Eero Puolanne
Department of Food Technology/Meat Technology
University of Helsinki

Riitta Kivikari
Department of Applied Chemistry and Microbiology/Food Chemistry
University of Helsinki

DETERMINATION OF THE BUFFERING CAPACITY OF POSTRIGOR MEAT

Abstract

Since 1938 several studies on buffering capacity of postrigor meat have been presented. As the methods used have varied considerably it is important to know how to compare the results. The method of titration, mainly the amount of dilution used, has a significant effect on the shape of the obtained buffering capacity curve. When a dilute solution is used, the curve has distinct maximum and minimum points. With less dilution, the buffering capacity curve approaches a shape with no distinct minimum and maximum points in pH range 5.5-7.0. However, it seems possible to estimate the buffering capacity of meat from data based on titrations made with different dilutions. A mean value for buffering capacity valid in pH range 5.5-7.0 can be estimated from titrations made with dilution ratios 1:10 and 1:1. The mean buffering capacity values in pH range 5.5-7.0 were for beef *m. longissimus* muscle 51 mmol H^+/(pH*kg), for pork *m. longissimus* 52 mmol H^+/(pH*kg), for beef *m. triceps brachii* 48 mmol H^+/(pH*kg) and for pork *m. triceps brachii* 45 mmol H^+/(pH*kg). For broiler breast and broiler leg-thigh muscles the corresponding values were 58 and 41 mmol H^+/(pH*kg).

Keywords: buffering capacity, meat

1. Introduction

The first analysis of the buffering capacity of meat was published by Bate-Smith in 1938. In that study buffering capacities of several muscles from different species were determined by titrating with dilute acid or base. The roles of proteins, carnosine and orthophosphate in buffering capacity were also discussed. Since then several authors have presented values for buffering capacity (BC) of meat, and many related variables have been studied, including changes in BC during post mortem reaction sequence (Hamm, 1959; Sayre *et al*., 1963), effect of heating (Hamm and Deatherage, 1960), different pig breeds (Sayre *et al*., 1963), different halothane types (Henckel *et al*., 1992), several species of fish, land and marine mammals (Castellini and Somero, 1981), light and dark beef muscles (Rao and Gault, 1989), and normal and PSE pigs (Bendall and Wismer-Pedersen, 1962). Table 1 summarizes the findings of results of these studies, although the varying methods used sometimes make it difficult to compare the results.

Light muscles usually have notably better buffering capacity than dark muscles. This is consistent, because they are comprised primarily of white muscle fibers, which have a high content of glycolytic enzymes. The end product of glycolytic metabolism is lactic acid, which tends to lower the pH. Thus, white fibers need a more effective buffering mechanism than red ones. Buffering prolongs the time of effective fiber activity. The principal difference in the buffering capacity of different types of muscles is due to the fact that white fibers have a higher content of histidine compounds than red ones do (Olsman and Slump 1981).

The same compounds which regulate pH in a living muscle fiber also regulate it in postrigor meat. The compounds that most affect the buffering capacity in the pH range 5.5-7.0 are 1) phosphate compounds having pK_a values between 6.1-7.1; 2) histidylimidazole residues of myofibrillar proteins and 3) the dipeptides carnosine and anserine. Buffering capacity in this pH range caused by compounds other than the dipeptides can be considered constant between samples of varying fiber type compositions and also between species (Sewell *et al.,* 1992). Consequently, variation in buffering capacity can be explained by variations in the amounts of dipeptides.

Table 1. Buffering capacities of meat and myofibrils.

reference	material	method[a]	buffering capacity[b]
Bate-Smith 1938	ox thigh pork psoas	dr: moistened with saline ↔ range: pH 5.5-7.5	range 6-7 ox BC 56 pork BC 57
Hamm and Deatherage 1960	beef LD[c]	dr: 1:1 ↔ range: pH 3-8	pHmax 5.4 BCmax 52
Honikel and Hamm 1974	beef LD	dr: 1:1 adj. to pH 9 range: pH 4-9	pHmin 5.5 BCmin 42 pHmax 6.5 BCmax 57
Sayre *et al*. 1964	pork LD 3 different breeds	dr: 1:10 adj. to pH 4.8 range: pH 4.8-7	range 5.2-6.5 BC 55
Monin and Sellier 1985	pork LD 4 different breeds	dr: 1:10 adj. to pH 4.8 range: pH 4.8-7	range 5.2-6.5 BC 57
Henckel *et al*. 1992	pork LD different genotypes	dr: 1:10 range: pH 6-7	BC 60-64
Castellini and Somero 1981	pork adductor beef temporalis	dr: 1:2 range: pH 6-7 temp: 37°C	pork BC 50 beef BC 52
Rao and Gault 1989	beef LD	dr: 1:9 range: ult.pH - 3	pHmin 5.0 BCmin 49
Bendall and Wismer-Pedersen 1962	pork myofibrils	dr: 1:4 ↔ range: pH 1.8 -11	no minimum no maximum
Connell and Howgate 1964	beef and tuna myofibrils	dr: 2-3% solut. ↔ range: pH 2-12	no minimum no maximum

[a] dr: dilution ratio
adj.: pH adjusted to the pH value indicated before titration
↔ : two separate titrations starting from intrinsic pH of the sample
range (in column 'method'): titrated pH range
[b] The unit for buffering capacity (BC) is mmol H^+/(pH*kg meat) and range indicates the pH range for which the BC value is valid.
For other abbr.: see later
[c] LD = *longissimus*

The present study focused on determining the buffering capacity of some dark and light beef, pork and poultry muscles and the effect of dilution on the buffering capacity curve.

2. Materials and methods

Buffering capacity was determined from the *m. longissimus* (LD) and *m. triceps brachii* (TB) muscles of ten porcine and ten bovine carcasses. The samples were excised from the carcass one day after slaughter, then homogenized with a Moulinette cutter (Moulinex, Italy) and stored frozen until measurement. Samples from different animals were assayed separately and single titrations were carried out.

Breast muscles from four broilers were homogenized to form one sample (B), as were the leg-thigh muscles (L). Titrations were carried out in triplicate.

Each sample was homogenized in a Moulinette cutter (Moulinex, Italy), then two 10 g aliquots were weighed out and separately homogenized with distilled water using a Ultra-Turrax T25 (Janke & Kunkel, Germany). Sample/water ratios used were 10 g sample/100 ml water (1:10), 10 g sample/10 ml water (1:1) and 10 g sample/0 ml water (1:0). The homogenates were titrated using 0.1 N HCl and 0.1 N NaOH. Additions of 1 ml at two minutes intervals were used. The homogenates were stirred during titration. Titrations were carried out at room temperature. Electrodes used were Ross Sure-Flow 8172BN (Orion Research AG, Switzerland) and Ingold LoT406-M6-DXK 'Xerolyt' (Ingold Messtechnik GmbH, Germany)

The titration curve for the pH range 4-9 was obtained by combining data from the two titrations. Buffering capacity was calculated for each increment of acid and base as described by Hill *et al*. (1985).

$$Bc_n = \Delta A / \Delta pH ,$$

where

ΔA = the increment of acid or base,

ΔpH = the corresponding change in pH, and

BC_n = the average buffering capacity for the range between two successive observations.

BC_n values were plotted against the midpoint of each respective pair of pH values. Curves were fitted using the spline smoothing procedure (SAS/GRAPH 'GPLOT' subroutine). The pH and BC values for the minimum and maximum points were read from the BC curve. The consumption of the titrant was read from the titration curve. The accuracy for reading the coordinates of the minimum and maximum points was for the buffering capacity curve: BC values ±0.1 [mmol H^+/(pH*kg meat)] and pH values ±0.01, and for the titration curve: consumption values ± 1 [mmol H^+/(pH*kg meat)].

Averages of pH values, not hydrogen ion concentrations, were used in calculations. The difference between successive pH measurements was usually about 0.1-0.2 units. With this level of difference no substantial error arises, even if the average is calculated using pH values and not hydronium ion concentrations (Hofmann, 1973).

In the tables, the following buffering capacity curve parameters appear:

initpH	the pH of diluted sample and initial pH for titration
pHmin	the pH value of the minimum point of the buffering capacity curve at pH range 5-6
BCmin	the BC value of the minimum point of the buffering capacity curve [mmol H^+/(pH*kg meat)] at pH range 5-6
pHmax	the pH value of the maximum point of the buffering capacity curve at pH range 6.5-7
BCmax	the BC value of the maximum point of the buffering capacity curve [mmol H^+/(pH*kg meat)] at pH range 6.5-7
cons	consumption of titrant measured from the titration curve in pH range 5.5-7.0, [mmol H^+/kg meat].

3. Results and discussion

3.1. Pork, beef and broiler

Tables 2-4 show the parameters of the BC curves for beef, pork and broiler samples. The difference in BC between LD and TB muscles in both pork and beef (Tables 2 and 3) were small, but in accordance with the expectation based on fiber type composition. Differences between corresponding muscles in beef and pork, respectively, were also small.

The differing amounts of carnosine and anserine explain the observed differences in BC. Carnagie *et al*. (1982) give a dipeptide concentration of 25 mmol/kg for LD muscle of pig. The corresponding value for TB muscle is not given, but they give a value of 14 mmol/kg for *m. trapezius*, a muscle resembling *m. triceps brachii* in fiber type composition (Ruusunen, 1994) and anatomical location. Based on these values, a difference of about 6.5 mmol H^+/(pH*kg) in the BCmax values of these muscles could be expected, which is 81% of the observed difference 8 mmol H^+/(pH*kg).

The difference in the buffering capacity maximum value (BCmax) between beef muscles was very small. The BCmax of LD muscle was 3 mmol H^+/(pH*kg) higher than that of the TB muscle. Also in other studies (Bendall *et al*., 1976; Bendall, 1979; Talmant *et al*., 1986; Rao and Gault, 1989), the observed differences in the buffering capacity of beef LD and TB muscles are small. Differences between beef muscles in the content of chemical compounds affecting buffering capacity are so small that no great difference in buffering capacity is to be expected on that basis. Rao and Gault (1989) give a dipeptide concentration of 25 mmol/kg for LD muscle and 20 mmol/kg for TB muscle of beef. Based on these values, a difference of about 2.9 mmol H^+/(pH*kg) in the BCmax values of these muscles could be expected.

Table 2. The means and standard deviations of the parameters of the buffering capacity curves of pork samples. (N=10, different animals)

	pork LD 1:10	pork TB 1:10
pHinit	5.44^{b} ±0.06	5.90^{a} ±0.14
pHmin	5.56^{b} ±0.04	5.64^{a} ±0.04
BCmin	38.9^{a} ±2.2	32.2^{b} ±1.9
pHmax	6.65^{b} ±0.06	6.69^{a} ±0.0
BCmax	65.4^{a} ±3.6	57.4^{b} ±4.0
cons	84^{a} ±5	70^{b} ±4

	pork LD 1:1	pork TB 1:1
pHinit	5.49^{b} ±0.02	5.85^{a} ±0.11
pHmin	5.70^{b} ±0.05	5.85^{a} ±0.05
BCmin	48.9^{a} ±1.8	40.3^{b} ±1.2
pHmax	6.69^{b} ±0.05	6.78^{a} ±0.04
BCmax	57.2^{a} ±2.1	48.8^{b} ±2.0
cons	82^{a} ±3	69^{b} ±3

^{a,b} Means within a row with different superscripts are significantly different ($p<0.05$).

Table 3. The means and standard deviations of the parameters of the buffering capacity curves of beef samples. (N=10, different animals)

	beef LD 1:10	beef TB 1:10
pHinit	5.77 ±0.30	5.84 ±0.32
pHmin	5.56 ±0.06	5.60 ±0.04
BCmin	40.5 ±3.2	37.6 ±3.6
pHmax	6.68 ±0.1	6.70 ±0.09
BCmax	61.3 ±3.8	58.2 ±4.8
cons	80 ±2	75 ±6

	beef LD 1:1	beef TB 1:1
pHinit	5.71 ±0.29	5.78 ±0.30
pHmin	5.83 ±0.13	5.82 ±0.14

BCmin	50.9[a] ±2.9	47.2[b] ±4.0
pHmax	6.66 ±0.12	6.69 ±0.11
BCmax	57.3 ±3.8	53.7 ±4.4
cons	82 ±5	77 ±6

[a,b] Means within a row with different superscripts are significantly different ($p<0.05$).

Table 4. The means and standard deviations of parameters of the buffering capacity curves of broiler breast (B) and leg-thigh (L) muscles. (N=3)

	B 1:10	L 1:10
pHinit	5.84[b] ±0.06	6.58[a] ±0.07
pHmin	5.60 ±0.06	5.61 ±0.01
BCmin	38.3[a] ±0.7	30.4[b] ±0.5
pHmax	6.95 ±0.06	6.92 ±0.04
BCmax	77.8[a] ±6.1	50.8[b] ±4.3
cons	88[a] ±5	63[b] ±4

	B 1:1	L 1:1
pHinit	5.77[b] ±0.03	6.54[a] ±0.04
pHmin	5.79 ±0.03	6.00 ±0.30
BCmin	48.9[a] ±2.2	39.5[b] ±2.6
pHmax	6.92 ±0.05	6.88 ±0.22
BCmax	71.1[a] ±2.8	44.7[b] ±3.3
cons	88[a] ±3	64[b] ±4

[a,b] Means within a row with different superscripts are significantly different ($p<0.05$).

Broiler breast and leg muscles differ greatly in their physiological characteristics. Broiler breast muscle contains almost exclusively white fibers. Papinaho *et al*. (1996) gives the following distributions for broilermuscles: *m. pectoralis* red fibers 0%, intermediate 0% and white fibers 100% and *m. biceps femoris* red fibers 12%, intermediate 0.5% and white fibers 87.5%. For the *m. sartorius* muscle in broiler leg the fiber type distribution is red fibers 25-30%, intermediate 40-50% and white 20-30% (Aberle and Stewart, 1983; Aberle *et al*., 1978).

The lactic acid content of postrigor broiler breast muscle is about 100 mmol/kg and that of leg muscle about 50 mmol/kg. But the effect of lactic acid on buffering capacity in the pH range studied is small, because the pK value of lactic acid does not coincide with pH range studied. The difference in lactic acid concentration only accounts for a difference of less than 1 mmol H^+/(pH*kg) in buffering capacity at pH 6.9.

The large difference in muscle physiology is also apparent in the large difference in dipeptide contents (Plowman and Close, 1988). We observed a difference of approximately 27 mmol H^+/(pH*kg) in BCmax values between leg-thigh and breast muscles with both dilution ratios. On the basis of the dipeptide contents as indicated by Plowman and Close (1988), a difference of approximately 21 mmol H^+/(pH*kg) could be expected. As a result, the difference in dipeptide content of the muscles accounts for about 80% of the difference in BCmax value.

3.2. The effect of dilution on buffering capacity curve

Table 5 shows the parameters of buffering capacity curves obtained using different dilutions. Sample/water ratios used were 10 g sample/100 ml water (1:10) and 10 g sample/10 ml water (1:1). In addition, titrations with no preceding dilution were carried out.

Table 5. The parameters of the buffering capacity curve for a beef sample at different dilution ratios. Values are means ± S.D.(1:0 titration was begun without added water. Titrations were carried out with 0.1 N solutions, such that when the titration was concluded (pH values 4.4 and 7.8), the dilution was approximately 1:1).

	1:10 (N=4)	1:1 (N=4)	1: 0 (N=5)
pHinit	5.67[b] ±0.04	5.60[c] ±0.00	5.73[a] ±0.02
pHmin	5.59[b] ±0.06	5.74[ab] ±0.08	5.82[a] ±0.05
BCmin	33.2[b] ±1.8	43.2[a] ±2.3	46.5[a] ±2.4
pHmax	6.98[a] ±0.09	6.87[ab] ±0.08	6.72[b] ±0.04
BCmax	64.6 ±10.5	60.2 ±5.1	55.2 ±1.5
cons	74 ±5	78 ±6	76 ±1

[a,b,c] Means within a row with different superscripts are significantly different ($p<0.05$).

As a summary of Table 5 the following trends can be seen:

- initial pH — no systematic differences between dilution ratios
- pHmin — lower with greater dilution
- BCmin — smaller with greater dilution
- pHmax — higher with greater dilution
- BCmax — greater with greater dilution
- consumption — no systematic differences.

Titrations with different dilutions revealed the following trends. When less diluted, the 'hump' in the titration curve grew smaller and the titration curve became straighter. Changes in the titration curve were small and difficult to observe, but in the buffering capacity curve, which is the reciprocal of the derivative of the titration curve, changes were clearly visible. Fig 1 shows a typical example, a set of three individual buffering capacity curves of a same sample obtained with different dilutions.

The buffering capacity value at the maximum point on the curve was greater when more water was used, and the buffering capacity value at the minimum point was correspondingly smaller. The buffering capacity (BCmax) at the maximum point on the buffering capacity curves was approximately 5-8 mmol H^+/(pH*kg) greater using a dilution ratio of 1:10 than with a dilution ratio of 1:1. The buffering capacity (BCmin) at the minimum point on the buffering capacity curve was approximately 10 mmol H^+/(pH*kg) smaller using a dilution ratio of 1:10 than with a dilution ratio of 1:1.

The pH values of the minimum and maximum points on the buffering capacity curve also changed as the amount of dilution changed. The pH value at the minimum point on the buffering capacity curve (pHmin) obtained by the dilution ratio 1:10 was approximately 0.15 pH units lower than with the dilution ratio 1:1. The pH value at the maximum point on the buffering capacity curve (pHmax) obtained by the dilution ratio 1:10 was approximately 0.1 pH units higher than with the dilution ratio 1:1.

pK_a values are dependent on ionic strength. The pK_a value of phosphoric acid decreases and the pK_a value of imidazole increases when ionic strength increases (Freifelder, 1985). This dependance of activity coefficients and ionic strength is valid only in dilute solutions and should not be applied as such to a concentrated solution or to solutions containing macromolecules.

However, the results obtained in this study seem to indicate a similar change in pK_a values. When the sample was less diluted, the maximum in the buffering capacity curve broadened, BCmax droped, and buffering capacity in the range pHmin-pHmax increased. These changes can be explained by assuming that the difference in pK_a values of the compounds forming the buffering capacity maximum in the pH range 6.5-7 increased.

As a buffering capacity curve has a specific shape based on its mathematical equation, spline smoothing is not the best way to analyse this data, because it does not fit a curve of this specific shape and thus fails to reveal the overlapping of peaks. More information could be gained from the titration data if it were analysed by a peak-fitting program (de Levie *et al.,* 1998).

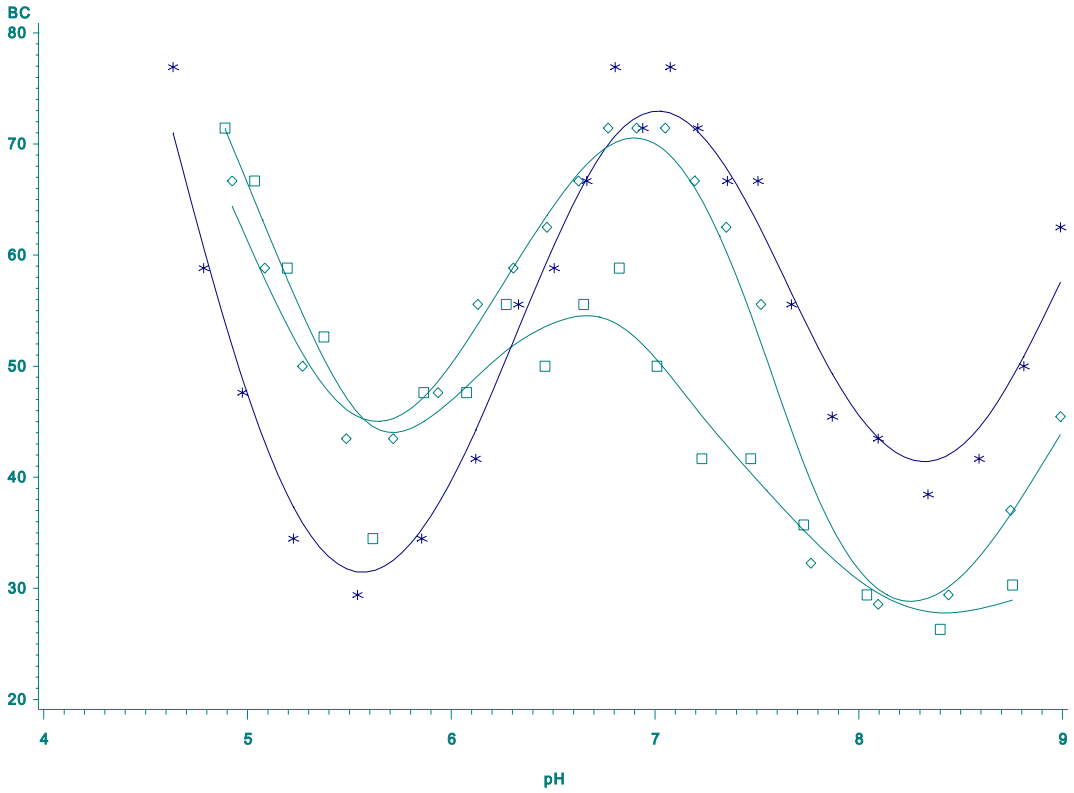

Fig 1. Buffering capacity curves obtained with different dilutions.

Sample-water ratios 1:10 (*);

1:1 (◊);

1:0 (□);

sample: beef,

horizontal axis: pH

vertical axis: buffering capacity, mmol H^+/(pH*kg).

3.3. The average buffering capacity values

Dilution makes it easier to carry out titrations of meat samples, but when using results obtained in this way, one needs to be sure that they are applicable to the situation in question. The buffering capacity of meat as it is, with no dilution, is often of interest. For example, the fall in pH during post mortem reaction sequence is a function of both the amount of lactic acid produced and of buffering capacity. The ultimate pH of a muscle is a function of these two independent factors.

It seems possible to estimate the buffering capacity of the original meat sample from the buffering capacity curves of diluted samples. When a dilution ratio of 1:10 was used for determining the buffering capacity curve, the curve obtained was pronounced curving. But when the sample was not diluted the buffering capacity remained quite constant in the pH range 5.5-7.0. When a single value is needed, as an estimate of buffering capacity, a suitable estimate in the pH range 5.5-7.0 is the mean of the BCmax and BCmin values determined using dilution ratio 1:10. Calculated in this way the buffering capacities of the different sample types studied (beef LD, beef TB, pork LD, pork TB, broiler breast, broiler leg-thigh) do not differ very dramatically from each other (Table 6). These values are valid in the pH range 5.5-7.0. In the pH range < 5.5 the buffering capacity strongly increases, e.g. if buffering capacity at pH 5.5 is 50 mmol H^+/(pH*kg), at pH 5.0 it is approximately 70 mmol H^+/(pH*kg) and at pH 4.5 80-90 mmol H^+/(pH*kg).

Table 6. Mean buffering capacities in pH range 5.5-7.0.

	BC mmol H^+/(pH*kg)
beef LD	51
beef TB	48
pork LD	52
pork TB	45
broiler breast	58
broiler leg-thigh	41

4. Conclusions

1. The method of titration, mainly the amount of dilution used, greatly affects the shape of the obtained buffering capacity curve. When a more dilute suspension is used, the curve has more distinct maximum and minimum points, and the difference of BCmax and BCmin is greater than when a more concentrated suspension is used. All in all, it appears that the less added water is used when titrating, the more linear the titration curve becomes. As a consequence, the buffering capacity curve approaches a shape with no distinct maximum or minimum points. When no added water is used, the buffering capacity seems to be quite constant in the pH range 5.8-6.5.

2. Calculated as a mean of BCmax and BCmin, the buffering capacity values in pH range 5.5-7.0 were for beef *m. longissimus* muscle 51 mmol H^+/(pH*kg), for pork *m. longissimus* 52 mmol H^+/(pH*kg), for beef *m. triceps brachii* 48 mmol H^+/(pH*kg) and for pork *m. triceps brachii* 45 mmol H^+/(pH*kg). For broiler breast and broiler leg-thigh muscles the corresponding values were 58 and 41 mmol H^+/(pH*kg). Differences in buffering capacity between the muscles can be explained by the variation in dipeptide content.

3. Consequently, it does not seem to be possible to relate the variation in technological properties of postrigor meat (e.g. water binding capacity) to variation in buffering capacity. Differences in buffering capacities between different kinds of samples are too small to give a solid basis for expecting differences in their pH patterns.

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