Product and Redox Potential Analysis of Sauerkraut Fermentation

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The relationships between the redox potential of the brine, during fermentation of white cabbage into sauerkraut of two early and two late fermentation processes, and the changes in the amount of sugars, organic acids, the redox potential of the brine and of the ascorbic acid redox couple, and pH are described. The trend in the change of the redox potential of the brine is the same for all four fermentation processes studied. In the first phase a sharp decrease in redox potential is followed by an increase in redox potential. In the second phase the redox potential is rather constant. This second phase is followed by another decrease in redox potential, which stabilizes at a minimum value, the third phase. It was observed that sugar fermentation and acid production mainly took place during the first and third phases, probably representing, respectively, the heterogeneous and homogeneous fermentation processes.

Keywords: *Sauerkraut; fermentation; redox potential; sugars; organic acids*

INTRODUCTION

Fermentation processes in the food industry are being used for several purposes. One of these purposes is the preservation of cabbage and other vegetables by spontaneous fermentation. The fermentative production of sauerkraut from shredded and salted white cabbage (*Brassica oleracea*) has been known for centuries. The product of this fermentation process can be consumed within several weeks or can be kept in the fermentation tank for up to a year. The fermentation process of white cabbage into sauerkraut is rather well described both from a biotechnological (Fleming et al., 1988) and from a microbial point of view (Stamer et al., 1971).

The sauerkraut fermentation process is a spontaneous process caused by the microorganisms present on the leaves of the cabbage. It is initiated by damaging the plant tissue, thereby increasing the total surface and concomitantly liberating nutrients for the microorganisms, allowing them to proliferate under anaerobic conditions. The addition of salt favors the growth condition of the desired microorganisms by increasing the ionic strength and the osmolarity of the medium, thereby extracting water and additional nutrients from the tissue. The fermentation process is characterized by an initial heterofermentative process mainly caused by *Leuconostoc mesenteroides*, followed a homofermentative process mainly caused by *Lactobacillus plantarum* (Stamer et al., 1971). Dependent on variety, season, fermentation temperature, and eventually added starter cultures (Daeschel and Fleming, 1984), the fermentation develops in time in a rather uncontrolled way.

During this fermentation process the saccharides sucrose, fructose, and glucose are either fermented into organic acids, ethanol, or carbon dioxide, or converted into mannitol. The main organic acids produced are lactic and acetic acid. For lactic acid a biphasic production in time was observed, whereas acetic acid was mainly produced in the first phase (Fleming et al., 1988). In the first instance the sugar mannitol was produced followed by consumption (Hughes and Lindsay, 1985).

Previously, attention has been paid to the role of ascorbic acid in the fermentation of white cabbage into sauerkraut. It is assumed that especially ascorbic acid prevents the sauerkraut from discoloration reactions (Stamer et al., 1973; Gierschner and Buckenhueskes, 1983; Bohrer et al., 1984). This suggests that a relationship should exist between the redox potential as indicated by the ascorbic acid redox couple and the quality of the sauerkraut. A redox potential describes a thermodynamic state of a (complex) product, in this case a solid-state fermentation. For this reason the redox potential of the brine was analyzed during the course of the fermentation processes studied. This was done to research if (i) the redox potential could be associated with discriminative phases in the sauerkraut fermentation process, (ii) the redox potential of the fermentation process could be linked to the ascorbic acid redox potential, and (iii) changes in the redox potential could be related to changes in either the consumption of sugars and/or the production of abundant organic acids (lactic and acetic acid).

MATERIALS AND METHODS

Chemicals Used. Acetonitrile, methanol, oxalic acid, phosphoric acid, potassium ferrocyanide, potassium hydroxide, sodium acetate, sodium azide, triethanolamine, Tris, zinc acetate, and tetrabutylammonium hydrosulfate were from Merck (Darmstadt, Germany); DL-homocysteine and calcium EDTA were from Fluka (Buchs, Switzerland); citric acid, fumaric acid, succinic acid, dl-lactic acid, acetic acid, and malic were from Sigma (Zwijndrecht, The Netherlands). Food grade sodium chloride was from KNZ (Hengelo, The Netherlands).

Cabbage Handling and Fermentation. Cabbages were obtained from the Institute of Applied Research for Arable Farming and Field Production of Vegetables (Lelystad, The Netherlands). The outer leaves and the central core of fresh cabbages were removed followed by slicing to a thickness of 1 mm with a Komen en Kuin slicer. To the sliced cabbage was

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added granular, food grade sodium chloride (3% w/w). In total 45 kg of sliced and salted cabbage was packed in vessels of 60 L (30 × 36 × 60 cm). After the fermentation vessels had been filled, the cabbage was kept below the brine using a plastic bag filled with water. This water-filled bag also ensured anaerobiosis. The fermentation processes were performed at 15 °C.

For the continuous measurement of the redox potential of the brine at ~25 cm from the bottom of the fermentation vessel, a hole ($\Phi = 2.1$ cm) was made in the wall of the vessel and equipped with a polypropylene tube of 4 cm length. To this tube the electrode was added to continuously measure the redox potential of the brine. The redox potential measurements were performed with a combined Ingold P14805ATO-DLO-S&/120 Ag/AgCl electrode (Wilmington, USA). The redox potential was continuously monitored on a Salm & Kipp recorder (Breukelen, The Netherlands).

Samples of the fermentation broth were taken with a special device. A hollow, rustproof steel cylinder ($\Phi = 4$ cm; h ranged from 8.5 to 12.5 cm), ending in a sharp point ($h = 12.5$ cm), was equipped with a moveable lid on top of the cylinder. Both the cylinder and lid were fixed to a rustproof steel bar of 100 cm height. This device was pushed through the fermentation broth. When the sampling device was lifted upward, the lid closed and the sample remained in the inner part of the cylinder. From these samples the pH and the amounts of saccharides, organic acids, and ascorbic acid were measured. During the first 10 days of the experiment the pH values were measured twice a day. After this period the measurements were performed once a day.

Two sets of experiments were performed. In the first set of experiments, which started October 1994, two cabbage varieties, Rinda and Almanac, were fermented; these were the "early" fermentations. In January 1995, two other cabbage varieties, Massada and Structon, were fermented; these were the "late" fermentations. Throughout this paper the varieties Rinda, Almanac, Massada, and Structon are indicated with R, A, M, and S, respectively.

Analytical Methods. After sampling, the samples were homogenized in liquid nitrogen, divided into two equal portions, and stored at −20 °C until further analysis. From each portion the dry matter, organic acid, sugar, and ascorbic acid contents were determined. The values determined from these two portions were considered duplicates.

During the fermentation process, the ratio between the liquid and solid phases of the samples varied (see Results). For this reason, the amounts of all the components analyzed are expressed as moles per kilogram of dry material.

Dry Matter Content. Samples were respectively dried for 16 h at 70 °C followed by 3 h of drying at 105 °C. After cooling in a desiccator to room temperature, samples were weighed and the dry matter content was calculated.

Ascorbic Acid (AA) and Dehydroascorbic Acid (DHA) Analyses. The analyses of AA and DHA were performed according to the method of Keijbets and Ebbenhorst-Seller (1990), with some minor modifications. To 20 g of the frozen pulp were added 10 mL of 0.8 M oxalic acid, 10 mL of methanol, and 30 mL of Milli-Q water. This mixture was homogenized with an Ultra Turrax (Ika Labortechnik, T25, Jane & Kunkel) for 30 s at maximal speed. Milli-Q water was added to a total volume of 100 mL. This slurry was clarified by filtering it over an S&S 595 $^1/_2$ filter (Schleicher & Schull, Keene, NH). For the determination of AA, to 10 mL of the filtrate was added 15 mL of Milli-Q water. After ultrafiltration (filter pore size = 0.22 μm), 10 μL of the last mixture was injected into the HPLC.

Total ascorbic acid (TAA; TAA = AA + DHA) was determined by adding to 10 mL of the filtrate a 2 M KOH solution until pH 5; the pH was adjusted to pH 7 by adding a 0.05 M Tris solution. The total volume was brought to 25 mL. To 1.0 mL of this final solution was added 1.0 mL of a 0.8% DL-homocysteine solution. The reduction reaction of DHA into AA was completed within 15 min. Ten microliters of this solution was analyzed after ultrafiltration (filter pore size = 0.22 μm). The amount of DHA was calculated by subtracting the determined amount of AA from the TAA. Care was taken that the analysis of ascorbic acid was performed in a dark environment given the light susceptibility of ascorbic acid.

Analysis of Organic Acids. Organic acids were determined according to the method of Luning et al. (1994). To 5 g of frozen pulp was added 45 mL of Milli-Q water. This mixture was homogenized with an Ultra Turrax for 30 s at maximal speed. The slurry was filtered over a Whatman GF/C filter. To 2.5 mL of the filtrate was added 2.5 mL of 21 mM H_3PO_4. This solution was cleansed with an activated Sep-Pak C_{18} column. Ten microliters of this solution was injected into the HPLC system, which consisted of a Waters 717 autosampler, a Waters 510 HPLC pump, a Waters 15207 guard column in a Waters column oven containing two H^+ charged Shodex KC 881 cation exchange columns in series, a Waters 486 tunable absorbance detector at 210 nm, and a Waters Millennium data acquisition system. The eluent was 21 mM H_3PO_4, the flow rate was 0.7 mL min^{-1}, and the column oven temperature was set at 65 °C.

Analysis of Sugars. Sugars were analyzed according to the method of Luning et al. (1994). To 5 g of frozen pulp was added 50 mL of Milli-Q water. This mixture was homogenized with an Ultra Turrax for 30 s at maximal speed. Next, 1.0 mL of Carrez I solution (0.25 M potassium ferrocyanide) was added, followed by the addition of 1.0 mL of a Carrez II solution (0.8 M zinc acetate) to remove protein. After mixing, the final volume was brought to 100 mL. This solution was filtered over an S&S 595 $^1/_2$ filter. One milliliter of the filtrate was, dependent on the concentration of the analyte, diluted two to five times with a solution containing 0.6 mM Ca-EDTA. After ultrafiltration over a sieve with pore size of 0.22 μm, 10 μL of the analyte was injected into the HPLC system, which consisted of a Waters 510 pump, equipped with a U6K injector, a Waters Sugar-Pak I column with a sugar guard column, a Waters 410 differential refractometer, a column oven, and a Waters Millennium data acquisition system. The flow rate was 0.5 mL min^{-1}, and the column oven temperature was set at 90 °C.

Analysis of the Data. The calculation of the redox potential of the brine and of the ascorbic acid/dehydroascorbic acid redox couple as well as the statistical analysis of the data is presented under Theory.

THEORY

Redox Potential Measurements. If in a redox reaction protons are involved, the redox potential of the system, $E_\mathbf{h}$ (V), changes toward a reference redox potential, E'_{ref}, with

$$E_\mathrm{h} = E'_{\mathrm{ref}} + (RT/nF)\ln[\mathrm{H}^+] \quad (1)$$

Here R is the gas constant (J K^{-1}), T is the measuring temperature (K), F is the Faraday constant (C mol^{-1}), and n is the number of electrons involved in the redox reaction; in case of protons $n = 1$.

Because the pH changes during the fermentation, this pH change is also sensed by the working electrode, which at a temperature of 15 °C equals

$$E_\mathrm{h} = E'_{\mathrm{ref}} - 0.0571 \times \mathrm{pH} \quad (2)$$

By definition the redox potential of the hydrogen/proton redox couple $E'_{\mathrm{ref}} \equiv 0$ at pH 0. In that case $E_\mathbf{h} = -0.0571 \times$ pH.

Ascorbic Acid/Dehydroascorbic Acid Redox Couple. The AA/DHA redox couple can be described according to Clark (1960) by

$$E_\mathrm{h} = E'_0 + \frac{RT}{2F}\ln\frac{[\mathrm{DHA}]}{[\mathrm{AA}]} + \frac{RT}{2F}\ln([\mathrm{H}^+]^2 + K'_\mathrm{r}[\mathrm{H}^+]) \quad (3)$$

which, at 15 °C, can be rewritten as

$$E_h = E'_0 + 0.0286 \log\frac{[DHA]}{[AA]} + 0.0571 \times pH + 0.0286 \log(K'_r[H^+]) \quad (4)$$

in which E'_0 is the standard potential of the AA/DHA redox couple at pH 0. This value of E'_0 equals 0.39 V at $T = 15$ °C. K'_r is the protonation constant of the carboxylic group of (dehydro)ascorbic acid. This value is 9.1×10^{-5} M^{-1} (Clark, 1960).

From eq 3 two limiting conditions can be described. The first one refers to the situation that $[H^+] \gg K'_r$. In that case the third term of eq 3 reduces to $RT \ln([H^+])/F$, which, at 15° C, equals $-0.0571 \times$ pH. In other words, if the pH drops 1 unit, the redox potential of the system becomes 57.1 mV more positive.

In the case that $[H^+] \ll K'_r$, the third term of eq 3 reduces to $RT \ln(K'_r[H^+])/2F$, which at 15 °C, equals $-0.0286 \times pK'_r - 0.0286 \times$ pH. Becuase the value of $pK'_r = 4.04$, this implies that at pH values substantially higher than pH 4 the redox potential of the ascorbic acid/dehydroascorbic acid redox couple changes by 57.1 mV per pH unit and at pH substantially below 4, by 28.6 mV per pH unit.

All redox potentials given are against the normal hydrogen electrode (NHE).

Statistical Analysis of the Data. *Estimated Variance of Sugars and Organic Acids Analysis.* For the statistical analysis of the data for the sugars and organic acids determined, it is assumed that (i) the standard deviation remains the same during the fermentation process and (ii) the standard deviation is independent of the four fermentation processes studied.

In that case the estimated standard deviation, σ, for one analyte for all four fermentation processes together in the case of duplicate measurements equals

$$\sigma = \sqrt{\frac{s^2_{1,1} + s^2_{2,1} + \ldots + s^2_{m_1,1} + s^2_{1,2} + \ldots + s^2_{m_4,4}}{m_1 + m_2 + m_3 + m_4}} \quad (5)$$

where $s_{i,j}$ is the estimated standard deviation of component x of fermentation process i analyzed at day j and m_i represents the number of days at which the amount of component x was >0 for fermentation process i.

Estimated Variance of the Redox Potential of the AA/DHA Redox Couple. In the case of vitamin C the reduced form is analyzed directly. To determine DHA, DHA is reduced and the total amount (T) is determined. By simple subtraction, the amount of DHA is calculated (DHA = T − AA). This implies that AA and DHA are not stochastically dependent. To determine the standard deviation of the redox potential of the AA redox couple, the distribution of the stochastic variable

$$Z = \frac{RT}{F} \ln\frac{[DHA]}{[AA]} = \frac{RT}{F} \ln\frac{[T - AA]}{[AA]} \quad (6)$$

has to be determined. This distribution for Z, and as a consequence its standard deviation, sd(Z), can be approximated using the δ method (Rao, 1973). By applying this δ method sd(Z) can be approximated by

$$sd(Z) = \frac{RT}{F} \sqrt{\frac{\mu^2_T\sigma^2_{AA} - 2\mu_T\mu_{AA}\sigma_{T,AA} + \mu^2_{AA}\sigma^2_T}{\mu_{AA}(\mu_T - \mu_{AA})}} \quad (7)$$

For day j of fermentation process i, μ_T and μ_{AA} are the averages and σ^2_T and σ^2_{AA} are the variances of, respectively, T and AA, and $\sigma_{T,AA}$ is the covariance of AA and T. If the standard deviation of Z for each day j and fermentation process i is known, the standard deviation of the redox potential of the four fermentations can be calculated using eq 7.

RESULTS AND DISCUSSION

Dry Matter Content. During the course of the fermentation, the dry matter content of the samples changed in a nonsystematic way (data not shown). The average values of the dry matter content for the two early fermentations R and A were, respectively, 75.5 ± 8.1 ($n = 14$) and 80.1 ± 5.6 ($n = 14$) g of dry weight (kg of raw material)$^{-1}$; for the two late fermentations M and S the values were, respectively, 96.9 ± 10.4 ($n = 14$) and 103 ± 12 ($n = 14$) g of dry weight (kg of raw material)$^{-1}$. The relatively large variance in the values for the dry weight is probably due to the sampling procedure, in combination with the changes occurring in the fermentation vessel during the fermentation process. Due to this variance in dry matter content the determined amounts of sugars and organic acids are expressed as moles per kilogram of dry weight.

It was noticed that after ~5 days, fermentation R started to form off-odors, as indicated by the ATO expert taste panel on sauerkraut (data not shown). During the course of fermentation R the intensity of this off-odor formation increased substantially, making the resulting product unacceptable for consumption. Probably microbial contamination (not controlled; beyond the scope of this study) caused this off-flavor formation. Despite this, the information obtained from fermentation R was used throughout this study to quantify potential differences between proper (fermentations A, M, and S) and contaminated (fermentation R) sauerkraut fermentation processes.

Redox Potential, AA, and pH Measurements during the Fermentation Process. In Figure 1 the change in redox potential of the system, E_h, corrected for the change in pH (see Theory), during the fermentation of the four varieties is shown. For all four fermentations, the following pattern in the change in redox potential is observed. After a large decrease in the value of the redox potential, during the first 5−7 days of the fermentation processes, a minimum value in redox potential is observed (E_1). Next, the value of the redox potential increased and reached a plateau value after ~10 days. This plateau lasted for 2−7 days for the early fermentations (R and A) and 12−18 days for the late fermentations (M and S). E_2 indicates the start and E_3 the end of this plateau. After this plateau, a second decrease in redox potential is observed which stabilizes at around −210 to −240 mV (E_4). The results of the observed changes in redox potential suggest that from zero time through E_1 until E_2, a first burst of fermentative activity, probably the heterofermentative phase, is observed. A transient, metabolic quiet phase, connecting the heterofermentative with the homofermentative phase, is probably associated with the plateau region ranging between E_2 and E_3. From E_3 to E_4 the main burst of activity of the homofermentative process is observed. A compilation of the values of E_1−E_4 and the fermentation time required to reach these values for the four fermentations studied are given in Table 1.

Because vitamin C is an important constituent of sauerkraut, its presence is assumed to prevent undesired discoloration processes (Gierschner and Bucken-

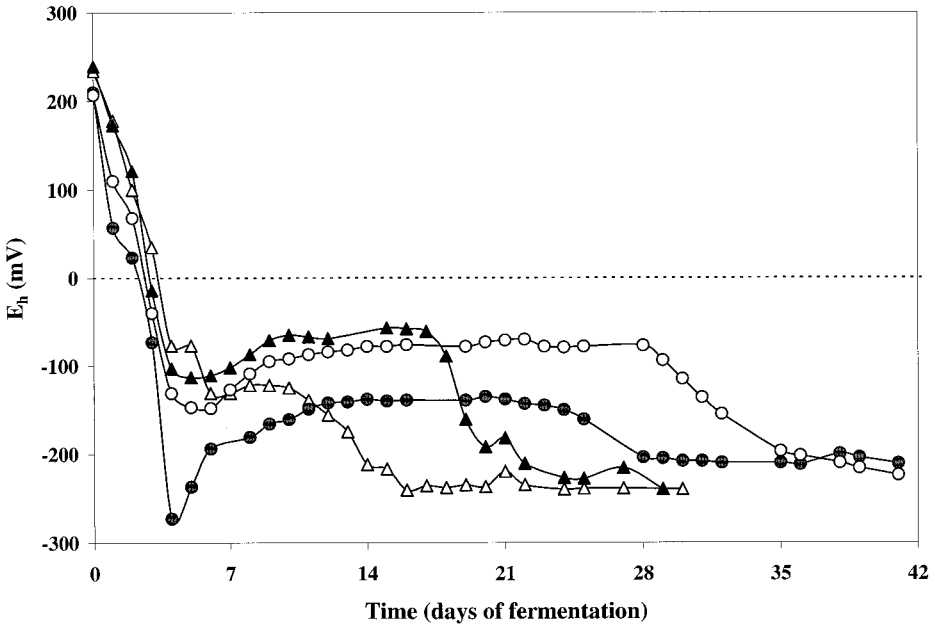

Figure 1. Change in redox potentials during fermentation. Changes in the measured redox potential of the brine, E_h, are corrected for the change in pH, during the course of the fermentation process for the early fermentations R (▲) and A (△) and the late fermentations M (●) and S (○).

Table 1. Redox Potentials (mV versus NHE) of the Brine, Corrected for the pH: Minimum and Maximum Values during the Fermentation Processes

	E_1 (mV) 1st minimum		E_2 (mV) onset plateau		E_3 (mV) end plateau		E_4 (mV) start 2nd minimum	
sample	day	$E_{h,brine}$	day	$E_{h,brine}$	day	$E_{h,brine}$	day	$E_{h,brine}$
R	7	−132	8	−122	10	−125	16	−236
A	5	−119	9	−71	16	−72	22	−240
M	5	−273	12	−142	22	−143	28	−204
S	5	−147	12	−84	28	−77	35	−200

hueskes, 1983; Bohrer et al., 1984); both AA and DHA concentrations were determined during the fermentation process. On the basis of the ratio of AA over DHA and the pH of the brine, the redox potential of the AA redox couple can be calculated (see Theory). In Figure 2 the redox potential of the AA/DHA redox couple, E_h, for the four fermentations is shown. The standard deviation of the calculated redox potential of the AA/DHA redox couple for all measurements (see Theory) was 13.9 mV ($n = 43$). In addition, the development of the pH during the fermentation process is shown for one early (A) and one late (M) fermentation. The pH development of the two other fermentations (R and S) is not shown, because they are almost identical to the ones shown in Figure 2.

For the four fermentations a steep increase in the value of E_h is observed for the AA redox couple during the first 5 days of the fermentation process. The increase in this value during this period is mainly caused by the decrease in pH, which amounts to ∼2.9 pH units. This decrease in pH causes an increase in the redox potential of the AA/DHA redox couple of ∼100 mV (see Theory). After this initial increase in redox potential, the redox potentials of the late fermentations M and S stabilize around 151 + 3.9 mV ($n = 13$) and 154 mV + 4.2 mV ($n = 11$), respectively. No redox potential could be assigned to the early fermentations (R and A) after ∼7 days of fermentation, because the amount of DHA was virtually zero. Whatever, during the fermentation process, the change in redox potential of the ascorbic redox couple (see Figure 2) is completely different from the change in redox potential of the brine (see Figure 1), both in magnitude and in shape. For these reasons, it can be concluded that they are not related one to each other. The underlying reason for the absence in this relation might be that the working electrode measures a reversible redox reaction, whereas the oxidation reduction process of vitamin C at a platinum electrode represents an electrochemically irreversible, and therefore not defined, redox reaction. Apparently, the redox potential as measured by the working electrode characterizes different redox processes as compared to the AA/DHA redox process.

During the first 4 days of fermentation a sharp decrease in pH was observed to pH ∼3.9. To the end of the fermentation process the pH showed a slow but continuous decrease to pH ∼3.5 for the early (R and A) fermatations and to pH ∼3.7 for the late (M and S) fermentations. In contrast to the redox potential, no discernible pattern could be observed either for the pH or for the protons formed per unit of time, other than a continuous decrease, respectively, continuous increase with time.

Changes in Sugars during the Fermentation Process. During the fermentation process the changes in amount of the most abundant sugars (glucose, fructose, saccharose, and mannitol) were analyzed (see Figure 3). The standard deviations for these four sugars for all measurements, as calculated according to the equations given under Theory, are 43 ($n = 43$), 34 ($n = 43$), 8 ($n = 23$), and 38 ($n = 41$) mmol (kg of dry weight)$^{-1}$ for glucose, fructose, saccharose, and mannitol, respectively. At the beginning of the fermentation process ($t = 0$) the amounts of glucose and fructose are independent of the variety used, respectively, 1.46 ± 0.02 and 1.17 ± 0.02 mol (kg of dry weight)$^{-1}$. The amount of saccharose at $t = 0$ is 39 ± 6 and 125 ± 6 mmol (kg of dry weight)$^{-1}$ for the early and late fermentations, respectively. During the course of the fermentation process the saccharose concentration became virtually zero after, respectively, 5 days for the

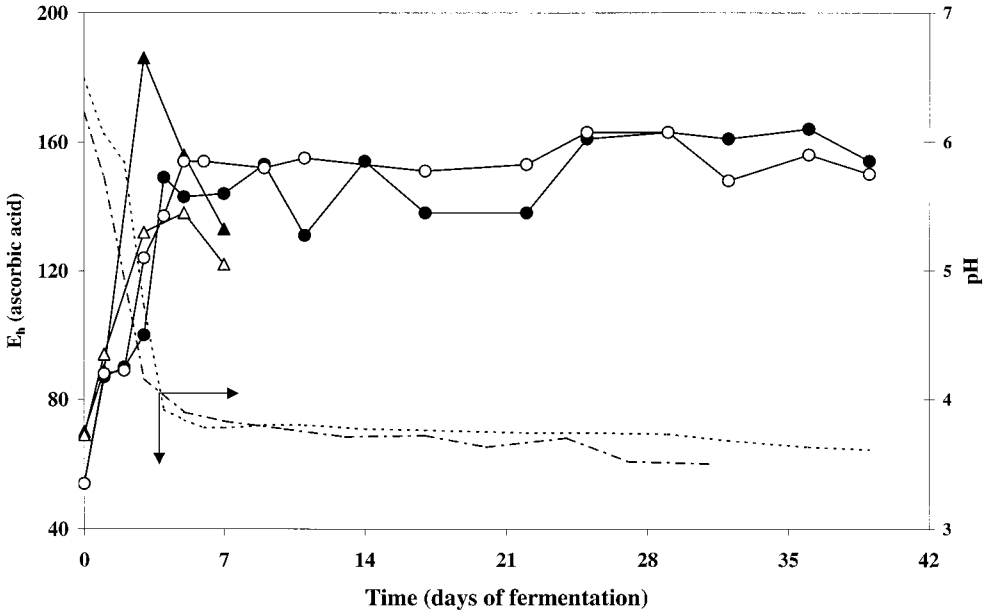

Figure 2. Change in pH and in the calculated redox potential of ascorbic acid during fermentation. Changes in the pH are shown for the early fermentation A (− · −) and late fermentation S (- - -). Changes in the calculated redox potentials, E_h, of the ascorbic acid redox couple, are corrected for the change in pH, during the course of the fermentation process for the early fermentations R (▲) and A (Δ) and the late fermentations M (●) and S (○).

early (R and A) fermentations and ~10 days for the late (M and S) fermentations. No mannitol could be detected at $t = 0$.

Because fermentation is a dynamic process, the changes in the amounts of these sugars are especially important. It was observed that for the four fermentation processes until E_3 (see Table 1) the sum of the amount of fructose and mannitol is constant (1270 ± 51 mmol kg^{-1}; $n = 37$). Because saccharose is hydrolyzed into glucose and fructose, taking the sum of saccharose, fructose, and mannitol until E_3, this value is also constant (1290 ± 52 mmol kg^{-1}; $n = 37$). This is nicely shown in Figure 3; the fructose consumption curve is the mirror image of the mannitol production curve. As suggested, from $t = 0$ until E_3, the heterofermentative phase takes place. During this phase, our results suggest that fructose becomes reduced to mannitol probably by mannitol dehydrogenase (EC 1.1.1.67), at the expense of NADH. If fructose is only converted into mannitol, this also suggests that, during the heterofermentative fermentation phase, glucose and probably also the glucose present in saccharose are the primary carbon sources for the fermentative conversion of sugars into organic acids, ethanol, and carbon dioxide. After about 20 days for the early fermentations (R and A) and 25 days for the late fermentations (M and S), more ore less coinciding with E_4 (see Table 1), mannitol is the only remaining sugar. After complete consumption of saccharose, fructose, and glucose, the amount of mannitol is ~1700 mmol kg^{-1}, independent of the fermentation processes.

For mannitol two production phases can be distinguished. During the first phase, until E_3, the bulk of the mannitol (~75%) is produced, probably at the expense of fructose. During the second phase an additional mannitol production takes place at the expense of glucose, because fructose is almost absent in this second phase. It seems therefore reasonable to assume that glucose is converted into mannitol by the combined action of a glucose isomerase and a mannitol dehydrogenase reaction. After complete exhaustion of both glucose and fructose, mannitol is the only remaining sugar. From this time onward the mannitol concentration slowly starts to decrease. This latter decrease was also observed by Hughes and Lindsay (1985). With regard to the fermentation of mannitol in the absence of glucose and fructose, it cannot be concluded whether mannitol is fermented directly or first converted into fructose (dehydrogenase reaction) followed by the isomerization into glucose, which is subsequently fermented.

In their study Fleming et al. (1988) observed a decrease in the amount of sucrose and fructose during the fermentation process. Glucose showed first an increase followed by a decrease in concentration. This difference in behavior with regard to glucose might be explained by the fact that in their study Fleming et al. (1988) performed the sugar analysis in the brine. In this study the analysis was performed on the basis of the dry matter content (brine plus solids). They also concluded that fructose was converted into mannitol. In addition, they also showed that the bulk of the mannitol production took place during the first 10 days of fermentation. However, on the basis of their results no second mannitol production phase can be distinguished.

With regard to changes in redox potential of the four fermentations studied (see Figure 1 and Table 1), the mannitol production tends to stops at E_2 (see Figure 3). At E_3 the second phase of the mannitol production tends to start. This production phase ends at E_4. For glucose and fructose the same pattern can be observed for all four fermentations in the sense that, instead of production, these sugars are consumed. The rate of consumption of these sugars for the first consumption phase tends to decline at E_1, and the consumption stops at E_2. The onset of the second consumption phase of these sugars restarts around E_3. At E_4 the remainders of glucose and fructose have been consumed. This suggests that E_1, E_2, E_3, and E_4, respectively, coincide with the onset of the decrease in activity, the end of the main activity of the first fermentation phase, the start of the second fermentation phase, and the maximum activity of the second fermentation phase.

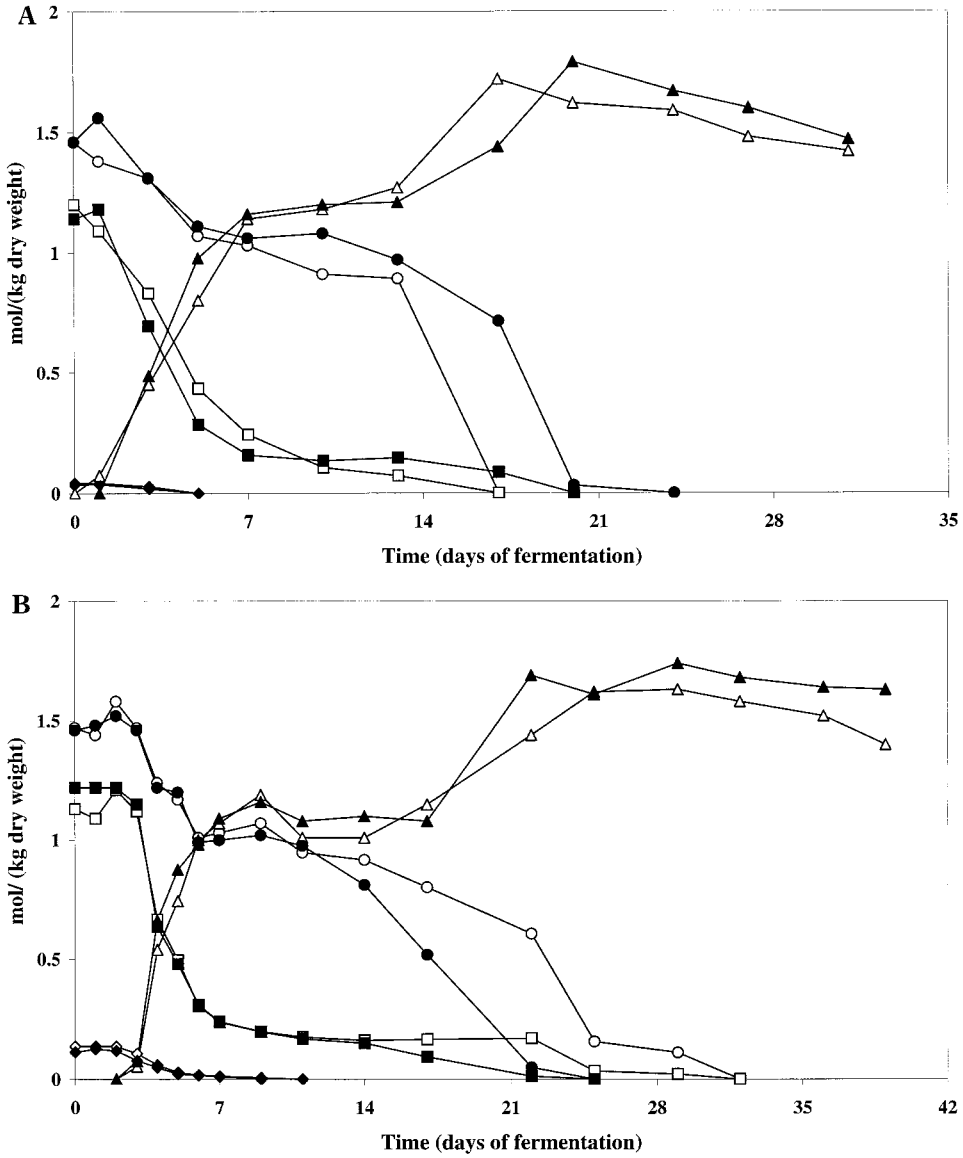

Figure 3. Change in the amount of sugars during the early (A) and late (B) fermentationss: mannitol (▲, Δ), glucose (●, ○), fructose (■, □), and saccharose (◆, ◇). In (A) open symbols represent fermentation R and solid symbols, fermentation A. In (B) open symbols represent fermentation M and solid symbols, fermentation S.

Changes in Organic Acids during the Fermentation Process. During the fermentation of white cabbage into sauerkraut, sugars are converted into organic acids, ethanol, and carbon dioxide. These latter two components were not analyzed in this study. At zero time some oxalic acid and fumaric acid were observed. The amount of these two acids never exceeded 5 mmol (kg of dry weight)$^{-1}$ during the course of the fermentation (data not shown). Also, citric acid [40 ± 8 (n = 8) mmol (kg of dry weight)$^{-1}$] and malic acid [99 ± 7 (n = 8) mmol (kg of dry weight)$^{-1}$] were present at t = 0 in the four cabbage varieties analyzed (data not shown). The amounts of these four acids were so low that the rest of the discussion is focused on the abundant lactic and acetic acids. At zero time, no acetic or lactic acid was present in the varieties M and A. Some lactic acid was present in varieties R and A, which might suggest that fermentation already has been initiated.

The main acids produced during the fermentation are lactic and acetic acid. In Figure 4A (early fermentations) and Figure 4B (late fermentations) the change in the amount of these acids during the fermentation process is shown. The standard deviations for these two acids for all measurements are, respectively, 23 and 12 mmol (kg of dry weight)$^{-1}$ for acetic and lactic acid, respectively.

For the lactic acid production, as for the production of mannitol, a biphasic behavior can be observed. For the four fermentations studied, the rate of production of lactic acid for the first production phase tends to decline at E_1, and the production stops at E_2. The onset of the second production phase of this acid restarts around E_3. At E_4 the lactic acid production seems to be at a maximum. This again suggests that E_1, E_2, E_3, and E_4, respectively, coincide with the onset of the decrease in activity, the end of the main activity of the first fermentation phase, the start of the second fermentation phase, and the maximum activity of the second fermentation phase.

The first phase for the acetic acid production is identical to the lactic acid production, in the sense that more lactic acid is produced than acetic acid. The rate of production of acetic acid for the first production phase tends to decline at E_1, and the production stops at E_2. For the four fermentations during this first production phase of organic acids, which lasts until E_2, the ratio

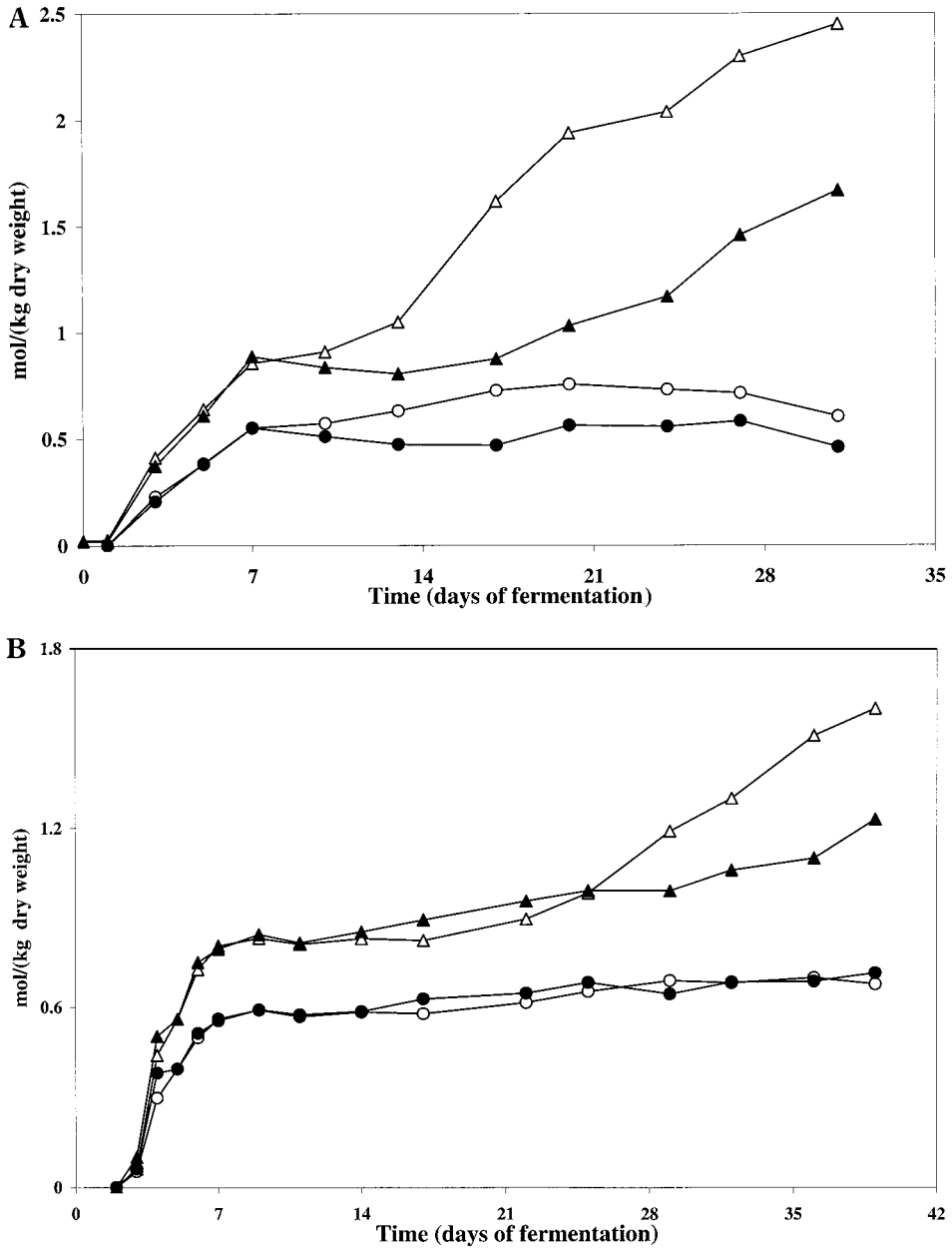

Figure 4. Change in the amount of organic acids during the early (A) and late (B) fermentations: lactic acid (▲, Δ) and acetic acid (●, ○). In (A) open symbols represent fermentation R and solid symbols, fermentation A. In (B) open symbols represent fermentation M and soild symbols, fermentation S.

between the amount of lactic acid and acetic acid is constant: 1.49 ± 0.09 ($n = 56$). This constant ratio suggests a strong coupling between the lactic acid and acetic acid production for the heterofermentative production phase. After this phase the amount of acetic acid tends either to be constant or to increase slightly. Given the small changes in the production of acetic acid in time after the first production phase, no links could be made with either E_3 or E_4. Whatever, the observed coupling between lactic acid and acetic acid production during the heterofermentative phase is lost during the homofermentative phase. In their study Fleming et al. (1988) also showed that after about one week the lactic acid concentration in the brine continuously increased while the acetic acid concentration slightly increased or remained constant.

The end pH values of the early fermentations were slightly more acidic than those of the late fermentations. However, no clear relationship could be obtained between the total amount of acids produced and the pH value at the end of the fermentation process.

Changes in Energy Potential during Fermentation. During sauerkraut fermentation sugars are either converted into lactic and acetic acid, as well as ethanol and carbon dioxide, or into each other. In this research it is attempted to relate transients in the measured redox potential of the brine with the consumption of sugars or the production of organic acids during the fermentation processes. It is, however, difficult to compare the underlying chemistries of the fermentation processes at different stages.

To obtain insight into this underlying chemistry, the potential energy contained in both the sugars and acids can be expressed in the general biological energy carrier ATP, as ATP equivalents, given their complete oxidative dissimilation. This implies that 1 mol of sucrose, glucose, fructose, mannitol, lactic acid, and acetic acid is equivalent to, respectively, 76, 38, 38, 41, 18, and 11 mol of

Table 2. Amount of ATP Equivalents during Fermentation

ref point	ATP equiv (mol/kg of dry wt) during fermentation process							
	R		A		M		S	
	sugars	acids	sugars	acids	sugars	acids	sugars	acids
start[a]	100	0.4	105	0.4	108	0.0	111	0.0
E_1	95.6	21.5	93.1	15.2	104	11.2	105	12.8
E_2	95.6	21.5	95.4	20.7	84.0	20.9	87.8	21.0
E_3	87.0	22.7	89.4	21.0	83.6	22.9	71.3	24.9
E_4	70.5	37.2	68.5	27.2	71.8	29.0	67.2	27.4
end	58.2	50.7	60.2	35.1	57.4	36.3	66.9	30.0

[a] Average value of days 0 and 1.

ATP. In making this transformation, the total energy contained in the sugars and formed acids can be followed and compared during the fermentation process. As discussed above, the points indicative of changes in redox potentials, E_1-E_4, are of importance. In Table 2, the ATP equivalents of the sugars and major organic acids (lactic and acetic acid) at the start, E_1-E_4, and end of the fermentation processes are given. From this table the following expected trends can be given. First, both the total sugar ATP equivalents decrease and the total organic acid ATP equivalents increase during fermentation. Second, the total ATP equivalents (sugars plus organic acids) decrease during fermentation due to carbon dioxide and ethanol production and microbial growth. With respect to the information presented in Table 2, two points are of major interest, E_2 and E_4. At E_2, the ratios of the ATP equivalents of the sugars over the organic acids are 4.45, 4.61, 4.02, and 4.18 for the fermentations R, A, M, and S, respectively. At E_4, the ratios of the ATP equivalents of the sugars over the organic acids are 1.90, 2.52, 2.47, and 2.45 for the fermentations R, A, M, and S, respectively. These latter ratios are, respectively, reached after about 15, 24, 28, and 36 days. These data indicate that the value of the ratio of the ATP equivalents of the sugars over the ATP equivalents of organic acids is constant, both at E_2 and at E_4, with the exception of E_4 for fermentation R. It is therefore suggested that E_2 and E_4 represent unique points in the sauerkraut fermentation process, where an energy balance exists, on the basis of ATP equivalents, between the sugars present and organic acids produced during the fermentation process. The observation that the value of this ratio at E_4 is lower for fermentation R, compared to the other fermentations, might be related to a difference in the fermentation process (microbiological spoilage) causing the strong off-odor formation of fermentation R. In addition, it should be noted that the value for the total ATP equivalents (sugars plus organic acids) is lower at the start of the experiment ($t = 0$) than at E_1. This difference might be caused by the fact that some sugar might be present in the form of, for example, starch, which was not analyzed.

Conclusion. The change in redox potential, as corrected for the pH, was related to changes in the amount of the most abundant sugars (glucose, fructose, and mannitol) and most abundant organic acids (acetic acid and lactic acid). Changes in the amount of sugars and acids mentioned are probably associated with a change in microbiological activity (heterogeneous and homogeneous fermentations). It is suggested that E_1, E_2, E_3, and E_4, respectively, coincide with the onset of the decrease in activity of the first fermentation phase, the end of the main activity of the first fermentation phase, the start of the second fermentation phase, and the maximum activity of the second fermentation phase. It is concluded that, on the basis of energetic considerations, both E_2 and E_4 represent unique points in the sauerkraut fermentation process, where an energy balance exists, on the basis of ATP equivalents, between the sugars present and the organic acids produced during the fermentation process.

A continuously performed redox potential measurement indicates the progress and state of the sauerkraut fermentation process. This progress and state is substantially better indicated by the redox potential of the system than by the pH. However, the redox potential as such is not a proper indicator of fermentations when off-odor formation takes place.

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