

Die pH-bestimmenden Substanzen in Rohwurst

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Im allgemeinen wird angenommen, dass pH in Rohwurst hauptsächlich durch die Wechselwirkung zwischen Milchsäure und Eiweissstoffe bestimmt wird. In einer Reihe von 6 Rohwurstreifungen wurde ein durchschnittlicher Fall von $0,88 \pm 0,14$ pH- Einheiten für eine Produktion von $5,1 \pm 0,9$ g Milchsäure/100 g Eiweissstoffe beobachtet. Dies ist erheblich höher als die Milchsäurequantität, nötig zur Herabsetzung des pH bei titrierung einer Wurstmenge. Eine statistische Wertbestimmung der analytischen Daten von 30 kommerziellen Rohwurstmarken hat gezeigt, dass nur 32% der Variation durch die Variation in Milchsäurekonzentration (mmoles/100 g brutto Eiweissstoffe) bestimmt war. Jedoch, Konzentrationsvariationen in Milchsäure (x_3) und Ammoniak (x_4) rechtfertigen 67% der pH-Variation (y) wie folgt

$$y = 5,04 - 0,0069 x_3 + 0,0331 x_4 \quad (100 R^2 = 67,4)$$

$$\text{wenn } X = \log \left(\frac{x_4}{x_3} 100 \right)$$

$$y = 3,52 + 1,1610 X \quad (100 R^2 = 68,5)$$

Die Inkorporation des Azetats in die Equation veränderte nicht im mindesten den Bestimmungskoeffizient. Es konnte entschlossen werden, dass pH in Rohwurst, insbesondere durch Konzentration des Ammoniaks und Milchsäures und durch die Pufferungsvermögen des Proteins, nach folgendem Modell bestimmt worden ist

$$\text{pH} = \text{pK} + \log \frac{\text{NH}_3}{\text{Milchsäure}}$$

Der niedrige Bestimmungskoeffizient ist möglicherweise teils mit Variationen in Ammoniak- und Milchsäuredissoziationen verbunden. Allerdings ist eine bedeutend negative Korrelation zwischen dem Wassergehalt und dem pH kalkuliert.

Compounds determining pH in dry sausage

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It is generally accepted that the pH of dry sausage is predominantly determined by the interaction of lactic acid and protein. In a series of 6 dry sausage ripenings, a mean decline of 0.88 ± 0.14 pH units was observed for a production of 5.1 ± 0.9 g of lactic acid/100 g of protein. This is much more than the amount of lactic acid needed to titrate a sausage mixture down over the same pH range. Statistical evaluation of analytical data from 30 commercial brands of dry sausage showed that only 32% of the variation in pH was determined by the variation in lactate concentration (mmoles/100 g of crude protein). Variations in both lactate (x_3) and ammonia (x_4) concentration (mmoles/100 g crude protein) however accounted for 67% of the variation in pH (y) following

$$y = 5.04 - 0.0069 x_3 + 0.0331 x_4 \quad (100 R^2 = 67.4)$$

$$\text{If } X = \log \left(\frac{x_4}{x_3} 100 \right), \text{ then } y = 3.52 + 1.1610 X \quad (100 R^2 = 68.5)$$

Incorporation of acetate into the equation did not change the determination coefficient.

It is concluded that pH in dry sausage is mainly determined by the concentration of ammonia and lactic acid and the buffering capacity of proteins following the model $\text{pH} = \text{pK} + \log \frac{[\text{NH}_3]}{[\text{lactic acid}]}$

The low determination coefficient is possibly and partly related to variations in ammonia and lactic acid dissociation. Indeed a significant negative correlation was calculated between water content and pH.

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Les substances déterminant le pH dans le saucisson sec.

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On accepte généralement que le pH dans de saucisson sec est déterminé principalement par l'interaction entre l'acide lactique et les protéines. Dans une série de 6 productions de saucisson secs, une chute moyenne de $0,88 \pm 0,14$ unités de pH correspondant avec la production de $5,1 \pm 0,9$ g de l'acide lactique/100 g de protéines bruts. Cette quantité est beaucoup plus grande que la quantité d'acide lactique nécessaire pour atteindre la même chute de pH dans une pâte fraîche de saucisson par titration.

Traitement statistique de données analytiques obtenus pour 30 marques de saucissons montrait que 32% de la variation du pH était déterminé par la variation de la concentration en acide lactique (mmoles/100 g de protéines bruts). Des variations dans la concentration de l'acide lactique (x_3) et ammoniac (x_4) néanmoins justifie 67% de la variation du pH (y) selon

$$y = 5,04 - 0,0069 x_3 = 0,0331 x_4 \quad (100 R^2 = 67,4)$$

$$\text{Si } X = \log \left(\frac{x_4}{x_3} 100 \right)$$

$$y = 3,52 + 1,1610 X \quad (100 R^2 = 68,5)$$

L'incorporation de l'acetate dans l'équation ne change pas le coefficient de détermination.

Il est conclu que le pH dans le saucisson sec est déterminé par les concentrations en ammoniac et acide lactique suivant le modèle

$$\text{pH} = \text{pK} + \log \frac{[\text{NH}_3]}{[\text{acide lactique}]}$$

Le coefficient de détermination bas est peut-être partiellement lié à la variation dans le degré de dissociation de l'ammoniac et de l'acide lactique, déterminé par la variation dans l'activité d'eau. En effet, une corrélation négative et significative a été observée entre l'humidité et le pH.

Compounds determining pH in dry sausage

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Introduction

According to Andersen and Ten Cate (1965) the fall in pH during ripening of fermented or dry sausage is predominantly determined by the interaction of lactic acid, formed from carbohydrate, with the buffering meat proteins. From their data on titration of fresh sausage mixtures with lactic acid, it can be calculated that a drop of 1 pH unit in sausage requires 2.67 g of lactic acid per 100 g of sausage crude protein. In a series of 16 sausage ripenings in our laboratory, an average pH drop of 0.93 ± 0.03 units (from 5.77 ± 0.03 to 4.84 ± 0.03) (mean value \pm S.E.) was observed, accompanied by the production of 4.18 ± 0.31 g of lactic acid per 100 g crude protein (mean value \pm S.E.) (Demeyer and Vandekerckhove, in preparation). This is significantly higher than the value calculated from the data presented by Andersen and Ten Cate (1965), indicating that in sausage ripening, compounds are formed buffering the lactic acid formed that increase pH. It is well known that in the later stages of the ripening period, later than the second week after filling, pH may increase by 0.2 - 0.3 units (De Ketelaere et al, 1974) (Wardlaw et al, 1973) (List and Klettner, 1978). During this period there is no correlation between lactic acid production and pH (List and Klettner, 1978) and it was suggested that the pH increase is related to proteolytic activity with formation of peptides, amino acids and ammonia (Cantoni et al, 1973) (Dierick et al, 1974). In this paper we have investigated the relative importance of various compounds, possibly affecting pH in dry sausage, using multiple linear regression analysis.

Materials and Methods

In a first analysis, the concentrations of water, crude protein, lactate, acetate, ammonia, α -NH₂-N and NaCl were related to pH, using data obtained in the analysis of 30 different sausage brands, purchased in local shops. Analytical data used, as well as methods of analysis were reported earlier (Vandekerckhove and Demeyer, 1975). In a second analysis the number of brands was increased to 56, but analysis was limited to data on pH, water, lactate, ammonia and crude protein content. The analytical data for the 26 additional brands are shown in table 1.

Results and Discussion

In the first analysis, sample linear regression analysis was applied between $Y = \text{pH}$ and respectively

$$\begin{aligned} X_1 &= \log [\text{H}_2\text{O}] && ([\text{H}_2\text{O}] = \text{g}/100 \text{ g of crude protein}) \\ X_2 &= \log [\text{lactate}] && ([\text{lactate}] = \text{mmoles}/100 \text{ g of crude protein}) \\ X_3 &= \log [\text{acetate}] && ([\text{acetate}] = \text{mmoles}/100 \text{ g of crude protein}) \\ X_4 &= \log [\text{ammonia}] && ([\text{ammonia}] = \text{mmoles}/100 \text{ g of crude protein}) \\ X_5 &= \log [\alpha\text{-NH}_2\text{-N}] && ([\alpha\text{-NH}_2\text{-N}] = \text{mmoles}/100 \text{ g of crude protein}) \\ X_6 &= \log [\text{NaCl}] && ([\text{NaCl}] = \text{g}/100 \text{ g of crude protein}) \end{aligned}$$

Logarithms of concentrations were used as $Y = \text{pH} = -\log [\text{H}^+]$. All concentrations were expressed per 100 g of crude protein, in line with the model of watersoluble compounds (X_2, \dots, X_6) determining pH by interaction with protein. Table 2 shows the equations and determination coefficients obtained.

It is clear that no factor alone explains more than 50% of the variation in pH, but five significant correlations were found involving, in order of decreasing significance, X_4 (log ammonia concentration), X_2 (log lactate concentration), X_6 (log NaCl concentration), X_1 (log H₂O concentration) and X_5 (log α -NH₂-N concentration). Multiple linear regression analysis, shows that X_4, X_2, X_6, X_1 and X_5 together explain 75% of the variation in Y. As there are highly significant correlations ($p < 0.01$) between X_4 and X_5 ($r = 0.66$), X_4 and X_1 ($r = -0.58$) and X_1 and X_6 ($r = 0.55$), multiple linear regression analysis was carried out using X_4 and X_2 only. It was found that X_2 and X_4 explain 66% of the variation in pH following $Y = 3.935 - 1.274 X_2 + 0.931 X_4$ with $100 R^2 = 65.8$

This relationship is not surprising as it reflects the classic equation for pH in water solutions

$$\text{pH} = \text{pK} + \log \frac{[\text{base}]}{[\text{acid}]}$$

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Table 1. Analytical data on dry sausages used in regression analysis

Sample no	pH	Lactate ^a	Ammonia ^a	Crude protein ^b	Dry matter (%)
First series	4.99 ^c	79.3	15.2	28.3	66.7
(n = 30)	+ 0.05	+ 3.8	+ 1.0	+ 0.5	+ 1.1
Second series					
(n = 26)					
1	5.30	81.00	28.22	18.89	85.07
2	5.10	83.49	14.17	21.80	82.33
3	5.05	86.57	13.08	20.33	81.46
4	5.20	54.70	15.88	26.51	75.75
5	5.20	98.20	16.33	17.21	85.22
6	5.35	62.45	9.96	23.70	72.92
7	4.60	118.18	15.27	22.00	62.72
8	4.55	107.34	16.95	24.78	62.70
9	4.70	82.32	14.73	25.39	61.75
10	4.60	71.98	13.93	32.37	75.06
11	5.20	66.17	23.99	22.97	80.93
12	5.13	83.47	21.96	31.15	59.90
13	5.30	75.29	19.82	29.22	71.47
14	5.28	76.66	21.17	28.96	74.65
15	4.73	89.57	16.42	37.40	58.19
16	4.60	102.80	13.49	27.14	61.42
17	4.66	127.08	16.32	20.46	63.78
18	4.60	126.30	13.39	25.02	62.06
19	4.90	90.43	11.21	30.52	59.92
20	5.05	54.40	11.92	25.92	72.44
21	4.62	126.42	11.63	23.73	67.68
22	5.53	65.90	14.84	17.45	85.10
23	5.54	34.73	32.36	19.87	82.50
24	4.90	98.62	10.53	20.99	67.13
25	5.92	48.25	9.74	32.54	79.10
26	6.63	33.45	7.49	38.56	76.84
Mean value	5.09	82.5	15.9	25.6	71.8
+ S.E.	+0.09	+ 5.2	+ 1.1	+ 5.7	+ 1.8

a : mmoles/100 g crude protein
 b : % of dry matter
 c : mean values + S.E. from table 1 (Demeyer and Vandekerckhove 1975)

Table 2. Simple linear regression analysis with data from table 1

Linear regression	Correlation coefficient	Determination coefficient (100 R ²)	F-value
Y = 7.531 - 1.131 X ₁	- 0.44	19.5	6.79 ^x
Y = 6.168 - 1.399 X ₂	- 0.55	30.6	12.35 ^{xx}
Y = 5.386 - 0.154 X ₃	- 0.03	1.0	<1
Y = 2.334 + 1.158 X ₄	0.66	43.8	21.85 ^{xx}
Y = 2.695 + 0.856 X ₅	0.39	15.1	4.98 ^x
Y = 8.053 - 2.389 X ₆	- 0.45	20.2	6.83 ^x

x : significant at p ≤ 0.05
 xx : significant at p ≤ 0.01

If $X = \log \left(\frac{X_4}{X_2} \cdot 100 \right)$ then it can be calculated that $Y = 3.52 + 1.161 X$ (I) with $100 R^2 = 68.5$

From these results it may be concluded that pH in dry sausage is predominantly determined by both lactate and ammonia concentration. In a second analysis, the original sample of 30 brands was enlarged to 56 brands, by addition of the data presented in table 1. From this table it can be seen that the second batch of 26 samples (collected in 1976) showed a higher D.M. content and a lower protein content than the first sample (collected in 1974). Also sausage size was much more variable in the second sample. When samples were pooled linear regression analysis was carried out between

$$Y = \text{pH} \quad \text{and} \quad X = \log \frac{[\text{ammonia}]}{[\text{lactate}]} \cdot 100 \quad \text{giving} \quad Y = 3.56 + 1.16 X \quad \text{with} \quad 100 R^2 = 39.$$

The similarity with equation (I) is satisfactory but it is clear that much less of the variation in pH is accounted for in this pooled sample.

In the pooled sample there was again a significant correlation between $\log [H_2O]$ and pH ($r = -0.616$) but there was no correlation between $\log [H_2O]$ and $\log [NH_3]$ or $\log [\text{lactate}]$. Therefore multiple regression analysis was applied using

$$Y = \text{pH}$$

$$X_1 = \log \left[\frac{\text{H}_2\text{O}}{\text{NH}_3} \right]$$

$$X_2 = \log \left[\frac{\text{NH}_3}{\text{lactate}} \right]$$

giving $Y = 4.317 - 1.152 X_1 + 0.751 X_2$ (II) with $100 R^2 = 54$

Again this equation may be compared with

$$\text{pH} = \text{pK} + \log \left[\frac{\text{base}}{\text{acid}} \right] \quad \text{(III)}$$

where $\text{pK} = -\log K$.

It is reasonable to assume that the theoretical value of pK decreases with increasing dissociation of electrolytes, or with increasing water content. Equation (II) may thus be seen as equation (III) where pK is corrected for electrolyte dissociation (or ionic strength). It is clear in conclusion that dry sausage pH is mainly determined by lactate, ammonia and water content, interacting with proteins. Lactate decreases pH whereas ammonia increases pH. The dissociation of both acid and base is lowered by decreased water content (longer drying periods) resulting in increased pH values.

Fig. 1 shows the relationship expressed by equation (II) and it is clear that even taking into account these three variables a large part of the variation remains to be explained. No doubt, considerable experimental error in analysis is involved here.

References

- Andersen G. and L. Ten Cate. *Fleischw.* 45, 599, 1965
 Cantoni C., M.A. Bianchi and G. Beretta. *Ind. Alim.* 12, 87, 1973
 De Ketelaere A., D. Demeyer, P. Vandekerckhove and I? Vervaeke. *J. Food Sci.*, 39, 297, 1974
 Dierick N., P. Vandekerckhove and D. Demeyer. *J. Food Sci.*, 39, 301, 1974
 List D. and P.G. Klettner. *Fleischw.* 58, 136, 1978
 Vandekerckhove P. and D. Demeyer. *Fleischw.* 55, 680, 1975
 Wardlaw F.B., G.C. Skelley, M.G. Johnson and J.C. Acton. *J. Food Sci.*, 38, 837, 1973

$$Y = 4.317 - 1.152 X_1 + 0.751 X_2$$

