.1	6.73	55.0	5.5	51	51	76
422 G	10/10	5.60	5.84	40.1	6.93	55.6	8.4	53	49	71

TABLE 2. Data from titrations with added salt.

1	2		3	4	5	6	7	8	9	10	11
362 F	10/100	1%	5.94	5.94	47.5	6.64	50.9	1.2	37	53	72
360 F	10/100	2%	5.88	5.99	52.5	6.57	55.5	0.9	31	54	81
367 F	10/100	3%	5.82	6.00	48.4	6.45	49.8	0.3	21	46	74
368 F	10/100	4%	5.80	5.98	53.8	6.32	54.5	0.1	16	47	80
364 F	10/100	5%	5.79	not det.	-	shoulder	-	-	-	-	76
363 F	10/10	1%	5.91	6.04	44.4	6.66	48.3	1.2	28	45	68
361 F	10/10	2%	5.84	6.04	41.8	6.70	48.5	2.2	30	45	69
365 F	10/10	3%	5.80	5.88	44.0	6.61	52.4	3.1	34	47	74
366 F	10/10	5%	5.74	5.94	37.9	6.69	48.0	3.8	33	44	65

Columns:
1. Code (Titrations coded with the same letter were performed using the same sample of meat.)
2. Sample/water (NaCl % Table 2)
3. Initial pH
4. BC-curve, minimum [pH]
5. BC, minimum [mmol H^+/pH/kg]
6. BC-curve, maximum [pH]
7. BC, maximum [mmol H^+/pH/kg]
8. Area A
9. Consumption [mmol H^+/kg] pH-range BC-minimum-maximum
10. Consumption/pH-unit [mmol H^+/kg]
11. Consumption [mmol H^+/kg] pH-range 5.5 -7.0

Figure 1. Titration curves obtained using two different sample/water -ratios.

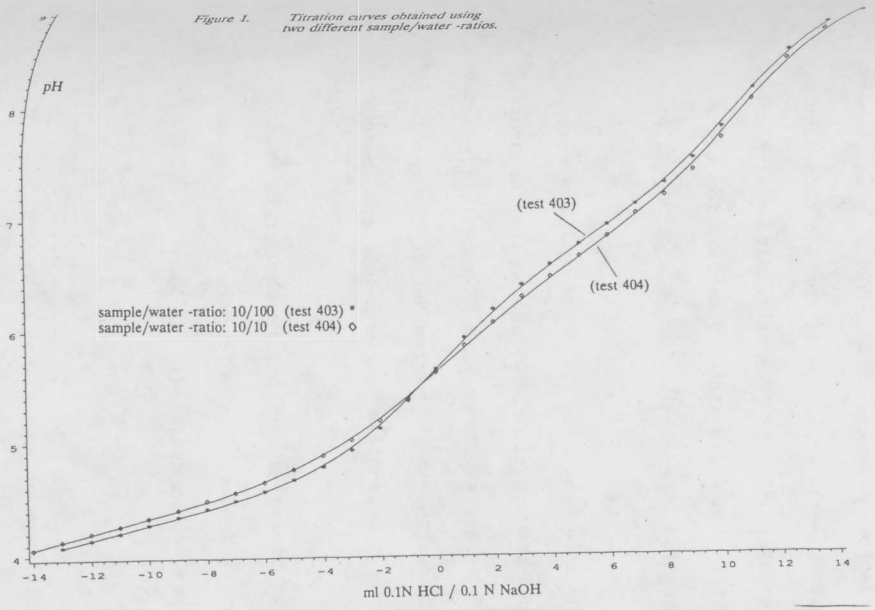

Figure 2. Buffering capacity curves for two different sample/water -ratios.

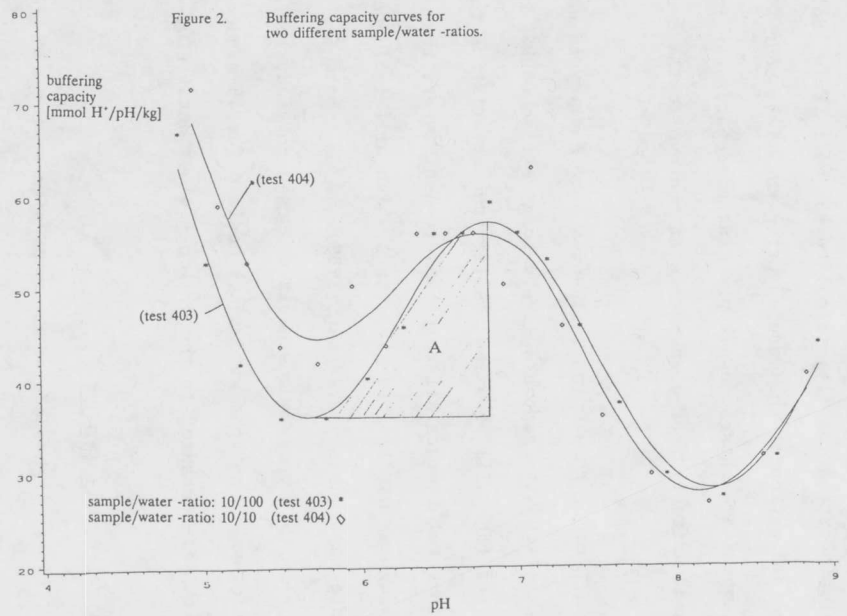

Figure 3. Titration curves from tests with different amounts of added salt.

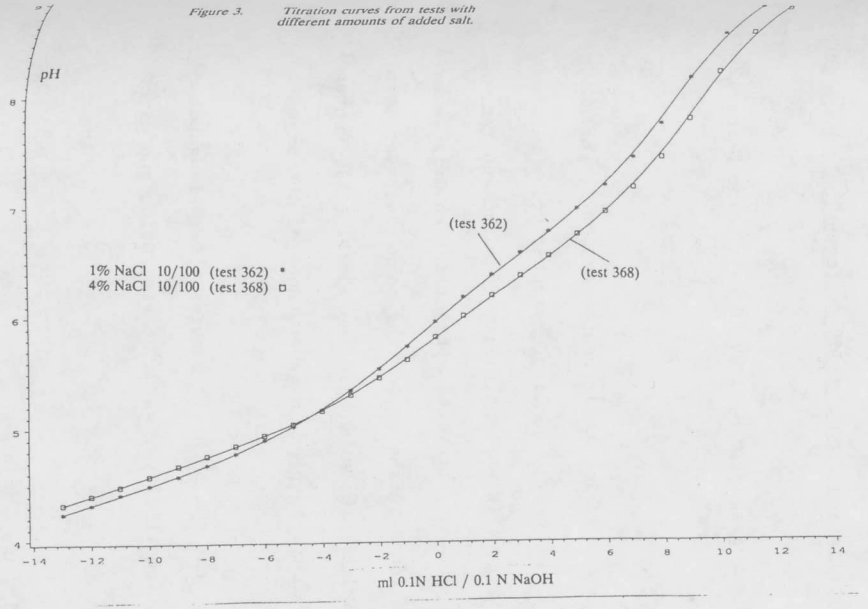

Figure 4. Buffering capacity curves from test with different amounts of added salt.