Comparison of two physical principles for determining the pH in meat

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Abstract

pH is one of the most important and most frequently measured physiochemical parameters in the meat industry, particularly with regard to shelf life and meat quality. A combination ion selective electrode (ISE) is used most often for this purpose. Ion-sensitive field-effect transistor technology (ISFET) is used in a newer type of pH electrode. This work uses the measurement of pork, poultry and fish to compare various parameters of pH determination using the two types of electrode. Differences were observed in the measured values of pH, the electrode stabilisation time and other parameters relating to practical aspects of their use. The ISFET electrode proved to be more stable with a significantly shorter stabilisation time and consistently higher measurement accuracy (lower variability) than the glass electrode. By excluding easily breakable materials, ISFET electrodes also make it possible to take measurements in solid materials without the risk of breakage and to perform sterilisation.

Food safety, glass electrode, MOSFET

Introduction

The pH is one of the most important and most frequently measured physiochemical parameters in the meat industry, particularly in regard to shelf life (Steinhauser et al. 2000) and meat quality (Knox et al. 2008; Kameník et al. 2014). A glass combination ion selective electrode (ISE) is currently used most often for measuring pH in the food industry. The standard pH meter is a voltmeter that converts potential into the pH value. At 20 °C, a difference of ± 1 pH represents a difference in potential of ± 58 mV, and at 25 °C, a difference in potential of ± 59 mV. A difference in temperature of ± 5 °C represents a difference in pH of ± 0.01. If the instrument we are using does not have automatic temperature compensation, we can expect a maximum error of 0.06 pH when determining the pH in warm (35 °C) and chilled (5 °C) halves of meat. The impedance of the pH-sensitive membrane is relatively high in a glass electrode, for which reason a large part of the surface of the glass membrane must be in contact with the sample to achieve reliable results. The physical properties of the material also limit the possible shape of a glass electrode. There is, of course, also the risk of breakage with glass, with consequent contamination of the food with splinters (Číhalík et al.1975).

An ISFET (ion-sensitive field-effect transistor) electrode is an entirely safe alternative in this regard. The first experiments using this technology were performed in 1970. It was developed on the basis of the MOSFET (metal-oxide silicon field-effect transistor) method. The basic principle of both MOSFET and ISFET technology is the control of a current between two semi-conductor electrodes. Two channels with conductivity N are created at a suitable distance from each other in the basic semi-conductor material, usually with conductivity P. One conductive channel is an electrode supplying electrons (known as S or "Source"); the second channel is an electrode receiving electrons (known as D or "Drain").

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An insulating layer of silicon oxide is created on the surface of the transistor. A conductive layer or gate (known as G or "Gate") is located above the insulation between the conductive channels. Supplying an electric potential to gate G results in a current beginning to flow between electrodes S and D. The extraordinarily high input resistance of the gate means that no great power input is required to control this current. In ISFET sensors, gate G is replaced by an ion-selective layer. This layer is in direct contact with the sample in which we are measuring the pH. It is impervious to ions in the sample. It merely creates the opposite response to the activity of the H^+ hydrogen ions, i.e. charging and discharging the surface in dependence on the pH value. The other electrode is part of a loop closing the electric circuit. A reference electrode is used for this purpose, as the precision and stability of pH measurement depends on a constant reference voltage, independent of the concentration of free hydrogen ions. Ions or other charged particles need not pass through the sensitive membrane that controls the voltage exclusively on the basis of electrostatic effects. To achieve the ion selectivity of the electrode we tested, the company Sentron uses aluminium oxides for the "Gate" electrode in its pH sensors. This produces the best results as an H^+ selective sensor. If the pH of the solution changes, the potential of the "Gate" electrode, and thereby that of the "Drain" electrode, also changes. At the same time, electronic feedback causes the charging of the reference electrode in such a way that the current on the "Drain" electrode is maintained as constant. The chemical process involved in the creation of a charge potential is described by the famous Nernst equation (Kalous 1975), similarly to the membranes of the glass electrodes mentioned above.

In contrast to glass electrodes, in which the pH-sensitive bulb must be filled with a buffer, ISFET semi-conductor technology offers a stable sensor with extremely rapid response and low impedance. An in-built microchip replaces the membrane of the glass electrode; it is housed in plastic in such a way that only its surface inlet comes into contact with the measured material – the meat. Sensors with ISFET technology contain a number of patented layers fitted selectively to a silicon chip. The upper layer shows an affinity only for hydrogen ions. The hydrogen ions present on the surface of the sensor or in the vicinity of the surface of the sensor cause a change in the electric signal which is detected and measured as the pH. This is also the basis of the technology of non-invasive contact probes. The probe also features ISFET technology sensitive to pH, a reference electrode and a temperature sensor in a robust plastic housing. The small dimensions of the ISFET system also allow for various kinds of probe without the need for modification, including minimisation to a probe size of 3 mm (Schaepman 2005).

Materials and Methods

The comparative determination of pH was performed by two different instruments with different electrodes. The first was a traditional Orion 250A pH meter (USA) with a glass Ross needle-probe electrode from Monokrystaly (Czech Republic). Calibration was performed on three buffers. The second instrument was a Sentron Argus pH meter with an attached ISFET needle-probe electrode of a total length of 223 mm, with possible immersion of 52 mm and calibration on two buffers.

Pork leg meat, chicken meat – skinned hind quarter, and fish meat (the European hake *Merluccius merluccius)* at a temperature of 6 °C were studied.

Ten measurements were taken with each probe in all cases on each sample of meat. The stabilisation time, which was signalled by the Orion pH meter with an acoustic signal, was subtracted. The time at which the pH became stabilised was recorded for the Sentron instrument. Basic statistical characteristics were calculated in the program Microsoft Excel, and a Student T-test with unequal variance for a level of significance of $\alpha = 0.05$ used for comparison of the results produced by the two electrodes

Results and Discussion

The ISFET electrode was seen to be more stable with a significantly shorter stabilisation time and consistently greater precision (lower variability) of measurement than the standard glass electrode which is characterised by shorter lifespan and was shown to be less suitable for measurements in large series due to the lengthening stabilisation time. A statistically significant difference in the stabilisation time of the two electrodes, and also between average measured values of pH in the majority of cases, was found in all the tested samples (Table 1). Rapid and large changes in pH and temperature have a smaller effect on measurement error in the case of the ISFET electrode thanks to the extremely rapid response of the sensor to any change in the chemical properties of the sample. The maximum stabilisation time (MEAN+SD) in the samples of meat we studied was up to 8 seconds for pork, up to 12 seconds for chicken, and up to 10 seconds for fish meat. The stabilisation time for the glass electrode is significantly longer in all cases (Table 1).

Table 1. Determination of pH and stabilisation time for pork, chicken and fish meat in dependence on the instrument and electrode used (arithmetic mean ± standard deviation)

Pork		Orion 250A	Sentron Argus
pH	Average ± SD	5.36 ± 0.05	5.40 ± 0.03
	V [%]	0.99	0.66
T-test	t	1.857	
	$t_{(18)}$ (α = 0.05)	2.131	
Stabilisation time [s]	Average ± SD	26 ± 11	6 ± 1
	V [%]	41.44	22.54
t-test	t	5.442	
	$t_{(18)}$ (α = 0.05)	2.262	
Chicken		Orion 250A	Sentron Argus
pH	Average ± SD	6.84 ± 0.08	6.59 ± 0.06
	V [%]	1.18	0.83
T-test	t	7.740	
	$t_{(18)}$ (α = 0.05)	2.120	
Stabilisation time [s]	Average ± SD	35 ± 11	9 ± 2
	V [%]	31.13	26.94
T-test	t	7.027	
	$t_{(18)}$ (α = 0.05)	2.228	
Fish		Orion 250A	Sentron Argus
pH	Average ± SD	7.06 ± 0.05	6.98 ± 0.05
	V [%]	0.64	0.66
T-test	t	3.931	
	$t_{(18)}$ (α = 0.05)	2.101	
Stabilisation time [s]	Average ± SD	35 ± 11	8 ± 2
	V [%]	32.29	22.50
T-test	t	7.060	
	$t_{(18)}$ (α = 0.05)	2.262	

PHP+ – Purasal HI Pure P Plus; PA – PuraQ® Arome NA4
V – variation coefficient [%]; α – level of significance; SD – standard deviation; t- calculated value of T-test; $t_{(18)}$ (α = 0.05) - critical value of T-test with unequal variance

An important property of the ISFET probe is the low measurement error which is less than 0.01 (at 25 °C) across a pH range of 1 to 13. The error is smaller than in glass electrodes for pH values lower than 1 and higher than 13.

The ISE must be conditioned before potentiometric measurement to obtain reproducible results. This is generally performed by immersing the electrode in a solution whose composition is most like the composition of calibration solutions, particularly so far as pH, ion strength and the presence of interferents is concerned. The simplest form of conditioning is the immersion of electrodes in an agitated buffer solution (Koryta and Štulík 1984). The company Monokrystaly s.r.o. states that drying-out does not matter if the sensitive membrane of the ISE electrode is made of high-quality glass. This cannot, however, be recommended in practice. A typical feature of the majority of pH-sensitive membranes is the destruction of the surface layer as a result of drying out, with the electrode then either not measuring at all or measuring extremely poorly with a long stabilisation time. An ISE electrode must, then, always be stored in a solution and used on a regular basis to ensure its reliable functioning.

Storage represents another difference between the two types of electrode. While glass electrodes must be stored in an aqueous solution (distilled water) or a buffer and kept hydrated for at least 24 hours before a planned measurement is taken (Dvořák et al. 1988), this is not necessary in the case of ISFET electrodes which can be stored dry, covered merely by their protective cap, with no risk of depreciation. Calibration before measurement or after between 30 and 50 measurements remains, however, essential for both types. Fig. 1 depicts deviations from the buffer of a pH value = 7, both positive, i.e. above 7, or negative, i.e. below 7. We can see evidently greater deviations in meat with a glass combined electrode from the company Monokrystaly attached to an Orion instrument. In absolute values, these deviations fall within a range from 0.09 to 0.14 pH. No dependence between the measured pH values and a positive or negative deviation from the buffer has been demonstrated. In Sentron electrodes, rapid and large changes in pH and temperature have a smaller effect on measurement error thanks to the extremely rapid response of the sensor to any change in the chemical properties of the sample. Their drawback is their higher purchase price.

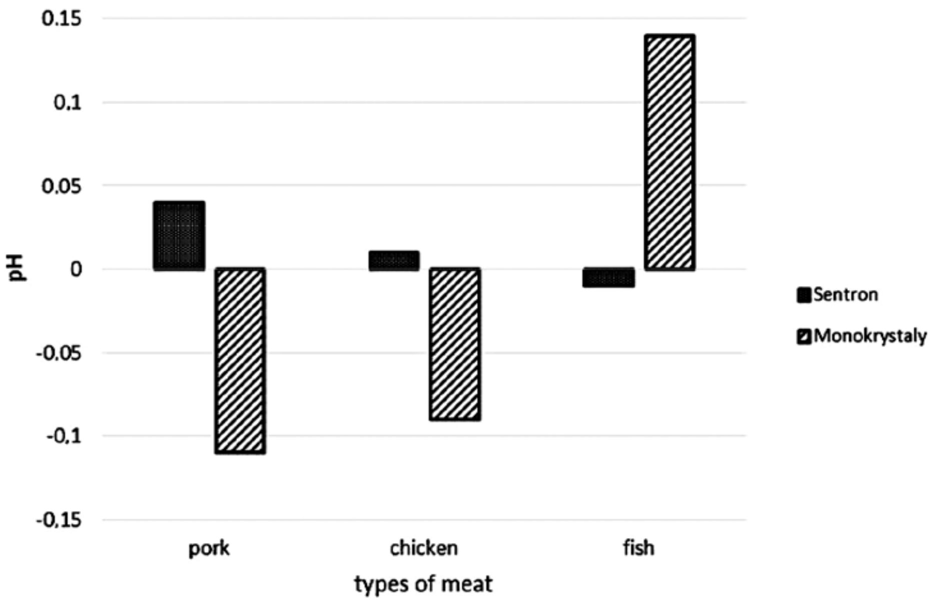

Fig. 1. Deviations from buffer following completion of measurement of various types of meat

The ISFET electrode is made of stainless steel. It contains an extremely robust sensor suitable for use in extremely tough materials that ensures reliable long-term operation. A brush and detergent are used for cleaning the sensor, and all that needs to be done after use is to put the protective sensor cap on. All these instruments are fitted with an integrated temperature sensor for effective temperature compensation. Membrane-free probes with no easily breakable materials allow for wide-ranging and convenient use, particularly under demanding operating conditions in the meat industry. Mobile instruments are equipped with a large back-lit LCD display and the measurement of pH, the electric potential in mV, temperature and conductivity. Laboratory instruments can be charged by a battery or from the electric network.

The ISFET electrode has shown itself to be more stable with a significantly shorter response time and consistently greater measurement precision than the standard glass electrode which is characterised by a shorter lifespan and has been shown to be less suitable for determining pH in large series in view of its long stabilisation time. A statistically significant difference was found in all the tested samples in the stabilisation time of the two types of electrode and, in the majority of cases, between the average pH values measured.

Another advantage of the ISFET electrode is that it can be sterilised. The only disadvantage of the specific transistor needle-probe electrode we tested was the larger diameter of the puncture marks in comparison with the thinnest glass needle electrodes.

Conclusions

The excellent mechanical properties of the ISFET pH probe predestine it for use in various branches of the food industry. It conforms to the hygiene requirements of the legal standards and FDA regulations.

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