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Effect of changes in pH on the release of flavour compounds from a soft drink-related model system

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Abstract

Citric acid and phosphoric acid were added in variable amounts to a soft drink model system to show their effect on the release of six flavour compounds. High concentrations of the acids decreased the release of esters, probably because of the presence of large amounts of the dissociated form of the acids. However, the same amounts of added citric acid had no effect on flavour release when pH was regulated with sodium hydroxide. Changes in pH values achieved by adding hydrochloric acid also had no effect on flavour release. These results indicate that pH values used in soft drinks do not affect the release of flavour molecules. It is more likely the citric acid and the phosphoric acid, particularly their dissociated forms, which decrease the release of the esters. The other flavour compounds were not affected. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Acids are important ingredients in soft drinks because they are necessary for balancing sweetness. They are used as flavour enhancers and as protectors against microbiological spoilage (Giese, 1992). All acids impart an acidic taste but the sensory character, intensity, aftertaste, etc., are unique for each acid (Dziezak, 1990).

Citric acid is the most commonly used acid in soft drinks. It lowers pH to enhance the taste and to provide microbiological stability. It is a bulky, highly polar molecule containing three carboxylic groups and a hydroxylic group (Ólafsson, 1995). This relatively strong organic acid ($pK_1 = 7.10 \times 10^{-4}$) is highly soluble in water and can deliver a ‘burst’ of tartness, which makes it suitable for use in flavour modification or enhancement. Citric acid is sometimes added to starter cultures in butter and margarine production to promote development of certain flavour compounds, such as diacetyl. It is also used in bakery products, soft drinks and confections to increase tartness levels and enhance fruit flavours (Dziezak, 1990).

Phosphoric acid is also used in soft drinks, mostly in cola drinks to give them their ‘tangy’ flavour. It also acts as a buffer in carbonated beverages to maintain a constant pH (Chang, 1991). Phosphoric acid is a strong acid ($pK_1 = 7.52 \times 10^{-3}$) that produces the lowest pH of all food acidulants and it is the only inorganic acid widely used in the food industry (Dziezak, 1990).

Some investigations into the effect of acids on flavour release have been made, mostly in the sensorial area. Valdés et al. (1956), for example, reported that apricot flavour was enhanced when a mixture of organic acids was added to fruit nectars and Pangborn et al. (1957) showed that citric acid increased the flavour intensity of fruit nectars. The aim of our study was to find out how the release of six flavour compounds (isopentyl acetate, ethyl hexanoate, *cis*-3-hexenyl acetate, linalool, l-menthone, and limonene) in a soft drink model system was affected by pH changes in the system. To a mixture of water and a tutti-frutti flavour, citric acid, phosphoric acid, hydrochloric acid, and sodium hydroxide were added in varying amounts and combinations. Static headspace and GC/MS were used to measure the headspace concentration of the flavour molecules.

2. Experimental design

The soft drink model system basically consisted of water and a non-commercial tutti-frutti flavour (donated

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Table 1
Concentrations of the measured flavour compounds in the tutti-frutti mixture and their hydrophobic fragmental constants (log *P*), calculated by a method described by Rekker (1977)

Flavour compound	Concentration (%) w/w	log *P*	log *P* (literature values)
Isopentyl acetate	0.40	2.12	
Cis-3-hexenyl acetate	0.10	2.63	
Ethyl hexanoate	0.12	2.76	2.76 (Nahon, Navarro y Koren, Roozen, & Posthumus, 1998)
l-Menthone	0.20	2.81	2.83 (Gunning, Parker, Ring, Rigby, Wegg, & Blake, 2000)
Linalool	1.20	3.06	
Limonene	0.10	4.28	4.35 (Gunning et al., 2000)

by Danisco Cultor, Aarhus, Denmark). The flavour contained 24 compounds with different properties, all dissolved in propylene glycol. Six of these compounds (Table 1) were chosen for this study because they had the best separation on the GC column, and therefore, were the most suitable for quantification. The tutti-frutti flavour was mixed with de-ionised water at 0.1% (v/w). Thereafter, 10 ml of the mixture was placed in a gas-tight vial (21 ml). The sample was equilibrated for 1 h at 30°C and the amount of flavour compounds in the gas phase was then determined. At equilibrium, 500 µl of the headspace was drawn from the vial using a gas-tight syringe and injected onto a gas chromatographic column. The flavour compounds were quantified and identified by GC/FID and gas chromatography combined with mass spectrometry (GC/MS). The analytical instrumentation consisted of a Hewlett Packard gas chromatograph HP 5890 equipped with a DB-FFAP (nitroterephtalic acid modified polyethylene glycol phase) 30 m×0.32 mm ID 0.25 µm column (J&W Scientific, Cologne, Germany), a Finnigan Incos 500 mass spectrometer and a flame ionisation detector. The temperature programme used was 40(5)–220(10) 4°C/min.

2.1. pH Regulation

To study the effect of pH changes on flavour release, four different experiments were performed (Table 2). First, citric acid was added in variable amounts (1.0, 0.1, 0.002, and 0 g) to a mixture of water (10 ml) and the tutti-frutti flavour (0.1% v/w) to obtain pH values of 2, 3, 4, and 5. Second, as a complementary study, 1 g of citric acid (pH 2) was added to the same water flavour mixture and sodium hydroxide was then added to adjust the pH values to 3, 4, and 5. Third, either hydrochloric acid or sodium hydroxide was added to samples containing water and the tutti-frutti flavour (0.1% v/w); hydrochloric acid was added to obtain pH values of 2 and 3 and sodium hydroxide was added to obtain pH values of 7 and 9. The water–tutti-frutti mixture has a pH value of 5 when no pH regulator is used. In the fourth experiment, phosphoric acid was added to the soft drink model system to obtain pH values of 2, 3, 4, and 5. All samples were tested in at least triplicate; some were analysed up to six times.

Table 2
An overview of the pH regulators used to get the various pH values

pH values	2	3	4	5	6	7	8
Citric acid	x	x	x				
Citric acid + NaOH		x	x	x			
Hydrochloric acid + NaOH	x	x				x	x
Phosphoric acid	x	x	x				

To determine if there was a difference in flavour release when both sugar and acid were used in the soft drink model system, invert sugar (10% w/w) was added to samples containing citric acid (pH 2, 3, 4, and 5) and also to samples containing both citric acid and sodium hydroxide (pH 3, 4, and 5).

All pH values were measured using a pH meter (PHM83 Autocal pH meter, Radiometer, Copenhagen, Denmark).

3. Statistical analyses

The Tukey HSD (Honestly Significant Difference) test was used to analyse individual differences between samples (O'Mahoney, 1986). The significance level was expressed as *P*-values.

4. Results

Citric acid and phosphoric acid are the acids most often used in the soft drink industry. To study their effect on flavour release they were added in variable concentrations to a soft drink model system containing water and a tutti-frutti flavour. When 0.002 g of citric acid (pH 4) was added to the model system, the esters (ethyl hexanoate, isopentyl acetate, and *cis*-3-hexenyl acetate) showed a significant ($P<0.05$) increase in headspace concentration compared to samples without citric acid (pH 5; Fig. 1). Samples containing 0.1 g of citric acid (pH 3) still showed a significantly ($P<0.05$) increased release of isopentyl acetate and ethyl hexanoate but not of *cis*-3-hexenyl acetate compared to samples with no added acid. *Cis*-3-hexenyl acetate and limonene also showed an increase in headspace concentration when 0.002 g of citric acid (pH 4) was added to the model system compared to samples containing 1 g of citric acid (pH 2). These results indicate that adding citric acid in small amounts increases the headspace concentration of the esters and limonene. However, when added in larger amounts such as 1 g (pH 2), citric acid does not have any significant effect on the release of any of the compounds.

To determine if the effect on flavour release from the esters was caused by the hydrogen ion concentration or the citric acid itself, 1 g of citric acid was added to the model system and then sodium hydroxide was added to adjust the pH values to 3, 4, and 5. The results showed no differences in the release of any of the flavour compounds in relation to the pH values, except for limonene (Fig. 2).

To further investigate the flavour release in relation to pH, hydrochloric acid and sodium hydroxide were added to the model system to obtain pH values of 2, 3,

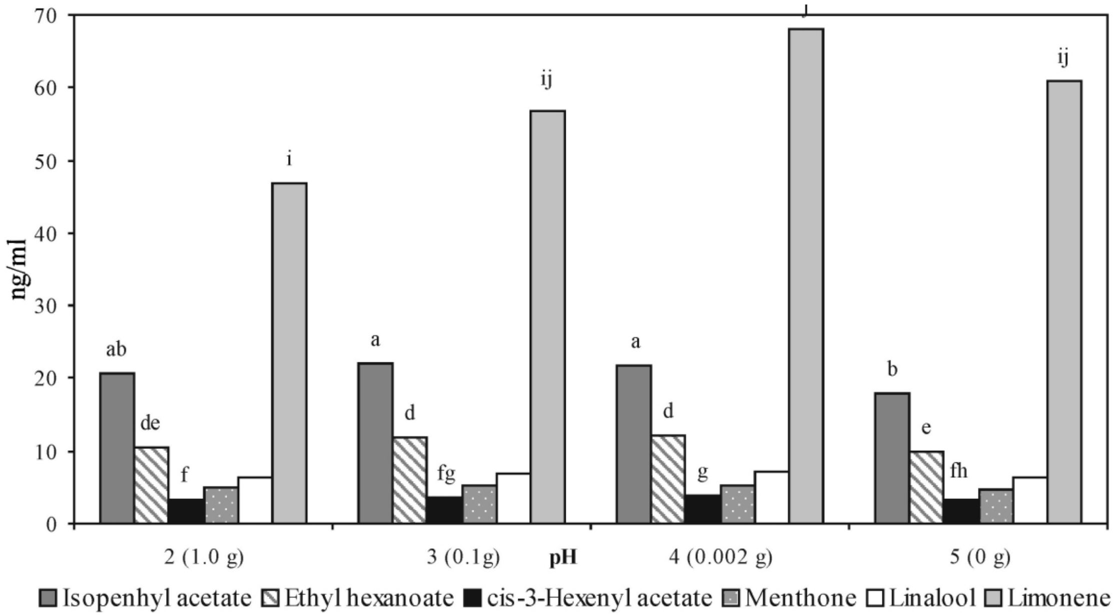

Fig. 1. Effect of different concentrations of citric acid on the headspace concentration of six flavour compounds. Superscripts indicate significant differences ($P < 0.05$) among concentrations of flavour compounds in the gas phase in relation to varying pH values.

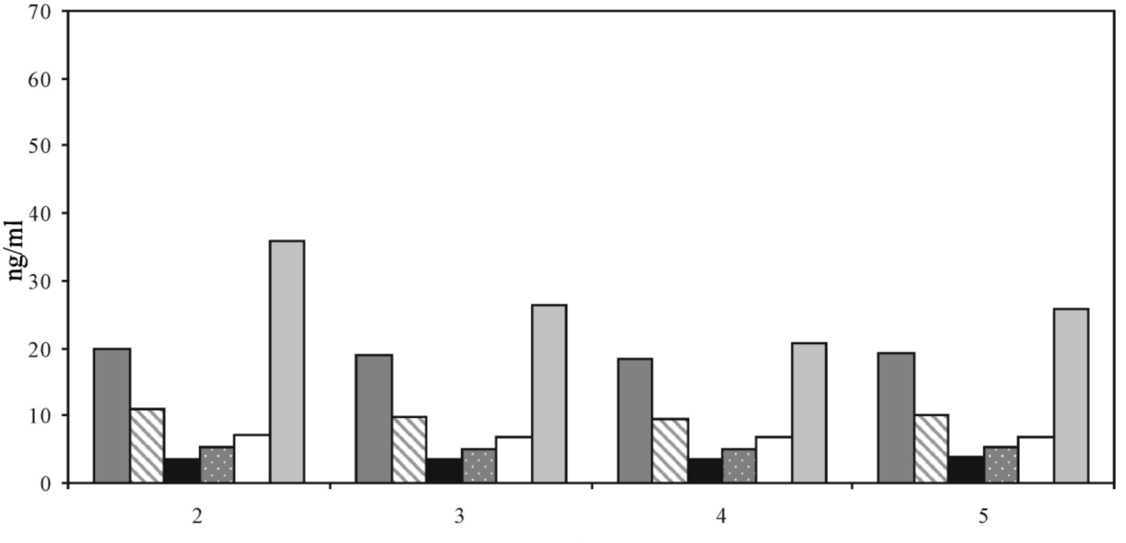

Fig. 2. Effect of adding 1 g of citric acid (pH 2) and sufficient sodium hydroxide to obtain pH values of 3, 4 and 5 on the headspace concentration of six flavour compounds.

5, 7, and 9. As before, the pH value of 5 was obtained by adding the tutti-frutti flavour to water, without adding any acid or base. Adding hydrochloric acid to obtain pH values of 2 and 3 did not produce any effect on the headspace concentration of the six flavour compounds (Fig. 3). The samples with sodium hydroxide added to obtain a pH above 5 showed a significant ($P<0.05$) increase in release of ethyl hexanoate at pH 7 and pH 9 compared to the sample with no added sodium hydroxide (Fig. 3). The release of the other flavour compounds was more or less unaffected.

Finally, phosphoric acid was added to the model system to adjust the pH values to 2, 3 and 4; a pH value of 5 was obtained using only the tutti-frutti flavour and water. The sample with phosphoric acid added to produce the pH value of 2 showed a decrease in release of the three esters, viz. isopentyl acetate ($P<0.05$), ethyl hexanoate ($P<0.1$), and *cis*-3-hexenyl acetate ($P<0.05$),

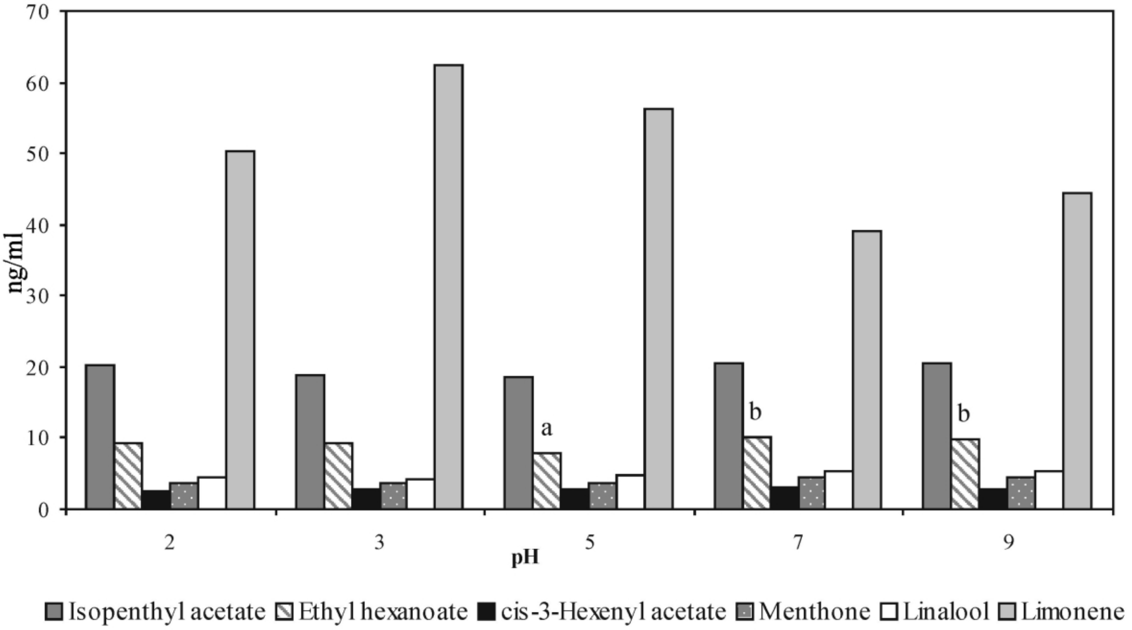

Fig. 3. Effect of variable pH values regulated by adding hydrochloric acid and sodium hydroxide on the headspace concentration of six flavour compounds. Superscripts indicate significant differences ($P<0.05$) among concentrations of flavour compounds in the gas phase in relation to varying pH values.

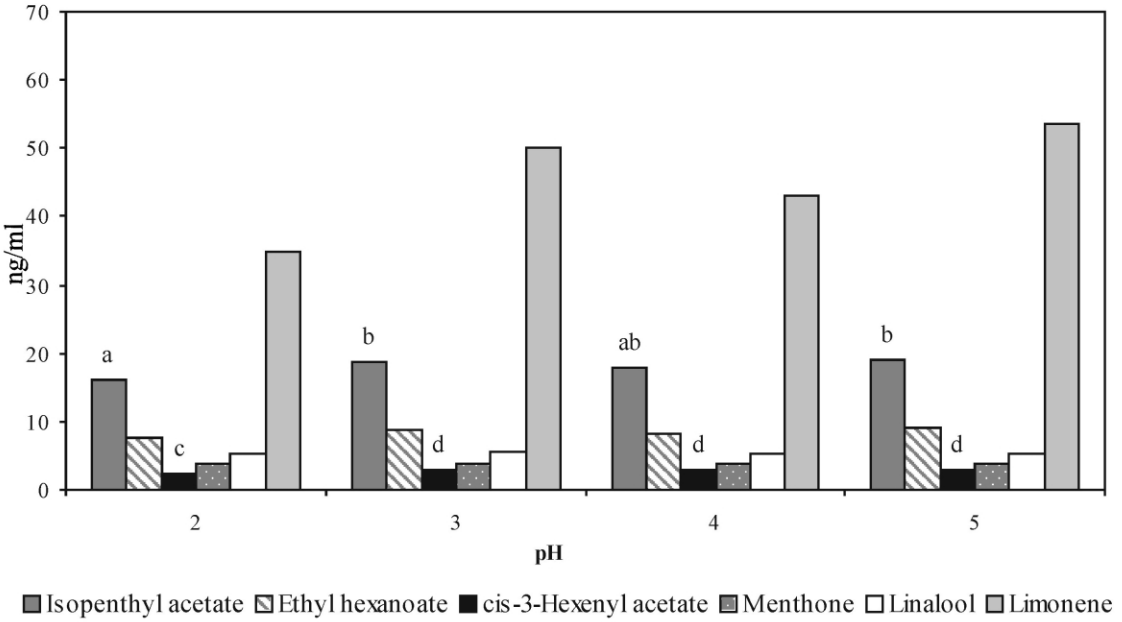

Fig. 4. Effect of different concentrations of phosphoric acid on the headspace concentration of six flavour compounds. Superscripts indicate significant differences ($P<0.05$) among concentrations of flavour compounds in the gas phase in relation to varying pH values.

compared to the sample containing no phosphoric acid (pH 5; Fig. 4). None of the other flavour compounds was significantly affected by the addition of phosphoric acid to pH 2. *Cis*-3-hexenyl acetate also showed a significant ($P<0.05$) decrease in headspace concentration at pH 2 compared to the release at pH values of 3 and 4. Isopentyl acetate showed a significant ($P<0.1$) decrease in headspace concentration at a pH value of 2 compared to pH 3. In general, the more phosphoric acid added to the model system, the smaller the amounts of esters become in the gas phase.

To make the model system more soft drink-related, 10% of invert sugar was added to the samples containing the varying amounts of citric acid (i.e. 0.002, 0.1, 1.0 and 0 g) used in the first experiment (Fig. 5). Headspace concentration was high for all compounds except limonene, irrespective of the amount of acid added, and no significant effects on flavour release related to changes in pH were noted.

Invert sugar was also added to the mixture containing citric acid and sodium hydroxide. Again, the headspace concentration was high for all compounds except limo-

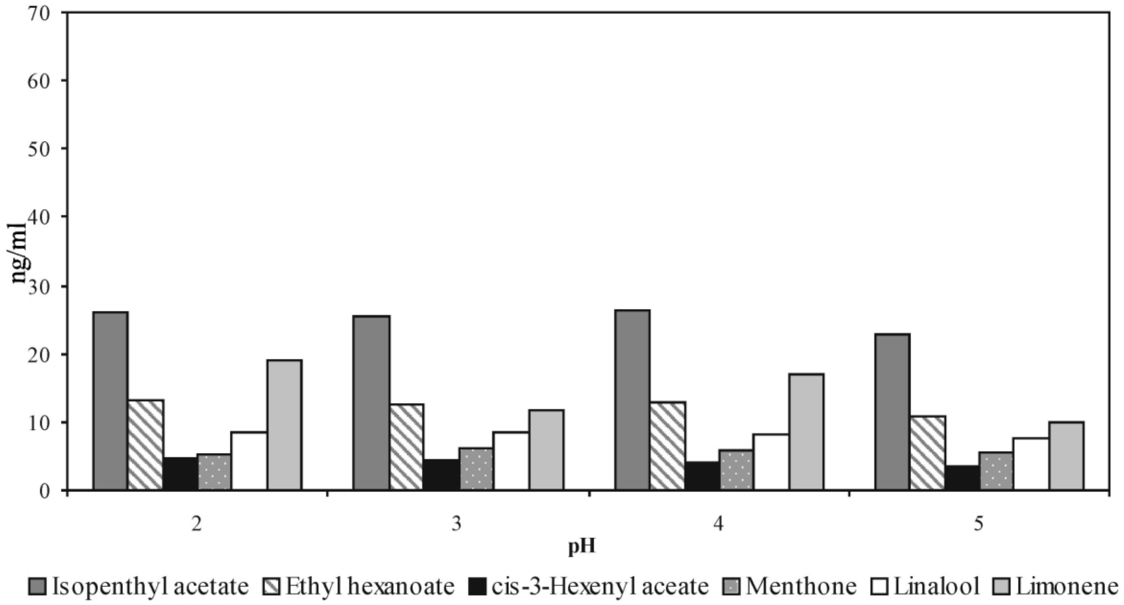

Fig. 5. Effect of citric acid in different concentrations and 10% (w/w) of invert sugar on the headspace concentration of six flavour compounds.

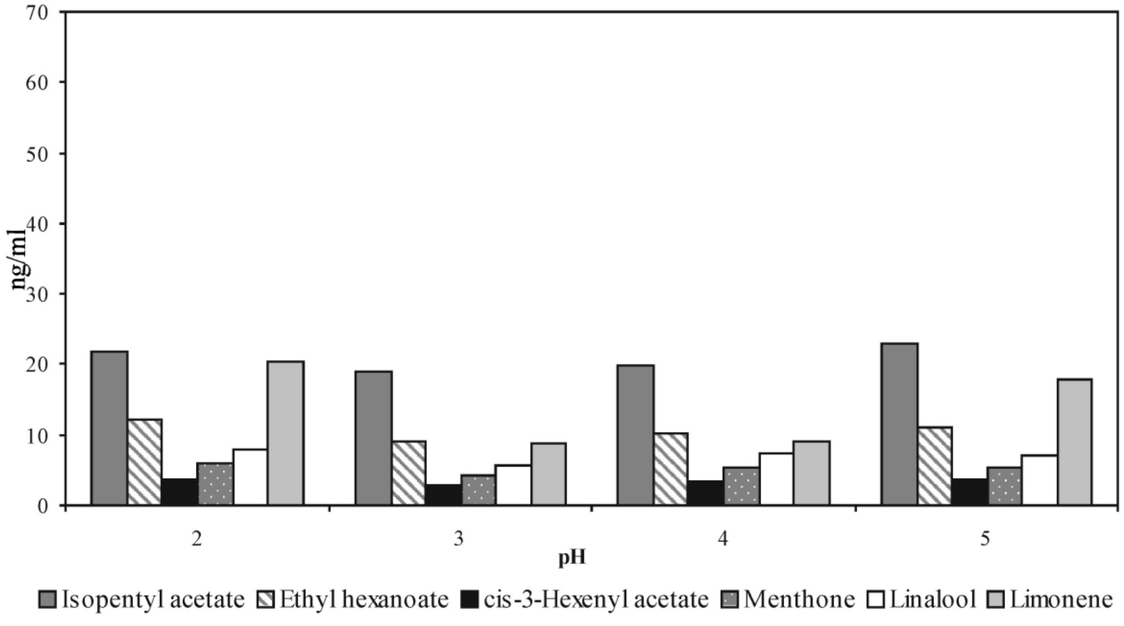

Fig. 6. Effect on headspace concentration of adding 1 g of citric acid, sufficient sodium hydroxide to obtain pH values of 3, 4, and 5, and 10% (w/w) of invert sugar.

nene and no significant effect on headspace concentration related to changes in pH was seen (Fig. 6).

5. Discussion

When small amounts (0.002 g) of citric acid were added to the soft drink model system, an increase in flavour release was observed for limonene and the esters. However, when 1 g of citric acid was added, there was a significant decrease in the release of *cis*-3-hexenyl acetate and limonene and a tendency for ethyl hexanoate and isopenthyl acetate to decrease as well. Water activity, which was measured, did not change when different amounts of citric acid were added to the system, demonstrating that the increased release of the flavour molecules does not depend on a 'salting-out' effect, in accordance with our earlier study (Hansson, Andersson & Leufvén, 2000). One could instead speculate that the increased release might be explained by a change in equilibrium between citric acid and its dissociated form. The pK_1 value when 50% of the acid is in the non-dissociated state (RCOOH) and 50% is in the dissociated state ($RCOO^-$) is 3.128 for citric acid. When a large amount of citric acid is added to the soft drink model system, a large amount of its dissociated form will be present. Citric acid is a triprotic acid and, according to calculations, the first form of dissociation is the dominating form in this system. This dissociated form might have a greater tendency than the non-dissociated citric acid to interact with chemical compounds. Citric acid is a chelating agent, which means that its dissociated form can form complexes with metal ions because of its unshared electron pair. Chelating agents such as citric acid and various phosphates are popular for stabilising foods (Lindsay, 1985). This chelating phenomenon might also be applicable to flavour compounds. The more citric acid added to the solution, the greater the amount of its dissociated form will be available to interact with the ester molecules. As a result, there are more flavour molecules in the gas phase of a sample at pH 3 and 4 compared to pH 2 since there is less dissociated citric acid in the water solution. Changes in pH might also affect the flavour compounds themselves. Each flavour compound has its own pK value and is affected to a different degree by a specific pH change (Bennett, 1992).

Increasing the pH will shift the equilibrium so that larger amounts of the citric acid are in the dissociated form, whereas lowering the pH leads to a larger amount of the non-dissociated form (Bennett, 1992). Therefore, when citric acid was added in equal amounts (1.0 g) and pH was varied to values of 3, 4, and 5 using sodium hydroxide, the sample with a pH value of 5 contained the largest amount of the dissociated form and the sample with a pH value of 2 contained the largest amount of the non-dissociated form. However, the amount of dissociated form is greater in all samples in this study compared to the samples containing citric acid only. No effect on release is seen in this study, which might be due to the high amount of the dissociated form of citric acid in all samples.

Adding hydrochloric acid to the soft drink model system to vary the pH value had no effect on the release of any of the flavour compounds. However, the addition of sodium hydroxide increased the release of ethyl hexanoate at the pH values of 7 and 9. This effect could be due either to changes in concentrations of hydroxyl groups or to a 'salting-out' effect of the sodium hydroxide itself. None of the other pH regulators have been added in amounts corresponding to pH values above 5, since this is not relevant to a soft drink model system. Therefore, the effect of basic pH values on flavour release was not clarified in this study.

A sample with added phosphoric acid in amounts corresponding to a pH value of 2 showed a decrease in the release of the esters compared to the sample with no added acid (pH 5). As for citric acid, this might be explained by interactions between the dissociated form of phosphoric acid and the esters, which keep the esters in the solution. The more phosphoric acid added to the soft drink, the more dissociated form would be present in the system, leading to larger amounts of esters remaining in the solution.

The concentrations of all flavour molecules in the gas phase except for limonene were highest in the samples containing citric acid and 10% invert sugar. The second highest flavour concentration was found in the gas phase of the samples containing citric acid, sodium hydroxide, and invert sugar (10%). This is in good agreement with our earlier study (Hansson et al., 2000), which showed an increased headspace concentration of the same flavour compounds in samples with added invert sugar compared to samples with no added sugar. For limonene, the concentration in the gas phase was highest when citric acid or hydrochloric acid were used as a pH regulator and no invert sugar was added to the system. This is because limonene is the most non-polar of the compounds (Table 1), and therefore, stayed in the water solution when invert sugar was added and the solution became more non-polar.

6. Conclusions

This study showed that adding one gram of citric acid per 10 ml of water-flavour mixture had a decreasing effect on release of esters and limonene, which might be explained by a change in equilibrium between citric acid and its dissociated form. A sample containing a large amount of citric acid also contains a large amount of its dissociated form, which might be able to complex-bind these flavour compounds. When phosphoric acid was added in larger amounts, a decrease in release of the esters was noted. This is probably because adding more phosphoric acid to the sample also adds more of its dissociated form and therefore binds more of the esters. However, addition of hydrochloric acid or citric acid in combination with sodium hydroxide to give an acidic pH, did not have any effect on release of the flavour compounds studied. There was, however, an increased release of ethyl hexanoate at pH values of 7 and 9. This indicates that changes of pH in the range of soft drinks do not affect the release of flavour molecules.

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