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ORIGINAL PAPER

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Acidity reduction in northern region berry juices by the malolactic bacterium *Oenococcus oeni*

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Abstract Acidity of juices from northern berries was reduced by inoculating with a malolactic microorganism, *Oenococcus oeni*. The berries were white and black currant, bilberry, lingonberry, and, for comparison, an apple cultivar. Malic acid was first converted to lactic acid in all fermentations, while soluble sugars and citric acid remained unattacked. Upon exhaustion of malic acid, sugar and citric acid degradations were initiated simultaneously. Sequential utilization of substrates by *O. oeni* offers a basis for multitude compositional changes in acidic juices. Elimination of malic acid alone led to a noticeable reduction of acidic taste in these berry juices.

Keywords Malolactic fermentation · Berry juices · Deacidification · *Oenococcus oeni*

Introduction

Malolactic fermentation (MLF) is a part of the traditional winemaking techniques for red and white wines. The term MLF refers to the most noticeable activity of the lactic acid bacteria, namely the conversion of L-malic acid to L-lactic acid and CO_2. This decarboxylation reduces the acidity of the juice or wine and can produce a more balanced wine. In addition to deacidifying, the MLF also affects the final aroma and taste balance of wine by modifying fruit derived aromas and producing aroma active compounds [1, 2, 3, 4, 5].

Transformation of L-malic acid to L-lactic acid is carried out by strains of lactobacilli, leuconostocs, oenococci, and pediococci. The most common malolactic-fermenting organism in wines is *Oenococcus oeni* (formerly *Leuconostoc oenos*). It is a heterofermentative coccus, which has in addition to malolactic activity, high tolerance to low pH and ethanol. The bacterium also converts citric acid into acetic acid and has the capacity to regenerate in the pH of wine. Furthermore, it produces small quantities of acetic acid without altering organoleptic characteristics as far as it does not ferment sugars [6, 7, 8].

Berries – wild or cultivated – are traditionally a part of the natural diet among northern Europeans. Northern berries are unpolluted and their nutritional value is high. They contain a lot of fiber, vitamins and minerals and wild berries in particular contain plenty of flavonoids. Bilberry, for example, has through the ages been used as a medical herb [9, 10, 11]. However, due to the short growing season, only a small portion of the berry harvest is consumed as fresh berries, and the rest is processed to various products. One of the problems in processing of the northern region berries is that they are rich in organic acids. The pH of berries is very low (pH 2.7–3.6) and due to the low sugar content the flavor is also very acidic. Much of this acidity results from the presence of citric acid and malic acid. Especially, the latter causes in high concentrations undesirable acidity in the product. The main sugars are glucose and fructose [12, 13].

The use of northern region berries could be markedly enhanced if the acidity of the juices could be controlled. However, deacidification of berry juices and wines by MLF has been less investigated. Therefore, the malolactic bacterium *Oenococcus oeni* was tested for its applicability in deacidification of four typical northern berry juices without a significant loss of sugar content.

Materials and methods

Microorganism

The microorganism used was *Oenococcus oeni* (formerly *Leuconostoc oenos*) obtained from the American Type Culture Collection (ATCC 39401). The strain was stored in 10% (v/v) glycerol at –60 °C until used.

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Berries and apple

Ripe Finnish black currants, white currants, bilberries, and lingonberries were obtained from Pakkasmarja Oy (Suonenjoki, Finland) and stored at –25 °C until used. The berries were allowed to digest for 2–3 d at +4 °C. The thawed berries were minced and the juice was extracted in a hydraulic press.

The apple variety used was Dutch Jonagold bought at a Finnish grocery store. The apples were peeled, chopped into small pieces and treated with Panzym® Super E-enzyme (Novo Nordisk Ferment Ltd.). After 1.5 h incubation at 30 °C the apple pieces were crushed and the juice was extracted in a hydraulic press.

Cultivation

The bacterium was first cultivated in an MRS medium supplemented with 2 g L^{-1} citric and 2 g L^{-1} L-malic acid. The pH was adjusted to 5.0. The tubes were incubated at 30 °C without stirring for 3–4 d, so that the final cell density was 10^8–10^9 CFU mL^{-1}.

To avoid reduction of the viable cells during the fermentations the bacteria were adapted to the different juices by cultivating in a second medium (adaptation medium) containing distilled water and the respective juice in a 1:1 ratio, supplemented with 5 g L^{-1} yeast extract. The pH was adjusted with NaOH to 3.6. From the supplemented MRS medium a 1.5 mL aliquot was transferred to 15 mL of the adaptation medium and incubated at 27 °C for 3–4 d, so that the final cell density was 10^7–10^9 CFU mL^{-1}.

Media used

Each berry juice (black currant, white currant, bilberry, lingonberry) and the apple juice were used as two different media (150 mL each), an autoclaved (12 min, 121 °C) 1:1 dilution of juice and the corresponding non-autoclaved juice. These media were inoculated by 15 mL of the fully developed adaptation medium. The inoculated media were plugged with aluminum foil and incubated without stirring in an incubator at 25 °C. Each bottle was inoculated in duplicate and an uninoculated bottle was used as a comparison.

Assay of acid, sugar, and ethanol concentrations

Organic acids, sugars, and ethanol were analyzed by using a Hewlett Packard (HP Series 1100) high-performance liquid chromatograph (HPLC) equipped with an Aminex HPX-87 H^+ column (300×7.8 mm, 9 µm) (Bio-Rad Laboratories). Column temperature was 35 °C and elution was carried out with 5 mM H_2SO_4. The flow rate was 0.6 mL min^{-1}. For benzoic acid analyses a Hypercil BDS C8 reverse phase column (250×4.6 mm, 5 µm) (Hypercil) was used. The column temperature was 30 °C, the eluent was 0.05 M phosphate buffer and methanol (1:1) and the flow rate was 1.0 mL min^{-1}. Acids were detected with a UV detector (Hewlett Packard) at 214 nm and the sugars with an RI detector (Hewlett Packard). Prior to sugar analyses the samples were exposed to a strong anion exchange column (Varian) to remove the acidic compounds. For quantification an internal standard with formic acid and xylitol was used. Before injection, all the samples were centrifuged and filtered through a membrane filter (pore size 0.2 µm). The quantification of L- and D-malic acid was performed by using commercial enzymatic test kits (Roche).

Determination of viable cells

Viable counts of *O. oeni* were expressed as colony-forming units (CFU) per mL of solution. For colony counting, MRS medium supplemented with agar (15 g L^{-1}) was used. The pH was adjusted to 5.0. The plates were incubated at 30 °C for 6–9 d.

Results and discussion

Acid and sugar contents of non-fermented juices

The average acid and sugar concentrations of berry and apple juices chosen for the study are presented in Tables 1 and 2.

The total concentration of acids in the berries and the apple appeared to be lower than has previously been re-

Table 1 The contents and relative proportions of major acids in different berry and apple juices

	Malic Acid	Citric Acid	Acetic Acid	Benzoic Acid	Total Acids
White Currant	3.7 g L^{-1}	30.5 g L^{-1}	–	–	34.2 g L^{-1}
	11%[a]	89%	–	–	100%
Black currant	3.8 g L^{-1}	34.2 g L^{-1}	–	–	38.0 g L^{-1}
	10%	90%	–	–	100%
Bilberry	2.6 g L^{-1}	6.3 g L^{-1}	0.7 g L^{-1}	–	9.6 g L^{-1}
	27%	66%	7%	–	100%
Lingonberry	0.8 g L^{-1}	12.6 g L^{-1}	4.4 g L^{-1}	0.9 g L^{-1}	18.7 g L^{-1}
	4%	67%	24%	5%	100%
Apple	3.9 g L^{-1}	–	–	–	3.9 g L^{-1}
	100%	–	–	–	100%

[a] The percentages are values of the total acids analyzed

Table 2 The contents and relative proportions of major sugars in different berry and apple juices

	Glucose	Fructose	Sucrose	Total Sugars
White Currant	51.5 g L^{-1}	52.3 g L^{-1}	–	103.8 g L^{-1}
	50%	50%	–	100%
Black currant	42.9 g L^{-1}	51.0 g L^{-1}	2.4 g L^{-1}	96.3 g L^{-1}
	45%	53%	2%	100%
Bilberry	34.6 g L^{-1}	46.0 g L^{-1}	–	80.6 g L^{-1}
	43%	57%	–	100%
Lingonberry	52.4 g L^{-1}	56.5 g L^{-1}	1.5 g L^{-1}	110.4 g L^{-1}
	48%	51%	1%	100%
Apple	21.5 g L^{-1}	71.7 g L^{-1}	24.0 g L^{-1}	117.2 g L^{-1}
	18%	61%	21%	100%

[a] The percentages are values of the total sugars analyzed.

ported in the literature [14, 15, 16]. For example, Matala [17] reports very high acid concentrations for black currant; 6 g L^{-1} malic acid and 40 g L^{-1} citric acid. On the other hand, other reports and food composition tables are in agreement with our results on acid contents [13, 15, 18, 19, 20].

The berries and apple contained a notably higher level of total sugar than reported by some authors [13, 14, 15, 17, 18, 19, 21, 22, 23, 24]. For example, according to Haila [12] the total sugar content of black currant is only 46.4–57.0 g L^{-1}. Furthermore, Souci et al. [13] report the glucose and fructose concentrations of lingonberry to be only 26.6–40.0 g L^{-1} and 7.4–33.6 g L^{-1}, respectively. However, there exist also data for different berries and apple which are in accord with present results on the total sugar content [13, 14, 15, 25].

Sucrose concentration varied widely from one juice to another. This is probably due to the enzymatic or chemical hydrolysis of sucrose to glucose and fructose that occurs due to disruption of the cellular structure during juice extraction [26]. Skrede [27] reports that during thawing as much as 70% of sucrose is degraded by invertase.

Regardless of these variations in reported and present data, malic acid and citric acid were the main acids in the investigated berries. Thus, most essential is the control and diminishing of these two acids by the malolactic fermentation. On the other hand, as the total sugar content of the berries is high, the loss of sugar during the malolactic fermentation should be avoided.

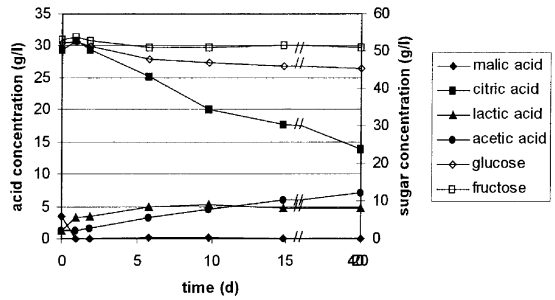

Fig. 1 Fermentation of autoclaved white currant juice

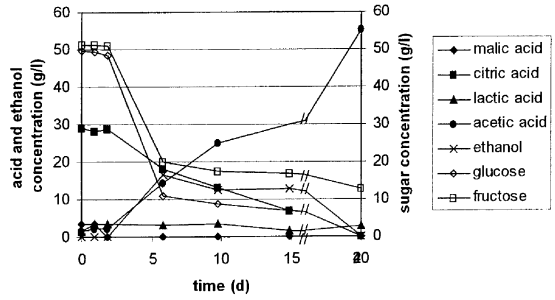

Fig. 2 Fermentation of non-autoclaved white currant juice

Fermentation of white currant juice

Autoclaved juice was inoculated by *O. oeni* to equal a viable cell count of 10^8 CFU mL^{-1}. This cell density has been reported as sufficient for proper malolactic activity in the medium [28, 29]. Fig. 1 indicates that malic acid was completely metabolized to lactic acid during the first day from inoculation without degradation of citric acid. The pH increased by about 0.1 units. Removal of malic acid occurred also without a significant loss of sugars. However, when malic acid was exhausted, citric acid and sugar degradations were initiated simultaneously. Citric acid was metabolized faster than glucose and fructose. After 40 d the citric acid content was diminished by about 12 g L^{-1} (40%) and the total sugars by only about 8 g L^{-1} (7%). The pH increased further by 0.3 units during the citric acid degradation and approximately 5 g L^{-1} acetic acid was formed. Because citric acid is degraded by citrate lyase into an equimolar amount of acetic acid, practically all the acetic acid came from the degradation of citric acid. Practically no changes in either sugar or acid concentrations were detected after 15 d, with the exception of the further degradation of citric acid. During the fermentations the viable cell density did not change. Overall, white currant juice was not a too difficult medium for *O. oeni*. Thus, the sequential malolactic utilization of substrates, which we have described in synthetic media [30], applied at least also to autoclaved white currant juice.

When the juice was fermented as above but without prior autoclaving, the malolactic reaction was still evident (Fig. 2). Malic acid was degraded within 2 d indicating a slight delay in the rate of fermentation. However, consumption of citric acid and sugars was arrested until malic acid was used up. Consequently, sequential degradation of the acids and sugars can be achieved also without sterilizing the juice. When the degradation of sugars initiated it proceeded faster than in the autoclaved juice. This faster sugar degradation suggests the presence of yeasts in the juice. The formation of ethanol during sugar utilization also supports this view. The fact that acetic acid was formed more (55 g L^{-1}) than expected from degradation of all the citric acid may reflect the presence of acetic acid bacteria. Plating of the non-autoclaved juices on MRS medium revealed the presence of wide range of microbes. Regardless of this, the MLF by *O. oeni* did occur.

The third set of fermentations consisted of non-autoclaved uninoculated juices (Fig. 3). The sequential utilization of fermentation substrates typical for the malolactic fermentation did not occur. Instead, Fig. 3 shows reactions typical for yeasts and acetic acid bacteria. Sugars were fermented to ethanol in about 10 d. Then, the acetic acid concentration increased. After 15 d, reactions typical for lactic acid bacteria occurred. Thus, it is evident that inoculation by *O. oeni* was required if the conver-

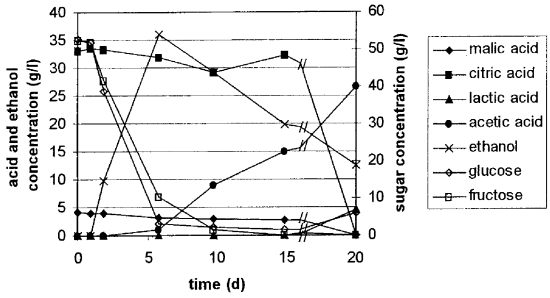

Fig. 3 Fermentation of non-autoclaved and uninoculated white currant juice

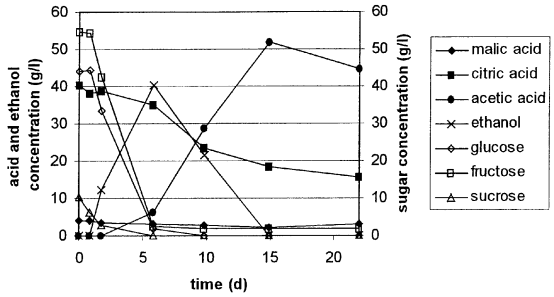

Fig. 5 Fermentation of non-autoclaved and uninoculated black currant juice

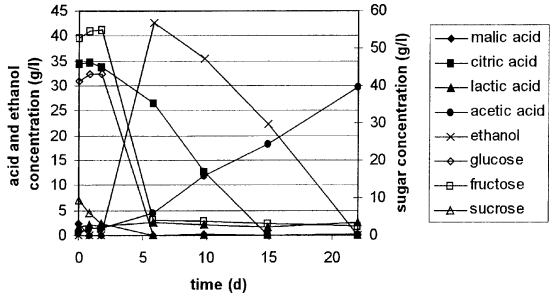

Fig. 4 Fermentation of non-autoclaved black currant juice

sion of malic acid into lactic acid is set as the primary purpose of fermentation.

Fermentation of black currant juice

Autoclaved black currant juices were fermented similarly as the white currant juices and after inoculation the cell content was about 10^8 CFU mL^{-1}. Malic acid degraded within 1 d into lactic acid while other fermentable compounds remained practically unchanged. The pH increased during this period by 0.1–0.2 pH-units. As malic acid was exhausted, citric acid and sugar degradations were again initiated simultaneously but the sugars were consumed at much slower rates than citric acid. During the degradation of citric acid the pH was raised further by 0.3–0.6 units. Thus, in spite of the differences in coloring matter in black and white currant juices, the fermentation and selectivity of substrate utilization were similar.

The fermentation of non-autoclaved black currant juice (Fig. 4) proceeded in outline as the fermentation of the non-autoclaved white currant juice. Malic acid was degraded first after the inoculation of *O. oeni* while the consumption of citric acid and sugars was arrested. However, when yeast-type sugar utilization was initiated, it proceeded faster and to a greater degree than in white currant juice. During about 6 d practically all sugars were fermented and ethanol was formed in three times the amount formed in the white currant juice. Ethanol formation was followed by acetic acid formation reflecting the presence of acetic acid bacteria. Slight differences in acid and sugar metabolism between these two berries suggest the presence of differing natural flora of the two berry juices. However, the natural microbial flora of the black currant juice did not prevent the selective degradation of malic acid by *O. oeni*.

The above typical features of malolactic fermentation were not detected in the fermentation of non-autoclaved, uninoculated black currant juice (Fig. 5). Practically no malic acid was degraded in 21 d and citric acid degradation was initiated immediately. Sugars were exhausted in about 6 d with concomitant formation of ethanol. After that ethanol was converted to acetic acid. This indicates that black currant juice has no malolactic activity and suggests that natural microbes, such as yeasts and acetic acid bacteria, are present.

Fermentation of bilberry juice

Bilberry was chosen for the fermentation tests as the relative content of malic acid in bilberry was two times higher than in currants (Table 1). Correspondingly, this berry contained less citric acid. Furthermore, bilberry contained also less sugar than the currants. Bilberry also has a very intensive color.

In the autoclaved juices, contrary to currant juices, malic acid degradation started first but did not proceed to completion in spite of the 10^8 CFU mL^{-1} inoculation. In about 2 d only half of the initial malic acid was consumed (Fig. 6). Nevertheless, during the period of malic acid degradation the degradations of the other substrates were arrested and the pH was raised by 0.1–0.2 units. When the rapid phase of malic acid degradation ended, citric acid and sugar degradations were initiated. The degradations proceeded slowly and only partially within 14 d. This led to the question as to why only half of the malic acid was utilized and did the residual malic acid inhibit the degradation of citric acid and sugars?

As only about 50% of malic acid was utilized and since *O. oeni* metabolizes only the L-form of the acid [1,

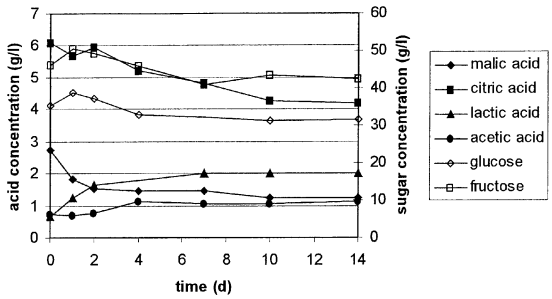

Fig. 6 Fermentation of autoclaved bilberry juice

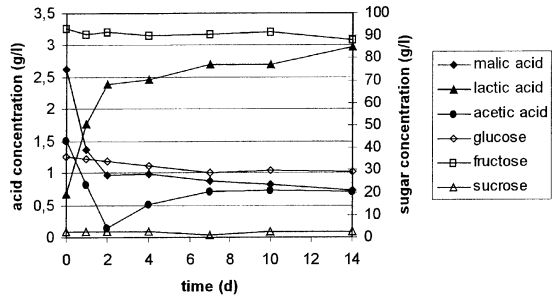

Fig. 7 Fermentation of autoclaved apple juice

4, 5] the possibility that the juice contained a racemic mixture could not be excluded. However, the juices did not contain detectable amounts of the D-form (detection limit 0.35 mg L^{-1}). A more probable explanation comes from the observation that the initial cell content (over 10^8 CFU mL^{-1}) was reduced to fewer than 10^6 CFU mL^{-1} within 6 d. This amount is not sufficient to maintain a malolactic fermentation [28, 29]. The very slow degradation of the acids and sugar was probably a reflection of both the weak residual activity of the malolactic bacterium and the inhibitory effect of the residual L-malic acid on the degradation of citrate and sugars.

Several reasons may account for the poor viability of *O. oeni* in bilberry juices. Amongst them may be natural antimicrobial compounds, low pH (3.0) or lack of essential nutrients.

However, malolactic fermentation may prove advantageous also for bilberry juice. Sufficient positive effects on pH or sensory values may be obtained even by partial reduction of malic acid, especially since during this period the degradation of sugars and citric acid can be arrested as with the currant juices. If, however, a more profound reduction in malic acid is sought for, renewal of the inoculum during the fermentation might prove to be sufficient.

In the non-autoclaved inoculated bilberry juice the rate of malic acid degradation was very slow and the consumption of sugars initiated from the beginning of the fermentation. Also ethanol formation was noticed. These phenomena were most probably due to the presence of yeasts in the juice.

In the non-autoclaved bilberry juice without the inoculation, degradation of malic acid or citric acid was not detected. Only slight degradation of sugars appeared. Thus, bilberry juice itself presumably does not contain any microorganisms with malolactic activity.

Fermentation of lingonberry juice

The most problematic compound in the fermentation of lingonberry juice is benzoic acid, which inhibits the yeast fermentative metabolism. According to Chipley [31], benzoic acid functions effectively also against bacteria if the concentration is 0.1% and the pH is low, as it is in lingonberry juice. However, it has not been proven that benzoic acid in lingonberry juice also inhibits the malolactic activity of *O. oeni*. In addition, the pH of lingonberry juice is very low (2.7–2.8). On the other hand, lingonberries are richer in sugar, color and flavor compounds than the sweeter-tasting bilberries, although acids cover the sweetness of lingonberries. Due to these facts lingonberry juice was a challenging material to be tested for malolactic fermentation.

The viable cell density of the inoculum decreased as early as the second adaptation cultivation, although the decrease was not drastic. However, during the fermentations of the autoclaved juices the cells died completely after 14 d. The low pH of the juice could not be solely responsible for this. The presence of benzoic acid is the most probable explanation for the cell death and for the observation that neither acids nor sugars were degraded. Thus, the pH did not change either. Only in the non-autoclaved and uninoculated juice could a trace degradation of sugars be detected due to other natural microbes probably more tolerable to benzoic acid.

Fermentation of apple juice

For comparison, MLF was also applied to apple juice. Its malic acid concentration is high (about 4 g L^{-1}), it lacks citric acid (Table 1), and contains over 100 g L^{-1} total sugars (Table 2).

The reactions in autoclaved apple juice are shown in Fig. 7. The malic acid degraded first to lactic acid in about 2 d to approximately half of the initial concentration. The degradation rate decreased but continued slowly during the 14 d of fermentation. However, the degradation did not proceed to completion. Lactic acid was the product and the pH was raised during the malolactic degradation by approximately 0.1–0.2 units. During the whole fermentation period the sugar concentrations remained practically unchanged, probably due to the presence of malic acid. Thus, the sequential utilization of malic acid and sugars was again noticed, even though citric acid was not present. The deceleration of the rate of degradation of malic acid after 2 d of fermentation may be due to the lowered content of living cells which by that time equaled 10^7 CFU mL^{-1}.

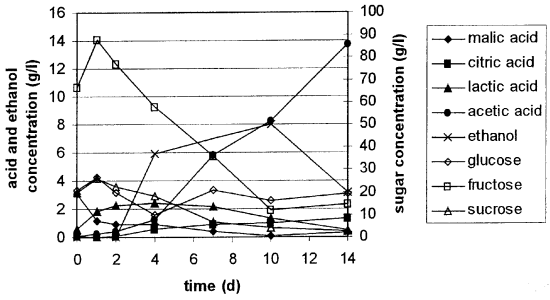

Fig. 8 Fermentation of non-autoclaved apple juice

The fermentation of non-autoclaved inoculated apple juice proceeded differently (Fig. 8). Malic acid was degraded by *O. oeni* to a larger extent and during this period the degradation of sugars was arrested. Following the malic acid degradation the sugar degradation commenced rapidly and also acetic acid was formed to a larger extent. Surprisingly, citric acid was formed after the fast initial period of MLF. In addition, slight formation of ethanol was noticed. All these reactions, other than malic acid degradation, were probably due to rich natural microbe flora present in the apple juice.

Without inoculation of the non-autoclaved apple juice, malic acid was degraded and its rate of consumption was faster than in any of the berry juices used. This indicates that apple juice itself contained MLF-active microbes in contrast to the berries. As in the non-autoclaved inoculated apple juice, also in the uninoculated juice, some citric acid was formed after the main period of malic acid degradation.

Apple juice was clearly a good medium for *O. oeni*. The pH was higher (pH 3.4–3.6) than in berry juices and the cells survived well in the juice. The concentration of the living cells even increased during the first week of the fermentation. Thereafter the cell counts decreased again, which can partially explain the deceleration of the malic acid degradation after about 3 d.

Conclusions

The results show that the typical malolactic reactions of *O. oeni* shown earlier in synthetic media [30] apply also to northern region berry juices. Malic acid can be eliminated with practically no consumption of sugar or citric acid and therefore the excess acidity typical for many berry juices can be attenuated. Malic acid is degraded in both sterilized and non-sterilized juices if inoculated with living cells of *O. oeni*. Even though the content of malic acid is low in comparison to other acids, its selective degradation by *O. oeni* causes a noticeable change in the acidity by these berries. Due to undesired reactions after the degradation of malic acid, proper timing of the duration of MLF is essential, especially if the natural microflora is not eliminated prior inoculation.

References

1. Beelman RB, Gallander JF (1979) Adv Food Res 25:1–53
2. Peynaud E (1984) Malolactic fermentation and lactic bacteria. In: Knowing and making wine. Wiley, New York, pp 120–138
3. McDaniel M, Henderson LA, Watson BT Jr, Heatherbell D (1987) J Sensory Studies 2:149–167
4. Henick-Kling T (1993) Malolactic fermentation. In: Fleet GH (ed), Wine microbiology and biotechnology. Harwood Academic Publishers, Chur, pp 289–326
5. Henick-Kling T, Acree TE, Krieger SA, Laurent M-H, Edinger WD (1995) Modification of wine flavor by malolactic fermentation. 100 Jahre Hefereinzucht in Geisenheim, pp 58–67
6. Sharpe ME (1981) The genus *Lactobacillus*. In: Starr MP, Stolp H, Trüper HG, Balows A, Schegel HG (eds), Prokaryotes, vol 2. Springer, Berlin Heidelberg New York, pp 1653–1679
7. Buckenhüskes HJ (1993) FEMS Microbiol Rev 12:253–273
8. Nielsen JC, Richelieu M (1999) Appl Environ Microbiol 65:740–745
9. Viberg U, Sjöholm I (1996) Livsmedesteknik 8–9:38–39
10. Häkkinen S, Heinonen M, Kärenlampi S, Mykkänen H, Ruuskanen J, Törrönen R (1999) Food Res Int 32:345–353
11. Jansa K (ed) (2000) Kotimaisia marjoja vai tuontihedelmiä? Helsinki: YLE–Finnish Broadcasting Company, Available from: www.yle.fi/ekoisti/arkisto1/27–09.htm, accessed Apr 12.
12. Haila K (1990) Markkinoilla olevien vihannesten, marjojen sekä omenien sisältämät sokerit ja orgaaniset hapot. MSc thesis, University of Helsinki, 103 p
13. Souci SW, Fachmann W, Kraut H (1994) Food composition and nutrition tables, 5th edn. Scientific Publishers, Stuttgart, 1091 p
14. Salo M-L, Suomi K (1972) Maataloustieteellinen Aikakauskirja 44:68–75
15. Fuchs G, Wretling S (1991) Vår Föda 43:425–438
16. Podsędek A, Wilska-Jeszka J, Anders B, Markowski J (2000) Eur Food Res Technol 210:268–272
17. Matala V (1999) Herukan viljely. Puutarhaliiton julkaisuja nro 306, opas nro 44, Helsinki, 267 p
18. Solberg Y (1980) Våre Nyttevekster 75:82–85
19. Haila K, Kumpulainen J, Häkkinen U, Tahvonen R (1992) J Food Comp Anal 5:108–111
20. Dueñas M, Irastorza A, Fernandez K, Bilbao A, Huerta A (1994) J Food Sci 59:1060–1064
21. Green A (1971) Soft Fruits. In: Hulme AC (ed) The biochemistry of fruits and their products. Academic press, London, pp 375–410
22. Southgate DAT, Paul AA, Dean AC, Christie AA (1978) J Human Nutr 32:335–347
23. Varo P, Laine R, Veijalainen K, Espo A, Wetterhoff A, Koivistoinen P (1984) J Agric Sci Finland 56:49–59
24. Livsmedelstabell (1993) Energi och näringsämnen. Statens Livsmedelsverk, Norstedts tryckeri, Uppsala, 213 p
25. Wrolstad RE, Shallenberger RS (1981) J Assoc Off Anal Chem 64:91–103
26. Plowman JE, Love JL, Herbert BR (1989) Int J Food Sci Technol 24:521–527
27. Skrede G (1983) J Food Sci 48:1094–1096
28. King SW (1985) Method for converting malic acid to lactic acid in wine. US Patent no. 4,562,099
29. Prahl C, Nielsen J (1993) A method of inducing malolactic fermentation in wine or fruit juice by direct inoculation with a non-activated starter culture. International Patent no. WO 93/20180
30. Viljakainen S, Laakso S (2000) Eur Food Res Technol 211:438–442
31. Chipley JR (1983) Sodium benzoate and benzoic acid. In: Branen AL, Davidson PM (eds), Antimicrobials in foods. Marcel Dekker Inc, New York, pp 11–35