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Synthesis of higher alcohols during cider processing

Rajko Vidrih*, Janez Hribar

Department of Food Science and Technology, Biotechnical Faculty, Jamnikarjeva 101, 1111 Ljubljana, Slovenia

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Abstract

Ciders from two apple cultivars Gloster and Gala, were prepared. Apples were crushed and juice was extracted, immediately after crushing or after 24 h of maceration. Juice was allowed to ferment at 15°C and analysed for higher alcohols and sugars during fermentation. All higher alcohols analysed increased during fermentation as a result of yeast activity. More higher alcohols were synthesised in cider made from Gala cultivar than in cider from Gloster cultivar. Maceration increased the synthesis of iso-amyl alcohol, 2-phenyl ethanol, *n*-propanol, *n*-butanol, iso-butanol and methanol. Sensory evaluation classified cider made from non-macerated Gloster cultivar as the best followed by non-macerated Gala cultivar and macerated Gala cultivar. Sensory evaluation score correlated negatively to the sum of higher alcohols. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Apples; Maceration; Cider; Methanol; Higher alcohols; Sensory evaluation

1. Introduction

Cider is quite popular in northern Slovenia, where climatic conditions allow no wine production. Many compounds are known to contribute to the taste and aroma of cider. These compounds include several higher alcohols and their esters each synthesised during apple ripening, juice fermentation or later, during storing of the cider in casks or in bottles. Higher alcohols may influence certain sensory characteristics although they constitute a relatively lesser quantity of the total substances. Rapp and Mandery (1987) found the total higher alcohols in wine to be in the range 80–540 mg l^{-1}; concentrations up to 300 mg l^{-1} contribute to pleasant flavour, but concentrations above 400 mg l^{-1} provoke unpleasant flavour and harsh taste. Some higher alcohols, particularly iso-amyl alcohol, contribute to unpleasant flavour (Rous, Snow & Kunkee, 1983), although Jepsen (1978) reported a positive correlation of *n*-butanol to the scores of aroma quality of apple juice. Traditional cider manufacturing uses wooden mills to crush the fruit and batch mechanical presses to extract the juice. Fermentation is carried out in wooden casks with no temperature control. Wild microflora having their origin on fruit or, on the surface of processing equipment, bring the fermentation to an end. Employing the traditional methods may cause uncontrolled fermentation and variations in final cider quality. The malo-lactic fermentation, i.e. conversion of L-malate into L-lactate by lactic bacteria may follow when favourable conditions are met. A new approach in cider manufacturing involves the use of selected yeasts, fermentation temperature control as well as suitable apple-cider cultivars. Higher alcohols are synthesised during fermentation from oxo-acids having their origin in amino acids and sugar metabolism (Mangas, Cabranes, Moreno & Gomis, 1994). Riberau-Gayon, Peynaud, Riberau-Gayon and Sudraud (1975) report that 10% of total higher alcohols is synthesised from corresponding amino acids, 65% from other amino acids and 25% from sugars. According to (Reazin, Scales & Andreasen, 1973) *Sacharomyces cerevisae* yeast produces only amyl alcohol from the amino acid isoleucine, while it produces amyl, iso-amyl and *n*-propanol from the amino acid threonine. A major conversion of sugars during fermentation provides a higher concentration of higher alcohols in cider (Mangas et al.). Spontaneous clarification of apple juice increased higher alcohols in cider compared to enzymatic clarification (Mangas et al.). Fruit maturity influences the synthesis of higher alcohols during fermentation; their concentration in Semillon wine tends to be lower in wine made from later harvested grape (Ribereau-Gayon & Sudraud, 1991).

* Corresponding author.

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Blanco, Moran, Gutiérezz, Moreno, Dapena and Mangas (1992) reported an increase in total N during ripening of some Spanish cider apple cultivars. Total N correlates negatively with the synthesis of total higher alcohols during must fermentation (Ough & Bell, 1980). Maceration of white grapes in the presence of endogenous pectolytic enzymes yields wines with increased levels of iso-amyl alcohol and 2-phenyl ethanol and a lower level of *n*-propanol (Bosso, 1993). The removal of large particles before fermentation is necessary to obtain high quality wines. Must clarification, by means of precipitation and sediment removal, yields wines with less higher alcohols than to non-clarified musts (Ribereau-Gayon & Sudraud, 1991). Such wines are considered to be fresh, fruity and delicate (Klingshirn, Lui & Gallander, 1987). The same authors found wines made from turbid juice harsh by taste and higher in higher alcohols. Presence of apple juice insoluble particles increases the content of fusel oils; any removing of insoluble particles reduces fusel oils in cider (Beech & Carr, 1977). Fermentation does not significantly influence the concentrations of *n*-pentanol, *n*-octanol or benzyl alcohol (Moyano, Moreno, Millan & Medina, 1994), while the synthesis of acetic acid, iso-butanol and iso-amyl alcohol during fermentation depends primarily on yeast strain employed (Giudici & Kunkee, 1994; Kunkee & Vilas, 1994; Leguerinel, Mofart, Cleret & Burgeois, 1989). Beech and Carr suggest that yeast strain plays a decisive role in fusel oil synthesis. Giudici, Romano and Zamonelli (1989) and Giudici, Altieri and Gambini (1993) showed that production of higher alcohols during must fermentation depends on yeast strain and grape cultivar. According to Ough, Guymon and Crowell (1966) fermentation temperature affects the synthesis of higher alcohols; synthesis of iso-amyl alcohol is most abundant at 35°C, while the synthesis of *n*-propanol is the lowest at 35°C. Kosmerl and Kordis-Krapez (1997) found an increase of iso-butanol, amyl alcohol, iso-amyl alcohol and 2-phenyl ethanol with increasing temperature of Riesling must fermented at 10, 15 and 25°C, respectively. Some auxotrophic yeast mutants which require branched chain amino acids (valine, leucine, isoleucine) are known to produce 20% less total higher alcohols and 50% less iso-amyl alcohol than parent Montrachet yeast strain (Rous et al., 1983). Partial pressure of CO_2 during fermentation significantly influences the growth and metabolism of yeasts; high CO_2 partial pressure reduced the synthesis of ethyl acetate and total amyl alcohols during brewery fermentation (Renger, van Hateren & Luyben, 1992). *n*-Butanol is a primary aroma compound of apples (Beech & Carr), so its concentration does not depend on processing technology (Mangas et al., 1994), but is cultivar- and maturity-dependent (Plestenjak, Hribar, Vidrih & Simcic, 1994). Some technological procedures, such as aeration during maceration of red grapes increase the synthesis of higher alcohols (Guymon, Ingraham & Crowell, 1961). Mauricio, Moreno, Zea, Ortega and Medina (1997) found an increase of higher alcohols during fermentation of Pedro Ximénez must under semiaerobic conditions compared to anaerobic conditions. Beech and Carr report that juice aeration after addition of yeasts provokes faster completion of the fermentation and lower residual nitrogen content. *n*-Propanol is usually in correlation with nitrogen in must, while amyl, iso-amyl and iso-butyl alcohols correlate negatively with must total nitrogen (Ough & Bell, 1980). Synthesis of iso-butanol increases with increased juice nitrogen (Beech & Carr) as well as with increased temperature during fermentation (Ohkubo & Ough, 1987). Lactic acid bacteria (Bertrand & Suzuta, 1976) is known to produce iso-butanol from 2,3-butanediol and is found in low quality brandies when wine remained on lees for a long period. In addition to iso-butanol, lactic acid bacteria synthesise volatile metabolites such as iso-amyl alcohol, *n*-hexanol, 3(methyl-thio)-*n*-propanol and 2-phenyl ethanol (Edwards & Peterson, 1994). Herjavec and Tupajic (1998) found an increase of iso-amyl acetate, ethyl caproate, ethyl caprylate, ethyl lactate and diethyl succinate in wine after the completion of malolactic fermentation, but no increase in higher alcohols. According to Leguerinel et al. iso-butanol reduces the perception of cider sweetness, although the same authors also found an opposite effect. 2-Phenylethanol is produced by yeasts and is capable of hindering the growth of some bacteria (Guerzoni & Giardini, 1988), thus preventing normal malolactic fermentation.

Cider taste depends primarily on apple cultivar (Beech & Carr, 1977), while cider odour depends more on the processing technology (Gomis, Gutierrez & Moran, 1991) and yeast strain employed (Leguerinel et al., 1989).

The goal of our work is to evaluate the synthesis of higher alcohols in the cider from two different apple cultivars and their influence on sensory properties of cider.

2. Material and methods

2.1. Cider preparation

Apples were washed with water and milled. Part of the mash was immediately pressed to obtain juice, while the rest was left to macerate for 24 h at 20°C and pressed afterwards. Juice from either treatment was spontaneously clarified by precipitation at 10°C and decanted after 24 h. A mixture of selected yeasts from our laboratory (*S. cerevisae* strain ZIM 705) was cultured at 25°C in pasteurised apple juice for 48 h. 2% of thoroughly mixed inoculum was used to inoculate the apple juice. Fermentation was carried out at 15°C and allowed to continue to complete dryness. Cider was then racked to remove the yeasts and provided with SO_2 to reach 25 mg SO_2 l^{-1} (determined as free). Samples were taken during fermentation at intervals of 2–5 days and stored at −20°C till further use.

2.2. Analytical methods

Higher alcohols were analysed by GC (Hewlett-Packard 5890 II) equipped with HP-FFAP (25 m×0.2 mm×0.3 μm) column and FID detector. Temperature programming was as follows: 6 min isothermal at 40°C, then a linear temperature rise of 5°C/min to 200°C. Higher alcohol analyses were performed as follows: 100 ml of cider was distilled with Gibertini distillation equipment; 100 ml of distillate was collected in a volumetric flask at 0°C to prevent losses; a standard solution of higher alcohols was distilled in the same way to determine the recovery of each higher alcohol. 1 μl of distillate was injected onto the column and peaks were recorded.

Sugars were analysed by HPLC: 4-channel degassing unit, X-act, Jour research; HPLC pump, Maxi Star, Knauer; Marathon-XT autosampler, Spark-Holland; Bio-Rad AMINEX HPX-87C column at 85°C using bidistilled water at 0.6 ml min^{-1} and RI detector.

Total nitrogen was determined by means of Kjeldahl digestion method described by Hach, Bowden, Kopelove and Brayton (1987).

2.3. Sensory evaluation test

Sensory evaluation was performed 6 months after the completion of fermentation by six trained panellists. The system used was Bux-Baum where colour (2 points), clarity (2 points), smell (4 points) and taste (12 points) were evaluated.

3. Results and discussion

Juice made from Gloster cultivar contained more total nitrogen (227 mg l^{-1}) than juice from Gala cultivar (160 mg l^{-1}), while maceration of Gala cultivar provoked a slight increase in total N (163 mg l^{-1}). Fermentation of apple juice was monitored by the decrease of glucose, fructose and total soluble solids (Tables 1 and 2). In all fermentations, glucose decreased faster than fructose, although both sugars reached the same level at the end of fermentation—below 0.5 g l^{-1}. All fermentations were completed in 14 days, except for the Gala (macerated) trial, which took 17 days to bring the fermentation to an end.

Higher alcohols analysed have their origin in fruit, with the exception of ethyl acetate, iso-amyl alcohol and 2-phenyl ethanol, which are metabolised predominantly during fermentation as a result of yeast activity. The most dramatic was the 20-fold increase of iso-amyl alcohol in cider compared to apple juice. Synthesis of iso-amyl alcohol was higher in cider made from the 'Gala' cultivar compared to 'Gloster' cultivar (Fig. 1). Maceration enhanced the synthesis of iso-amyl alcohol slightly, probably due to higher aeration during maceration (Mauricio et al., 1997). The yeast strain (A.W.Y. 350R) produced more iso-amyl alcohol in aerated stirred batch cultures than under non-aerated conditions (Beech & Carr, 1977). Bosso (1993) found an increase in iso-amyl alcohol in white wine obtained from macerated grapes. Iso-amyl alcohol correlated negatively with total nitrogen; juice from Gloster cultivar was higher in total nitrogen, while the resulting cider had less iso-amyl alcohol compared to the Gala cultivar. According to Ohkubo and Ough (1987), synthesis of iso-amyl alcohol depends primarily on fermentation temperature and on juice total N content (Ough & Bell, 1980). Klingshirn et al. (1987) found a positive correlation

Table 2
Concentration of glucose (g l^{-1}), fructose (g l^{1}) and soluble solids (%) during fermentation of macerated Gala apples juice

Days	Glucose	Fructose	Soluble solids
0	48.5	52.6	12.6
3	25.5	50.3	11.3
5	12.8	45.0	9.8
7	7.1	30.6	7.6
10	5.6	20.8	5.7
12	0.5	19.6	6.5
17	0.5	0.7	4.3

Table 1
Concentration of glucose (g l^{-1}), fructose (g l^{-1}) and soluble solids (%) during fermentation of non-macerated juice from Gloster and Gala apples

Gloster non-macerated				Gala non-macerated		
Days	Glucose	Fructose	Soluble solids	Glucose	Fructose	Soluble solids
0	42.1	45.6	11.8	48.5	52.6	12.6
2	9.9	26.3	9.5	10.3	34.6	8.7
4	9.5	23.3	7.8	6.9	27.8	6.8
7	0.5	15.6	5.4	2	13.4	5.1
9	0.5	5.4	5.2	0.5	4.2	4.9
14	0.5	0.5	4.4	0.5	0.5	4.4

of juice insoluble solids with the synthesis of iso-amyl alcohol. Iso-amyl alcohol concentration is usually too low to influence the flavour of cider (Leguerinel et al., 1989), although it may contribute to an unpleasant flavour (Rous et al., 1983).

2-Phenyl ethanol was synthesised predominantly during the initial phase of the fermentation (Fig. 2). Mash maceration before fermentation provoked an increase of 2-phenyl ethanol, which is in agreement with the observation of Bosso (1993). Cider made from cultivar 'Gloster' contained less 2-phenyl ethanol than cider from 'Gala' cultivar. That supports the observation of Beech and Carr (1977) that 2-phenyl ethanol decreases with increasing total juice nitrogen. Lea (1995) attributed the higher level of 2-phenyl ethanol to low nutrient status. Apple juice from Gala apples contained less N than Gloster apples, which agrees with the observation of Lea. Some authors believe that 2-phenyl ethanol does not derive de novo from yeasts but originates from a glycosidically-bound form in the fruit, which is liberated during fermentation (Schwab & Schreier, 1990) as a result of enzyme activity.

Iso-butanol level was low in apple juice but increased steadily during fermentation (Fig. 3) as a result of yeast

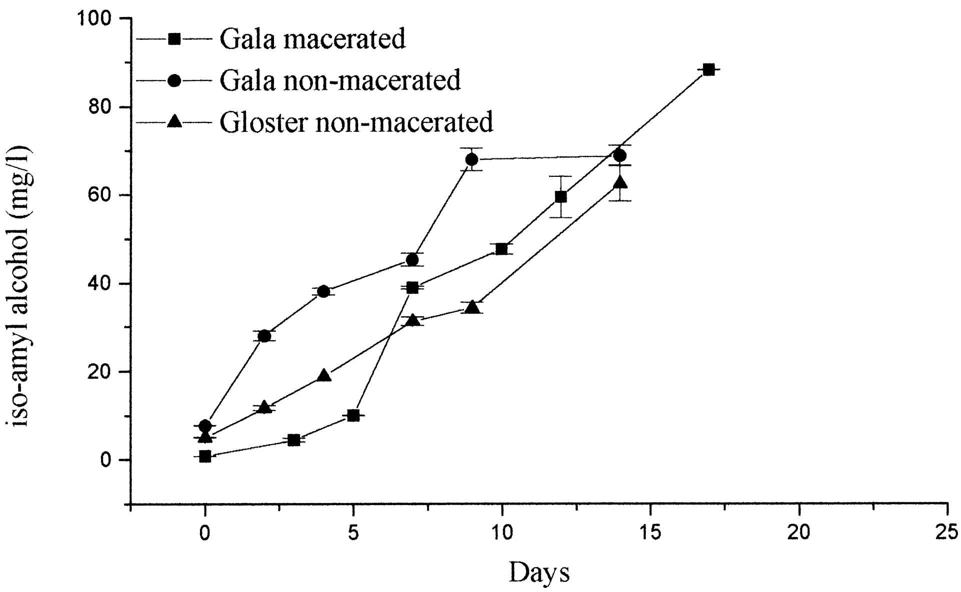

Fig. 1. Synthesis of iso-amyl alcohol during fermentation of apple juice from Gala and Gloster apples. Data represent means of three replicates ±SD.

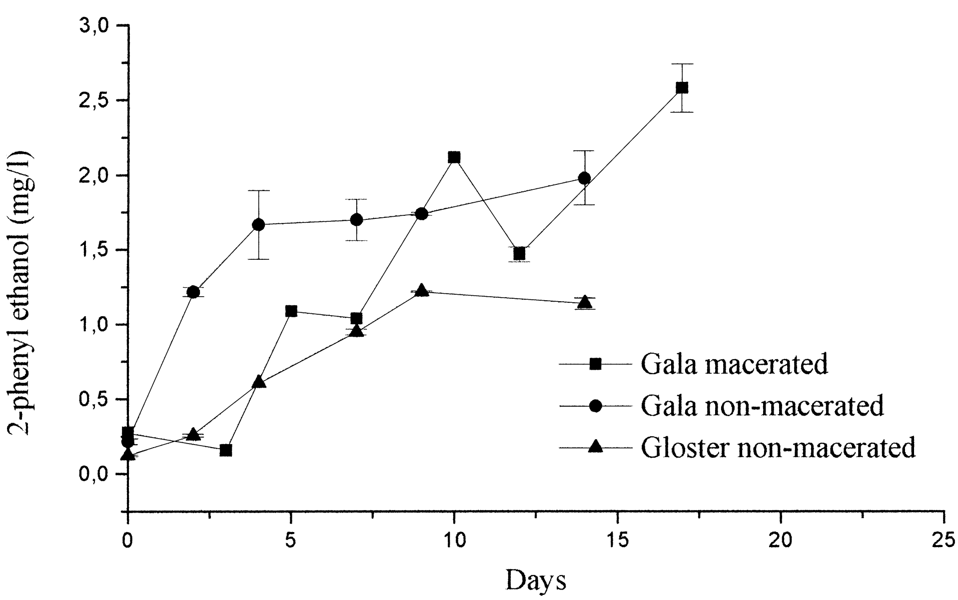

Fig. 2. Synthesis of 2-phenyl ethanol during fermentation of apple juice from Gala and Gloster apples. Data represent means of three replicates ±SD.

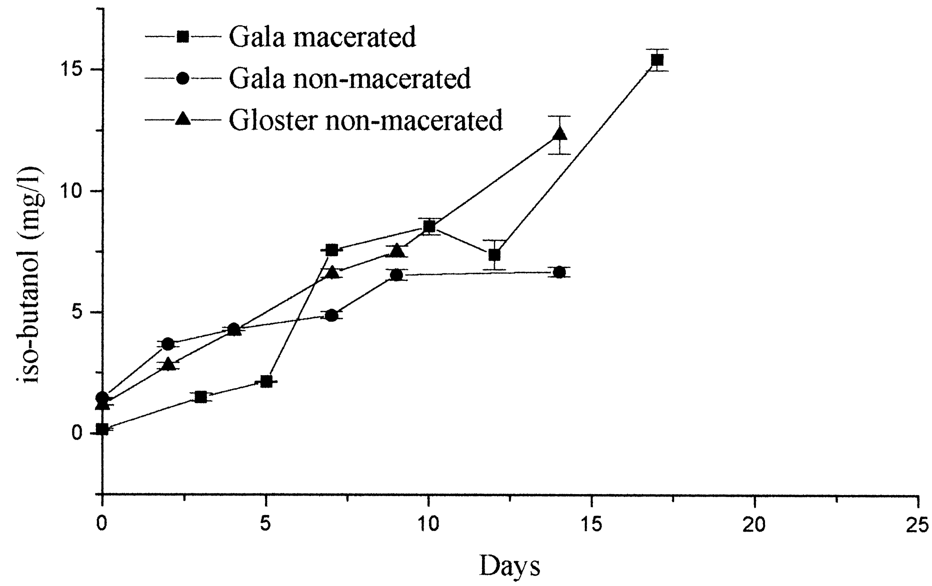

Fig. 3. Synthesis of iso-butanol during fermentation of apple juice from Gala and Gloster apples. Data represent means of three replicates ±SD.

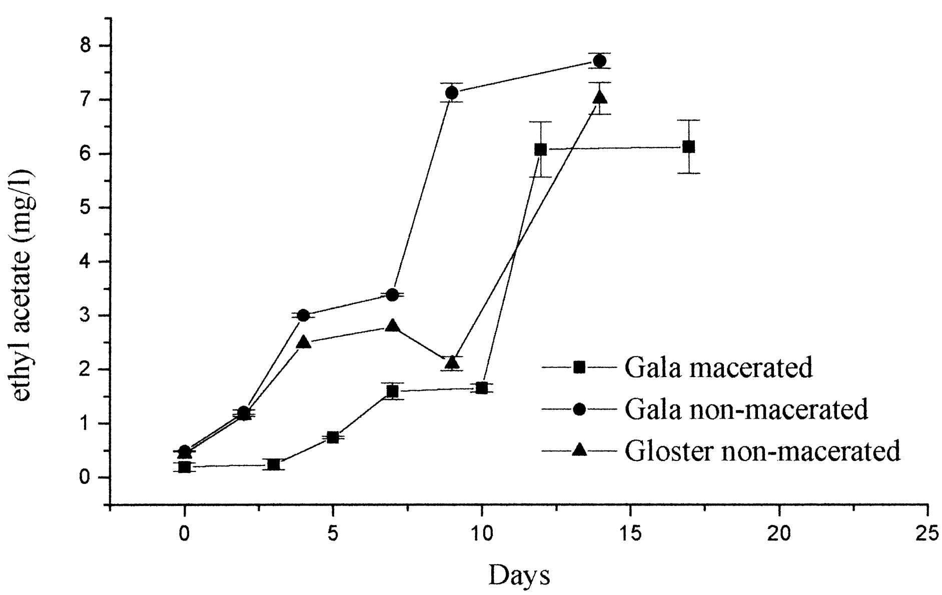

Fig. 4. Synthesis of ethyl acetate during fermentation of apple juice from Gala and Gloster apples. Data represent means of three replicates ±SD.

activity. More iso-butanol was found in cider from the Gala cultivar which also contained less total N. This observation agrees with that of Ough and Bell (1980) and Beech and Carr (1977). Maceration, which allows a certain oxygen diffusion in the mash, increased the iso-butanol synthesis twofold. Mauricio et al. (1997) found an increase of iso-butanol during must fermentation under semiaerobic conditions, compared to strictly anaerobic conditions. The removal of insoluble solids from juice before fermentation significantly reduces the synthesis of iso-butanol during fermentation (Klingshirn et al., 1987) which may be the reason why maceration increases its synthesis. Klingshirn et al. found a positive correlation between juice insoluble solids and the concentration of iso-butanol, amyl and iso-amyl alcohol. Their results also proved a positive correlation between the size of insoluble particles and the concentration of total higher alcohols. Fermentation temperature and total must N do not affect the synthesis of iso-butanol significantly (Ough & Bell, 1980; Ough et al., 1966), although Kosmerl and Kordis-Krapez (1997) observed an increase of its synthesis with higher temperature during must fermentation. According to Leguerinel et al. (1989), iso-butanol may mask the perception of sweetness in cider, although Montedoro and Bertuccioli (1984) found to have a positive effect on the quality of red wines.

Ethyl acetate was not present in apple juice, but was synthesised de novo during fermentation, independently of technological process or apple cultivar (Fig. 4). It seems that neither apple cultivar nor maceration could influence its synthesis. Ethyl acetate concentration rose during fermentation: more pronounced increase was detected in the final phase of fermentation. Our results are in agreement with those of Pajunen, Jääskeläinen and Mäkinen (1977), who observed the synthesis of ethyl acetate in the last phase of beer fermentation. Herjavec and Tupajic (1998) observed a slight increase in ethyl acetate during malolactic fermentation of red wine.

n-Propanol was present in fresh fruit but fermentation provoked further increase (Fig. 5). *n*-Propanol started to accumulate at the very beginning of fermentation. Maceration caused an increase of *n*-propanol, probably because of larger O_2 entrapment (Mauricio et al., 1997). Ough and Bell (1980) reported a positive correlation of *n*-propanol to total N content in grape must. Cider made from 'Gloster' cultivar contained less *n*-propanol than cider made from 'Gala' cultivar, which is not in accordance with the observation of Ough and Bell. Beech and Carr (1977) found a decrease of *n*-propanol in aerated batch cultures. Giudici and Kunkee (1994) found *n*-propanol production to be strongly yeast

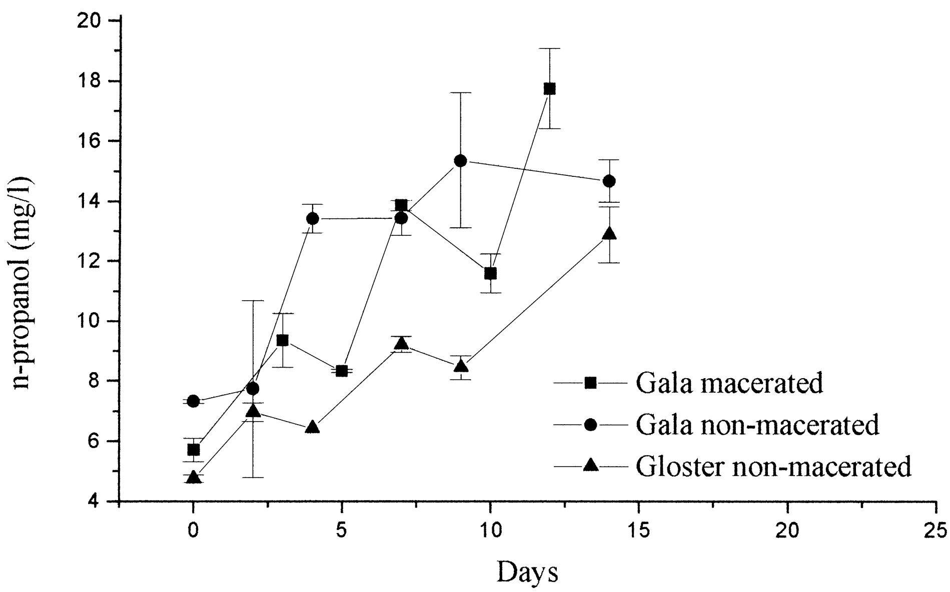

Fig. 5. Synthesis of *n*-propanol during fermentation of apple juice from Gala and Gloster apples. Data represent means of three replicates ±SD.

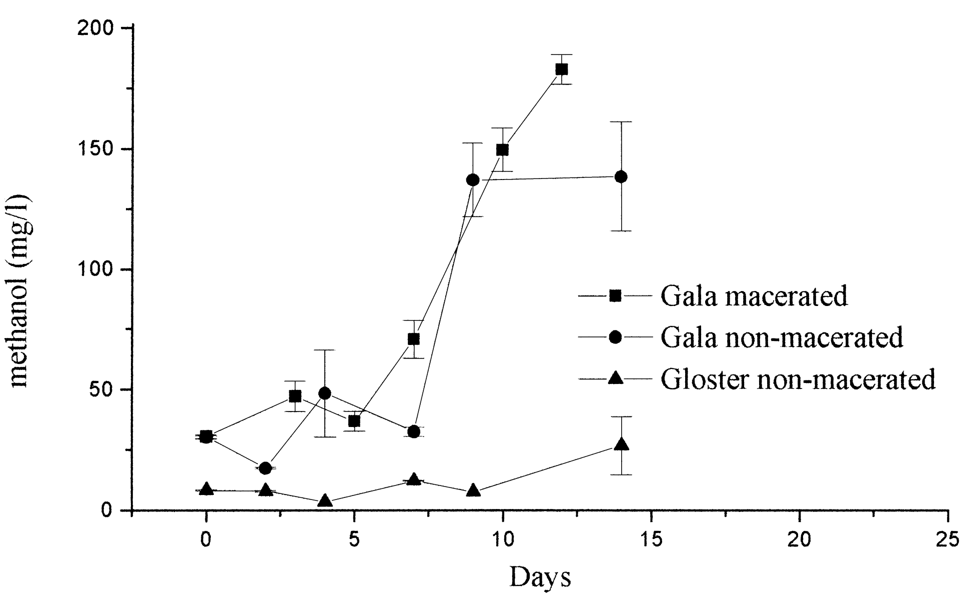

Fig. 6. Synthesis of methanol during fermentation of apple juice from Gala and Gloster apples. Data represent means of three replicates ±SD.

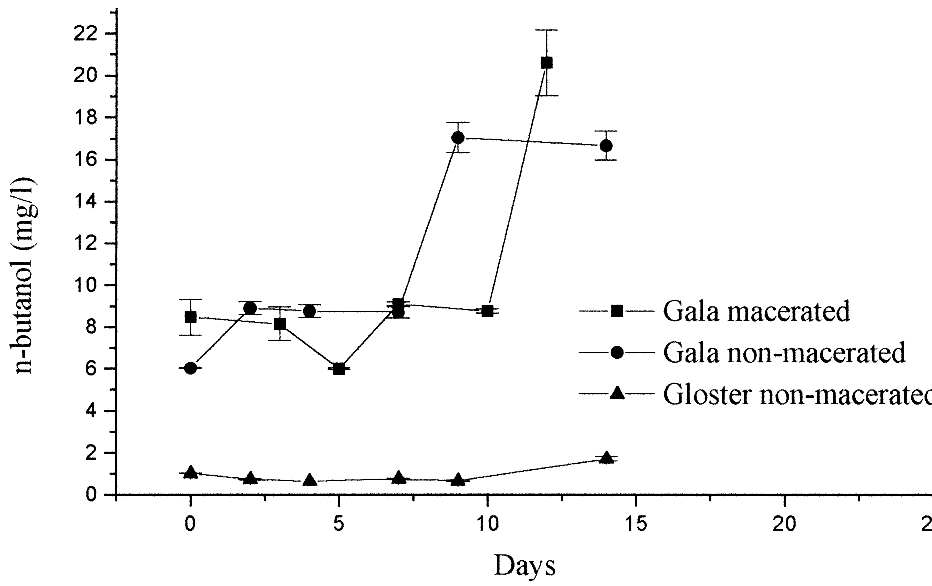

Fig. 7. Synthesis of *n*-butanol during fermentation of apple juice from Gala and Gloster apples. Data represent means of three replicates ±SD.

Table 3
Scores of sensory evaluation of cider[a]

	Colour	Clarity	Smell	Taste	Sum	Total higher alcohols[b]
Gala macerated	2.0	2.0	2.0	6.7	12.7	151
Gala non-macerated	2.0	2.0	2.7	6.2	12.9	108
Gloster non-macerated	2.0	2.0	3.2	8.6	15.8	95.8

[a] Data represent means of six panellists.
[b] Sum (mg l^{-1}) (*n*-butanol, iso-butanol, 2-phenyl ethanol, iso-amyl alcohol, *n*-propanol) 6 months after the completion of fermentation.

Table 4
Concentration of higher alcohols (mg l^{-1}) in cider 6 months after the completion of fermentation[a]

	Isoamyl	2-phenyl ethanol	iso-Butanol	*n*-Propanol	Methanol	*n*-Butanol
Gala macerated	91.4±3,8	2.4±0,1	16.8±0,6	18.3±0,9	181±3,4	22.3±2,1
Gala non-macerated	67.6±1,8	2.1±0,3	7,1±0,3	15.4±1,1	147±8,2	15.9±1,9
Gloster non-macerated	64.7±3,6	1.3±0,2	14.5±0,6	13.2±1,2	28.6±4,5	2.1±0,2

[a] Data represent means of three replicates±SD.

strain-dependent; strain 6527 always produced low amounts of *n*-propanol irrespective of the total N in synthetic medium; strain 6392 responded strongly to the amount of N in the medium. Methanol is present in fresh fruits as a result of its liberation from pectin by means of pectin methyl esterase. Enzyme activity is known to increase in the final phase of fruit ripening but may be prolonged well into the phase of maceration. Mash maceration increased the methanol content in cider (Fig. 6) due to the activity of endogenous pectin methyl esterase. Less methanol was found in cider from 'Gloster' apples than in cider from Gala apples.

Fresh fruit contained *n*-butanol that was synthesised during fruit growing and ripening. According to Jepsen (1978) *n*-butanol is known to correlate positively to the scores of aroma quality in apple juice. Its concentration rose slightly during fermentation but a more pronounced increase took place during the final phase of fermentation (Fig. 7). 10-fold less *n*-butanol was found in the cider from 'Gloster' cultivar, while maceration increased its synthesis by 25%. 'Gala' cultivar is regarded as an aromatic one, and is undoubtedly more aromatic than the 'Gloster' cultivar.

Maceration provoked an increase of iso-amyl alcohol, 2-phenyl ethanol, *n*-propanol, *n*-butanol, iso-butanol and methanol compared to the cider made from non-macerated juice. Synthesis of iso-butanol seems to be particularly enhanced by maceration; its content is nearly double in cider from macerated juice. More iso-amyl alcohol, 2-phenyl ethanol, *n*-propanol, *n*-butanol and methanol were found in cider made from 'Gala' cultivar. Cider made from non-macerated Gloster cultivar recieved the highest quality score, followed by Gala non-macerated and Gala macerated trial (Table 3). Total higher alcohols, measured 6 months after fermentation, were the lowest in cider made from non-macerated Gloster cultivar (Tables 3 and 4) and the highest in cider from the 'Gala' macerated experiment. This supports the thesis of Rapp and Mandery (1987) that higher alcohols negatively influence the quality score.

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