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Effect of tissue wounding on the results from calorimetric measurements of vegetable respiration☆

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Abstract

When plant tissue is wounded a number of protective processes start. We have made a study by isothermal calorimetry of the heat production response of root and tuber tissue to wounding. Samples with different surface to volume ratios were prepared from carrots, potatoes and swedes (rutabaga) and the thermal power was measured in closed glass ampoules in a TAM Air isothermal calorimeter. The evaluation was made by assuming that a certain heat production rate per volume tissue was associated with the normal metabolic activity, and that another heat production rate per surface area was associated with the wound response. The results showed that the wound response part was high; in some cases almost half the heat came from the wound response and not from the ordinary metabolic activity. We also discuss and give some evidence that the effect seen is not caused by limited supply of oxygen to the core of our samples. This is an important factor to take into account when making calorimetry on cut biological samples. It also shows that isothermal calorimetry can be used for the study of wound response in vegetable tissue.

Keywords: Isothermal calorimetry; Potato; *Solanum tuberosum*; Carrot; *Daucus carota*; Swede; Rutabaga; *Brassica napus*; Bulk respiration; Wound response

1. Introduction

When calorimetry is made on biological samples like vegetables and leafs one often has to cut the material to get a sample of reasonable size. As it is well-known that cut plant tissue show wounding reactions of different sorts [17] it is possible that the heat generated by the wounding reactions will influence a calorimetric measurement thought to be made on bulk tissue. This paper describes a study that comes to the conclusion that the wounding effect can be a significant part of the result from a calorimetric measurement.

It has been found that respiration gradually increases after vegetable tissue has been wounded, e.g. Laties [12] found that 0.5 mm potato slices increased their respiration four- to five-fold during a day, and grated carrots show several times higher respiration rates compared to whole carrots [20]. It has also been found that wounded potato tissue gradually increase the alternative pathway of respiration [19], i.e. that the normal bulk respiration differs from the wound induced processes.

Processes occurring on wounding are of different types. Some, like decompartmentation of enzymes and substrates, are direct effects of the mechanical wounding, while others are metabolic changes designed to bring about membrane restoration [17]. These membrane repair processes may persist for as long as 10 days at 10 °C [15]. The cells that are close to the site of injury strengthen their cell walls by the secretion of additional structural components, e.g. lignin in carrots [18].

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Calorimetry has been used in the study of vegetable respiration since the beginning of the 20th century [8,11], but we have found only three calorimetric measurements on cut fruits and vegetables reported in literature. Firstly, Iversen et al. [10] studied spoilage of cut pineapple and found that thermal powers were within ±15% proportional to mass, and that the major metabolic events linked with spoilage were not those of the fruit itself, but rather microbiological spoilage starting at about 10 h after cutting. Secondly, Ordentlich et al. [14] studied the heat production in potato slices. Thirdly, Smith et al. [19] studied metabolic changes in 1 mm slices of potato and found that the heat production rate increased upto 30 h. They also found a change from lipid to starch as the substrate.

The aim of this work was two-fold. Firstly, to study the effect of wounding on calorimetric measurements to be able to give recommendation on how samples should be cut for calorimetric measurements. Secondly, to study the wound response in cut vegetables to quantify the metabolic response after cutting. We will use this for optimization of process parameters, which is of importance for the large market for minimally processed, ready to eat vegetables [20].

2. Materials

Sample cylinders of roots and tubers were excised with cork bores and the lengths and diameters were measured with a caliper. The mass of each sample was also measured. To get different surface/mass-ratios the samples were cut into smaller parts. All samples contained only flesh and no skin.

Three locally purchased materials were used:

1. Two carrot roots (*Daucus carota*) were used. From each carrot were taken four duplicate samples of phloem parenchyma tissue (a total of 8 samples). The cylindrical samples had a diameter of about 7.5 mm. The mass of the carrot samples was around 1.7 g. The maximally cut carrot sample was cut into 16 transversal slices resulting in slices with a thickness of about 2.5 mm.
2. From each of two potato tubers (*Solanum tuberosum*, cultivar Astrix) were made 8 sample cylinders with a diameter of about 10.3 mm. The mass of the potato samples were 2.1–4.0 g. The maximally cut potato sample was cut into four longitudinal parts that were cut into 15 transversal slices each. The smallest dimensions of the smallest potato parts was about 2.5 mm. The potato samples were taken without respect to the different parts of a potato.
3. One large swede root (rutabaga, *Brassica napus*) was used. A total of 24 cylindrical samples with a diameter of about 9.2 mm were taken. The masses of the swede samples were from 3.2 to 6.7 g. The maximally cut swede sample were cut into 37 transversal slices resulting in slices with a thickness of about 1.5 mm.

Even the smallest samples used were at least 15 times larger than the cells in the three vegetables. The large potato cells typically have 150 μm diameter [9], whereas the carrot and swede cells typically have diameters of 60 [23] and 94 μm [9], respectively.

3. Method

The measurements were made in a TAM Air isothermal calorimeter (Thermometric, Järfälla, Sweden) at 20 °C. This calorimeter has a sensitivity (precision) of ±10 μW [24]. All eight calorimeters were charged with samples with different masses and surface to volume ratios (see above). Baselines were taken before or after each measurement and the calorimeters were calibrated electrically. The measurements were made 100 min after the samples were cut and quickly charged into the calorimeter in sealed 20 ml glass ampoules (cf. Fig. 1). At 100 min the thermal power levels were quite constant.

If the measured thermal power P (W) from a wounded tissue can be seen as being the sum of thermal powers originating from the bulk (normal metabolic activity) and from the surface (wounding) we may write:

$$P = p_V V + p_A A \tag{1}$$

Here, V (m^3) and A (m^2) are the volume and surface area of the sample, and p_V ($W\,m^{-3}$) and p_A ($W\,m^{-2}$) are coefficients for the thermal power per unit volume and area. Eq. (1) can be reformulated by replacing volume by mass divided by tissue density ρ ($g\,m^{-3}$) and reorganizing:

$$\frac{P}{m} = \frac{p_V}{\rho} + p_A \frac{A}{m} \tag{2}$$

Here, the specific thermal power P/m is a linear function of the surface area divided by mass. The intercept is the volume heat production coefficient divided by the density and the slope is the surface heat production coefficient. From a plot of P/m as a function if A/m for a number of samples with different surface-to-area ratios one can therefore evaluate the two heat production coefficients. Note that all exposed area is equally counted even if it can be expected that the effect of wounding would be different for cuts in different directions in a root or tuber.

4. Results and discussion

Fig. 1 shows examples of measured thermal powers for eight swede samples (the other types of samples show qualitatively similar results). It is seen that the thermal powers are quite constant when the values used in the evaluation are taken (100 min). After this the values are time-dependent for all types of samples (not shown); in general they first increase and then decrease. The increase in respiration after wounding has been seen in different biological samples, e.g. in potatoes [12] and in carrots [20]. If the measurements are

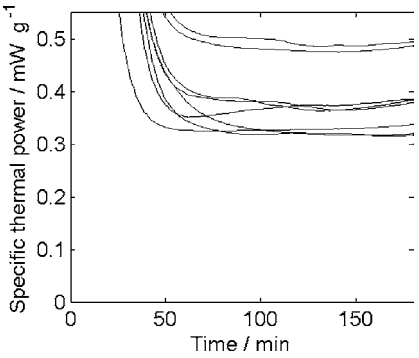

Fig. 1. Example of 8 swede measurements showing the initial part where the thermal power values were taken (100 min).

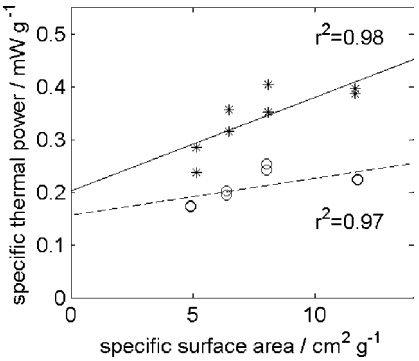

Fig. 2. Specific thermal power as a function of specific surface area from measurements with samples from two carrots. Lines are linear regressions. Carrot 1: stars and solid line; carrot 2: circles and dashed line.

continued long enough the thermal power drops to low values (not shown), indicating that oxygen is depleted.

Figs. 2–4 show experimental results evaluated with Eq. (2) for carrot, potato and swede samples and one can see a dependence of the measured specific thermal power on the specific surface. For the carrot samples the specific thermal power originating from the bulk of the sample is about 0.18 mW g^{-1} (zero surface area). When a sample has a surface area of 10 cm^2 g^{-1} the measured specific thermal power is 50–100% higher than for a corresponding sample with no surface. For potatoes the bulk thermal power was about 0.16 mW g^{-1} (zero surface area) and the measured thermal power was only increased about 30% when the surface area was 10 cm^2 g^{-1}. For the swede the bulk thermal power was about 0.28 mW g^{-1}. For carrot, potato and swede the mean coefficients p_A were about 14, 6, and 12 μW cm^{-2}, respectively.

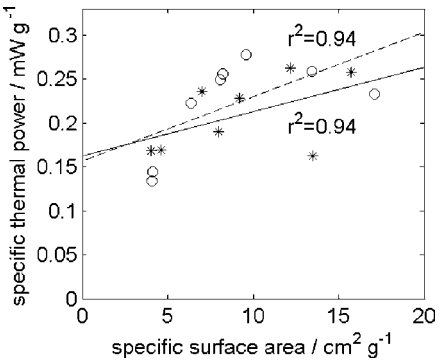

Fig. 3. Specific thermal power as a function of specific surface area from measurements with samples from two potatoes. Lines are linear regressions. Potato 1: stars and solid line; potato 2: circles and dashed line.

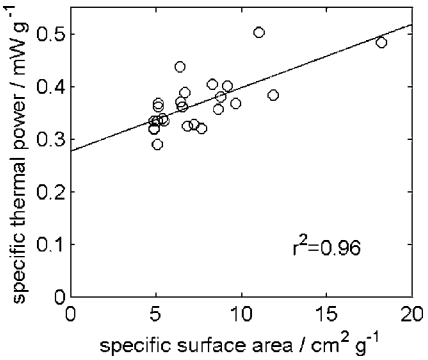

Fig. 4. Specific thermal power as a function of specific surface area from measurements with 24 sample from one swede. Line is a linear regression.

If one knows the two coefficients p_A and p_V one can for any sample calculate the fraction f of heat that comes from the wounding (not from the ordinary bulk metabolic activity):

$$f = \frac{p_A A}{p_A A + p_V V} \quad (3)$$

It is reasonable to put a limit to this fraction when designing an experiment, e.g. that less than 5% of the heat produced in a respiration experiment should come from the wounding, or that more than 50% of the heat in a wounding experiment should come from the wounding.

Above we have assumed that the increase in activity seen for wounded samples is caused by increased activity at the wounded surfaces. However, we may have got a similar result if low oxygen levels inside the larger samples limited their respiration. It is well-known that the oxygen levels in the center of large tissues like storage roots can decrease to too low values for the normal respiration to take place, e.g. during flooding [5] or at storage with too low oxygen supply [2]. Respiration is then replaced by deleterious anaerobic processes. It is also well-known that the skin of vegetables, fruits and other plant parts often presents a large diffusion resistance [1,3,25] so that the oxygen levels inside plant parts often is lower than the external oxygen level. However, there has been some controversy over whether the flesh of vegetables and fruits presents appreciable diffusion resistances that will give rise to large internal oxygen gradients. As an example, Burton [3] came to the conclusion that “the flesh of the [potato] tuber offered no serious resistance to slow oxygen diffusion”, while Rajapakse et al. [16] concluded that “Fruit flesh (apples, Asian pears, nectarines) was found to exert a

significant resistance to O_2-diffusion". However, what looks like different opinions may be based on similar data, but applied to different systems (species, plant part, developmental stage, size, temperature, etc.). As an example, Lytle et al. [13] found a significant increase in heat production rate when sample size was diminished for Voodoo lily appendix tissue and attributed this to lower oxygen levels in larger samples. This is a likely explanation as the Voodoo lily has an extremely high specific respiration rate (in the order of 1000 times higher than our vegetable samples) so that all oxygen is consumed by the outer cells in all but very small samples. However, this does not mean that all plant samples show this behavior. Below we will discuss this in some detail and make approximate calculations of the oxygen concentrations in the center of our samples.

In the cells the oxygen is used by mitochondria whose activity is in practice not limited by low oxygen pressures [22] as the binding of oxygen to cytochrome oxidase within the mitochondria is virtually saturated already at an oxygen concentration of about 0.1 μM [5] (the oxygen concentration in air-saturated aqueous solutions is about 250 μM [21], which corresponds to about 8 g m^{-3}). Small tissue samples have been found to maintain their respiration down to about 25 μM (0.8 g m^{-3} oxygen concentration) or lower [5]. If the core part of a vegetable sample reduces its respiration because of low oxygen pressures this is commonly because the external oxygen pressure is low, not because of internal diffusion resistances. As we have no measurements of the core oxygen pressures in our samples we will make probable that the sample cores are aerobic by a calculation. This can be done with the method of Warburg [26] for calculation of oxygen concentration gradients in respiring tissue. We have here written this equation for the core oxygen concentration c_c (g m^{-3}) in a cylinder with radius R (m) and a diffusion coefficient D (m^2 s^{-1}) and in which the oxygen is consumed at a rate η (g m^{-3} s^{-1}) [1]:

$$c_c = c_e - \frac{\eta R^2}{4D} \quad (4)$$

Here, c_e (g m^{-3}) is the surface oxygen concentration.

A typical specific thermal power of our samples is 0.3 mW g^{-1} and if we assume an (aerobic) respiration enthalpy of −470 kJ per mol oxygen [6] and a respiratory quotient of 1.00, we can recalculate our measured thermal powers to an oxygen consumption of about 0.02 g m^{-3} s^{-1}. We can also use the enthalpy and the respiratory quotient to calculate approximate external oxygen pressures in the ampoules after 100 min when the values used in the evaluation were taken. The result is surface oxygen concentration of about 6.5 g m^{-3} for the larger swede samples and about 7.5 g m^{-3} for smaller carrots and potato samples.

The main problem with this type of calculation is the value of the diffusion coefficient. In plant tissue gas diffusion takes place in both the aqueous phase (in the cell sap) and the gas phase (in intercellular air spaces; e.g. potatoes have intercellular spaces in the order of 1% [3]) [16,21]. If the diffusion was in the liquid phase and in the cell sap only, potato slices would be anoxic at distances little more than 1.0 mm below the surface [12]. As the gas phase diffusion is much higher than the liquid phase diffusion the intercellular voids become very important for the oxygenation of bulky plant tissue.

Unfortunately, there are only few actual measurements of vegetable or fruit tissue diffusion coefficients. The absolute majority of papers dealing with diffusion in vegetable and fruits only consider the skin resistance, considering the internal concentration to be uniform. Such studies have for example been made for peas [25], tomatoes [4] and potatoes [2]. We have only found a few papers giving diffusion coefficients (D) of the flesh of bulky plant tissues. Abdul-Baki and Solomos [1] found that the carbon dioxide concentration in the center of potato tubers of 27 mm radius were 14 kPa when the carbon dioxide pressure under the skin was 15.8 kPa. From this they calculated a D of carbon dioxide in potatoes to be about 25×10^{-9} m^2 s^{-1} (the coefficient for oxygen should be similar). Woolley [27] found slightly slower D of argon in potato tissue. Rajapakse et al. [16] found D of 30×10^{-9} and 110×10^{-8} m^2 s^{-1} for two cultivars of Asian pears that has a porosity of about 2% and slightly higher D for other fruits with higher porosities.

With the above given values of oxygen consumption rate, sample radii, and external oxygen concentrations, and a diffusion coefficient of 25×10^{-9} m^2 s^{-1}, the centers of our samples would have oxygen concentrations of about 2 g m^{-3} for potatoes and swede and about 4.5 g m^{-3} for carrots. These values are not so low as to decrease the respiration rates [5].

From the above calculations we find it reasonable to assume that the effect seen in Figs. 2–4 is indeed a direct response of the wound metabolism. However, the calculations are quite approximate, mainly because the diffusion coefficients of our samples are uncertain. The following points should also be noted:

- Eq. (4) is rather sensitive to small changes in the parameters, e.g. a 1 mm larger radius may give anoxic conditions (all other parameters constant).
- In the discussion above we have concentrated on lowered oxygen levels, but the simultaneously elevated carbon dioxide levels may also be important.
- The vegetables were taken from cold aerated storage before the measurements, but we did not control the initial oxygen and carbon dioxide levels in our samples.
- It is possible that the intercellular voids at the sample surfaces were filled with sap, and thus presents a higher diffusion resistance than normal tissue [27].

In previous experiments we have seen that very mild blanching seems to give more constant thermal powers, which may be due to that the blanching eliminated the reactions in the wounded cells [7].

5. Conclusions

Thermal power from wound processes can contribute a large part of the measured thermal power from an excised vegetable tissue. Measurements such as the ones shown here can be used to optimize experiments with respect to this problem.

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