MEAT FRESHNESS ASSESSMENT USING A BIOSENSOR ARRAY

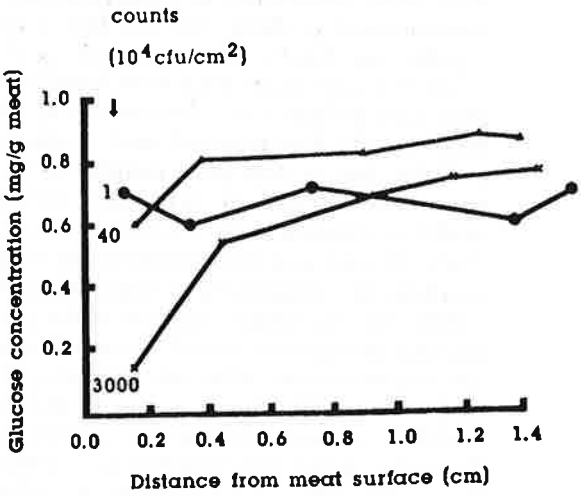
E. Kress-Rogers, J.E. Sollars, E.J. D'Costa[1], J.M. Wood, A.P.F. Turner[1], M.K. Knight, Leatherhead Food Research Association, Leatherhead, KT22 7RY, UK, [1]Cranfield Institute of Technology, Cranfield, Bedford, MK43 OAL, UK

SUMMARY

A sensor array has been developed for the assessment of meat freshness by a measurement of the glucose profile resulting from microbial activity. A trial for pork loins has shown that the sensor reading is correlated with the progress of spoilage. A configuration for the commercial prototype has been outlined and the data presented here for pork loin indicate that this could provide a prediction of the shelf-life from two days before the end of shelf-life onwards or earlier. Data for pork, beef and lamb suggest that this technique of rapid freshness assessment (approximately 1 minute) could be applied to a range of meat types and cuts.

INTRODUCTION

Measurement of microbe numbers by colony counting or by monitoring changes in the electrical impedance of a culture broth in order to assess the microbial status of a food requires incubation periods of days or hours. Measurement of bacterial ATP in a solid food requires prior extraction and separation procedures, resulting in an assay procedure of about 1 hour. None of these techniques is suitable for checking a lorry load of meat on receipt by shop-floor staff, for example. If, on the other hand, one can identify a marker chemical indicative of the microbial status and if one can then find a chemical sensor or a biosensor that can be adapted for direct use in meat, an instrument can be constructed that gives a reading within a minute or so and is simple and compact enough for shop-floor use. One of the amines produced in the microbial spoilage of meat (Slemr 1981; Wortberg and Woller 1982) would be an obvious candidate as a marker chemical, but their concentration in the meat reaches appreciable levels only once spoilage has set in. In order to assess meat freshness at the pre-spoilage stage, the depletion of meat glucose by the microbial surface flora of chilled meat was chosen as the indicator of impending spoilage.



Development of glucose profile in lamb joint with increasing bacterial surface load

Fig.1 : Development of glucose profile in lamb joint with increasing bacterial surface load by conventional assay technique.

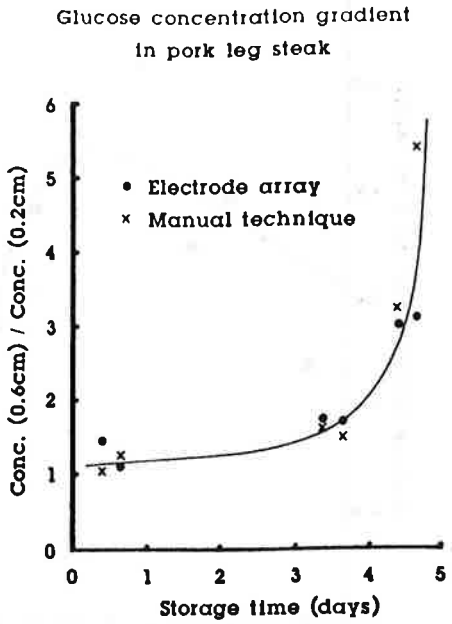


Fig.2 : Comparison of glucose concentration measurements in pork leg steak by electrode array and by the reference technique.

Workers in New Zealand (Gill 1976) showed that for lamb inoculated with controlled bacterial cultures, a gradient in the glucose concentration from the surface to 15 mm into the bulk of the meat developed as the microbial load increased from 10^4 to 10^7 cfu/cm^2.

DEVELOPMENT OF A MEAT FRESHNESS SENSOR

Experiments at the LFRA for lamb, beef and pork joints with their native bacterial flora exhibited broad agreement with Gill's results for the model system. For very fresh meat samples, however, the formation of a glucose gradient by the activity of microbes at the surface was masked by an increase of the glucose concentration throughout the meat within the first days post-mortem, probably due to a synthesis of glucose owing to the continuing activity of endogenous enzymes. This meant that the profile at 10^7 cfu/cm^2 was less pronounced than the earlier study for the inoculated lamb had suggested (see Fig.1).

Based on the specifications determined in the new study, the Leatherhead Food RA Fellow at Cranfield Institute of Technology developed a glucose sensor array for meat with four glucose probes spaced over 8 mm (Kress-Rogers and D'Costa 1986). This is based on a biosensor principle (amperometric mediated enzyme electrode, Cass et al. 1984), which is now available commercially (from MediSense) for use by diabetic patients in the form of disposable sensor strips plugging into a pen-shaped instrument. The patient obtains a digital read-out within 30 seconds of applying a drop of blood, without pre-treatment of the sample. The pH

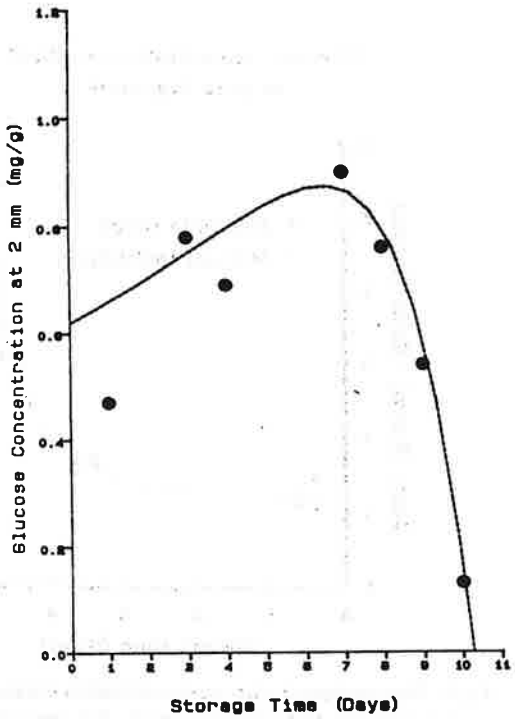


Fig.3 : Change of glucose concentration at 2 mm depth with storage time for pork loins measured with the electrode array. (o: data for one loin; ------: fitted curve for four loins.)

changes along the sampled distance were found to be too small to require compensation of the electrode reading.

EXPERIMENTAL TRIALS

The glucose sensor array developed for meat can be inserted into a meat joint (after making a small cut) for a direct reading of the glucose concentration profile over the sampling depth of 8 mm. An initial trial in pork leg steak (as purchased in a local butcher's shop) showed that

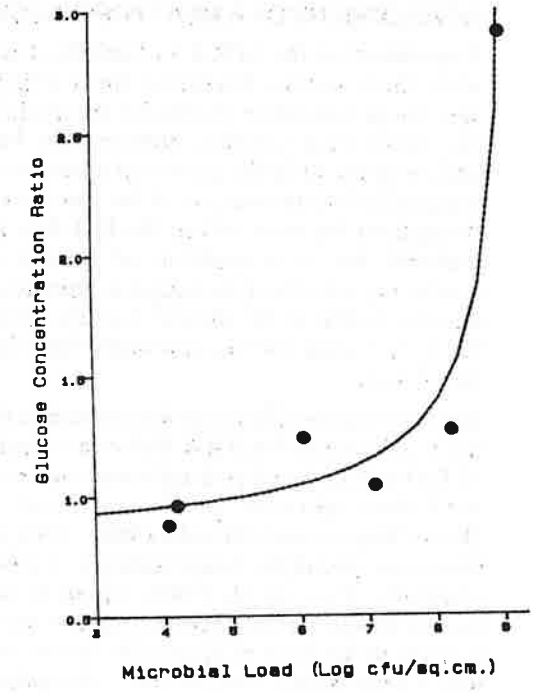


Fig.4 : Sensor response to the microbial deterioration of pork loins (o: data for one loin; ------: fitted curve for four loins).

the sensor array provided glucose concentration gradients in good agreement with results from Sigma kit enzymic assays carried out for meat samples adjacent to the sampling points of the electrode array (Fig. 2). but at a fraction of the time required for the Sigma assay.

For this study, two sections (each approximately 3 x 4 mm^2 cross-section) were cut from the meat adjacent to the sensor array. After freezing, this was cut into four slices each at positions between the individual electrode measuring points. These slices were analysed by the Sigma assay. One of the two top slices was used for the microbial analysis. This procedure was later modified for the trial described below to use a larger surface area (20 x 20 mm approx.) for the microbial analysis.

In the subsequent main trial, the deterioration of pork loins during chill storage at 2-4°C was followed over a period of two weeks, comparing the electrode array reading with results from Sigma assay, microbial load measurement and an organoleptic assessment. The pork muscles (*Longissimus dorsi*) were received fresh from a local slaughterhouse and had a microbial load of approximately 10^4 cfu/cm^2 on the first sampling day (1 day post-mortem). Initially, the glucose concentration increased throughout the sampled depth of the meat (consistent with the earlier LFRA experiments described in the introduction; see Fig.3). After 7 days, the microbial flora had reached an exponential growth pattern, with a load of 10^6 cfu/cm^2. The glucose concentration in the depth of the meat had stabilised at this time and a slight depletion of glucose at the surface relative to the bulk was first observed as a trend, although the present configuration did not allow a reliable measurement of the gradient increase at this stage (9%) with a single measurement. An increase of the ratio (concentration at 4 mm / concentration at 2 mm) by 44% was seen for a growth of the microbial flora from 10^4 cfu/cm^2 to 10^8 cfu/cm^2. For loads of over 10^8 cfu/cm^2 (for storage times of over 9 days), when the organoleptic properties of the meat were considered as objectionable, the glucose concentration gradient over the first 4 mm increased rapidly (see Fig.4). The electrode readings for the gradient deeper in the meat were found to be much less pronounced than those determined by the reference assay on the homogenised meat sections. This was probably due to drip juice forming in the cut before insertion of the electrode. It is expected that this problem would be eliminated by a commercially manufactured electrode array configured in the form of planar sensors on a knife tip and allowing a readout within half a minute . Moreover, the greater stability and reproducibility of this type of enzyme electrode when manufactured on an optimised commercial line (the clinical equivalent can be used without calibration as a one-shot disposable sensor) would allow a much shorter measurement time, without the need to wait for an equilibrated response. These two factors are expected to increase the reliability of the measurement of the glucose concentration gradient considerably and thus should allow an assessment of the freshness of the meat from two days before the end of shelf-life onwards with a single one-minute measurement. An earlier prediction could be possible with more closely spaced electrodes, giving a

measurement of the glucose concentration gradient in the more immediate vicinity of the surface, where the microbes are consuming the meat glucose.

CONCLUSIONS

A sensor array for the assessment of meat freshness by a measurement of the glucose profile resulting from microbial activity has been developed based on data gathered with conventional glucose assay techniques. A trial for pork loins has shown that the sensor reading is correlated with the progress of spoilage as determined by microbial and organoleptic assessment. A configuration for the commercial prototype has been outlined and the data presented here for pork loin suggest that this could provide a prediction of the shelf-life from two days before the end of shelf-life onwards or earlier. The results taken in our earlier study with conventional assay techniques indicate that the development of the glucose profile as a function of spoilage follows a similar pattern in pork, beef and lamb although the absolute value of the glucose concentration varies between meat types and cuts.

Further trials will be necessary to see whether a single clear cut-off point in the glucose concentration gradient can be defined for the acceptance of meat as fresh for a wide range of meat types and cuts If obtainable, these are to be carried out with commercial prototypes. The instrument is envisaged as a hand-held probe with plug-in, multi-shot disposable sensor arrays in the form of a short plastic knife tip carrying the electrodes. The probes are to be inserted into the meat without sample preparation, giving a readout within approximately one minute, perhaps in the form of a simple fresh/acceptable/spoilt indication (green/amber/red lights, for example).

It is possible that the sensor will give a better prediction of shelf-life than a microbial assay if both the glucose concentration gradient and the absolute level of the glucose concentration at the surface and deeper in the meat are taken into account. This could be the case because the change in the microbial metabolism from the utilisation of glucose to that of amino acids with the formation of amines is dependent on the availability of glucose to the microbial flora. Meat deficient in glucose at the time of slaughter is known to spoil more rapidly than meat with a normal glucose level at the same microbial load.

The sensor can also be used to observe the diffusion of glucose through meat by injection of glucose solution into the meat a few centimetres from the point where the glucose sensor has been inserted . This could help in the assessment of diffusion rates, perhaps in the assessment of previously frozen meat .

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