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Sensory perception of different acidulants in flavoured sports drinks

By

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Submitted in partial fulfilment of the requirements for the degree

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DECLARATION

I hereby declare that this dissertation herewith submitted for the degree of MSc Food Science at the University of Pretoria, has not previously been submitted by me for a degree at any other University or institution of higher education.

Marise Kinnear

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DEDICATION

This work is dedicated to the following people:

My parents, F.X and Marietjie Jurgens

My husband, Gerhard

My little girl, Mia

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BACKGROUND TO THE STUDY

Mrs Retha Jacobs was a registered MSc (Agric) Food Science and Technology student with the Department of Food Science, University of Pretoria from 1997 to 2000. Her project titled "Comparison of sensory attributes of food acids in model systems" under the supervision of Dr. H.L. de Kock and Dr. H.C. Schönfeldt dealt with the following objectives

- To measure at which concentration each of six food acid solutions tasted equally sour compared to that of a 0.2% w/v citric acid in water solution.
- To compare the relative temporal taste properties of malic acid, tartaric acid, fumaric acid and three acid blends with those of citric acid at equi-sourness concentration levels in three different model solutions using a time-intensity sensory technique.

Unfortunately she did not complete the master degree because she died in 2002 as a result of cancer. The experimental work was completed. However the final dissertation was not fully completed.

This study follows on the work of Mrs Jacobs but deals with different objectives. The researcher had access to the research reports and do refer to some of the results in certain sections.

ABSTRACT

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by
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Supervisor: Dr H.L. de Kock
Department: Food Science
Degree: MSc Food Science

This study investigated the implications of substituting citric acid with other acidulants; malic acid, fumaric acid and tartaric acid and a combination thereof (Fruitaric® acid), in a flavoured sports drink. A trained sensory panel (n=10) compared the sourness intensity of the acidulants at both equal sour and equal weight concentrations in water and in a *Grape* and a *Lemon & Lime* flavoured sports drink. The sensory panel compared the temporal character of the acidulants at equal sour concentrations in water and both sports drink flavours. This included determining the rate, onset, duration and maximum intensity parameters of the perceived sourness. To determine if repeated exposure testing of sports drinks with the different acidulants added at equal sour concentrations would lead to hedonic adjustment, consumers (n=128) were repeatedly exposed to a *Lemon & Lime* flavoured sports drink over a period of 22 days.

Citric acid, the acidulant currently used in the sports drinks, served as a reference in all the comparisons. Previously determined equal sour concentrations of tartaric and Fruitaric® acids as determined in water was found to be equally sour to citric acid when applied to water and a *Lemon & Lime* flavoured sports drink but less sour than citric acid when compared in the *Grape* flavoured sports drink. Malic and tartaric acids were found to be equal sour to citric acid in water and both sports drink flavours. The application of equal sour concentrations seemed to be flavour specific.

Sourness of water and more complex solutions, such as flavoured sports drinks, seemed to be dependant on multiple factors including pH, titratable acidity, molecular weight, acidulant concentration and ^{o}Brix. The results from this study rejected anecdotal reports that acidulants differ in their temporal sensory profiles, although the lack of significant differences may be a function of the specific concentration level (0.2%) used.

Repeated exposure testing of *Lemon & Lime* flavoured sports drinks with different acidulants resulted in hedonic adjustment. Consumer preferences post exposure could not have been predicted with a traditional consumer taste test at the start of the study. The findings of this study surely challenge the validity of sensory evaluation test strategies that rely on single exposure testing to predict long term consumer preferences.

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CHAPTER 1 : INTRODUCTION

Food manufacturing companies make changes to food products to retain or improve their market share. The food manufacturer needs to overcome two hurdles to ensure that its product is a success on the market: immediate acceptance of the food product and ongoing purchase and consumption of the product (Moskowitz, 2000). One act of buying is usually not the goal of food manufacturers: a product is placed on the market to be bought repeatedly, and in the mean time tasted and eaten – to deserve its position in the market (Tuorila, Meiselman, Cardello & Lesher, 1998).

Acidulants provide sourness in foods and beverages, but also other functions, such as pH adjustment, preservation, stabilisation of colour and flavour and metal sequestration (Hansson, Andersson, Leufven & Pehrson, 2001). The most commonly added acid in beverages, especially juice-based ones, is citric acid, but claims have been made that citric acid does not give the best possible taste results in beverage applications (Lanton, 2004). Compared to citric acid, other acidulants e.g. malic acid, fumaric acid and tartaric acid or combinations thereof, may potentially provide superior sensory properties in beverage applications. However, the decision to substitute citric acid with another acidulant in a sports drink should be made with caution. Acidulants added at equal weight concentrations, vary in perceived intensity of sourness in decreasing intensity order from fumaric then tartaric, malic and citric acids (Berry, 2001). It is therefore not appropriate to substitute one food acid for another on equal weight basis. Limited research has been done on how the temporal characteristics of the different acidulants vary from each other. The rate, onset, duration and intensity of the perceived sourness of the different acidulants might have an impact on the final perceived flavour of products like sports drinks.

According to Stein, Nagai, Nakagawa & Beauchamp (2003) the superior taste profile of specific components of beverages may not be evident immediately after consuming such a product for the first time. The assumption that brief, once-off laboratory consumer sensory evaluation can predict long-term product success or failure may not be true (Stubenitsky, Aaron, Catt & Mela, 1999). Repeated exposure to specific food stimuli may lead to either an increase or a decrease in acceptance ratings. It is not known whether repeated exposure will lead to the identification of subtle differences in beverages with different acidulants. Subtle differences in the taste profiles may become more evident after repeated exposure and therefore influence liking. These potential changes in acceptance ratings may be explained by hedonic adjustment. The advantage of repeated exposure testing is that the data will provide a reflection of a real-life situation in which consumers repeatedly consume a product over a period of time. The disadvantage of this type of research is that it is time consuming and therefore expensive to conduct (Zandstra, Weegels, Van Spronsen & Klerk, 2004).

CHAPTER 2 : LITERATURE REVIEW

2.1 Physical and chemical properties of acidulants

Acidulants provide sourness in foods and beverages, but also other functions, such as pH adjustment, stabilisation of colour and flavour, prevention of non-enzymatic browning, acting as buffers to control acidity levels, and also acting as preservatives to restrict microbial growth by lowering pH and metal sequestration (Gardner, 1966; Hansson *et al.*, 2001; Shui & Leong, 2002). The principal acids used to enhance beverage flavours are citric, tartaric, fumaric and phosphoric acids. Citric acid is the most widely used acid while malic and tartaric acids are important natural compounds of fruits that are used along with fumaric acid in fruit-flavoured beverages. The chemical structure and properties of some organic acids are summarised in Table 2.1.

Table 2.1: Chemical structure and properties of selected organic acids

Acidulant	Chemical structure (Lindsay, 1985)	Ka-values* (Dzlezak, 1990)	pKa-values (Siebert, 1999)	Molecular weight (g/mol)
Citric acid	$HOOC\text{-}CH_2\text{-}CH(COOH)\text{-}CH_2\text{-}COOH$	$Ka_1 = 7.10 \times 10^{-4}$ $Ka_2 = 1.68 \times 10^{-5}$ $Ka_3 = 6.4 \times 10^{-7}$	$pKa_1 = 3.14$ $pKa_2 = 4.77$ $pKa_3 = 6.39$	192
Malic acid	$HOOC\text{-}CHOH\text{-}CH_2\text{-}COOH$	$Ka_1 = 3.9 \times 10^{-4}$ $Ka_2 = 7.8 \times 10^{-6}$	$pKa_1 = 3.40$ $pKa_2 = 5.11$	134
Tartaric acid	HOOC-CHOH-CHOH-COOH	$Ka_1 = 1.04 \times 10^{-3}$ $Ka_2 = 4.55 \times 10^{-5}$	$pKa_1 = 2.98$ $pKa_2 = 4.34$	150
Fumaric acid	HOOC-CH=CH-COOH	$Ka_1 = 9.30 \times 10^{-4}$ $Ka_2 = 3.62 \times 10^{-5}$	$pKa_1 = 3.03$ $pKa_2 = 4.44$	116

*Ka_1, Ka_2, Ka_3 – dissociation constant for the ionisation of the first, second and third proton

Sourness is one of the five major taste sensations: sour, salty, sweet, bitter and umami (Lawless & Heymann, 1998). The sour taste sensation depends on a number of factors which include pH, total titratable acidity, buffering effects of salts and presence of other stimulants such as salt and sugar (Gardner, 1972). Unlike the sensations of sweetness and bitterness, which can be evoked by a variety of substances, sourness is only evoked by the hydronium ion of acidic compounds.

In aqueous solutions, weak acids are slightly dissociated according to the equilibrium:

$Ka = [H^+].[A^-]/[HA]$, HA ------ $[H^+] + [A^-]$

$H^+ + H_2O$ ----- H_3O^+

Ka is the dissociation constant, which characterizes weak acidulants and A^- is the anion of the acidulant. The sour taste of a food product is contributed by the hydrogen ion (H^+) or hydronium ion (H_3O^+) (Ganzevles & Kroeze, 1987). Berry (2001) stated that sourness is also influenced by the concentration of the solution, pH and anions of the acid.

2.1.1 Citric acid

The most popular and commonly added acid in beverages, especially juice-based ones, is citric acid (Dzlezak, 1990). It accounts for more than 60 % of all acidulants consumed. In 2004 global production of citric acid reached 1.4 million tonnes (Soccol, Vandenberghe, Rodrigues & Pandey, 2006). Citric acid has been indicated as a standard for evaluating the effects of other acidulants in food products (Gardner, 1972). Citric acid is highly soluble in water and can deliver a “burst” or rapid built up of tartness, which makes it suitable for use in flavour modification or enhancement. Citric acid is used to increase tartness levels and enhance fruit flavours in soft drinks and confectionery (Hansson *et al.*, 2001). Citric acid also has strong metal chelating properties and has the widest buffer range of the food acids (2.5 – 6.5) (Dzlezak, 1990).

2.1.2 Malic acid

Malic acid is a naturally occurring acid in apples and other fruit. Malic acid provides a clean, smooth tart taste that lingers in the mouth without a “burst” in the flavour (Dzlezak, 1990). It is said to have unusual taste-blending and flavour-fixative qualities. Malic acid has very little character and is sometimes used in combination with citric acid. The citric portion gives the initial sharp taste of acid and its effect falls off rapidly. When malic acid is used, the lingering acid effect lasts longer, masking the initial sharp taste (Giese, 1995). Although malic acid has a similar ionization potential than citric acid, malic has a stronger apparent acidity that enables smaller amounts of it to be used in certain applications, such as hard candies and canned tomatoes, for the same taste effect (Dzlezak, 1990).

2.1.3 Tartaric acid

Tartaric acid occurs naturally in grapes (Coultate, 1996). Tartaric and malic acids account for 90% or more of the total acidity in grapes. Both tartaric acid and cream of tarter are produced from by-products of the wine industry. It is the most water-soluble of the solid acidulants and contributes a strong, tart taste that enhances fruit flavours (Dzlezak, 1990). Tartaric acid is used for pH reduction and for its flavour-enhancing effects in foods requiring tartness. Tartaric acid is sharper than citric acid and may be used at lower levels. Tartaric acid is often used in grape and lime-flavoured food products. Tartaric acid is said to have a higher sourness intensity than citric and malic acid. It also has a sharp initial impact (Giese, 1995).

2.1.4 Fumaric acid

Fumaric acid occurs naturally in rice, sugar cane, wine, plant leaves and mushrooms. Commercial production of fumaric acid is by fermentation of glucose or molasses with certain *Rhizopus* sp (Dzlezak, 1990). Fumaric acid is one of the most economical acidulants and can be used in smaller quantities than citric, malic, tartaric and lactic acids to achieve similar taste effects (Gardner, 1972; Dzlezak, 1990). Approximately 2 parts of fumaric acid can replace 3 parts of these acids depending on the product and formulation. It is stronger than citric and malic acid with a lingering acidity profile (Giese, 1995). One of its limitations for usage in beverage application is its low solubility in water (Gardner, 1972). Fumaric acid does not provide a "burst" in sour taste. Fumaric acid is said to enhance grape flavours (Gardner, 1972).

2.1.5 Fruitaric® acid

Acidulants are rarely used in a food application on their own, but rather in a combination of two or more acids. In nature, acids are rarely found on their own, and therefore the combination of two or more acidulants enables acidified food products to easier simulate natural flavour (Hartwig & McDaniel, 1995). Fruitaric® acid, the brand name of a combination of malic, tartaric and fumaric acid has been developed by Isegen SA. Fruitaric® acid is claimed to be 1.2 – 1.3 times stronger than citric acid. It is reported to contribute to superior flavour enhancement, especially with fruit flavours (Lanton, 2004).

2.2 Effect of pH on sour taste perception

In early studies (Harvey, 1920) it was found that the hydrogen ion concentration on its own was not a good prediction of sour taste and pH alone was therefore insufficient to predict sourness intensity of a solution. Ganzevles & Kroeze (1987) showed that acetic and citric acids are more sour than hydrochloric acid at the same pH. These authors also found that perceived sourness of carboxylic acids was positively correlated to the dissociation constant (Ka). Hartwig & McDaniel (1995) confirmed this, stating that acidulants with higher pKa values (low capacity to dissociate) were more sour.

Titratable acidity has also been hypothesized (Harvey, 1920 and Noble, Philbrick & Boulton, 1986) to be related to sourness while other researchers (Rubico & Straub according to Hartwig & McDaniel, 1995) reported that the two aspects were slightly or not at all related. Neta, Johanningsmeier & McFeeters (2007) defined titratable acidity (also known as total acidity) as "a measure of both bound and free hydrogen ions in solution". Titratable acidity is also known as the potential hydrogen concentration (Straub, 1992). Shallenberger (1996) proposed that sour taste is related to the potential hydrogen ion (titratable acidity) concentration of a solution. This would imply that acidulants with equal normalities will elicit equal sourness perception, as they have equal titration coefficients. It was also suggested that the chemical mechanism for sour taste perception could be related to the titration of an acid by a base to a neutral end point, the taste receptor serving as the "base" (Neta *et al.,* 2007). Lugaz, Pillias, Boireau-Decept & Faurion (2005) stated that titratable acidity rather than pH should be used to compare weak acids. Norris, Noble & Pangborn (1984) compared acidulant solutions that varied in pH (3.00, 3.25, 3.50 and 3.70) at a constant titratable acidity of 4.00 g/l as well as tartaric-fumaric acid mixtures varying in titratable acidity (3.70, 4.00, 4.30 and 4.60 g/l) at a constant pH of 3.5. The results showed that solutions with the lowest pH and highest titratable acidity had the maximum perceived sourness. Noble *et al.* (1986) compared the sourness intensity of six organic acids in binary solution at equal pH (3.5) and equal titratable acidity (4 g/l) using a paired comparison method. The authors found the sourness intensity of citric acid to be significantly lower than tartaric, malic, succinic, fumaric or lactic acids. Pangborn (1963) found no significant correlations between pH, titratable acidity and the relative sourness of citric, lactic, tartaric and acetic acids.

Buffer capacity is expressed as the molar concentration of a strong acid or strong base which causes variation of the pH of a buffer solution by 1 unit (Neta, 2005). Early studies by Kenrick (as cited by CoSeteng, McLellan & Downing, 1989) and Beatty & Cragg (1935) suggested that the buffer capacity of an acid solution is an indication of the sourness intensity. The results indicated that the sourness of acidulants is correlated with the respective amounts of phosphate buffer needed to adjust an acidulant solution to a pH of about 5. This is better known as the buffer-titration mechanism. Noble *et al.* (1986) compared the sourness of six organic acids and found the significant differences in sourness to be related to the buffer-titration mechanism. Ganzevles & Kroeze (1987) found that the titration volume rank-order of citric, tartaric, formic, lactic, acetic and propionic acids differs from the sour taste intensity rank order.

2.3 Effect of chemical structure on sour taste perception

The results of a study by Moskowitz (1971) concluded that after measuring the psycho-physical sourness functions of 24 organic acids, no simple relationship between sourness and the physico-chemical properties of the acids, such as molecular weight, polarity and optical form, existed. In a study by Siebert (1999) principal component analysis (PCA) was applied to 11 physical and chemical properties of 17 organic acids in beer. Four properties; the number of polar groups, the number of double bonds, molecular size and solubility in non-polar solvents, could predict threshold concentrations in beer.

Gardner (1980) found relations between intensity of the sour taste of weak acids and lipid solubility. The author suggested that absorption of an acid into the taste cell membrane plays a role in the perception of the sour taste. It was therefore suggested that the hydrophobicity of the acid anion were correlated with the sourness intensity. Results from a study by Noble *et al.* (1986) showed that the sour taste of several sets of binary acid solutions at equal pH and titratable acidity were not related to the hydrophobicity of the acidulants. Norris *et al.* (1984) found that hydrophobicity was insufficient to predict sour taste intensities of citric, fumaric and tartaric acids.

CoSeteng *et al.* (1989) correlated sourness intensity of organic acids to the number of carboxylic groups of the acids. Mono-carboxylic acids were found to be more sour than dicarboxylic acid which were more sour than tricarboxylic acids. For the dicarboxylic acids, sourness increased with increasing molecular weight and number of polar substituents. In addiction they reported that molecular weight and polarity were important factors in sourness perception, demonstrating that increasing molecular weight and hydrophobicity of an acid molecule increased sourness intensity.

2.4 Effect of the acidulant concentration on sour taste perception

Organic acids at equal concentrations vary in the degree or intensity of sourness. Rubico & McDaniel (1992) found that acidulants at equal weight (w/v or v/v) differ in their flavour and taste dynamics. In a study by Makhloaf & Blum (1972) acidulants at a range of concentrations were ranked according to the amount of saliva elicited. The rank order appeared to be proportional to the dissociation constant (Ka): hydrochloric > tartaric > citric > succinic > lactic > acetic > proprionic acid. The hydronium ion was found to be insufficient to determine the sourness intensity of a specific acidulant. Fabian and Blum (1943) reported the sourness rank order to be HCL > lactic > malic > tartaric > acetic > citric acid. Watline as cited by Berry (2001) found the intensity of sourness to be fumaric > tartaric > malic > acetic > citric > lactic > gluconic acids. Beuchsenstein & Ough (1979) compared relative sourness of citric, dl-malic and fumaric acids in distilled water solutions and in wine. The results showed that equal molar concentrations were found to be equal in sourness intensity (equi-sour). Relative sourness of the three acidulants at equal weight concentrations were found to be fumaric > dl-malic > citric. In a study by CoSeteng *et al.* (1989) a trained sensory panel ranked the sourness of citric, malic, tartaric, lactic and acetic acid solutions at equal weight concentrations (4g/l). The results of the sourness intensity were: acetic > malic > tartaric = lactic > citric. Ough (1963) compared equal molar concentrations of acids in wine and found the perceived sourness intensity to be citric > tartaric = fumaric > adipic.

Sowalsky & Noble (1998) evaluated the separate effects of concentration, pH and anion species on intensity of sourness and astringency of four organic acids (citric, malic, tartaric and lactic acid). In the first experiment, a trained sensory panel rated the sourness of three concentrations of each acidulant (0.02N, 0.06N and 0.10N) at three different pH levels (2.8, 3.4 and 4.0). A total of nine test solutions for each acid were compared. The results showed that the intensity of the sourness increased with decreasing pH, while at each pH level, sourness increased with increased acid concentration (expressed as normality). During the second experiment twelve binary solutions with equal pH and titratable acidity were formulated for all possible pairwise combinations. Using contrast statements to directly compare the sourness differences for each binary pair, only one significant difference was found. For the lactic-citric pair, the sample with lactic acid as the major anion and citric as the minor one was rated 20% more sour than the sample in which citric acid was the dominant anion. This is consistent with previous results (Noble *et al*., 1986). The authors concluded that sourness is independently influenced by concentration, pH and anion species of the acid.

Johanningsmeier, McFeeters & Drake (2005) analysed data from their own research together with data from Hartwig & McDaniel (1995) and Sowalsky & Noble (1998) and hypothesized that sourness intensity is linearly related to the molar concentration of all organic acid species with at least one protonated carboxyl group plus the molar concentration of free hydrogen ions. The hypothesis implies that, on a molar basis, different organic acids will be equally sour, provided at least one carboxyl group is protonated. Neta *et al.* (2007) concluded that molar concentration is insufficient to predict sour taste perception. The diversity of results when ranking the sour taste perception by molar concentration may be explained by different ranges in concentration and different pH values of the solutions used in the different experiments.

2.5 Physiology of sour taste perception

The mechanisms behind the perception of sweet, salt, bitter and umami taste sensations are better understood compared to the mechanism behind sour taste perception (Neta *et al.*, 2007). The physiology of sour taste perception remains controversial and significant diversity among different species exists with regards to cellular schemes used for detection of stimuli. Sour and salty tastes are caused by substances that ionize in solution while sweet and bitter tastes are elicited by substances that do not ionize (Shallenberger, 1996).

Taste perception is initiated by the interaction of a chemical stimulus with receptor sites located on microvilli. The stimuli may bind to a membrane receptor or protein, it may pass through a channel, or it may activate or block an ion channel (Lindemann, 1996; Neta *et al.*, 2007). DeSimone, Lyall, Heck & Feldman (2001) suggested that proton movements through paracellular pathways may contribute to sour taste transduction.

Sourness is evoked by dissociable H+ ions. Previous studies found that the hydrogen ion concentration on its own was not a good prediction of sour taste and pH alone was therefore insufficient to predict sourness intensity of a solution (Harvey, 1920; Beatty & Cragg, 1935; Ganzevles & Kroeze, 1987). Therefore taste receptor cells could not be defined as extracellular pH detectors (Lyall, Alam, Phan, Ereso, Phan, Malic, Montrose, Chu, Heck, Feldman & DeSimone, 2001). Ganzevles & Kroeze (1987) proposed that different receptor systems are responsible for sour taste transduction of hydrogen ions and protonated acid species. DeSimone *et al.* (2001) stated that intracellular pH changes may also play an important role during acid taste perception and that weak organic acids can permeate cell membranes as undissociated molecules. The authors also found a linearly relation between intracellular pH and extracellular pH which resembles the characteristics of acid sensing cells in the brain and carotid bodies. Lyall *et al.* (2001) confirmed this, stating that a decrease in the intracellular pH of taste receptor cells acted as stimulus in rating sour taste transduction. DeSimone *et al.* (2001) proposed that the mechanisms for sour taste perception are different among strong and weak acids. Lyall *et al.* (2001) found that responses to chorda tempani nerve were positively correlated with a decrease in intracellular pH and that weak acids were more effective than strong acids in acidifying the cell.

A significant diversity exist among different species with regards to the mechanisms used to perceive sour taste. Kinnamon & Roper (1988) found that acid blocked K^+ channels located in the apical membrane of *Necturus* species mediated sour taste perception. Miaymoto *et al.* according to DeSimone *et al.* (2001) found that HCl depolarized taste receptor cells via apical H^+ gated channels permeable to Ca^{2+} and Na^+ ions in bullfrogs. Shimada, Ueda, Ishida, Yamamoto & Ugawa (2006) stated that sour and salty tastes are detected by ion channels in rats and identified acid-sensing ion channel 2a (ASIC2a) and ASIC2b as candidates for rat sour-sensing channels. These acid sensing ion channels are located on the apical and basolateral membranes. Recently, a polysistic-kidney-disease-like ion channel (PKD2L1) was identified as a possible sour taste sensor (Huang, Chen, Hoon, Chandrashekar, Guo, Trankner, Ryba & Zuker, 2006; Ishimaru, Inada, Kubota, Zhuang, Tominaga & Masunami, 2006). Huang *et al.* (2006) genetically modified mice to lack PKD2L1 expressing cells and found that these animals did not respond to sour stimuli, while reactions to sweet, salty, bitter and umami were unaffected.

2.6 Time intensity

Descriptive sensory evaluation involves the evaluation of sensory attributes of food products and is able to describe how changes in the product formulation (e.g. change to a different acidulant) will influence the sensory profiles of products. The method is insufficient to provide information on how perceived sensory properties change over time. Panellists are usually instructed to rate the intensity of an attribute on a scale, resulting in a single (unipoint) measurement. This method requires the panellist to "time-average" the changing perceived sensation to a single value, without reporting the changes in the perceived intensity over time. Unipoint or a single time point measurement is insufficient to distinguish between products with different temporal characteristics (Fradin, 1999). Unipoint scaling of attribute intensities have been shown to correlate well with maximum intensity (I_{MAX}) values of a time intensity curve (Lundahl, 1992; Lawless & Heymann, 1999) indicating that taste intensity judgment depends strongly on peak-intensity and to a lesser degree on other time intensity parameters. The measurement of time intensity parameters of a perceived intensity is especially useful when lingering aftertastes are compared (Straub, 1992).

Time intensity (TI) is "the measurement of the intensity of a sensation over time in response to a single exposure to a product or other sensory stimulus" (ASTM according to Fradin, 1999). TI is therefore an extension of the traditional scaling method, as used during descriptive sensory evaluation, due to the fact that it provides temporal information about perceived sensations.

Several studies to compare the time intensity properties of sweet and bitter taste sensations have been conducted. The effect of different sweeteners at equi sweet concentrations on the temporal quality of sweet taste intensities have been studied by Schiffman, Sattely-Miller & Bishay (2007). Cliff & Noble (1990) studied sweetness and fruitiness using TI in solutions varying in sugar and flavour concentrations. Guinard, Hong & Budwig (1995) investigated the TI profiles of sweet and bitter stimuli at equi-intense concentrations. Leach & Noble (1986) studied the temporal bitterness elicited by equi bitter concentrations of caffeine and quinine. Bonnans & Noble (1993) compared the sweetness, sourness and fruitiness of orange flavoured solutions at equi-sweet levels.

Few studies have considered the time intensity parameters of sour compounds. Claims have been made that each acidulant has a particular set of taste characteristics, which include rate of sourness development, the intensity of sourness, the lingering effect as well as the aftertaste of the acidulants (Berry, 2001; Lanton, 2004). Gardner (1966) stated that shape of time intensity curves of different acidulants differs. The taste of malic acid was said to build up more slowly than citric acid, reached a lower sourness intensity but persisted much longer. Berry (2001) described the taste of citric acid as a sharp, clean sour taste with little persistence on palate; the taste of malic acid as a strong but smooth acidic taste, persisted longer on the palate; the taste of tartaric acid as a sharp and bitter acidic taste of short duration and the taste of fumaric acid as a strong metallic taste, very lingering on the palate. However, these verbal descriptions are not supported by scientifically published evidence of experimental results.

Limited research has been done on how the temporal characteristics of the different acidulants vary from each other. A TI study conducted by Straub (1989) compared the sourness and astringency of seven acidulants at equal sour concentrations at two different sourness levels (0.041%citric acid and 0.083% citric acid). No significant differences were found for the time to reach maximum intensity (T_{MAX}) values between citric, malic, tartaric and fumaric acids at both sourness levels. Fumaric acid had a significantly higher I_{MAX} and area under curve value (AUC) compared to citric, malic and tartaric acids at the lower sourness level. The acids did not differ significantly in duration (DUR) at the lower sourness level.

At the higher sourness level no significant difference was found between the I_{MAX} for malic and tartaric acids, while citric acid had the lowest and fumaric acid the highest I_{MAX} value ($p<0.0001$). Fumaric acid had a significantly higher AUC value compared to citric, malic and tartaric acids at the higher sourness level. Citric and malic acids did not differ in AUC and malic and tartaric acids did also not differ in the AUC value. The sourness duration for fumaric acid was significantly longer than that of tartaric acid. Citric, malic and tartaric acids did not differ in duration at the lower sourness level, while fumaric, malic and tartaric acids did not differ in duration of sourness at higher sourness level.

Jacobs (2001 - unpublished) studied the effect of seven acidulants on TI parameters at equal sour concentrations. These concentrations were added at weight/volume basis and were 0.2% citric acid, 0.14% malic acid, 0.121% tartaric acid, 0.101% fumaric acid and 0.108% Fruitaric® acid. When comparing the acidulants in water, no significant differences were found in I_{MAX}, T_{MAX}, AUC and DUR between citric, malic, tartaric, fumaric and Fruitaric® acids for sour taste perception. The same result was found when these acidulants were compared in a sweetened, unflavoured model (Table

2.2). When the seven acidulants were compared in a sweetened, flavoured model no significant differences were found for the DUR between citric, malic, tartaric, fumaric and Fruitaric® acids. The author found that tartaric acid had the lowest total sourness taste impact. Fumaric acid had a slightly lower mean AUC value. Malic acid had a similar mean AUC compared to citric acid. I_{MAX} for sourness intensity of Fruitaric® acid was significant higher than that of tartaric acid.

In a study by Norris *et al.* (1984) a trained sensory panel compared the perceived sourness of solutions of citric + fumaric acids and citric + tartaric acids at pH 3.5 and titratable acidity of 4.0 g/l using the time-intensity method. The maximum sourness intensity of citric acid was found to be significantly lower than that of tartaric and fumaric acids. Lugaz *et al.* (2005) evaluated the time intensity of acidulants at equal molar concentrations and found that the I_{MAX} to be citric > malic > lactic > acetic.

Table 2.2: Mean values of TI parameters for acidulants at equal sour concentrations in a sweetened, flavoured model (adapted from Jacobs, 2001 – unpublished).

	I_{MAX}	DUR	AUC
Citric Acid	46.65^{ab}	37.65^{a}	961.8^{b}
Malic Acid	47.08^{ab}	38.88^{a}	959.1^{b}
Tartaric Acid	41.96^{a}	35.74^{a}	828.7^{a}
Fumaric Acid	44.92^{ab}	35.94^{a}	906.7^{a}
Fruitaric® Acid	53.04^{b}	37.21^{a}	1043.1^{c}

abc: Average values in a column with different letters differed significantly ($p<0.05$)

2.7 Consumer Sensory Evaluation

In the food industry it is often necessary to make small changes to the formulation of a well-known food product. Chung & Vickers (2007a) stated that such changes to a food product could affect its long term acceptability. It is easier to change the sensory profiles of some food products without losing loyal consumers, than it is to change it of other products (Zandstra *et al*., 2004).

Traditional consumer sensory evaluation methodology does not always provide an accurate measure of repeated/extended use responses to a product. The measurement is normally restricted to first time impressions and acceptance of products after repeated exposure is rarely considered. The assumption that brief, once-off laboratory consumer sensory evaluation can predict long-term product success or failure may not be true (Stubenitsky *et al*., 1999). Repeated exposure to specific food stimuli may lead to either an increase or a decrease in acceptance ratings (Köster, Couronne, Léon, Lévy & Marcelino, 2002). The first phenomenon has been defined as the "mere exposure effect". The concept of mere exposure as introduced by Zajonc (1968) states that "the more a consumer is exposed to a certain stimulus the more he/she will gain in liking or preference for the specific stimuli". According to Mela (2000) the phenomenon of "I got used to it" is also explained by the mere exposure effect. Pliner (1982) suggested that the mere exposure effect may play a role in the acquisition of food product acceptance.

The decline of acceptability with repeated exposure is related to concepts like food boredom and monotony. The effects of loss of initial curiosity, the development of food boredom which could lead to product irritation, are often the cause of product failure in the food market (Köster *et al.,* 2002). Hetherington, Pirie & Nabb (2002) defined food monotony as "lowered acceptance of a food as a function of the number of times a food product is consumed". Zandstra *et al.* (2004) defined the phenomenon of boredom as "a neural/physiological response with a decrease in actual liking caused by satiation with specific attributes of the consumed food". The theories of both monotony and food boredom indicate the existence of a link between familiarity and preference (Lévy & Köster, 1999). According to Miller, Bell, Pelkman, Peters & Rolls (2000) the definition for sensory-specific satiety is the decrease in pleasantness of the sensory properties (taste, texture, odour and appearance) while consuming a food product, causing a decrease in desire to continue to eat the product within a meal. Sensory specific satiety is used to describe the decrease in acceptance immediately after consumption. Sensory specific satiety is thus an explanation of short-term boredom.

Rolls & de Waal (1985) reported long term sensory-specific satiety in an Ethiopian refugee camp. Refugees that had been staying in the camp for six months and others that have just arrived at the camp rated their liking of three regular camp foods and three foods not consumed in the camp before. The refugees liked the foods they have been eating for 6 months significantly less than the new foods, while the new refugees liked both types equally. Chung & Vickers (2007a) suggested that the long term sensory-specific satiety could be the same phenomenon as boredom or monotony. Porcherot & Issanchou (1998) stated that the effect of repeated exposure to a specific food product depends on the familiarity level of the product, the initial preference and also the complexity level of a food product.

These changes in acceptance ratings over time may be referred to as hedonic adjustment. Hedonic adjustment potential (HAP) was defined as "the predictive measure of the potential changes in like/dislike perceptions of consumers to the sensory properties of food stimuli after repeated exposure" (De Kock & Kinnear, 2003).

2.7.1 Familiarity with food product

Several studies have linked consumer preferences to familiarity (Lévy & Köster, 1999; Stallberg-White & Pliner, 1999; Hetherington, Bell & Rolls, 2000; Hetherington *et al*., 2002). Porcherot & Issanchou (1998) found that the most familiar flavours are usually also the most preferred when consuming it for the first time. Pliner (1982) stated that unfamiliar food products increase in liking with repeated exposure, while familiar food products lead to monotony or boredom.

The optimal arousal level theory of Berlyne (1970) states that experience with a specific product influences consumers' attitude towards this product. Experience with the product may lead to either an increase on a decrease in acceptability. These changes in acceptability may depend on how high the arousal potential of the initial experience is compared to a person's optimal arousal level. A high arousal potential will lead to increase liking and low arousal level will lead to decreased liking.

2.7.2 Perceived complexity and preferences

Köster *et al.* (2002) stated that as a consumer is repeatedly exposed to a more complex product than the product he/she prefers, the complexity of the new product will become less complex and might lead to increases in liking perceptions over time. Berlyne (1970) stated that acceptability of more complex products will increase after repeated exposure to the product, while the opposite will occur if less complex products become less novel after repeated exposure. The pacer theory of Dember (1970) predicts that consumers learn to appreciate more complex stimuli during experience. Exposure to stimuli which are slightly more complex than optimal leads to an increase in the person's optimal arousal level.

Stubenitsky *et al*. (1999) stated that "simple" food stimuli become boring more quickly than "complex" stimuli. Köster (1981) stated that consumers would prefer food products that are more complex (have more information) if they are only exposed to the product for a short while, because the consumer probably will not have been able to analyze all the information contained in the product. If consumers are exposed to the product long enough to make a more thorough analysis consumers tend to prefer products of moderate complexity. This indicates that initial preference testing tends to be influenced by the complexity of the food product and might not be a clear indication of the actual preference for the food product. For complex food products it is therefore risky to rely on initial consumer preferences if the consumers did not have time to familiarize themselves with the complexity.

In a study by Porcherot & Issanchou (1998) consumers were exposed to different flavours of salty crackers for a period of 3 months. Consumers were asked to rate the samples hedonically as well as rate the perceived complexity of and their familiarity with each sample. A significant positive correlation was found between the hedonic ratings and the familiarity levels. A significant negative correlation was found between the hedonic ratings and the perceived complexity. The consumers also commented that the more complex products were more difficult to describe.

2.7.3 Initial preferences for the food product

Data of Schutz and Pilgrim, according to Mela (2000) found that it is appropriate to consume certain foods every day (e.g. bread, cereals, etc.) and thus some products are more resistant to be associated with boredom. This indicated that staple foods are more resistant to boredom than other foods. In a study by Zandstra, de Graaf & van Trijp (2000b) repeated consumption did not change the pleasantness of staple foods such as dairy products, bread, or coffee, whereas other foods such as meat and vegetables followed a decline in preference. A laboratory based study of food boredom done by Siegel & Pilgrim, according to Hetherington *et al.* (2000) found that food products with initial high preference ratings slowed the development of boredom, while preference ratings of food products that were low in preference declined steeply with repeated exposure.

In a study by Zandstra, De Graaf, Mela & Van Staveren (2000a) consumers were exposed to bread for a period of five successive days. The breads varied in salt concentrations (low, medium and high) and were used as food products differing in levels of pleasantness. Mean pleasantness ratings remained unaltered for all the samples throughout the study. Desire to eat increased for the unpleasant bread sample over the five day period. The difference in results between the desire-to-eat and pleasantness ratings indicated that the pleasantness or hedonic ratings for a specific food product do not predict the desire-to-eat that product or if the consumer will purchase this product once it is on the market.

Kroeze (1982) defined habituation as the decrease in acceptance ratings of an unfamiliar food product after repeated exposure. Ernest & Epstein (2002) reported a correlation between the rate of habituation and the intensity of the stimulus (quicker habituation to weaker stimuli) and a correlation between rate of habituation and frequency of stimulation (habituation develops more quickly when more frequently exposed to a stimuli). The theory of habituation was explained as the repeated exposure to food stimuli which results in decreased acceptance/preference ratings to the sensory properties of the food product, which could result in satiation for that product (Ernest & Epstein, 2002). Kroeze (1982) stated that habituation is different from adaptation in that adaptation is a peripheral brain process compared to habituation which is a central cognitive process.

According to Köster (1981) the human senses loose some of their sensitivity after repeated or prolonged stimulation to specific stimuli. This phenomenon is called sensory adaptation. Prolonged stimulation to a specific sensory attribute may also result in habituation. Köster (1981) defined habituation as "a loss of interest in the information presented". New information of new food products always attracts the attention of consumers and therefore consumers are interested in buying the new food products. As the consumer is exposed to the product repeatedly the new food product looses its information value, consumers loose interest as this product also looses its novelty value. After a while the consumers might stop buying the product completely. Köster *et al.* (2002) stated that when novelty dominates in the initial preference testing and this influence are changed due to new characteristics that are explored later on, preference changes will be largest for new and unfamiliar products.

2.7.4 Types of repeated exposure tests to determine hedonic adjustment potential

To predict whether a food product will be a success on the market it was suggested that products should be evaluated to determine long-term aspects of preference and that consumption behaviour under domestic conditions be measured. There are two methods of measuring the consumption behaviour. The first method is to observe the consumption of the food product in a natural context, i.e. in a dining hall or at home. The second method is a diary method in which participants have to complete a diary at home each time they consumed the product (Porcherot & Issanchou, 1998).

Köster, according to Porcherot & Issanchou (1998) developed a laboratory boredom test to predict long-term acceptability/preference of food products under well-controlled conditions. The aim of the boredom test is to determine if boredom is caused by different prototypes of the same product. This test consists of many sessions, one session for each variant. During these sessions, participants are asked to rate their liking of at least fifteen samples. Although the samples are exactly the same, the participants are informed that the samples differ slightly. It was suggested that the total mass/volume of the number of samples that are evaluated one after the other should be at least three times the suggested market portion to observe a boredom effect while avoiding complete satiety or aversion.

Porcherot & Issanchou (1998) suggested that it would be better to allow participants to consume the quantity of the products they wanted and examine the change of the consumed amount over the number of samples after repeated exposure to that product rather than examine the change in liking ratings. Different boredom effects between the products could be determined by these consumption measurements.

Time-preference measurement allows product developers to quickly determine if repeated consumption of a food product may lead to boredom. In a time-preference test, as described by Moskowitz (2000), the consumers consumed the food product three times at home and are then asked to estimate their degree of interest in the food product. They are asked to imagine that they had not use the product for 3 days, 7 days, 14 days and 30 days, respectively. For each time period, the consumer rates how interesting they would consider the product.

In a study by Lévy & Köster (1999) consumers were subjected to three different soft drinks, differing in familiarity levels. After the laboratory measurements, the consumers could choose samples to consume at home. The results indicated that the most familiar sample received the highest ratings under laboratory conditions. With repeated exposure the unfamiliar drink were chosen more often. It was also stated that the more experience a consumer gets with a range of stimuli, the less likely he is to return to the more familiar one. The results indicated a link between the hedonic ratings and the choices made by the consumer. More than half of the consumers chose according to their hedonic ratings. The rest of the consumers were not loyal and did not make the choices according to the hedonic ratings. This was especially true for the more unfamiliar samples.

One of the reasons why changes in consumer choices occur is to satisfy their need for variety. Chung & Vickers (2007b) defined variety seeking behaviour as the “tendency for a person to switch away from an item consumed during the last occasion”. Individuals differ in the way that they make food choices. In variety seeking the focus is more on the attitude of the individual than on the food product itself (Meiselman, 1996).

Van Trijp & Steenkamp according to Meiselman (1996) developed a questionnaire (Table 2.3), which measures variety seeking and which consists of eight items with five-point agree-disagree scales. They have based the variety seeking on the motivational concept of optimal stimulation level, which can modify the extent to which consumers explore new food products.

Studies have attempted to use sensory specific satiety as a rapid predictor of long term acceptability (Vickers & Holton, 1998; Chung & Vickers, 2007a). In a typical sensory specific satiety test the consumers will taste and rate their liking of several samples of food, including the test food, eat a serving of the test food, then finally retaste and re-rate their liking of all the food samples. Both the amount of the test food consumed and the change in liking of the test food from before to after consumption has potential to serve as indicators of long term acceptability as they incorporate the effects of adaptation, habituation and ingestion (Vickers & Holton, 1998).

Table 2.3: The items constituting the VARSEEK-scale (Van Trijp & Steenkamp, according to Meiselman, 1996)

	Please indicate your feeling about each statement, using the following scale; 1 = Disagree strongly, 9 = Agree strongly
1	When I eat out, I like to try the most unusual items, even if I am not sure I would like them
2	While preparing foods or snacks, I like to try out new recipes
3	I think it is fun to try out food items one is not familiar with
4	I am eager to know what kind of foods people from other countries eat
5	I like to eat exotic foods
6	Items on the menu that I am unfamiliar with make me nervous
7	I prefer to eat food products I am used to
8	I am curious about food products I am not familiar with

Chung & Vickers (2007a) studied the effect of repeated exposure on teas with different sucrose levels. Subjects tasted and evaluated the samples in a taste test; repeatedly consumed the teas over a 6 week period and again during a taste test. A sensory specific satiety test was also conducted. Liking ratings for the lower concentration sucrose tea increased after repeated exposure becoming equal to the optimally sweetened tea at the end of the study. Sensory specific satiety measured in a single session was insufficient to predict the long-term acceptance for the products.

2.7.5 Effect of repeated exposure on acceptance of beverages with small differences in flavour profiles

In a study by Stein *et al.* (2003) consumers in a laboratory evaluation setting evaluated amongst other products a commercially available bittersweet beverage. The participants consumed the beverage once daily for a period of seven days in a home use environment, and returned to the laboratory to evaluate the beverage again. During the laboratory evaluation the participants were instructed to rate the hedonic, intensity and familiarity levels of the products. At the end of the first laboratory evaluation participants were told that as a reward, they could take home up to 14 bottles of the beverage for their own consumption. The number of bottles (0-14) requested by each participant was recorded. The results from the second laboratory evaluation showed an increase of 68% in the hedonic ratings for the beverage. There was also a significant increase in the familiarity ratings from the first laboratory session to the second laboratory session. The relationship between familiarity and hedonic evaluations was established. The results also suggested that repeated tasting might be necessary to identify flavour qualities that may not be perceived on first tasting. Repeated tasting resulted in redistribution of perceived taste qualities e.g. a product that was initially perceived to be very bitter, was after repeated exposure perceived to be slightly sweet with medium bitterness.

In a study by Luckow, Seehan, Delahunty & Fitzgerald (2005) four orange juice samples (one control and three juices fortified with probiotic microorganisms) were evaluated. During a laboratory test, consumers (n=75) rated the overall acceptability (on a 100 mm line scale) of the four juice samples (40 ml) and then ranked the four samples according to preference. At the end of the laboratory session the consumers were divided in 4 groups. Each group received one of the juice samples for the home exposure. Each group received seven 100 ml bottles to consume at home and was instructed to consume one bottle per day and return for another laboratory session in seven days time. The results showed a significant increase in overall acceptance for the more unfamiliar orange juice samples after the exposure. The group that was exposed to the control sample rated the control sample significantly lower after exposure. The authors concluded that exposure to and familiarity of probiotic juice helps to increase consumer acceptance of these samples.

Vickers & Holten (1998) studied the effect of repeated exposure on consumer acceptability of ice teas with different strengths. At the start of the trial the consumers preferred the tea with high flavour concentration compared to the ice tea with the lower concentration. After repeatedly consuming the ice tea *ad lib* on 12 different sessions, the consumers found the weaker tea to be more acceptable than the stronger tea. The authors found that sensory specific satiety testing may better measure long term acceptance as it incorporate more effects of adaptation, habituation and ingestion. In a study by Weijzen, Zandstra, Alfieri & De Graaf (2008) consumers (N=66) were exposed to three soups for 14 days and to four snack samples for 5 days (N=61). The results from this study showed that sensory specific satiety testing were insufficient to determine long term product acceptance.

Chung & Vickers (2007b) measured consumers' choice and changes in choice of three types of tea, each at two sweetness levels. In a taste test the consumers ranked the six samples according to preference after which they attended 20 sessions as part of the long-term choice experiment. During the long term choice test, consumers could choose one of the samples to drink, indicated their liking for the product, if they were tired of it and their satisfaction with having chosen it. The authors observed four choice patterns: constant-switcher, acquired-liker, non-switcher and systematic-switcher. Liking ratings of the low sweetened tea increased and the tiredness ratings of the optimum-sweet tea increased.

Luckow, Seehan, Fitzgerald & Delahunty (2006) studied the effect of repeated exposure on consumer acceptability of probiotic juices. Probiotic juices may cause off-flavours which often lead to consumer dissatisfaction. The authors attempted to mask the off flavours of the juice by altering the formulations.

After the consumers evaluated the three samples (control, mask and no mask) in a laboratory, they were divided in three experimental groups each taking 7 bottles of one of the juices home for consumption at home. After the home exposure the consumers returned to the laboratory and evaluated the overall liking of the three samples again. Exposure had a significant effect on the consumer acceptability. Consumer acceptance increased for the no mask juice after exposure. Although not significant, exposure lead to a decrease in acceptability for the control juice which could be attributed to sensory specific satiety. The authors found that it might be that the control juice was consumed until satiety.

2.8 Conclusion

Organic acids at equal concentrations vary in the perceived degree or intensity of sourness. Physical and chemical properties such as pH, molecular weight, pKa values, chemical structure and acidulant concentration were reported to clarify the differences in sourness intensities of acidulants at equal weight concentrations. Claims have been made that each acidulant has a particular set of taste characteristics, which include rate of sourness development, the intensity of sourness, the lingering effect as well as the aftertaste of the acidulants. Limited research has been done on how the temporal characteristics of the different acidulants vary from each other.

Traditional consumer sensory evaluation methodology does not always provide an accurate measure of repeated/extended use responses to a product. Repeated exposure to specific food stimuli may lead to either an increase or a decrease in acceptance ratings. Factors that might influence changes in consumer acceptance include familiarity, perceived complexity and initial preferences for the food product.

CHAPTER 3 : HYPOTHESES AND OBJECTIVES

3.1 Hypotheses

It is hypothesized that lower concentrations of the acidulants (malic, tartaric, fumaric and Fruitaric® acid) will taste equally sour than a 2% citric acid solution, both in water and in a flavoured sports drink. Acidulants (malic, tartaric, fumaric and Fruitaric® acids) at equal weight concentrations in water will be perceived as more sour compared to a 2% citric acid solution. Previous studies showed that acidulants at equal weight concentrations vary in sourness intensity. These differences were said to be contributed by multiple variables, both physical and/or chemical properties, i.e. differences in molecular weight (CoSeteng *et al.,* 1989), pKa values (Rubico & McDaniel, 1992), chemical structure (Moskowitz, 1971; CoSeteng *et al*., 1989; Siebert, 1999; Johanningsmeier *et al.,* 2005), the pH of the solution (Norris *et al*., 1984; Noble *et al.,* 1986; Ganzevles & Kroeze, 1987; Sowalsky & Noble, 1998) or the titratable acidity of the solution (Harvey, 1920; Shallenberger, 1996; Lugaz *et al.,* 2005).

Although claims have been made that each acidulant has a particular set of taste characteristics, which include the rate of sourness development, the intensity of sourness, the lingering effect as well as the aftertaste of the acidulants (Berry, 2001; Lanton, 2004), it is hypothesized that the temporal sensory profiles of the flavoured sports drinks, containing different acidulants at equal sourness concentrations to a 0.2% citric acid solution, will not differ. Previous studies showed that equal molar concentrations were found to be equal in sourness intensity (Ough, 1963; Beuchenstein & Ough, 1979; Sowalsky & Noble, 1998) which might lead to equal temporal impact.

It is hypothesized that consumers will initially not be able to clearly distinguish that different acidulants have different taste qualities in application but after repeated exposure the different taste qualities will become more evident and influence liking. Previous studies have shown that slight differences in the taste profiles of comparative food products only became clearly noticeable after repeatedly tasting the products (Stein *et al.*, 2003; Chung & Vickers, 2007a). Repeated exposure to specific food stimuli may lead to either an increase or a decrease in acceptance ratings (Köster *et al.*, 2002) i.e. hedonic adjustment.

3.2 Objectives

To determine the sourness intensity of malic, tartaric, fumaric and Fruitaric® acids at equal sour and equal weight concentrations compared to a 0.2% solution of citric acid in water, as well as in a flavoured sports drink.

To determine the temporal perceived sourness of different acidulants (citric, malic, tartaric, fumaric and Fruitaric® acids) in water and flavoured sports drinks at equal sour concentrations. The rate, onset, duration and intensity of the perceived sourness will be compared.

To determine if repeated exposure testing of sports drinks with different acidulants (citric acid, malic acid, tartaric acid, fumaric acid and Fruitaric® acid) added at equal sour concentrations would lead to hedonic adjustment.

CHAPTER 4 : RESEARCH

The three objectives of this study will be investigated in the style of three research articles.

Sensory perception of acidulants at equal weight and equal sour concentrations (4.1)

Temporally perceived sourness of different acidulants in water and flavoured sports drinks at equal sourness concentrations (4.2)

Would repeated consumption of sports drinks wit different acidulants lead to hedonic adjustment? (4.3)

4.1 SENSORY PERCEPTION OF ACIDULANTS AT EQUAL WEIGHT AND EQUAL SOUR CONCENTRATIONS

4.1.1 Introduction

Organic acids at equal concentrations vary in the perceived degree or intensity of sourness (Fabian and Blum, 1943; Ough, 1963; Makhloaf & Blum, 1972; Beuchsenstein & Ough, 1979; CoSeteng *et al.,* 1989; Rubico & McDaniel, 1992; Johanningsmeier *et al.,* 2005). It is therefore not appropriate to substitute one food acid for another on equal weight basis. Claims have been made that citric acid does not give the best possible taste results in beverage applications (Lanton, 2004). Compared to citric acid, other acidulants e.g. malic acid, fumaric acid and tartaric acid or combinations thereof, may potentially provide superior sensory properties in beverage applications. When used in food products, acidulants differ in the perceived sourness elicited when consuming these foods. Berry (2001) suggested the following percentages for replacing citric acid, fumaric acid (67 – 72%), malic acid (78-94%) and tartaric acid (80-85%). Several studies were conducted to determine the equal sourness concentrations of different acidulants. Beatty & Cragg (1935), Pangborn (1963), Straub (1992) and Jacobs (2001 - unpublished) used paired comparison tests to assess sourness intensity of different acidulants. Beatty & Cragg (1935) used hydrochloric acid as reference while the other studies used citric acid as reference. Previously the equal-sourness of an acid was defined as that concentration at which at least 50% of responses given by a trained sensory panel indicated it to be more sour than citric acid (Beatty & Cragg, 1935; Pangborn, 1963).

The assumption could be made that equal sourness concentrations as determined in water solutions would be the same in a more complex beverage. Pangborn (1963) found that this was not true. Equal-sourness concentrations as determined in water were therefore also compared in beverage applications. During this study the equal weight and equal sourness concentrations of citric acid, malic acid, tartaric acid, fumaric acid and Fruitaric® acid were compared when added to water and also when added to two flavours (*Grape* and *Lemon & Lime*) of a commercial sports drink. The objective of this study was to compare the sourness intensity and chemical properties of malic, tartaric, fumaric and Fruitaric® acids at equal sour and equal weight concentrations to a 0.2% solution of citric acid in water, as well as in a flavoured sports drink.

4.1.2 Methodology

Samples

Five acidulants were included in the study. DL malic acid, fumaric acid (hot water soluble) and Fruitaric® acid (a composite of malic acid, tartaric acid and fumaric acid) from Isegen South Africa (Pty) Ltd. Citric acid (anhydrous) and tartaric acid from C.J. Petrow Chemicals (Pty) Ltd. were also used. The acidulants were dissolved in deionised water and also in concentrated sports drink syrups.

Previously, equal-sourness levels of malic, fumaric and tartaric acids compared to 0.2% w/v citric acid in deionised water were determined (Jacobs, 2001 - unpublished) using paired comparison tests incorporating 5 to 7 concentrations of each acid. The concentration of 0.2% (w/v) was selected to investigate acid characteristics as this level is often used for beverages.

The acidulants were added at either equal-sour (as determined in water) or equal weight concentrations. These concentrations were added on a weight/volume (w/v) basis. The equal sour concentrations added to water were 0.2% citric acid, 0.14% malic acid, 0.121% tartaric acid, 0.101% fumaric acid and 0.108% Fruitaric® acid, respectively. The sports drink was supplied as a concentrated syrup without any acidulants added and were provided by a South African manufacturing company. The sports drink is a well-known and popular sports drink in South Africa.

The ingredients of the sports drink were: water, sucrose, dextrose, flavour, salt, cloudifier, sodium citrate, vitamin C, monopotassium phosphate, colourant, sodium benzoate and sorbic acid. Two flavours of the sports drink, namely *Grape* and *Lemon & Lime* were included in the study. The sports drink usually contains citric acid at a concentration of 0.21% w/v. The equal-sourness concentrations as determined in water were therefore adapted (by numerical calculation) when added to the sports drink and were 0.21% citric acid, 0.147% malic acid, 0.127% tartaric acid, 0.106% fumaric acid and 0.113% Fruitaric® acid, respectively. The sports drink concentrate was diluted (1:4) with deionised water. All the samples were prepared in clean 500 ml volumetric flasks the day before testing. The samples were kept at 4^{o}C overnight. The samples were left at room temperature (± 25^{o}C) before serving them to the sensory panel at 13 ± 3 ^{o}C.

Sensory panel

The sensory panel consisted of ten trained panellists (2 male, 8 female). The panellists were students and staff members from the University of Pretoria. The panellists were non-smoking and self-reported healthy individuals. Prior to participation, potential panellists were screened for recognition of the basic tastes and ability to discriminate small flavour differences.

The best estimate detection threshold (BET) value for sour taste of the individual panellists was determined during the screening phase of the study.

This was conducted according to determination of odour and taste thresholds by a forced-choice ascending concentration series method of limits defined by ASTM Practice E 679-79 (ASTM, 1991). This protocol uses the 3 Alternative Forced Choice (3-AFC) test method. A set of three samples, two deionised water samples and one water sample with citric acid (test sample), was presented and the panellist was requested to identify the test sample. A total of six sets of three samples each were presented to each panellist. The sets were arranged in order of increasing concentrations of the test samples. The citric acid concentrations used during the test were 0.0041 % w/v to 0.0125 % w/v with a dilution factor of 0.803 per step (Jacobs, 2001 - unpublished). The panellists started with the lowest concentration step. These evaluations were conducted to determine the detection thresholds for citric acid. After selecting the most sensitive panellists, four training sessions of one hour each were conducted to familiarise the panellists with different sourness intensities and usage of the scale. The panellists were paid for their participation.

Sensory testing protocol

The trained sensory panel compared the sourness of malic, tartaric, fumaric and Fruitaric® acids to citric acid using a labelled magnitude scale (Green, Shaffer & Gilmore, 1993). The sourness intensity ratings on the labelled magnitude scale was captured using Compusense® *five* data collection software (Compusense® *five,* release 4.6, Compusense Inc., Guelph, ON, Canada). During each evaluation session the panellists received four sets of acidulants in water solution (20 ml each). Each set consisted of citric acid (0.2%) and another acidulant, either at equal weight or equal sour concentration. Citric acid was always served first. The panellists were instructed to put the whole sample in their mouth, swirl it around for about 10 seconds and expectorate. The instruction to the panellists was to rate the sourness of the sample in the context of the strongest imaginable sensation they have ever experienced using the labelled magnitude scale (LMS). The acidulant solutions (20ml) were served to the panellists in glass polytops (25 diameter x 75 mm height). The polytops were covered with plastic lids and coded with three-digit random codes. Deionised water was used as a mouth cleanser. The panellists were instructed to wait ten minutes in between evaluating each set to avoid fatigue. Each set was evaluated in duplicate. A maximum of four sets were evaluated during an evaluation session. The panellists evaluated the different sets in the same order and the order of the sets served was randomised over the different evaluation sessions. All of the evaluations were conducted in the sensory laboratory at the Department of Food Science, University of Pretoria.

Measurement of pH

The measurement of the pH of the sports drinks and acid-in water solutions was conducted with the use of an electronic pH meter (pH211 microprocessor meter, HANNA® products, Italy) with a combination electrode. The use of commercial pH meters is based on the AOAC Official Method 973.41 pH in water (AOAC, 2000). pH levels were measured in triplicate.

Titratable acidity

Determination of the titratable acidity of the sports drinks and acid-in-water solutions was conducted using the AOAC Official Method 942.15 (Horwitz, 2002). Ten millilitres of sample were pipetted into a 250 ml Erlenmeyer flask. Distilled water (90 ml) was added, together with 2 drops of phenolphthalein indicator. The prepared solution was then titrated with 0.1 M NaOH until a persistent pink colour appeared. Conversion factors used for citric acid monohydrate were 0.070, for malic acid 0.067 and for tartaric acid 0.075, for fumaric acid 0.058 and for Fruitaric® acid 0.0634 (Horwitz, 2002; Robert Fowlds, Managing Director of Isegen South Africa (Pty) Ltd, 2004 - personal communication). Titratable acidity values were measured in triplicate at approximately 20 ^{o}C.

Determination of ^{o} Brix (soluble solids)

The ^{o} Brix of the sports drink samples was determined by using the Pocket PAL-1 refractometer (Atago, USA). ^{o} Brix were measured in triplicate at ±

15°C. Brix/acid ratio was calculated by dividing the °Brix with the concentration of the acidulant (w/v).

4.1.3 Statistical Analysis

The individual threshold values for citric acid of each panellist were determined by taking the geometric mean of the last incorrect concentration and the first correct concentration. The group threshold was calculated as the geometric mean of the individual panellists' thresholds.

The sourness intensities of sample pairs were analysed using a t-test (independent by groups) to test for the effect of sample on sourness using Statistica version 7.1 (StatSoft, Inc. (2006). In all cases a significance value (p-value) of < 0.05 was used as the criteria to determine if a significant difference existed or not. One way analysis of variance (ANOVA) was performed to determine whether there were differences in the pH levels, titratable acidity values, °Brix and Brix/acid ratio ($p < 0.05$) of the sports drinks and/or water solutions with different acidulants at equal sour and equal weight concentrations. The Fisher Least Significant Difference (LSD) test was used to investigate the nature of the differences.

Perceptual maps were plotted using principal component analysis (PCA) to investigate which physical and chemical properties (molecular weight; acidulant concentration, expressed as normality, molarity and weight/volume; pH; °Brix; titratable acidity, Brix/acid ratio) as well as the sourness intensity of the acidulants) had the most influence on the differences between the samples at both equal sour and equal weight concentrations in water and both sports drink flavours. Please note that the w/v concentrations were not included in the PCA of the acidulants at equal weight concentrations, as these were exactly the same. For inclusion in the PCA the average sourness intensities of citric acid in the different applications were calculated. The calculation of molarity and normality of the acidulants in water solutions are presented in Table 4.1.

Table 4.1: Molarity, normality and weight/volume concentrations of acidulants when dissolved in water

Acidulant	Equal weight concentrations			Equal sour concentrations		
	Weight/volume (g/l)	Molarity (M)	Normality (N)	Weight/volume (g/l)	Molarity (M)	Normality (N)
Citric Acid	2.0	0.010	0.031	2.0	0.010	0.031
Malic Acid	2.0	0.014	0.030	1.4	0.010	0.021
Tartaric Acid	2.0	0.013	0.027	1.21	0.008	0.016
Fumaric Acid	2.0	0.017	0.034	1.01	0.009	0.017
Fruitaric® Acid	2.0	0.015	0.032	1.08	0.009	0.017

4.1.4 Results

The panellists in the group were ranked in an ascending order according to BET (Table 4.2). This represents the panellists in ranked order of sensitivity, from most sensitive (low threshold value) to the least sensitive (high threshold value).

Table 4.2: Ranking of panellists according to their best estimated threshold (BET) values in decreasing order of sourness sensitivity

Ranked Order	Gender	BET for sour (% w/v citric acid)
1	Female	0.0037
1	Female	0.0037
1	Female	0.0037
1	Male	0.0037
1	Female	0.0037
1	Female	0.0037
1	Male	0.0037
1	Female	0.0037
2	Female	0.0046
2	Female	0.0057
3	Male	0.0057
	Group	0.0041

Sourness intensity as measured by labelled magnitude scaling

Using LMS no significant differences in sourness intensity were found for malic, fumaric, tartaric and Fruitaric® acid water solutions when compared to citric acid water solution at equal sour concentration (Table 4.3). Added at equal weight, fumaric and Fruitaric® acid solutions were found to be significantly more sour ($p<0.05$) compared to citric acid. The panel did not find a significant difference ($p=0.46$) between citric and tartaric acid and between citric acid and malic acid ($p=0.2$) in water solutions.

No significant differences ($p>0.05$) were found in the LMS ratings for *Grape* flavoured sports drinks with added malic or fumaric acid at equal sour concentration compared to the drink with added citric acid, respectively (Table 4.3). The panel found the *Grape* flavoured sports drinks with tartaric acid and Fruitaric® acid (at equal sour concentrations) to be significantly less sour ($p<0.05$) than the sports drink with citric acid. When compared at equal weight concentrations, no significant difference was found between the LMS ratings for the *Grape* flavoured sports drinks with malic acid compared to the sports drink with added citric acid. *Grape* flavoured sports drinks with tartaric, fumaric and Fruitaric® acid respectively were significantly more sour ($p<0.05$) than the sports drink with citric acid.

When comparing the *Lemon & Lime* flavoured sports drink with citric acid to the others, no significant differences ($p>0.05$) were found between the respective LMS ratings for malic, tartaric, fumaric and Fruitaric® acid *Lemon & Lime* flavoured sports drinks at equal sour concentrations (Table 4.3). When the acidulants were added at equal weight concentrations in a *Lemon & Lime* flavoured sports drink, no significant differences ($p>0.05$) were found between the sourness ratings for the sports drinks with malic and tartaric acids compared to the sports drink with citric acid, respectively. The panel found the sourness of the *Lemon & Lime* flavoured sports drink with fumaric and Fruitaric® acids to be significantly more sour than the drink with citric acid.

Table 4.3: Average ratings on the labelled magnitude scale (0=No sensation, 1.98=Strongest imaginable) comparing the sourness of acidulants in water and flavoured sports drinks at equal sour and equal weight concentrations

EQUAL SOUR CONCENTRATIONS				EQUAL WEIGHT CONCENTRATIONS			
	n	Average LMS rating	p-value		n	Average LMS rating	p-value
			Water				
Citric Acid 0.2%	21	1.38	0.1	Citric Acid 0.2%	21	1.38	0.2
Malic Acid0.14%	21	1.28		Malic Acid 0.2%	21	1.48	
Citric Acid 0.2%	21	1.34	0.16	Citric Acid 0.2%	21	1.42	0.46
Tartaric Acid 0.121%	21	1.21		Tartaric Acid 0.2%	21	1.49	
Citric Acid 0.2%	22	1.30	0.31	Citric Acid 0.2%	22	1.35	0.004
Fumaric Acid 0.101%	22	1.22		Fumaric Acid 0.2%	22	1.56	
Citric Acid 0.2%	22	1.36	0.06	Citric Acid 0.2%	22	1.36	0.001
Fruitaric® Acid 0.108%	22	1.21		Fruitaric® Acid 0.2%	22	1.56	
			***Grape* flavoured sports drink**				
Citric Acid 0.21%	16	1.26	0.08	Citric Acid 0.21%	16	1.28	0.13
Malic Acid 0.147%	16	1.02		Malic Acid 0.21%	16	1.43	
Citric Acid 0.21%	16	1.22	0.02	Citric Acid 0.21%	16	1.25	0.001
Tartaric Acid 0.127%	16	0.90		Tartaric Acid 0.21%	16	1.54	
Citric Acid 0.21%	15	1.21	0.16	Citric Acid 0.21%	15	1.20	0.0002
Fumaric Acid 0.106%	15	0.99		Fumaric Acid 0.21%	15	1.61	
Citric Acid 0.21%	15	1.17	0.04	Citric Acid 0.21%	15	1.17	0.001
Fruitaric® Acid 0.113%	15	0.85		Fruitaric® Acid 0.21%	15	1.55	
			***Lemon & Lime* flavoured sports drink**				
Citric Acid 0.21%	19	1.19	0.18	Citric Acid 0.21%	19	1.05	0.16
Malic Acid 0.147%	19	1.0		Malic Acid 0.21%	19	1.26	
Citric Acid 0.21%	19	1.16	0.42	Citric Acid 0.21%	19	1.21	0.28
Tartaric Acid 0.127%	19	1.04		Tartaric Acid 0.21%	19	1.34	
Citric Acid 0.21%	17	1.07	0.17	Citric Acid 0.21%	17	1.06	0.0006
Fumaric Acid 0.106%	17	1.05		Fumaric Acid 0.21%	17	1.49	
Citric Acid 0.21%	17	1.10	0.21	Citric Acid 0.21%	17	1.08	0.02
Fruitaric® Acid 0.113%	17	0.92		Fruitaric® Acid 0.21%	17	1.38	

pH results

At equal sour concentrations, the average pH of the water solution with added citric acid was significantly lower than that of the solutions with malic and tartaric acids, respectively (Table 4.4). The malic, fumaric and Fruitaric® acid water solutions did not differ in terms of pH at equal sour concentrations. At equal weight concentrations, fumaric and Fruitaric® acid had the lowest pH when dissolved in water, followed by tartaric acid, then malic acid and then citric acid, which had the highest pH. No significant difference was found in the pH of fumaric and Fruitaric® acid water solutions at equal weight concentrations in water. Although the pH of the different acid water solutions differed statistically, the range of values was very small (2.84 – 2.89).

In the *Grape* flavoured sports drink at equal sour concentrations, the citric acid sample had the lowest pH ($p<0.05$), followed by the sports drink with tartaric acid, then sports drink with Fruitaric® acid, while the sports drink with malic acid had the highest pH value (Table 4.4). No significant differences were found in the pH of the *Grape* flavoured sports drinks with tartaric and fumaric acid, as well as between fumaric and Fruitaric® acid sports drink samples, respectively. When added at equal weight concentrations to the *Grape* flavoured sports drink no significant differences were found in the pH values of tartaric, fumaric and Fruitaric® acid samples. The pH values of these sports drink samples were significantly lower than that of the sports drinks containing citric and malic acid at equal weight concentrations. The *Grape* flavoured sports drink with added malic acid had a significantly higher pH than that of the sports drink with citric acid. When dissolved in the *Grape* flavoured sports drink the samples with tartaric, fumaric and Fruitaric® acid had significantly lower pH values compared to the *Grape* flavoured sports drinks with citric and malic acids. The pH range difference in the *Grape* flavoured application was much greater (2.68 – 2.87) than in water.

The *Lemon & Lime* flavoured sports drink with added citric acid had the lowest pH, followed by the sports drink with tartaric acid and fumaric acid, then by the sports drink with Fruitaric® acid, while the *Lemon & Lime* flavoured sports drink with malic acid had the highest pH at equal sour concentrations (Table 4.4). At equal weight concentrations, the *Lemon & Lime* flavoured sports drink with added fumaric acid had the lowest pH, followed by the sports drink with Fruitaric® acid, then the sports drink with tartaric acid, then the sports drink with citric acid while the *Lemon & Lime* flavoured sports drink with malic acid had the highest pH.

Titratable acidity

At equal sour concentrations the fumaric acid water solution had the lowest titratable acidity, followed by Fruitaric® acid, then citric acid, while the malic acid solution had the lowest titratable acidity (Table 4.5). The titratable acidity of the tartaric acid solution did not differ from that of Fruitaric® and citric acids, respectively. At equal weight concentrations the malic acid water solution had a significant lower titratable acidity than the tartaric acid, fumaric and Fruitaric® acid solutions. The citric acid, fumaric and Fruitaric® acid water solutions had intermediate titratable acidity values, and these three water solutions did not differ in terms of titratable acidity at equal weight concentrations.

The *Grape* flavoured sports drink with added citric, malic or tartaric acid had the highest titratable acidity at equal sour concentrations (Table 4.5). The titratable acidity of these sports drinks did not differ from each other. The *Grape* flavoured sports drink with Fruitaric® acid had a significantly lower titratable acidity than these samples, while the titratable acidity of the *Grape* flavoured fumaric acid sports drink was the lowest. At equal weight concentrations, the *Grape* flavoured sports drink with added tartaric acid had the highest titratable acidity, while the fumaric acid *Grape* sports drink had the lowest value. The titratable acidity of the *Grape* flavoured sports drinks with citric, malic and Fruitaric® acids did not differ at equal weight concentrations and were significantly lower than the sports drink with tartaric acid, but significantly higher than the sports drink with fumaric acid.

Table 4.4: Average pH measurements (± standard deviation) of the five acidulants in water and in two flavours of a sports drink at equal sour and equal weight concentrations

EQUAL SOUR CONCENTRATIONS		EQUAL WEIGHT CONCENTRATIONS	
Water			
p-value	<0.0001	p-value	<0.0001
Citric Acid 0.2%	2.84[a] (± 0.02)	Citric Acid 0.2%	2.82[d] (± 0.02)
Malic Acid 0.14%	2.88[bc] (± 0.01)	Malic Acid 0.2%	2.79[c] (± 0.02)
Tartaric Acid 0.121%	2.89[c] (± 0.01)	Tartaric Acid 0.2%	2.76[b] (± 0.01)
Fumaric Acid 0.101%	2.86[abc] (± 0.02)	Fumaric Acid 0.2%	2.70[a] (± 0.01)
Fruitaric® Acid 0.108%	2.86[ab] (± 0.01)	Fruitaric® Acid 0.2%	2.70[a] (± 0.01)
***Grape* flavoured sports drink**			
p-value	<0.0001	p-value	<0.0001
Citric Acid 0.21%	2.68[a] (± 0.02)	Citric Acid 0.21%	2.71[b] (± 0.02)
Malic Acid 0.147%	2.87[d] (± 0.03)	Malic Acid 0.21%	2.79[c] (± 0.03)
Tartaric Acid 0.127%	2.76[b] (± 0.02)	Tartaric Acid 0.21%	2.68[a] (± 0.02)
Fumaric Acid 0.106%	2.79[bc] (± 0.01)	Fumaric Acid 0.21%	2.65[a] (± 0.01)
Fruitaric® Acid 0.113%	2.82[c] (± 0.02)	Fruitaric® Acid 0.21%	2.67[a] (± 0.01)
***Lemon & Lime* flavoured sports drink**			
p-value	0.01	p-value	<0.0001
Citric Acid 0.21%	2.70[a] (± 0.05)	Citric Acid 0.21%	2.69[d] (± 0.01)
Malic Acid 0.147%	2.90[d] (± 0.01)	Malic Acid 0.21%	2.73[e] (± 0.01)
Tartaric Acid 0.127%	2.80[b] (± 0.01)	Tartaric Acid 0.21%	2.66[c] (± 0.01)
Fumaric Acid 0.106%	2.80[b] (± 0.01)	Fumaric Acid 0.21%	2.60[a] (± 0.01)
Fruitaric® Acid 0.113%	2.84[c] (± 0.01)	Fruitaric® Acid 0.21%	2.63[b] (± 0.01)

[a,b,c,d,e] Average values with the same superscript in the column of each subsection do not differ significantly (p>0.05)

Table 4.5: Average titratable acidity values (g acid per 100g) (± standard deviation) of the five acidulants in water and two flavours of a sports drink at equal sour and equal weight concentrations

EQUAL SOUR CONCENTRATIONS		EQUAL WEIGHT CONCENTRATIONS	
Water			
p-value	<0.0001	p-value	0.006
Citric Acid 0.2%	0.25^{c} (± 0.02)	Citric Acid 0.2%	0.24^{ab} (± 0.02)
Malic Acid 0.14%	0.29^{d} (± 0.02)	Malic Acid 0.2%	0.23^{a} (± 0.02)
Tartaric Acid 0.121%	0.21^{bc} (± 0.06)	Tartaric Acid 0.2%	0.27^{c} (± 0.01)
Fumaric Acid 0.101%	0.16^{a} (± 0.03)	Fumaric Acid 0.2%	0.26^{bc} (± 0.02)
Fruitaric® Acid 0.108%	0.20^{b} (± 0.02)	Fruitaric® Acid 0.2%	0.26^{bc} (± 0.02)
***Grape* flavoured sports drink**			
p-value	<0.0001	p-value	<0.0001
Citric Acid 0.21%	0.27^{c} ± 0.01	Citric Acid 0.21%	0.28^{b} (± 0.01)
Malic Acid 0.147%	0.26^{c} ± 0.02	Malic Acid 0.21%	0.28^{b} (± 0.01)
Tartaric Acid 0.127%	0.27^{c} ± 0.02	Tartaric Acid 0.21%	0.33^{c} (± 0.01)
Fumaric Acid 0.106%	0.16^{a} ± 0.02	Fumaric Acid 0.21%	0.25^{a} (± 0.01)
Fruitaric® Acid 0.113%	0.22^{b} ± 0.01	Fruitaric® Acid 0.21%	0.27^{b} (± 0.02)
***Lemon & Lime* flavoured sports drink**			
p-value	0.01	p-value	0.008
Citric Acid 0.21%	0.25^{c} ± 0.03	Citric Acid 0.21%	0.27^{ab} (± 0.01)
Malic Acid 0.147%	0.21^{b} ± 0.01	Malic Acid 0.21%	0.29^{bc} (± 0.03)
Tartaric Acid 0.127%	0.22^{b} ± 0.02	Tartaric Acid 0.21%	0.30^{c} (± 0.03)
Fumaric Acid 0.106%	0.15^{a} ± 0.02	Fumaric Acid 0.21%	0.25^{a} (± 0.02)
Fruitaric® Acid 0.113%	0.16^{a} ± 0.02	Fruitaric® Acid 0.21%	0.27^{ab} (± 0.01)

^{a,b,c,d} Average values with the same superscript in the column of each subsection do not differ significantly ($p>0.05$)

At equal sour concentrations the titratable acidity of the *Lemon & Lime* flavoured sports drinks with fumaric and Fruitaric® acid did not differ significantly from each other and were the lowest (Table 4.5). The malic and tartaric acid *Lemon & Lime* sports drinks had significantly higher titratable acidity values than these samples but significantly lower values than the sports drink with citric acid, which had the highest titratable acidity. At equal weight concentrations, the titratable acidity of *Lemon & Lime* flavoured sports drink with added fumaric acid was significantly lower compared to sports drinks with malic and tartaric acid, respectively. The titratable acidity of the *Lemon & Lime* flavoured sports drink with added malic, citric and Fruitaric® acids was intermediate and did not differ from each other. The titratable acidity of citric, Fruitaric® and malic *Lemon & Lime* flavoured sports drinks did not differ, and the values for the malic and tartaric *Lemon & Lime* drinks also did not differ.

Soluble solids content (^{o}Brix)

No significant differences were found in the soluble solids (expressed as ^{o}Brix) content of the *Grape* flavoured sports drink samples at both equal weight and equal sour concentrations (Table 4.6). At equal sour concentrations the *Lemon & Lime* flavoured sports drink with added malic acid had significantly less soluble solids compared to the *Lemon & Lime* flavoured sports drink with added citric, tartaric, fumaric and Fruitaric® acids, respectively. The soluble solids content of the *Lemon & Lime* flavoured sports drink with added citric, tartaric, fumaric and Fruitaric® acids did not differ significantly from each other at equal sour concentrations. The fumaric acid *Lemon & Lime* flavoured sports drink had significantly less soluble solids, while the sports drinks with added citric and malic acid had the most soluble solids at equal weight concentrations. The sports drinks with tartaric and Fruitaric® acids had intermediate soluble solids content at equal weight concentrations.

Brix/acid ratios

Description of the taste or sourness of fruit juices is commonly done by Brix/acid ratio. The ratios for the flavoured sports drinks are presented in Table 4.7. At equal sour concentrations the *Grape* flavoured sports drink with added citric acid had the lowest Brix/acid ratio, followed by the sports drink with malic acid, then the sports drink with tartaric acid, then the sports drink with Fruitaric® acid, while the sports drink with fumaric acid had the highest Brix/acid ratio. No significant differences were found between the *Grape* flavoured sports drink with acidulants added at equal weight concentrations as expected from the ^{o}Brix results.

At equal sour concentrations the *Lemon & Lime* flavoured sports drink with added citric acid had the lowest Brix/acid ratio, followed by the sports drink with malic acid, then the sports drink with tartaric acid, then the sports drink with Fruitaric® acid, while the sports drink with fumaric acid had the highest Brix/acid ratio. The fumaric acid *Lemon & Lime* flavoured sports drink had the lowest Brix/acid ratio, while the sports drinks with added citric and malic acid had the highest Brix/acid ratio when compared at equal weight concentrations. The Brix/acid ratio of the Fruitaric® acid *Lemon & Lime* flavoured sports drink was significantly higher than the fumaric acid sports drink but significantly lower than the sports drinks with added citric and malic acid.

Table 4.6: Average ^{o}Brix values (± standard deviation) of the five acidulants in two flavours of a sports drink at equal sour and equal weight concentrations

EQUAL SOUR CONCENTRATIONS		EQUAL WEIGHT CONCENTRATIONS	
***Grape* flavoured sports drink**			
p-value	0.401	p-value	0.295
Citric Acid 0.21%	8.00 ^{a} (± 0.44)	Citric Acid 0.21%	7.80 ^{a} (± 0.10)
Malic Acid 0.147%	7.80 ^{a} (± 0.26)	Malic Acid 0.21%	7.80 ^{a} (± 0.10)
Tartaric Acid 0.127%	7.93 ^{a} (± 0.29)	Tartaric Acid 0.21%	7.90 ^{a} (± 0.10)
Fumaric Acid 0.106%	7.80 ^{a} (± 0.17)	Fumaric Acid 0.21%	7.53 ^{a} (± 0.64)
Fruitaric® Acid 0.113%	7.53 ^{a} (± 0.12)	Fruitaric® Acid 0.21%	8.17 ^{a} (± 0.32)
***Lemon & Lime* flavoured sports drink**			
p-value	0.004	p-value	0.004
Citric Acid 0.21%	8.33 ^{b} (± 0.06)	Citric Acid 0.21%	8.47 ^{c} (± 0.25)
Malic Acid 0.147%	7.97 ^{a} (± 0.06)	Malic Acid 0.21%	8.40 ^{c} (± 0.20)
Tartaric Acid 0.127%	8.27 ^{b} (± 0.06)	Tartaric Acid 0.21%	8.30 ^{bc} (± 0.20)
Fumaric Acid 0.106%	8.20 ^{b} (± 0.17)	Fumaric Acid 0.21%	7.40 ^{a} (± 0.20)
Fruitaric® Acid 0.113%	8.33 ^{b} (± 0.06)	Fruitaric® Acid 0.21%	7.90 ^{b} (± 0.44)

^{a,b,c} Average values with the same superscript in the column of each subsection do not differ significantly (p>0.05)

Table 4.7: Average Brix/acid ratio (± standard deviation) of the five acidulants in two flavours of a sports drink at equal weight concentrations

EQUAL SOUR CONCENTRATIONS		EQUAL WEIGHT CONCENTRATIONS	
	***Grape* flavoured sports drink**		
p-value	<0.001	p-value	0.295
Citric Acid 0.21%	38.10 [a] (± 2.08)	Citric Acid 0.21%	37.14 [a] (± 0.48)
Malic Acid 0.147%	53.06 [b] (± 1.80)	Malic Acid 0.21%	37.14 [a] (± 0.48)
Tartaric Acid 0.127%	62.47 [c] (± 2.27)	Tartaric Acid 0.21%	37.62 [a] (± 0.48)
Fumaric Acid 0.106%	73.59 [e] (± 1.63)	Fumaric Acid 0.21%	35.87 [a] (± 3.06)
Fruitaric® Acid 0.113%	66.67 [d] (± 1.02)	Fruitaric® Acid 0.21%	38.89 [a] (± 1.53)
	***Lemon & Lime* flavoured sports drink**		
p-value	<0.001	p-value	0.004
Citric Acid 0.21%	39.68 [a] (± 0.27)	Citric Acid 0.21%	40.32 [c] (± 1.20)
Malic Acid 0.147%	54.20 [b] (± 0.39)	Malic Acid 0.21%	40.00 [c] (± 0.95)
Tartaric Acid 0.127%	65.09 [c] (± 0.45)	Tartaric Acid 0.21%	39.52 [bc] (± 0.95)
Fumaric Acid 0.106%	77.36 [e] (± 1.63)	Fumaric Acid 0.21%	35.24 [a] (± 0.95)
Fruitaric® Acid 0.113%	73.75 [d] (± 0.51)	Fruitaric® Acid 0.21%	37.62 [b] (± 2.08)

[a,b,c,d,e] Average values with the same superscript in the column of each subsection do not differ significantly ($p>0.05$)

Multivariate analysis of chemical properties and sourness intensities of acidulants

Multivariate PCA maps of the acidulants at equal sour and equal weight concentrations when applied in water and both sports drink flavours are presented in Figures 4.1 – 4.6. These maps described between 74% and 93% of the variance between the acidulants at equal sour and equal weight concentrations when applied in water and both sports drink flavours.

The first two principal components explained 88% of the differences among the acids when added to water at equal sour concentrations (Figure 4.1). At equal sour concentration in water, citric acid is distinguished from the other acidulants based on higher normality, molecular weight, molarity and weight/volume addition. From this map it is evident that malic acid seems to be the most similar to citric acid compared to the other acidulants. Principal component 2 (note less influential) shows fumaric and Fruitaric® acids to be more similar to citric acid based on lower titratable acidity and pH values. At equal weight concentrations (Figure 4.2), the picture looks completely different, which explain the different results found for equal weight and equal sour concentrations. Figure 4.2 shows that principal component 1 (69%) separates fumaric and Fruitaric® acid water solutions from citric, malic and tartaric acids water solutions based on higher sourness intensity, molarity, normality and titratable acidity values.

Principal component 1 (Figure 4.3) described 72% of the variance in the perception of acidulants added to *Grape* flavoured sports drink at equal sour concentrations. Tartaric, malic, fumaric and Fruitaric® acid *Grape* flavoured sports drink samples grouped together and were more similar compared to the citric acid sports drink sample. This separation was based on the citric acid *Grape* flavoured sports drink's higher titratable acidity, ^{o}Brix, molecular weight, acidulant concentration (expressed as weight/volume), normality, and molarity and sourness intensity. Principal component 2 (17%) shows that the fumaric acid *Grape* flavoured sports drink was more similar to the citric acid sample at equal sour concentrations, based on lower pH, titratable acidity and °Brix. Figure 4.4 indicates that for principal component 1 (49%), *Grape* flavoured sports drinks with added citric, malic and tartaric acids were separated from the fumaric and Fruitaric® *Grape* flavoured sports drink samples at equal weight concentrations. Citric, malic and tartaric acid *Grape* flavoured sports drink had higher °Brix, Brix/acid ratio, titratable acidity, pH and molecular weight values. Principal 2 (25%) shows that the malic and fumaric acid *Grape* flavoured sports drink were more similar to the sports drink with added citric acid based on higher molecular weights and pH values.

At equal sour concentrations, the citric acid *Lemon & Lime* flavoured sports drink was separated (PC1 = 72%) from the sports drinks with added malic, tartaric, fumaric and Fruitaric® acids based on higher sourness intensity, molecular weight, acidulant concentration (expressed as weight/volume, normality, and molarity) and titratable acidity (Figure 4.5). Principal component 2 (17%) separated malic acid *Lemon & Lime* flavoured sports drink from the other samples based on higher pH values and lower °Brix values. At equal weight concentration principal component 1 (76%) the citric acid *Lemon & Lime* flavoured sports drink was identified as being more similar to malic and tartaric acid sports drinks but separated from sports drink with fumaric and Fruitaric® acid, based on higher titratable acidity, molecular weight, pH values, °Brix and Brix/acid ratio (Figure 4.6). Principal component 2 (17%) separated citric acid from the other *Lemon & Lime* flavoured sports drinks based on higher molecular weight, normality and molarity.

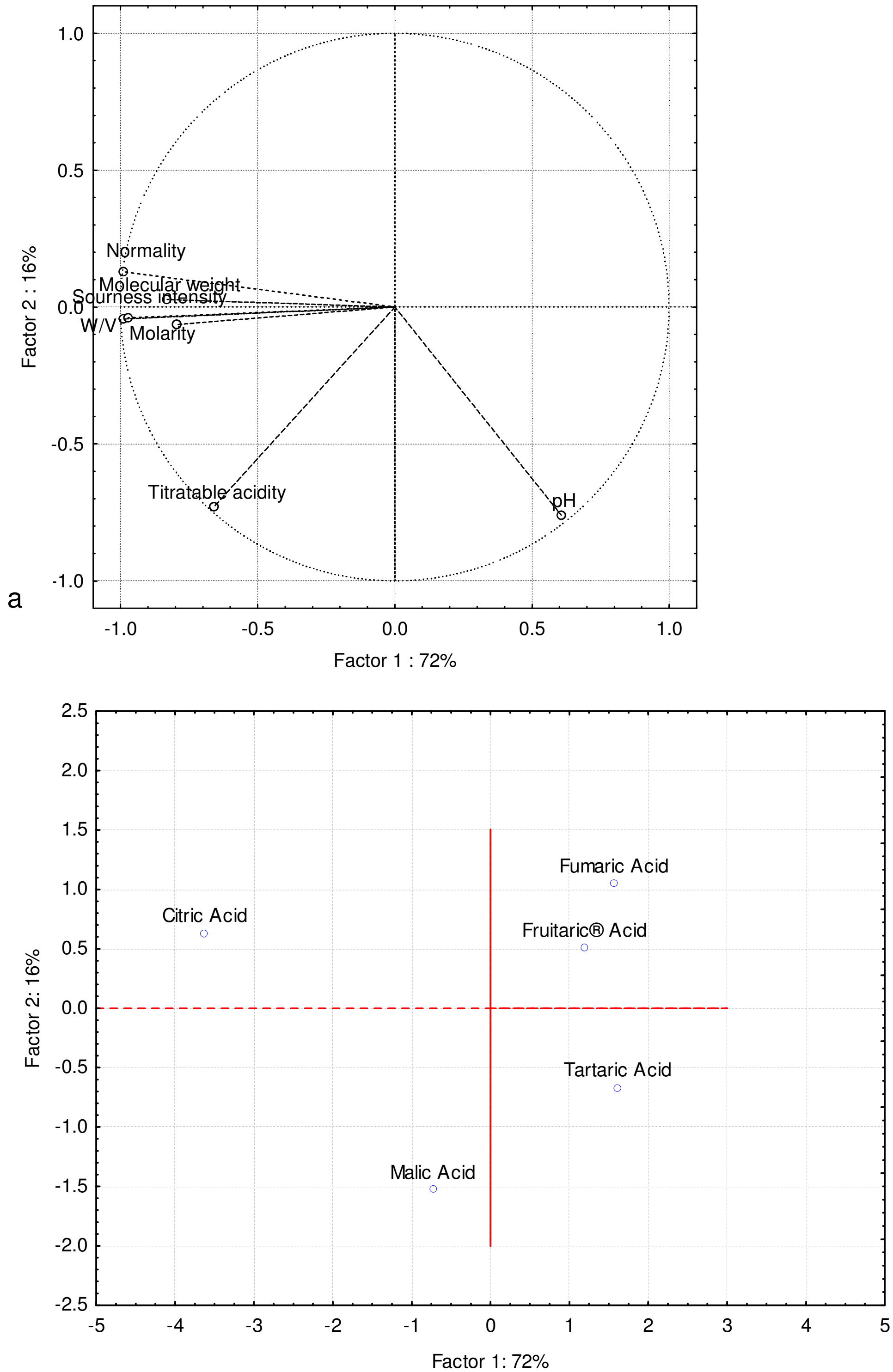
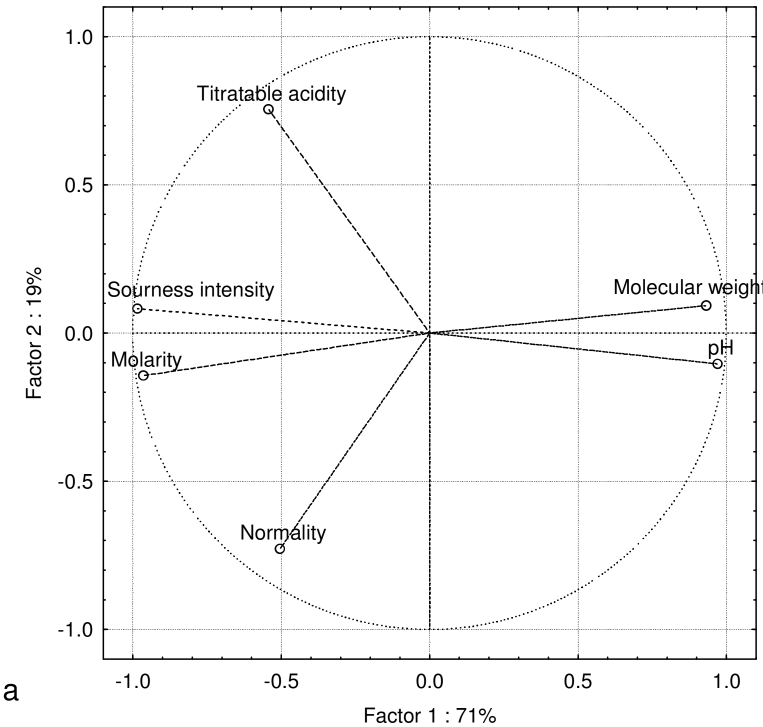
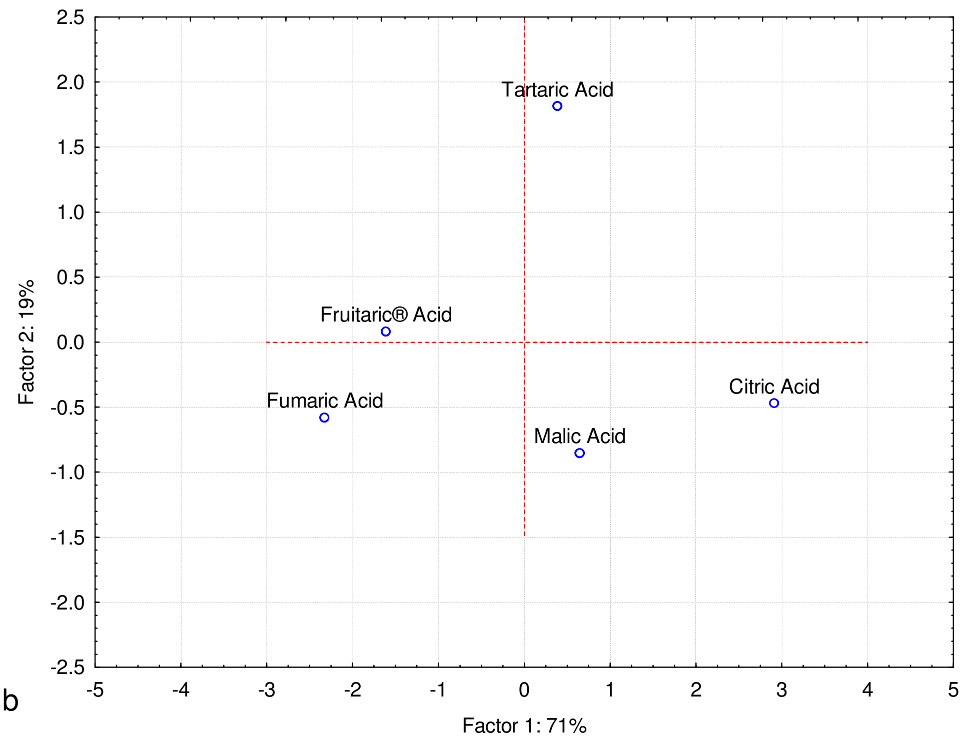


Figure 4.1: Principal component analysis (PCA) loadings for (a) physical, chemical and sensory properties and (b) acidulants in water solutions at equal sour concentrations

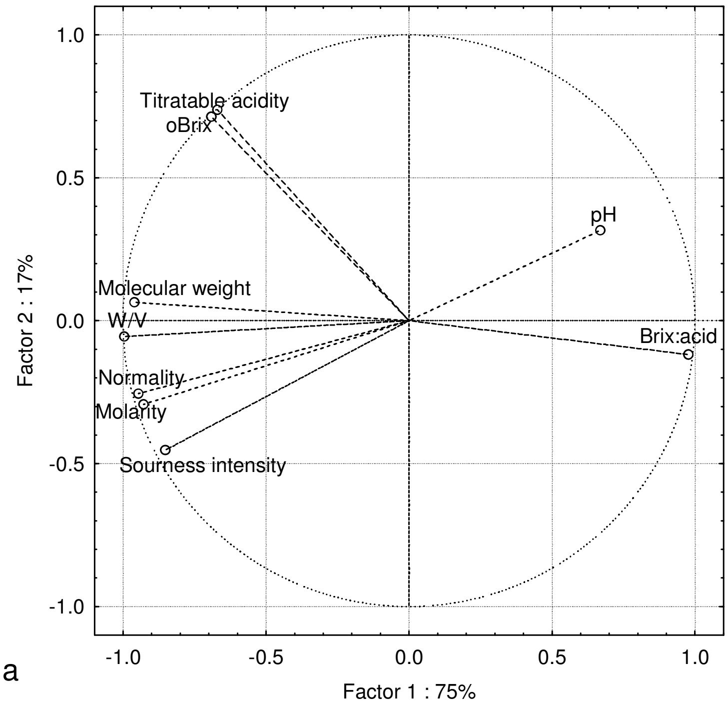


a



b

Figure 4.2: Principal component analysis (PCA) loadings for (a) physical, chemical and sensory properties and (b) acidulants in water solutions at equal weight concentrations



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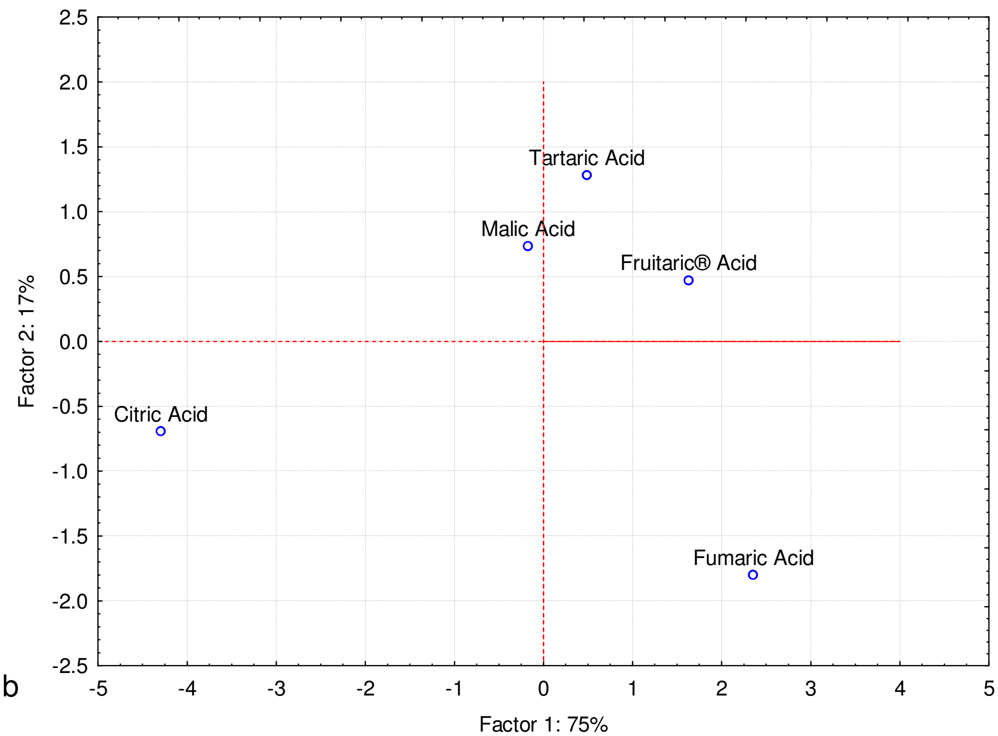
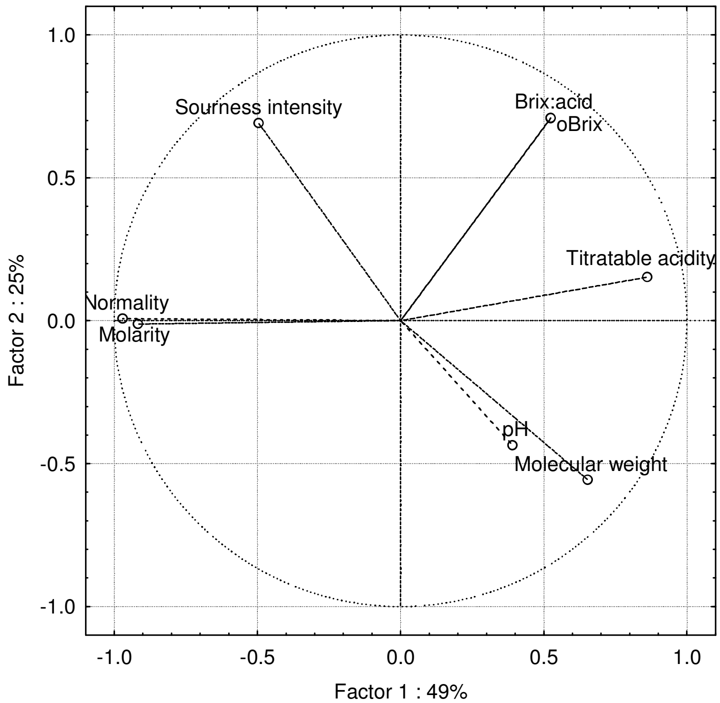


b

Figure 4.3: Principal component analysis (PCA) loadings for (a) physical, chemical and sensory properties and (b) acidulants in *Grape* flavoured sports drink at equal sour concentrations



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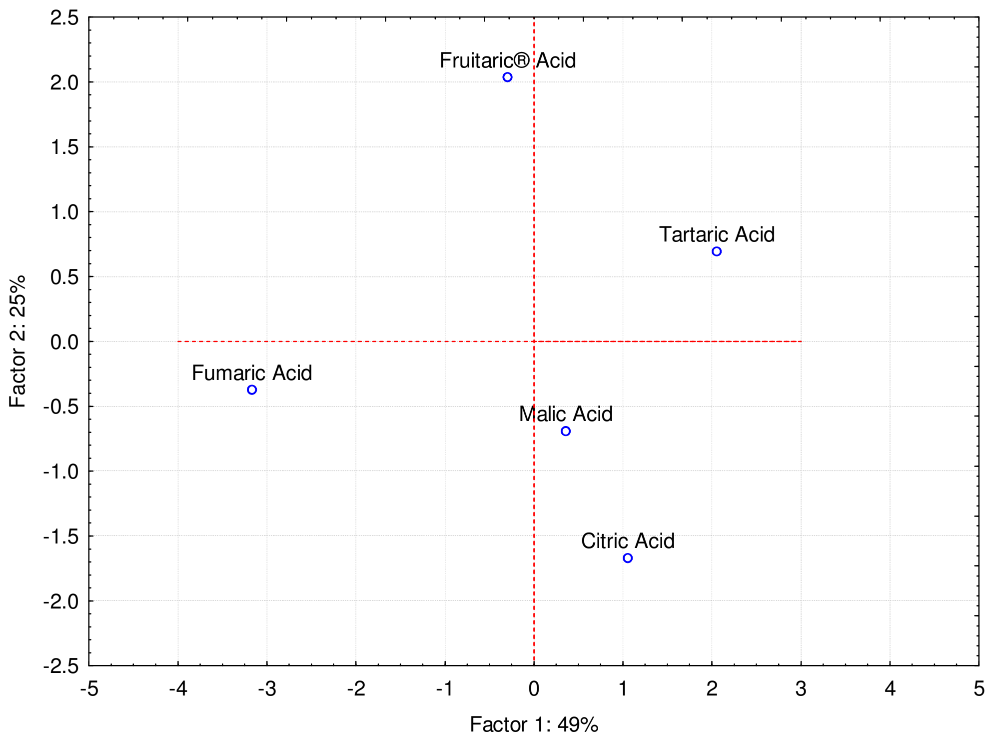
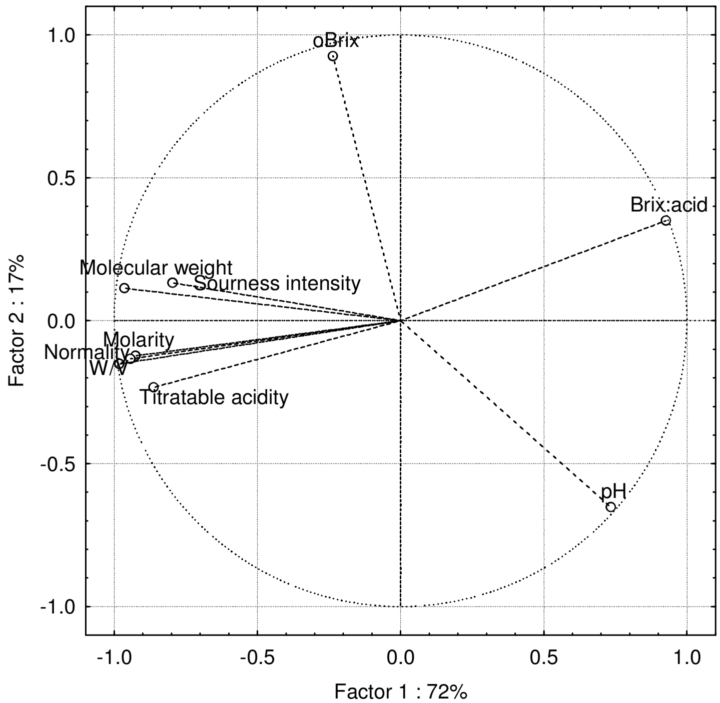


b

Figure 4.4: Principal component analysis (PCA) loadings for (a) physical, chemical and sensory properties and (b) acidulants in *Grape* flavoured sports drink at equal weight concentrations



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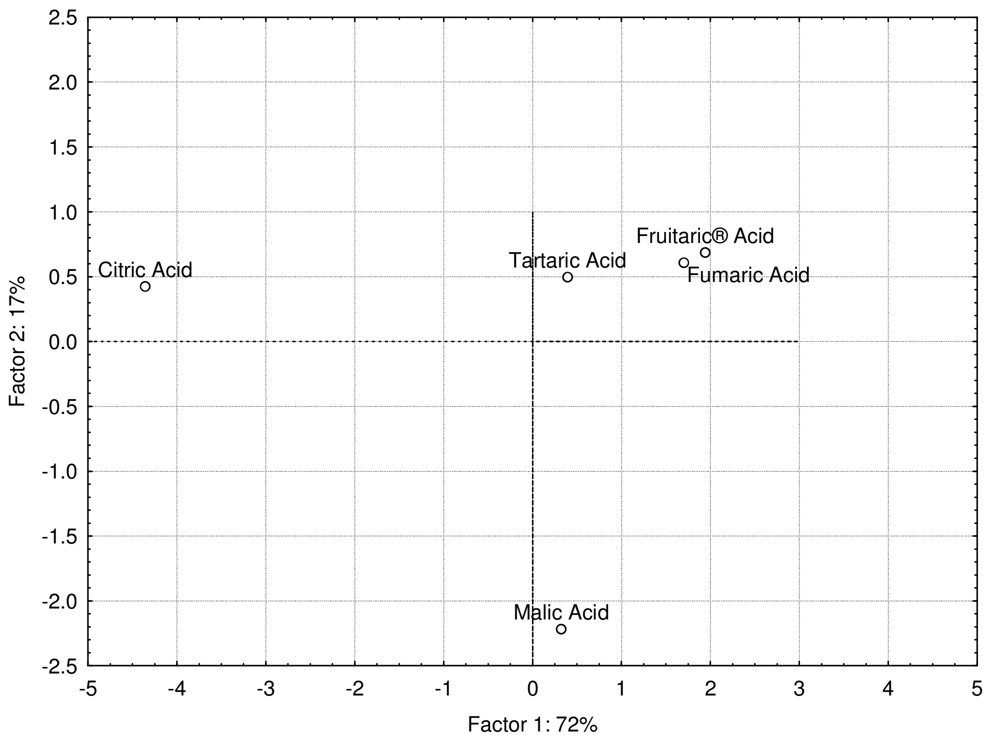
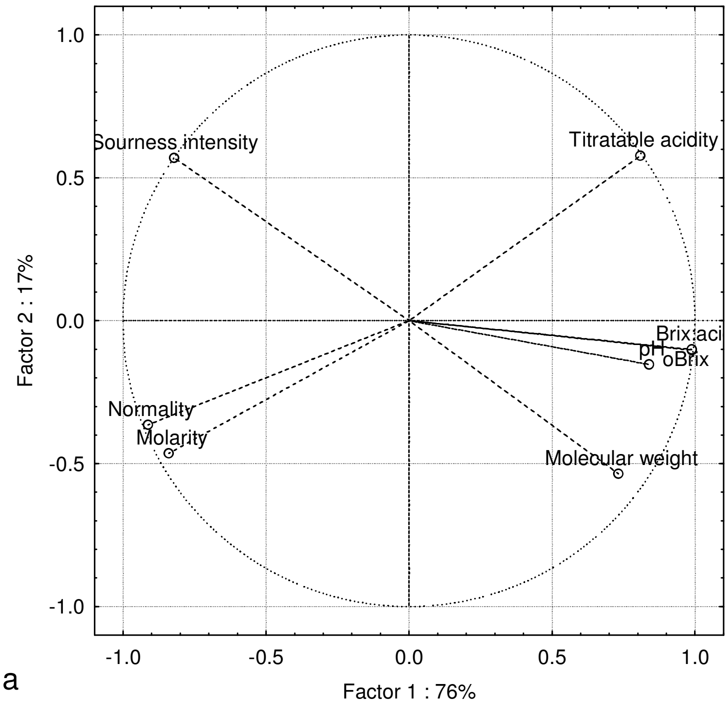


b

Figure 4.5: Principal component analysis (PCA) loadings for (a) physical, chemical and sensory properties and (b) acidulants in *Lemon & Lime* flavoured sports drink at equal sour concentrations



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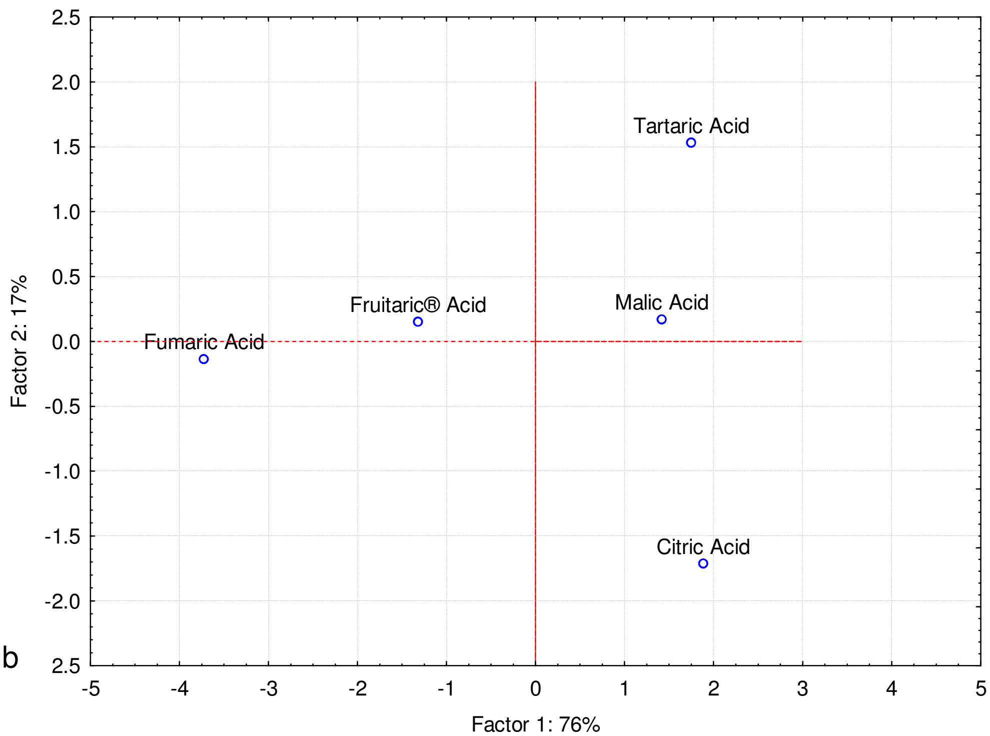

b

Figure 4.6: Principal component analysis (PCA) loadings for (a) physical, chemical and sensory properties and (b) acidulants in *Lemon & Lime* flavoured sports drink at equal weight concentrations

4.1.5 Discussion of results

The trained panel's group threshold for citric acid in water was calculated as 0.0041 g citric acid/100 ml solution. Jacobs (2001 - unpublished) reported a group BET value of 0.0054 g/100 ml solution. Jellinek (1985) reported mean threshold levels of 0.0022 g citric acid/100 ml solution. Pangborn (1963) reported thresholds of between 0.0223 and 0.00096 g citric acid/100 ml solution. Fabian & Blum (1943) reported a sensitivity threshold of 0.0042 citric acid/100ml solution. The BET values for the selected panellists were therefore within the range of reported values. The acidulant concentrations used during this study were considerably higher than the calculated group threshold, thus the panel was sensitive enough to evaluate the sourness intensity of acidulants at the concentrations used during the study.

At equal sour concentrations added to water and the *Lemon & Lime* flavoured sports drink, no significant differences in sour intensity ratings were found when comparing citric acid to malic, tartaric, fumaric and Fruitaric® acids, respectively. The equal-sourness concentrations of the acidulants in water as determined by a previously trained panel (Jacobs, 2001 - unpublished) using paired comparison tests were thus confirmed by this trained panel using a different method (LMS). Equal sour concentrations of acidulants used in this study were approximately equal molar. Ough (1963) found that citric, tartaric, fumaric and adipic acids differed in sourness intensities at equal molar concentrations. In contrast, Buechenstein & Ough (1979) also found that acidulants at equal molar concentrations were equal-sour. Neta *et al.* (2007) stated that molar concentration of acidulants is insufficient to predict sour taste perception. The authors stated that different ranges in concentration and pH levels contributed to different rank orders of perceived sourness intensities.

Comparing malic acid to citric acid at equal weight concentrations, no significant differences were found in the perceived sourness when added to water and to both sports drink flavours. This is in contrast to results by Fabian

& Blum (1943), Buechenstein & Ough (1979), CoSeteng *et al.* (1989) and Noble *et al.* (1986) who found malic acid to be more sour than citric acid at equal weight concentrations. It should however be noted that Noble *et al.* (1986) and CoSeteng *et al.* (1989) compared the acidulants at higher concentrations than the present study. Fabian & Blum (1943) and Buechenstein & Ough (1979) used concentrations similar to the present study. The reason for the differences in results is probably due to different assessment methodologies used e.g. use of LMS scale versus paired comparison tests. PCA also showed that malic and tartaric acids were most similar to citric acid when added to water and both sports drink flavours.

At equal weight concentration the *Grape* flavoured sports drink with tartaric acid were found to be significantly more sour than citric acid, while no significant difference in ratings were found when the two acidulants were compared in water and *Lemon and Lime* flavoured sports drink. CoSeteng *et al.* (1989) found tartaric acid to be more sour than citric acid, while Ough (1963) found citric acid to be more sour than tartaric acid. Claims have also been made that apart from sourness intensity, acidulants also differ in duration and mouthfeel properties. The taste of citric acid was described as a sharp, clean sour taste with little persistence on palate, while malic acid is known to have a slow build up but lingering sour taste (Berry, 2001). Tartaric acid is reported to have a sharp initial impact without the lingering effect. The panel was only exposed to the acidulants in solution for a short period (approximately ten seconds) and therefore the time could have been insufficient to evaluate the lingering properties of the acidulants. These temporal differences could explain why tartaric acid *Grape* flavoured sports drink was more sour than the citric acid sports drink. Fumaric and tartaric acid is said to enhance grape flavoured products (Gardner, 1968; Giese, 1995) which could explain why the *Grape* flavoured sports drink with tartaric acid was found to be more sour than the citric acid sports drink.

At equal weight concentrations, fumaric and Fruitaric® acid was found to be more sour than citric acid in all three applications. Tartaric acid was found to be more sour than citric acid when compared in the *Grape* flavoured sports drink. No significant differences were found between malic and citric acids in all three applications. CoSeteng *et al.* (1989) stated that perceived sourness of a solution could be correlated with the chemical structure of the acidulant. Monocarboxylic acidulants are more sour than dicarboxylic acidulants which are more sour than tricarboxylic acidulants. In the present study only tricarboxylic acids (citric acid) and dicarboxylic acids (malic, tartaric and fumaric acids) were included. It was therefore expected and found that the perceived sourness of tartaric and fumaric acids at equal weight concentrations would be more intense compared to citric acid. The authors also reported that molecular weight and polarity were important factors in sourness perception, demonstrating that increasing molecular weight and hydrophobicity of an acid molecule may potentially increase sourness intensity. The molecular weight of citric acid (192g/mol) is higher than malic acid (134g/mol), tartaric acid (150g/mol) and fumaric acid (116g/mol). Increasing molecular weight did not increase sourness intensity of the acidulants at equal weight concentrations.

Fumaric acid was found to be more intense in sourness compared to citric acid in all three applications. Fumaric acid was the only unsaturated acid included in this study, compared to the saturated acids (citric, malic and tartaric acids) (Lindsay, 1985). Fumaric acid is therefore more hydrophobic and is expected to elicit a more sour taste compared to the other acidulants used in this study (Moskowitz, 1971). The perceptual maps at equal weight concentrations also confirmed that fumaric acid was one of the most intense sour samples in all three applications.

A positive correlation between the pKa values and perceived sourness intensity of organic acids has been reported (Ganzevles & Kroeze, 1987; Hartwig & McDaniel, 1995). pKa values of malic acid is higher than that of citric acid, while the pKa values of tartaric acid and fumaric acid are lower than that of citric acid. Therefore, it cannot be concluded that the sourness intensities of the acidulants could have been predicted using the pKa values of the acids.

Equal sour concentrations, as determined in water solutions, were confirmed in the *Lemon & Lime* flavoured sports drink. In the *Grape* flavoured sports drink, malic and fumaric acid added at equal sour concentrations, were found to be equally sour to citric acid while the sourness of tartaric and Fruitaric® acids were suppressed. The *Grape* flavoured sports drink with added tartaric and Fruitaric® acids were found to be less sour than the sports drink with added citric acid. It is not clear why the sourness of only these two acids were suppressed but not the others. Mixture suppression is the tendency of mixtures of different tastes/flavours to show partially inhibitory or masking interactions (Lawless & Heymann, 1998). In beverages the sourness of acidulants can be partially masked by the sweetness from the sugar added to the beverage. Possible ingredient interactions cannot be specified because the specific formulations of the different flavours were unknown.

The sourness intensities were higher for all of the acidulants when evaluated in a water solution compared to the sports drinks. The panel perceived the different acidulants therefore to be more sour in water application than when added to the sports drink. The sports drink contained ingredients such as sugar, mono potassium phosphate and sodium citrate that masked the sourness of the acid in the sports drink and therefore it was more difficult to distinguish the sourness of the acids in sports drinks compared to water solutions.

When compared at equal weight concentrations, in all three applications the samples with fumaric acid had the lowest pH value. At equal sour concentrations, the average pH values were the lowest in all three applications with added citric acid. In both the *Grape* and *Lemon & Lime* flavoured sports drinks the sample with added citric acid had the lowest pH value, while the samples with malic acid had the highest pH value. Note that malic acid was the only acid where practically no difference in pH was observed when applied at equal sour concentration to citric acid in water and the two beverage applications. Although statistical significant differences were found in the pH values for the acidulants when dissolved in water at equal sour concentrations, the pH range was quite small (2.84 – 2.89). If sour taste intensities of acidulants were dependant on the pH of the solution, no significant differences in the pH would have been expected. In both water and *Lemon & Lime* flavoured sports drinks the equal sour concentrations of the acidulants were proved, but significant differences were found for the pH of the relevant samples. Previous studies (Harvey, 1920; Beatty & Cragg, 1935; Ganzevles & Kroeze, 1987) also found pH to be insufficient to predict sourness intensity of a solution. Sowalsky & Noble (1998) evaluated the separate effects of concentration, pH and anion species on intensity of sourness and astringency of citric, malic, tartaric and lactic acids. A trained sensory panel rated the sourness of three concentrations of each acidulant (0.02N, 0.06N and 0.10N) at three different pH levels (2.8, 3.4 and 4.0). The results showed that the intensity of the sourness increased with decreasing pH, while at each pH level, sourness increased with increased acid concentration (expressed as normality). The other ingredients in the sports drink such as sugar, mono potassium phosphate and sodium citrate which could have acted as buffering agents, influenced the pH of the solution. The results from this study showed that the use of pH on its own to predict the sourness intensity of the water and sports drinks with different acidulants was insufficient.

Titratable acidity has been hypothesized to be related to sourness (Harvey, 1920; Noble, *et al.*, 1986; Shallenberger, 1996; Lugaz *et al.*, 2005), while other researchers (Pangborn, 1963; Rubico and Straub according to Hartwig and McDaniel, 1995) reported that the two aspects were slightly or not at all related. The titratable acidity of fumaric acid in water and both flavours of sports drink was the lowest, while the titratable acidity of citric acid (in *Grape* and *Lemon & Lime* flavoured sports drinks) and malic acid (in water and *Grape* flavoured sports drinks) was highest at equal sour concentrations. The range of titratable acidity of the acidulants in water was slightly lower (0.23 –

0.27) compared to the values for the *Grape* flavoured sports drink (0.25 – 0.33) and *Lemon & Lime* flavoured sports drink (0.25 – 0.3). Norris *et al.* (1984) found that acidulant solutions with the lowest pH and highest titratable acidity had the highest perceived sourness. The fumaric acid solution was of the most sour intense samples at equal weight concentrations in water and both sports drink flavours. At equal weight concentrations the fumaric acid samples (in water and both sports drinks flavours) had the lowest pH and in both sports drink flavours the titratable acidity of the fumaric acid samples was the lowest. Shallenberger (1996) stated that if titratable acidity was related to sourness of a solution, this would imply that acidulants with equal normalities will elicit equal sourness perception, as they have equal titration coefficients. The normalities of the acidulants at equal weight concentrations were generally larger (0.027-0.034) than the normalities at equal sour concentrations (0.016-0.031) (Table 4.1). The results from the present study showed that the hypothesis of Shallenberger (1996) could therefore be rejected.

Soluble solids (%), which are largely sugars, are generally expressed in degrees Brix, which relates specific gravity of a solution to an equal concentration of pure sucrose (Potter & Hotchkiss, 1995). As the acidulant added to the sports drink (either at equal sour or equal weight concentrations) was the only variable, no differences were expected in the ^{o}Brix results. Other factors that also contributed to the ^{o}Brix of a solution are minerals and acidulant contents. Shachmann (2005) referred to the Brix/acid ratio as the "sweetness to sourness" relationship of the solution. The higher the ^{o}Brix the greater the concentration of sugars in the solution, the higher the Brix/acid ratio the sweeter and less sour is the solution (Potter & Hotchkiss, 1995). In both sports drink flavours, the sports drink with added citric acid had the lowest Brix/acid ratio and was therefore expected to be the least sweet but the most sour, while the sports drink with added fumaric acid had the highest Brix/acid ratio and was expected to be the most sweet and less sour, at equal sour concentrations. When comparing the Brix/acid ratios at equal weight concentrations, the fumaric acid *Lemon & Lime* flavoured sports drink would be expected to be the least sweet but the most sour while the citric acid *Lemon & Lime* flavoured sports drink would be expected to be the most sweet and least sour sample. This is also confirmed by the results from the LMS scaling, where fumaric acid was found to be one of the most sour acidulants in both water and the sports drink flavours and citric acid was one of the least sour acidulants at equal weight concentrations. Although the equal sourness concentrations in the *Lemon & Lime* flavoured sports drink was confirmed for all the acidulants and for malic and fumaric acids in the Grape flavoured sports drink. The sourness intensities of the acidulants in application were only compared with citric acid and the sourness intensities of other acidulants, i.e. the sourness intensity of malic vs. tartaric acid was not compared. Although these concentrations were equally sour to citric acid, differences in sourness intensities between malic and tartaric acids could have existed. In addition to sugar other ingredients in the sports drinks, i.e. mono potassium phosphate, sodium citrate and acidulant used also contributed to the ^{o}Brix of the solutions (Schachman, 2005).

4.1.6 Conclusion

Using LMS, the panel confirmed equal sour concentrations of malic, tartaric, fumaric and Fruitaric® acids in water, as previously determined using traditional paired comparison tests. The equal sour concentrations were approximately equal molar and also applied to a *Lemon & Lime* flavoured sports drink. However the concentrations of tartaric and fumaric acids were not perceived as equal sour in the *Grape* flavoured sports drink. Sourness of complex solutions seems to be dependant on multiple factors including pH, titratable acidity, molecular weight, acidulant concentration, ^{o}Brix. However, the use of either pH or titratable acidity to predict sourness intensity of a solution was found to be insufficient. No clear relationships between sourness intensity and pH and titratable acidity of the solution could be established. Differences in sourness intensities were found when comparing tartaric, fumaric and Fruitaric® acids to citric acid at equal weight concentrations. Substituting citric acid with tartaric, fumaric, and Fruitaric®

acids at equal weight would make products perceptually more sour and potentially less acceptable. Lower concentrations of malic, tartaric, fumaric and Fruitaric® acid compared to citric acid can be used in some applications and still result in equal sourness perception, which could result in cost reduction.

4.2 TEMPORALLY PERCEIVED SOURNESS OF DIFFERENT ACIDULANTS IN WATER AND FLAVOURED SPORTS DRINKS AT EQUAL SOUR CONCENTRATIONS

4.2.1 Introduction

Previously, equal sour concentrations of different acidulants were determined and confirmed in both water and a sports drink application (Chapter 4.1). Lower concentrations of malic, tartaric, fumaric and Fruitaric® acid were needed to achieve sourness perception equalling a 0.2% citric acid solution. Claims have been made that each acidulant has a particular set of taste characteristics, which include rate of sourness development, the intensity of sourness, the lingering effect as well as the aftertaste of the acidulants (Berry, 2001; Lanton, 2004). Gardner (1966) stated that shape of time intensity curves of different acidulants differs but did not support the statement with evidence of experimental results. The taste of malic acid was said to build up more slowly than citric acid, reached a lower sourness intensity but persisted much longer. Limited research has been done on how the temporal characteristics of the different acidulants vary from each other (Norris *et al.,* 1984; Straub, 1992; Jacobs, 2001 – unpublished; Lugaz *et al.,* 2005). The time to reach maximum intensity, onset, duration and intensity of the perceived sourness of the different acidulants might have an impact on the final perceived flavour of products like sports drinks.

Replacing one acidulant with another at equal sourness concentration in a sports drink might not result in the same flavour profile for that specific product. Descriptive sensory evaluation involves the evaluation of sensory attributes of food products and is able to describe how changes in the product formulation (e.g. change to a different acidulant) will influence the sensory profile of the product. The method is insufficient to provide information on how perceived sensory properties change over time during consumption.

Panellists are usually instructed to rate the intensity of an attribute on a scale, resulting in a single (unipoint) measurement. This method requires the panellist to "time-average" the changing perceived sensation to a single value, without reporting the changes in the perceived intensity over time. Unipoint or single time point measurement is insufficient to distinguish between products with different temporal characteristics (Fradin, 1999). Unipoint scaling of attribute intensities have been shown to correlate well with maximum intensity (I_{MAX}) values of a time intensity curve (Lundahl, 1992; Lawless & Heymann, 1999) indicating that taste intensity judgment depends strongly on peak-intensity and to a lesser degree on other time intensity parameters. Time intensity (TI) is "the measurement of the intensity of a sensation over time in response to a single exposure to a product or other sensory stimulus" (ASTM according to Fradin, 1999). TI is therefore an extension of the traditional scaling method, as used during descriptive sensory evaluation, due to the fact that it also provides temporal information about perceived sensations. The objective of this study was to determine the temporally perceived sourness of different acidulants (citric, malic, tartaric, fumaric and Fruitaric® acids) in water and flavoured sports drinks at equal sour concentrations. The time to reach maximum intensity, onset, duration and intensity of the perceived sourness were compared.

4.2.2 Methodology

Samples

Five acidulants were included in the study: DL malic acid, fumaric acid (hot water soluble) and Fruitaric® acid (a composite of malic acid, tartaric acid and fumaric acid) from Isegen South Africa (Pty) Ltd. Citric acid (anhydrous) and tartaric acid from C.J. Petrow Chemicals (Pty) Ltd. were also used. The acidulants were dissolved in deionised water and also in concentrated sports drink syrups. The sports drink base (in the form of concentrates) was provided by a South African manufacturing company. The sports drink is a well-known and popular sports drink in South Africa. The sports drink concentrates (*Grape* and *Lemon & Lime* flavoured) were diluted (1:4) with deionised water. The acidulants were added at equal-sour (as determined in water) concentrations. These concentrations were added at weight/volume basis and were 0.2% citric acid, 0.14% malic acid, 0.121% tartaric acid, 0.101% fumaric acid and 0.108% Fruitaric® acid, respectively. The sports drink usually contains citric acid at a concentration of 0.21% w/v. The equal-sourness concentrations as determined in water were therefore adapted (by numerical calculation) when added to the sports drink and were 0.21% citric acid, 0.147% malic acid, 0.127% tartaric acid, 0.106% fumaric acid and 0.113% Fruitaric® acid, respectively. All the samples were prepared in clean 500 ml volumetric flasks the day before testing. The samples were kept at 4°C overnight. The samples were left at room temperature (± 25°C) before serving them to the sensory panel at 16 °C.

Sensory panel

The sensory panel consisted of ten trained panellists (2 male, 8 female). The panellists were students and staff members from the University of Pretoria. The panellists had previous experience with sourness evaluation and participated in the study concerning the equal sour and equal weight determinations of different acidulants in water and sports drink applications (section 4.1). Additional training sessions (6 hours) were conducted to familiarise the panellists with the TI method using Compusense® five data collection software (Compusense® five, release 4.6, Compusense Inc., Guelph, ON, Canada.). Water solutions were used to demonstrate temporal sourness perceptions of the different acidulants. The flavoured sports drinks were not included in the training phase of the TI method.

Time intensity protocol

The perceived sourness of the acidulants in water and sports drink applications was evaluated by using the TI method. The acidulant solutions (20ml) were served to the panellists in glass polytops (25 mm diameter x 75 mm height). The polytops were covered with plastic lids and coded with random three-digit codes. Compusense® five data collection software was used for data acquisition. Panellists rated the perceived sourness intensity on a horizontal 150 mm unstructured line scale. A 2% citric acid in water solution served as a reference and its maximum intensity was agreed to be 75% on the line scale.

At zero time the panellists clicked on start while simultaneously taking the whole 20 ml sample in their mouths. The recording of the responses of the panellists started immediately. The panellists were instructed to move the cursor to the right if the intensity of the sourness increased and to move the cursor to the left when the sourness intensity decreased. During the training phase, the panellists agreed that the reference sample reached baseline during the 80 second evaluation. After fifteen seconds, a message appeared on the screen, indicating to the panellists to swallow the sample. The sourness intensity of the different samples was recorded for 80 seconds and data were collected every 0.5 seconds (160 data points in total were recorded). Panellists were instructed to breathe normally and to not hold their breath. The panellists evaluated six samples per session, first the reference sample and then another five samples (i.e. citric acid, malic acid, tartaric acid, fumaric acid and Fruitaric® acid) in random order. There was a five minute break between evaluating each sample to minimise carry-over effects and to avoid fatigue. Only one application, either water or one of the sports drink flavours, was evaluated during a session. The panellists received deionised water and carrots as palate cleansers between evaluating the different samples. Four replications of each acidulant in each application were conducted.

4.2.3 Statistical Analysis

Four parameters were extracted from the individual TI curves: T_{MAX} (the time, in seconds, required to reach maximum intensity), I_{MAX} (maximum intensity, the highest point on the curve as recorded by each panellist), DUR (the total duration time in seconds from the time the attribute is first detected to the finish of the test) and AUC (the total area under the curve). The generalized linear model (GLM) ANOVA was used to analyse the main effects of panellist, acidulant and sports drink flavour as well as interaction effects of the four parameters using SAS ® version 8.2 (SAS Institute Inc. Cary, NC, USA). Note that panellist 4 did not participate in the *Grape* flavoured sports drink evaluation. The analysis of the acidulants in water was conducted separately from the flavoured sports drinks. Here only the main and interaction effects of panellist and acidulant were included. The Fisher Least Significant Difference (LSD) test was used to investigate the nature of the differences of the TI parameters.

4.2.4 Results

There was a significant panellist effect ($p<0.001$) for all the parameters, both in water and the two sports drink flavours (Table 4.8 and 4.9). The average time intensity parameters for sourness of acidulants in water and two flavours of a sports drink at equal sour concentrations are presented in Table 4.10 and 4.11. Individual time intensity curves as evaluated by the different panellists are presented in Figure 4.7. This figure presents, for each panellist, the average curve for each of the applications (water and two sports drink flavours).

In water

In water no significant differences were found between the different acidulants in terms of T_{MAX}, I_{MAX}, DUR and AUC (Table 4.8). No interaction effect was found for panellist x acid, showing consistency among panellists in rating the sourness of the different samples in the same way.

In sports drinks

The sourness of *Grape* and *Lemon & Lime* flavoured sports drinks did not differ significantly in T_{MAX}, I_{MAX} and DUR (Table 4.9). The panellists recorded a significantly higher AUC for Grape flavoured sports drink compared to the *Lemon & Lime* flavoured sports drink across all the acidulants. There was a significant panellist x flavour effect for T_{MAX}, I_{MAX} and AUC, indicating that respective panellists perceived differences between *Grape* and *Lemon & Lime* flavoured sports drinks differently for these parameters. However the reason for the interaction effect can be traced to one panellist deviating from the others. Only panellist 9 recorded a significantly larger AUC for *Grape* flavoured sports drink compared to the *Lemon & Lime* flavoured sports drink across all the acidulants (Figure 4.8). The other panellists found no significant differences in the AUC values for *Grape* and *Lemon & Lime* flavoured sports drinks. Panellist 9 also found the time to reach the maximum intensity (T_{MAX}) of *Lemon & Lime* to be significantly longer than the Grape flavoured sports drink across all the acidulants (Figure 4.9). The other panellists found no significant differences in the T_{MAX} values for *Grape* and *Lemon & Lime* flavoured sports drink. The majority of the panellists recorded no significant differences in I_{MAX} for the *Grape* and *Lemon & Lime* flavoured sports drink. Only panellists 1 and 2 recorded a significantly higher maximum intensity (I_{MAX}) for *Grape* compared to *Lemon & Lime* flavoured sports drinks (Figure 4.10).

No significant difference was found between the acidulants for T_{MAX}, I_{MAX} and DUR. Citric acid was found to have a significantly larger AUC value than tartaric and Fruitaric® acids. AUC for sports drinks with tartaric, fumaric, Fruitaric® and malic acids did not differ significantly, while AUC for drinks with fumaric acid, malic acid and citric acid was also equal (Figure 4.11). No interaction effects were found for flavour x acid, panellist x acid and flavour x panellist x acid, respectively for any of the parameters (Table 4.9).

Table 4.8: F-values for the time-intensity parameters for sourness of acidulants in water at equal sour concentrations

	T_{MAX}	I_{MAX}	DUR	AUC
R^2 of model	0.35	0.44	0.49	0.37
Panellist	4.30***	7.54***	9.32***	5.07***
Acid	0.22	0.72	0.86	1.27
Panellist x Acid	0.61	0.58	0.65	0.48

*, **, *** refer to p<0.05, p<0.01, p<0.001, respectively

Table 4.9: F-values for the time-intensity parameters for sourness of acidulants in two flavours of a sports drink at equal sour concentrations

	T_{MAX}	I_{MAX}	DUR	AUC
R^2 of model	0.42	0.69	0.63	0.72
Flavour (*Grape*/*Lemon & Lime*)	3.62	2.17	0.05	7.70**
Panellist	7.60***	50.73***	34.59***	53.08***
Acid	2.03	2.06	0.42	2.85*
Panellist x Flavour	3.71***	4.13***	0.52	4.51***
Flavour x Acid	0.86	1.00	1.47	1.35
Panellist x Acid	0.79	0.94	1.10	1.14
Flavour x Panellist x Acid	0.62	0.69	1.12	1.00

*, **, *** refer to p<0.05, p<0.01, p<0.001, respectively

Table 4.10: Average values for time intensity parameters for sourness of acidulants in water at equal sour concentrations

	T_{MAX} (s)	I_{MAX} (%)	DUR (s)	AUC
Citric acid 0.2%	23.03	64.28	60.30	2010.18
Malic acid 0.14%	21.82	56.00	59.24	1560.52
Tartaric acid 0.121%	22.47	59.91	55.04	1692.55
Fumaric acid 0.101%	19.86	61.91	53.06	1717.21
Fruitaric® acid 0.106%	21.79	63.85	56.55	1992.80
Average	21.79	61.19	56.84	1794.65
Standard error (±)	2.52	3.97	3.21	175.90

Table 4.11: Average values for time intensity parameters for sourness of acidulants in two sports drink flavours at equal sour concentrations

	T_{MAX} (s)	I_{MAX} (%)	DUR (s)	AUC
***Grape* flavoured sports drink**				
Citric acid 0.21%	18.05	36.81	48.46	977.41
Malic acid 0.147%	14.14	31.16	47.67	730.04
Tartaric acid 0.127%	15.57	29.43	45.19	540.89
Fumaric acid 0.106%	15.44	35.00	50.74	883.99
Fruitaric® acid 0.113%	15.38	26.48	50.66	641.15
Average	15.72	31.78	48.54	754.70
Standard error (±)	1.98	2.74	3.80	101.92
Lemon & Lime flavoured sports drink				
Citric acid 0.21%	20.42	32.18	45.14	692.48
Malic acid 0.147%	16.85	31.04	54.59	668.95
Tartaric acid 0.127%	20.88	31.81	50.51	544.36
Fumaric acid 0.106%	13.86	28.05	44.88	503.89
Fruitaric® acid 0.113%	17.89	25.69	44.99	515.30
Average	17.98	29.75	48.02	585.00
Standard error (±)	1.77	2.45	3.40	91.16

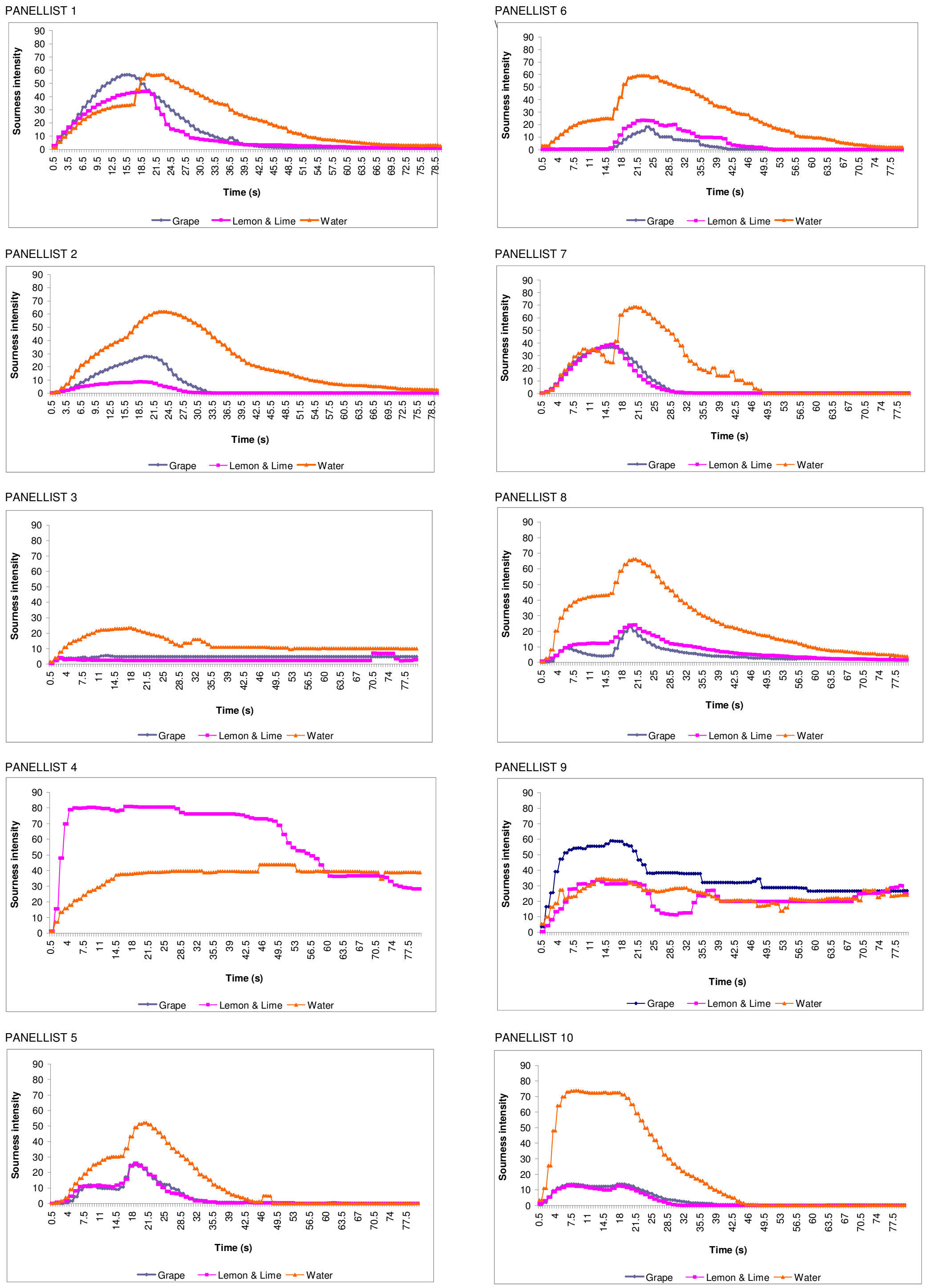

Figure 4.7: Average time intensity curves for each of the applications (water and two sports drink flavours) as evaluated by the different panellists.

* Note that panellist 4 did not participate in the *Grape* flavoured sports drink evaluation

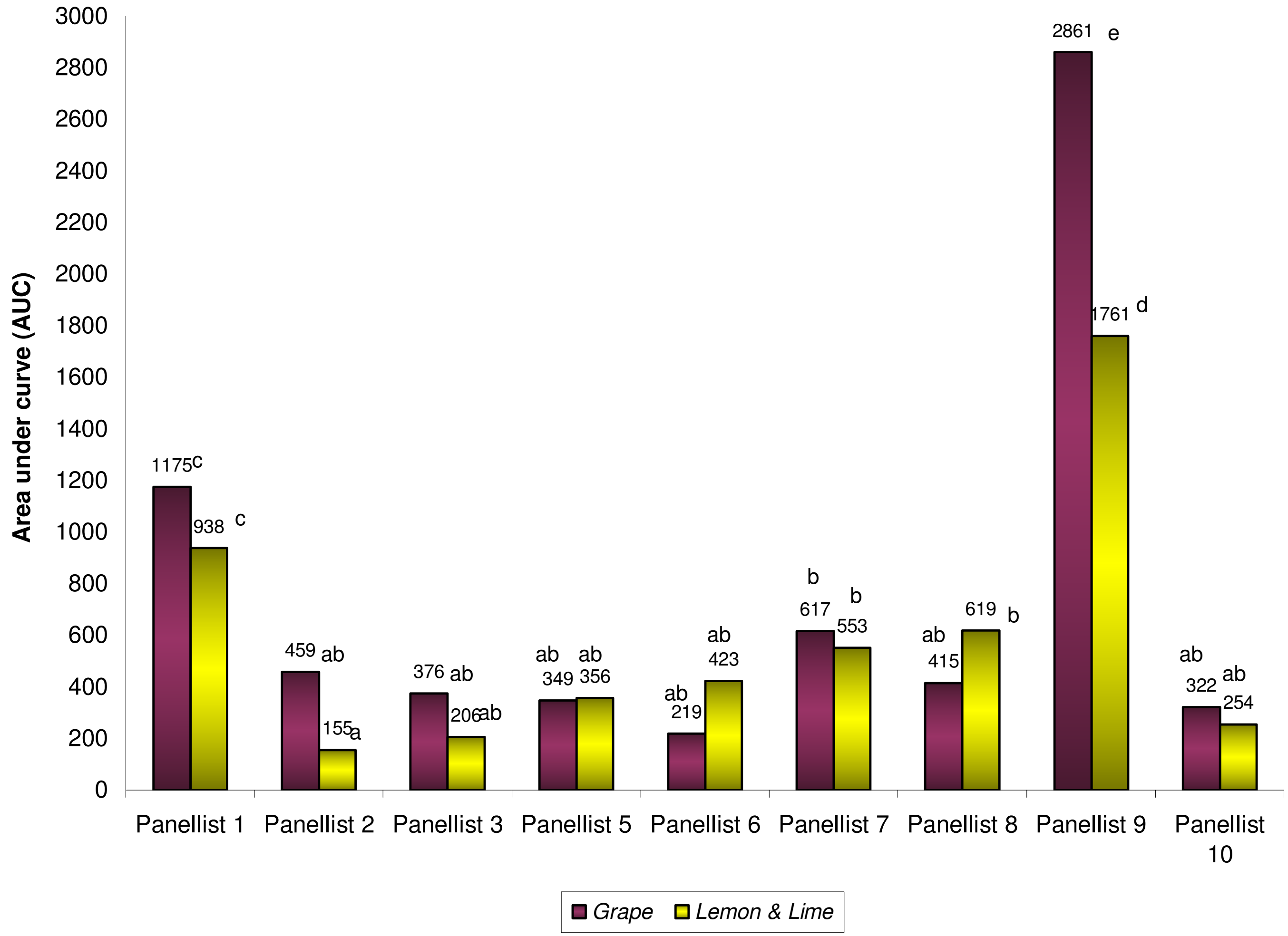

Figure 4.8: ***Area under the curve (AUC) values for*** **Grape** ***and*** **Lemon & Lime** ***flavoured sports drinks as evaluated by the different panellists***

abcde = values with different letters differed significantly ($p < 0.05$).

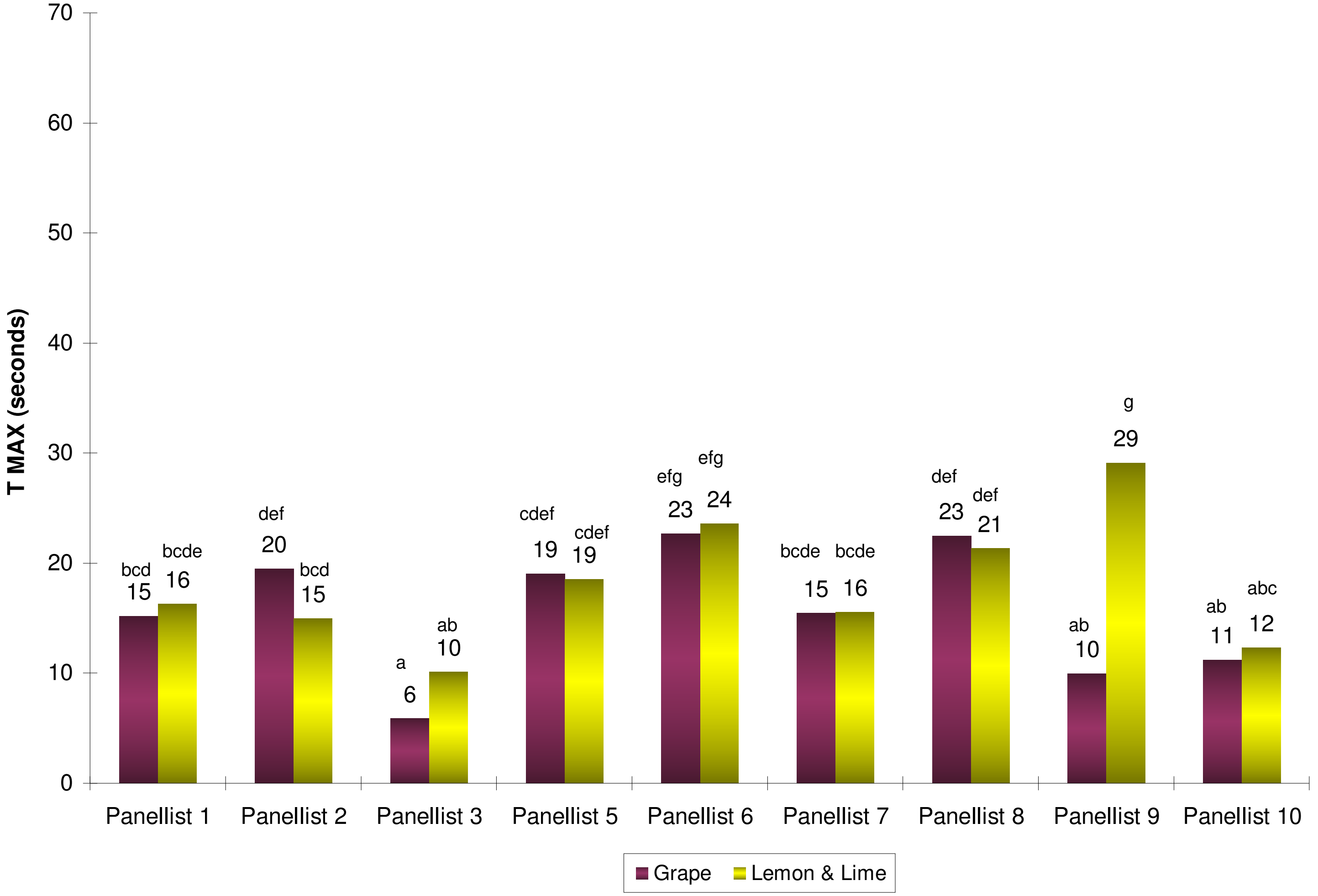

Figure 4.9: T_{MAX} **values for *Grape* and *Lemon & Lime* flavoured sports drinks as evaluated by the different panellists**

abcdef = values with different letters differed significantly ($p < 0.05$).

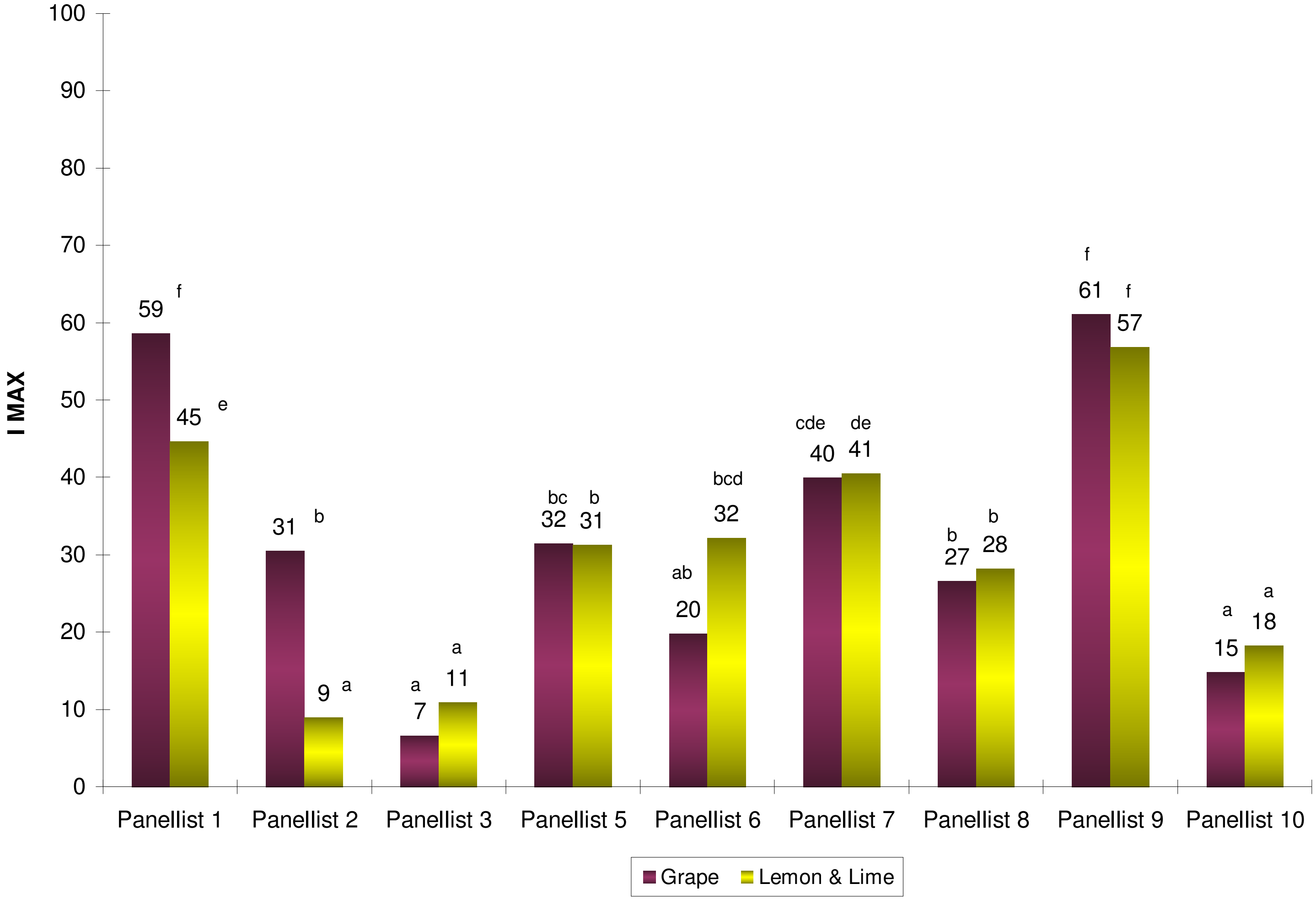

Figure 4.10: I_{MAX} values for *Grape* and *Lemon & Lime* flavoured sports drinks as evaluated by the different panellists

abcdefg= values with different letters differed significantly ($p < 0.05$).

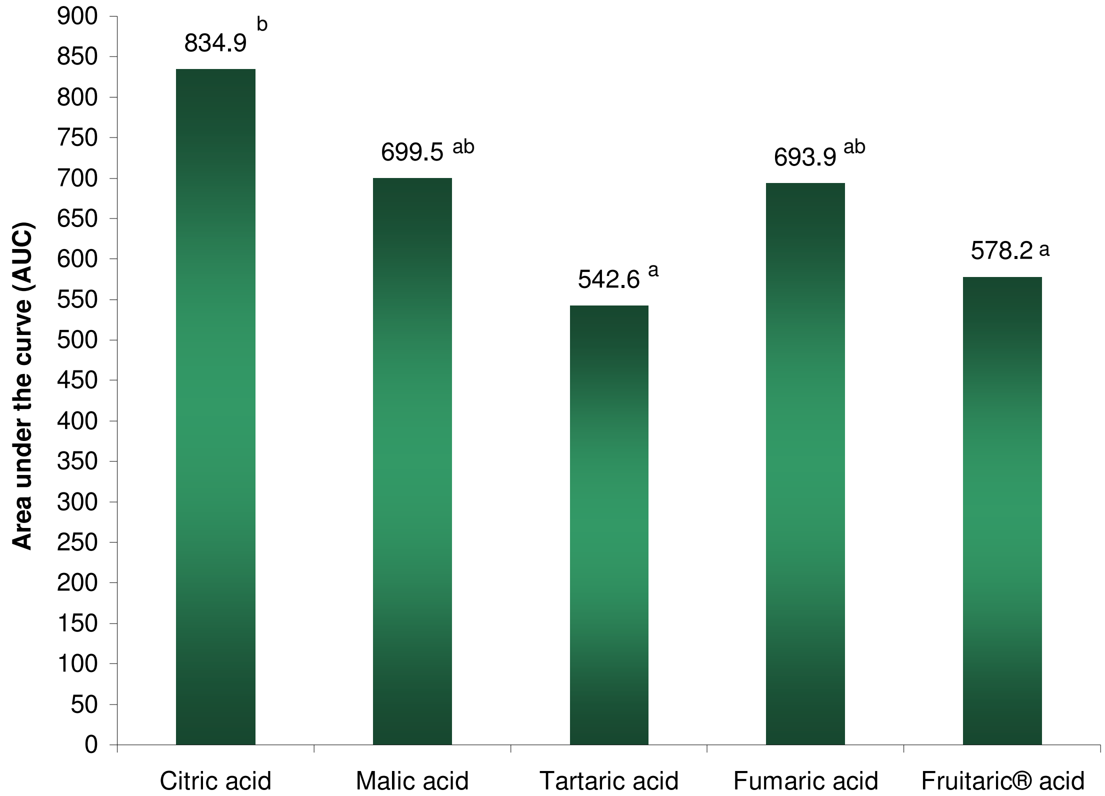

Figure 4.11: Area under the curve (AUC) values for the different acidulants in a flavoured sports drink

ab= values with different letters differed significantly ($p < 0.05$).

4.2.5 Discussion of results

The significant panellist effect for all parameters in both water and flavoured sports drinks indicated that the different panellists used the scale differently. However, only two panellists (panellists 4 and 9) clearly responded differently to the rest of the panel when rating the sourness of the acidulants. Panellist 3 gave much lower values compared to the other panellists. According to these two panellists, the sourness intensity of the acidulants in solution, including the reference citric acid sample did not return to the zero baseline after the 80 second evaluation. For the TI method to work effectively the time duration for evaluation need to be long enough for the stimulus sensation to dissipate completely i.e. to return to baseline. The inclusion of a reference sample to standardise all the panellists was therefore not sufficient to calibrate all the panellists. In future experiments it may be necessary to extent the evaluation time to beyond 80 seconds. Fradin (1999) stated that the inclusion of a reference sample during the training phase of TI experiments is vital, but the use of reference sample during the evaluation phase is not standard practice. The time intensity curves as evaluated by the different panellists indicate extreme diversity between the different panellists. The panellists' time intensity curves differ in height, shape and size. This phenomenon is called panellist's signature (Dijksterhuis & Piggott, 2001) and could be explained by various physiological factors, such as different salivary flow characteristics, differences in anatomy and oral manipulation habits (Lawless & Heymann, 1998; Fradin, 1999).

The AUC values calculated for the TI curves, indications of the total sour taste impact of samples were significantly different for the flavoured sports drinks tested. Overall, the *Grape* flavoured sports drink was perceived to have a greater sour taste impact compared to the *Lemon & Lime* flavoured sports drink. Fumaric and tartaric acid is said to enhance grape flavoured products (Gardner, 1968; Giese, 1995) which could perhaps vaguely explain why the *Grape* flavoured sports drink was found to have a significantly higher overall sour taste impact compared to the *Lemon & Lime* flavoured sports drink.

Differences in product formulations, overall flavour profile impact and possible ingredient interactions could possibly explain these differences but cannot be specified because the specific formulations of the different flavours are unknown.

No significant differences were found in the TI parameters of the acidulants when evaluated in water. This showed that the acidulants, added at equal sour concentrations, led to an equal TI impact. In the flavoured sports drinks citric acid was found to have a significantly higher overall sourness impact (AUC) than tartaric and Fruitaric® acids, while fumaric acid was found to have an intermediate sour taste impact. A TI study conducted by Straub (1992) compared the sourness and astringency of seven acidulants at equal sour concentrations at two different sourness levels. Citric acid was used as reference for the equal sour concentrations. At the first acid level citric acid was used at 0.041% w/v and at 0.083% for the second level. Straub (1992) found that citric, malic and tartaric acids did not differ from one another in their sourness characteristics at both sourness levels. Straub (1992) found fumaric acid to have a significantly higher maximum sourness intensity and overall sour taste impact (AUC) compared to citric, malic and tartaric acids at the higher sourness level. These results were based on lower concentrations than those in the present study. Citric acid is said to deliver a “burst” of sourness, compared to malic acid which is said to have a smooth, flat taste with little character (Gardner, 1972; Berry, 2001). No evidence could be found that the claims made by these authors were based on research data or whether the acidulants were compared at equal weight or equal sour concentrations. The higher AUC, an indication of overall sour taste, of citric acid might be explained as the “burst” in flavour. Jacobs (2001 - unpublished) studied the effect of seven acidulants on TI parameters at equal sour concentrations. The acid concentrations were the same as in the present study. When comparing the acidulants in water as well as in a sweetened, flavoured water model, citric, malic, tartaric, fumaric and Fruitaric® acids did not differ in their sourness characteristics. When the seven acidulants were compared in a sweetened, flavoured model no significant difference was found for the DUR among citric, malic, tartaric, fumaric and Fruitaric® acids.

The author found that tartaric acid had the lowest total sourness taste impact (AUC). Fumaric acid had a slightly higher mean AUC value compared to tartaric acid. Malic acid had a similar mean AUC compare to citric acid. I_{MAX} for sourness intensity of Fruitaric® acid was significant higher than that of tartaric acid (Table 2.2).

The acidulants, both in water and the flavoured sports drinks, did not differ in the DUR of the perceived sour taste. Claims that malic and fumaric acids are more lingering than some of the other acidulants, i.e. tartaric acid (Gardner, 1977; Berry, 2001) can therefore, at least at equal sour concentrations, be rejected. Straub (1992) found fumaric acid to be more lingering, i.e. had a significantly longer duration, than tartaric acid. However samples assessed were at a lower concentration than what was used in the present study. The temporal sourness intensities were perceived to be higher in water application than when added to the flavoured sports drinks. Savant & McDaniel (2004) stated that suppression or partial masking of sourness regularly occurs in complex flavoured food systems. The other ingredients in the sport drink, i.e. sugar, mono potassium phosphate and sodium citrate could have caused flavour masking, leading to the perceived temporal sourness intensity of the sports drinks to be lower compared to the intensity in water.

4.2.6 Conclusion

The acidulants, when added at equal sourness in water, displayed an equal temporal impact. The flavoured sports drinks with added citric acid was found to have a higher total sour impact compared to tartaric and Fruitaric® acid. When applied at equal sour concentrations to *Grape* and *Lemon & Lime* flavoured sports drinks malic, tartaric, fumaric and Fruitaric® acid did not differ in terms of their sourness characteristics. Anecdotal reports that acidulants display differences in temporal character, such as the so called lingering effect of malic and fumaric acid, were not confirmed.

4.3 WOULD REPEATED CONSUMPTION OF SPORTS DRINKS WITH DIFFERENT ACIDULANTS LEAD TO HEDONIC ADJUSTMENT?

4.3.1 Introduction

In the food industry it is often necessary to make small changes to a formulation of a well-known food product. These formulation changes could influence long term consumer acceptance of the product (Vickers & Chung, 2007a). Zandstra *et al.* (2004) stated that it is easier to change the sensory profiles of some food products without losing loyal consumers, than it is to change it of other products. The decision to change from using citric acid to another acidulant in a sports drink can be a major decision for a manufacturer. Other acidulants, e.g. malic acid, tartaric acid, fumaric acid and Fruitaric® acid could be used at lower concentrations which could be more cost effective. Subtle differences in the taste profiles of different acidulants have however been reported (Rubico & McDaniel, 1992; Giese, 1995; Berry, 2001).

Traditional consumer evaluation does not always provide an accurate measure of repeated/extended use responses to a product. The measurement is normally restricted to first time impressions and acceptance of products after repeated exposure is rarely considered. Previous studies have shown that slight differences in the taste profiles of comparative food products may only become clearly noticeable after repeatedly tasting the products. In a study by Stein *et al.* (2003), 27 consumers evaluated a bittersweet beverage in a sensory laboratory test. The consumers rated the hedonic acceptability, taste intensity and familiarity levels of the beverage. After repeatedly consuming the beverage at home for a period of 7 days the consumers returned to the laboratory to evaluate the samples again. The results showed an increase of 68% in the hedonic ratings for the beverage over time. There was also a significant increase in the familiarity ratings from the first laboratory session to the second laboratory session. The results also suggested that repeated tasting might be necessary to identify flavour qualities that may not be perceived on first tasting. The superior taste profile of specific components of beverages was not evident immediately when consuming the product for the first time. Repeated tasting resulted in redistribution of perceived taste qualities e.g. the product that was initially perceived to be very bitter, was after repeated exposure perceived to be slightly sweet with medium bitterness.

Chung & Vickers (2007a) studied the effect of repeated exposure on teas with different sucrose levels. Liking ratings for the lower concentration sucrose tea increased after repeated exposure becoming equal to the optimally sweetened tea at the end of the study. The results showed that sensory specific satiety measured in a single session were insufficient to predict the long-term acceptance of the products. Chung & Vickers (2007b) measured consumers' choice and changes in choice of three types of tea, each at two sweetness levels. The authors observed four consumer choice pattern behaviours: constant-switcher, acquired-liker, non-switcher and systematic-switcher. Liking ratings of the low sweetened tea increased and the tiredness ratings of the optimum-sweet tea increased.

The assumption that brief, once-off laboratory consumer sensory evaluation can predict long-term product success or failure may not be true (Stubenitsky *et al.*, 1999). Repeated exposure to specific food stimuli may lead to either an increase or a decrease in acceptance ratings (Köster *et al.*, 2002). These changes in acceptance ratings over time may be referred to as hedonic adjustment. Hedonic adjustment potential (HAP) was defined as "the predictive measure of the potential changes in like/dislike perceptions of consumers to the sensory properties of food stimuli after repeated exposure" (De Kock & Kinnear, 2003). The purpose of this study was to determine if repeated exposure testing of sports drinks with different acidulants would lead to hedonic adjustment.

4.3.2 Methodology

Preparation of samples

Five sports drinks, each containing the same base concentrate, but with a different acidulant (citric acid, malic acid, tartaric acid, fumaric acid and Fruitaric® acid) added were included in the evaluation. The sports drink base (in the form of a concentrate) was provided by a South African manufacturing company. The sports drink was a well-known and popular sports drink in South Africa. The sports drink concentrate (*Lemon & Lime* flavoured) was diluted (1:4) with deionised water. DL malic acid, fumaric acid (hot water soluble) and Fruitaric® acid (a composite of malic acid, tartaric acid and fumaric acid) from Isegen South Africa (Pty) Ltd. and citric acid (anhydrous) and tartaric acid from C.J. Petrow Chemicals (Pty) Ltd. were used The acidulants were added at equal-sour (as previously determined in water) concentrations. These concentrations were added at weight/volume basis and were 0.2% citric acid, 0.14% malic acid, 0.121% tartaric acid, 0.101% fumaric acid and 0.108% Fruitaric® acid. The prepared samples were stored at 5 ^{o}C until the day of evaluation.

General design of experiment

The consumers attended an evaluation session in the sensory laboratory (i.e. laboratory session) once a week for a period of three weeks. The laboratory sessions were conducted in the sensory laboratory of the Department of Food Science, University of Pretoria. It is equipped with individual tasting booths and uses computers equipped with Compusense® five data collection software (Compusense® five, release 4.6, Compusense Inc., Guelph, ON, Canada) to display the evaluation criteria and allow for direct electronic data capturing. The laboratory sessions were held four days a week with three sessions (12:30, 13:30 and 14:30) per day. Between 12 and 16 consumers attended a laboratory session at a specific time.

During each laboratory session consumers received a set with five sports drinks (each sports drink, 50 ml, contained one of the five acidulants) served in 125 ml polystyrene cups at ± 10 °C. The polystyrene cups were covered with plastic lids. Each sample was coded with a randomly selected three-digit code. Sample presentation order was also randomised over the group. Tap water served as a palate cleanser. The consumers were asked to rank the five samples in order from 1 to 5 where 1 represented "Like it the most" and 5 represented "Like it the least". Ties were not allowed in this test. At the end of the first and second laboratory sessions each participant received a bag that contained 6 sports drink bottles to consume at home. The consumers were screened to ensure that they frequently consumed sports drinks (at least one 500 ml bottle a week). The consumers were divided in six experimental groups. The individual consumers were randomly assigned to the different experimental groups. Each group received one of the sports drinks with a different acidulant added to consume at home. One of the groups served as the control group and received no sports drinks to evaluate at home and was only asked to attend the next laboratory session. The samples for consumption at home were distributed in 500 ml translucent polypropylene bottles, covered with white caps. The consumers had to drink the sports drinks (500 ml each), one a day over six days until the next laboratory session. The bottles for the home exposure were also coded with randomly selected three-digit codes. The consumers were instructed to return the empty bottles at the next laboratory session. The consumers were not informed that the six bottles they received contained in fact exactly the same sports drink. During the first laboratory session the consumers were asked to indicate their activity level, using a scale from 1=slightly active to 5=extremely active. The consumers were also asked to indicate their favourite sports drink given a choice of five (Game, Powerade, Energade, Lucozade, USN Energ) as well as an "other" option.

Consumers

The consumer evaluation included 128 consumers. The profile of the consumers that participated in the study is summarised in Tables 4.12 and 4.13. All consumers were recruited among the staff and students of the University of Pretoria, Pretoria, South Africa. The consumers received gift packs containing sports drinks, water bottles and energy bars at the end of the study. Lucky draws also took place and consumers stood the chance of winning movie tickets, snacks, restaurant vouchers and soccer balls.

Table 4.12: Age group distribution (%) of consumers (n=128) in the six experimental groups that participated in the study

Age	Citric Acid (n=20)	Malic Acid (n=24)	Tartaric Acid (n=22)	Fumaric Acid (n=22)	Fruitaric® Acid (n=21)	Control group (n=19)	Total group n=128
15-20 yrs	50	42	46	23	19	47	37
21-25 yrs	45	50	45	55	48	37	47
26-30 yrs	0	8	9	5	9	11	7
31-40 yrs	5	0	0	4	9	0	4
41-50 yrs	0	0	0	9	5	0	3
50+ yrs	0	0	0	4	10	5	2

Table 4.13: Gender distribution (%) of consumers (n=128) in the six experimental groups that participated in the study

Gender	Citric Acid (n=20)	Malic Acid (n=24)	Tartaric Acid (n=22)	Fumaric Acid (n=22)	Fruitaric® Acid (n=21)	Control group (n=19)	n=128
Male	50	63	50	77	48	53	53
Female	50	37	50	23	52	47	47

4.3.3 Statistical Analysis

To analyse the results for preference ranking of samples, the Friedman Rank Sum test was performed (Compusense® *five*) using a significance level of 95% ($p \leq 0.05$) to determine whether differences in consumer preferences for the samples were found during each laboratory session for the total group and separately for the subgroups. The rank sum totals were divided by the number of consumers that evaluated the samples. The Least Significant Ranked Difference (LSDR) values were calculated to determine which sample/s was significantly preferred over others. One way analysis of variance (ANOVA) was conducted to test the effect activity levels of the consumers had on the different experimental groups.

4.3.4 Results

There was no significant difference in the activity levels of the different experimental groups (p-value = 0.82). Tables 4.14 and 4.15 indicate the activity level and favourite sports drink of the consumers. Powerade and Energade was mostly indicated as the most favourite sports drink in most of the groups. The tartaric acid group included a large group of consumers that preferred Game. The majority of the group members that were exposed to citric and fumaric acids indicated the test product (Energade) to be their favourite sports drink.

During the first laboratory evaluation session (pre-exposure) the consumers (n=128) significantly preferred the sports drink with citric acid to the sports drinks with fumaric acid and Fruitaric® acid, respectively (Table 4.16). Preference for sports drinks with citric acid, malic acid and tartaric acid did not differ significantly, while preference for drinks with malic acid, tartaric acid, fumaric acid and Fruitaric® acid were also equal. No significant difference in preferences for the five different sports drink samples was found during the second laboratory evaluation (mid-exposure). The results from the third laboratory session (post-exposure) showed a significant preference for sports drinks with tartaric, fumaric, and Fruitaric® acids compared to the sports drink with citric acid. Preferences for drinks with citric acid and malic acid did not differ significantly, while preferences for sports drinks with malic acid, tartaric acid, fumaric acid and Fruitaric® acid were also equal.

Table 4.14: Distribution of consumers (%) (n=128) in activity categories in the six experimental groups that participated in the study

Level of activity	Citric Acid (n=20)	Malic Acid (n=24)	Tartaric Acid (n=22)	Fumaric Acid (n=22)	Fruitaric ® Acid (n=21)	Control group (n=19)	n=128
Extremely active (4)	0	4	9	9	0	0	4
Very active (3)	45	21	9	27	33	26	27
Moderately active (2)	45	63	73	55	57	69	60
Slightly active (1)	10	12	9	9	10	5	9
Mean ± std dev	2.7±0.7	2.8±0.7	2.8±0.7	2.6±0.8	2.8±0.6	2.8±0.5	2.8±0.7

Table 4.15: Favourite sports drink distribution (%) of consumers in the six experimental groups that participated in the study

Favourite sports drink	Citric Acid (n=20)	Malic Acid (n=24)	Tartaric Acid (n=22)	Fumaric Acid (n=22)	Fruitaric® Acid (n=21)	Control Group (n=19)	n=128
Game	0	0	**45**	9	19	0	5
Powerade	35	**46**	23	36	38	**42**	**40**
Energade	**50**	29	14	**45**	24	26	33
Lucozade	10	17	4	5	10	11	11
USN Energ	5	8	14	5	5	11	5
Other	0	0	0	0	4	10	6

Values in bold indicate a sports drink brand that was selected by more than 40% of consumers in a specific group

Table 4.16: Preference ranking results for five sports drinks for the overall population of consumers (n=128). Products ranked closest to "1" were more liked; products ranked closest to "5" were less liked.

Exposure	Citric Acid	Malic Acid	Tartaric Acid	Fumaric Acid	Fruitaric® Acid
Pre	2.6^{a}	3.1^{ab}	3.0^{ab}	3.2^{b}	3.1^{b}
Mid	3.2^{a}	3.0^{a}	3.0^{a}	2.9^{a}	2.9^{a}
Post	3.4^{b}	3.0^{ab}	2.8^{a}	2.9^{a}	2.9^{a}

^{a,b} Values with different superscripts in a row differ significantly ($p<0.05$).

The consumers that were exposed to sports drinks with citric acid (n=20) during the home exposure periods had no significant preference for any of the five sports drink samples during laboratory sessions 1 and 2 (pre and mid exposure) (Table 4.17). Post exposure, the sports drink with citric acid was significantly less preferred compared to the sports drink with tartaric, fumaric and Fruitaric® acids. The malic acid group (n=24) did not show significant preferences ($p>0.05$) for the five sports drink samples during each of the laboratory sessions. The group of consumers that were exposed to sports drinks with tartaric acid (n=22) initially significantly preferred ($p<0.05$) sports drinks with tartaric acid, citric and Fruitaric® acid over the sports drink with malic acid. After exposure to sports drinks with tartaric acid at home no significant difference in preferences was found.

Pre exposure, the fumaric acid group (n=22) significantly preferred sports drinks with citric acid, malic acid and tartaric acid to the sports drink with Fruitaric® acid (Table 4.17). No significant preferences were found during the second laboratory evaluation (mid exposure). Post exposure the consumers preferred the sports drink with malic acid significantly more than the sports drinks with citric, tartaric, fumaric and Fruitaric® acids.

The consumers that were exposed to Fruitaric® acid (n=21) during the home exposure period showed no significant preferences for any of the five sports drink samples during the first and second laboratory evaluation. The results from the third laboratory evaluation indicated a significant preference for the sports drink with tartaric acid over the sports drink with citric acid.

The control group (n=19) did not receive any sports drink samples to evaluate at home. The results showed that the consumers from this group significantly preferred sports drinks with citric acid, malic acid and tartaric acid compared to sports drink with Fruitaric® acid during the first laboratory session. No significant preferences were found during the second and third laboratory session. For this latter group although not significant, there was a trend showing that consumer preferences for the sports drink with citric acid was lower than the other samples after repeated exposure.

Table 4.17: Preference ranking results (rank averages) for the five sports drink, by exposure group (1=Like it the most, 5=Like it the least)

Exposed to sports drink with added	N	Exposure	Citric Acid	Malic Acid	Tartaric Acid	Fumaric Acid	Fruitaric® Acid
Citric acid	**20**	**Pre**	3.0^{a}	2.6^{a}	3.5^{a}	3.2^{a}	2.8^{a}
		Mid	3.0^{a}	2.9^{a}	3.3^{a}	2.8^{a}	3.1^{a}
		Post	3.6^{b}	3.6^{b}	3.2^{ab}	2.3^{a}	2.5^{a}
Malic acid	**24**	**Pre**	2.6^{a}	3.5^{a}	2.9^{a}	3.3^{a}	2.7^{a}
		Mid	2.6^{a}	3.0^{a}	3.2^{a}	3.2^{a}	3.1^{a}
		Post	3.2^{a}	3.6^{a}	2.5^{a}	2.9^{a}	2.9^{a}
Tartaric acid	**22**	**Pre**	2.5^{a}	4.1^{b}	2.6^{a}	3.3^{ab}	2.5^{a}
		Mid	3.4^{a}	2.9^{a}	3.0^{a}	2.9^{a}	3.0^{a}
		Post	2.9^{a}	3.0^{a}	3.0^{a}	3.0^{a}	3.2^{a}
Fumaric acid	**22**	**Pre**	2.5^{a}	2.7^{a}	2.5^{a}	3.3^{ab}	4.0^{b}
		Mid	3.4^{a}	3.1^{a}	2.8^{a}	2.9^{a}	2.9^{a}
		Post	3.5^{b}	2.0^{a}	3.1^{b}	3.3^{b}	3.1^{b}
Fruitaric® acid	**21**	**Pre**	2.5^{a}	3.0^{a}	3.6^{a}	3.2^{a}	2.8^{a}
		Mid	3.4^{a}	3.1^{a}	2.5^{a}	2.9^{a}	3.0^{a}
		Post	3.8^{b}	3.1^{ab}	2.3^{a}	2.8^{ab}	3.0^{ab}
Control group	**19**	**Pre**	2.4^{a}	2.6^{a}	2.9^{a}	3.2^{ab}	4.0^{b}
		Mid	3.4^{a}	3.1^{a}	3.6^{a}	2.6^{a}	2.3^{a}
		Post	3.9^{a}	2.9^{a}	2.7^{a}	2.8^{a}	2.7^{a}

^{a,b,c} Rank totals in a row with different superscripts differ significantly ($p<0.05$)

4.3.5 Discussion of results

During the first laboratory evaluation session the results showed that the consumers (n=128) significantly preferred the sports drink with citric acid to the sports drinks with fumaric acid and Fruitaric® acid respectively. No significant difference in preferences for the five different sports drink samples was found during the second laboratory evaluation. The results from the third laboratory session showed a significant preference for sports drinks with tartaric, fumaric, and Fruitaric® acids compared to the sports drink with citric acid. A clear shift in preferences occurred. The number of exposures to a sports drink with a specific acid or time on trial seemed to have influenced consumer preferences for the sports drink samples.

Throughout the study, no significant differences in consumer preferences were found during the second laboratory session. This was also the case for all of the experimental subgroups. In a study by Pliner (1982) consumers were exposed to unfamiliar fruit juices for different numbers of time (20, 10, 5 or 0 times). The results indicated that frequency of exposure had an influence on consumer acceptability, the more an individual was exposed to the fruit juices, the better it was liked. During the second laboratory session, consumers have only been exposed to the sports drinks seven times (once during the first laboratory session and six times during the home exposure) compared to fourteen times at the third laboratory session. It was therefore expected and confirmed by this study that more clear differences in preferences would occur during the third laboratory session compared to the second laboratory session.

Citric acid is the acidulant that is currently used in the commercial sports drink that was used as a base in this study and the sports drink with citric acid is therefore the most familiar sample in the experiment. Several studies have linked consumer preferences to familiarity (Lévy & Köster, 1999; Stallberg-White & Pliner, 1999; Hetherington *et al.*, 2000 & Hetherington *et al.*, 2002). Porcherot & Issanchou (1998) found that the most familiar flavours are usually also the most preferred when consuming it for the first time in a test situation. Pliner (1982) stated that unfamiliar food products increase in liking with repeated exposure, while familiar food products lead to monotony or boredom. The results from this study confirmed this theory. The more familiar sample (sports drink with citric acid) was the most preferred at the beginning of the trial, but after repeated exposure it was the least preferred. Thus, repeated exposure to the familiar sample probably led to food boredom. The rest of the acidulants that were added to the sport drink were unfamiliar for this specific sports drink. Malic acid is used in some beverage applications (Berry, 2001). Giese (1995) reported that the use of tartaric acid in grape and lemon flavoured beverages is preferred because it enhances the flavour. Fumaric acid is reported to contribute a strong, metallic sour taste that is lingering on the palate (Berry, 2001). Theoretically the sports drinks with fumaric and Fruitaric® acids were therefore considered to be the most unfamiliar samples in this study. As expected these unfamiliar samples were less preferred at the beginning of the study, but the most preferred after repeated exposure. This increase in liking could be explained by the mere exposure theory (Pliner, 1982). Zajonc (1968) stated that the effect of mere exposure occurs more often with unfamiliar food products. The pacer theory of Dember (1970) predicts that consumers learn to appreciate more complex stimuli with experience. Exposure to stimuli which are slightly more complex than optimal leads to an increase in a person's optimal arousal level. Köster *et al.* (2002) stated that as a consumer is repeatedly exposed to a more complex product than the product he/she prefers, the complexity of the new product will become less complex and might lead to increases in liking perceptions over time. The metallic, lingering sour taste of fumaric acid could have been perceived as more complex compared to the citric acid.

When comparing the results of the different experimental groups, the results from the group that was exposed to the sports drink with citric acid (n=20) were similar to the overall results (n=128). Repeated exposure to the sports drink with citric acid resulted in this sports drink to become the least preferred during the third laboratory evaluation, while there was no significant preference for a specific sports drink at the beginning of the trial. The repeated exposure to the sports drink containing citric acid led to boredom. In a study by Luckow *et al.* (2005) consumers were exposed to probiotic orange juice samples. The results showed that the sub group that was exposed to the untreated control sample rated it significantly lower after exposure. Citric acid is the acidulant currently used in the sports drink and could therefore be considered as the control sample of this study.

The malic acid group (n=24) did not show significant preferences for any of the five sports drink samples during each of the laboratory sessions. Malic acid is said to have a smooth, flat taste (Berry, 2001) which could be explained by its taste-blending and flavour-fixative qualities (Dzlezak, 1990). Giese (1995) claimed that the taste of malic acid has very little character. This could have caused malic acid to make no significant contribution to the sour taste of the sports drink and therefore the repeated exposure to the sports drink with malic acid did not influence consumer preferences. The group that was exposed to the sports drink with added fumaric acid also showed signs of food boredom after repeated exposure. At the end of the trial the sports drink with fumaric acid was one of the least preferred sample, while it was intermediate in terms of acceptance pre exposure. Contrary to the increase in liking for the fumaric acid sports drink reported earlier and explained by the pacer theory, the strong, metallic, lingering sour taste of fumaric acid (Berry, 2001) possibly caused irritation and contributed to the fact that the consumers preferred the sports drink with malic acid, which is said to have a smooth, flat taste, after repeated exposure rather than the sports drink with added fumaric acid. Berlyne (1970) stated that acceptability of more complex products will increase after repeated exposure to the product, while the opposite will occur if less complex products become less novel after repeated exposure. The strong, metallic, lingering sour taste of the fumaric acid could have been perceived to be more complex than the malic acid.

A higher percentage of consumers that were included in the groups that were exposed to the sports drinks with citric and fumaric acids, was users of the sports drink that was included in the trial. The optimal arousal level theory of Berlyne (1970) states that experience with a specific product influences consumers' attitude towards that product. These groups had the most experience with the specific sports drink and this might explain why these were the only subgroups that behaved according to predictable theories.

The results from the groups that were repeatedly exposed to sports drinks with added tartaric and Fruitaric® acids are less clear. Tartaric acid has a sharp, bitter sour taste (Giese, 1995 and Berry, 2001), without the lingering characteristic of fumaric acid. For the experimental group that was exposed to the sports drink with added tartartic acid, this drink was one of the preferred samples at the beginning of the trial. After repeated exposure to this sport drink, no significant differences were found in the consumer preferences for the five sport drink samples. No clear explanation can be given for this. Repeated exposure to the sports drink with Fruitaric® acid did not influence consumer preferences for this sports drink. At the end of the experiment, the sports drink with added tartaric acid was more preferred than the sports drink with added citric acid for this group. Fruitaric® acid is a combination of malic, tartaric and fumaric acids. Fruitaric® acid contains approximately equal quantities of malic and fumaric acids and only a very small percentage of tartaric acid (Robert Fowlds, Managing Director of Isegen South Africa (Pty) Ltd, 2004 - personal communication) and could not be the explanation of why it was the preferred sports drink with tartaric acid at the end of the trial.

Although previous studies showed that repeated exposure to products with small ingredient changes could result in changes in consumer acceptance (Stein *et al.,* 2003; Chung & Vickers, 2007a; Chung & Vickers, 2007b) it is possible that the differences in the sensory profiles of the sports drinks with the different acidulants were too small to have an impact on desire and liking. The question that was unfortunately not answered by the experimental design selected was whether the samples differed noticeably pre or post exposure. It seems that a shortcoming of this experiment is the lack of a repeated-use difference test to answer the former question.

4.3.6 Conclusion

Repeated exposure testing of sports drinks with different acidulants resulted in hedonic adjustment. Consumer preferences post exposure could not have been predicted with a traditional consumer taste test at the start of the study. It is possible that subtle differences in the taste profiles of the sports drinks with the different acidulants became more evident after repeated exposure and therefore influenced liking. Home use exposure to specific sports drinks mostly resulted in changes in preferences, at least partially according to predictable theories, either mere exposure or boredom.

One of the advantages of repeated exposure testing is that the data provide a reflection of real-life situation in which consumers repeatedly consume a product over a period of time. The disadvantage of this type of research is that is time-consuming and therefore expensive to conduct. Larger quantities of the product are needed, which is sometimes difficult during the early stages of product development. It is more challenging to recruit consumers for longer term participation. Information supplied to the consumers could influence results; if the consumer knew what the researcher were trying to measure it could influence their responses. Also, no standard method to measure the effects of repeated exposure has been developed yet, which challenges experimental design. As sensory scientists we need to critically evaluate the validity of our standard single exposure small sip consumer tests to predict long term consumer perception. Additional research is needed to better understand changes in acceptability that could lead to hedonic adjustment.

CHAPTER 5 : CRITICAL REVIEW OF EXPERIMENTAL DESIGN, METHODOLOGIES USED AND RESULTS

The sourness perception of five acidulants, i.e. citric acid, malic acid, tartaric acid, fumaric acid and Fruitaric® acid were compared during this study. A trained sensory panel compared the sourness intensity of the acidulants at equal sour and equal weight concentrations when applied to water and two sports drink flavours (*Grape* and *Lemon & Lime).* The effects of the different acidulants on some physico-chemical properties of the water solutions and sports drink samples were compared. These included pH, titratable acidity and ^{o}Brix. The sensory panel evaluated the temporally perceived sourness of the acidulants in water and the flavoured sports drinks at equal sour concentrations. The time to reach maximum intensity, duration and intensity of the perceived sourness were compared. Consumers (n=128) participated in a consumer test to determine if repeated exposure testing of *Lemon & Lime* flavoured sports drinks containing different acidulants, added at equal sour concentrations, would lead to hedonic adjustment.

Aspects that complicated the interpretation of results included the following. A commercially available sports drink base was used for all the analyses during this study. The sports drink (in the form of concentrates) was provided by a South African manufacturing company. The sports drink concentrates (*Grape* and *Lemon & Lime* flavoured) were diluted (1:4) with deionised water. Although the acidulant was the only independent variable during the analysis, the specific formulation of the sports drink base was unknown, which makes it difficult to identify and discuss possible ingredient interactions which might have had an influence on sourness perception. It is for example well-known that sucrose and sweeteners suppresses the sourness of acidulants e.g. citric acid (Bonnans & Noble, 1993; Pelletier, Lawless & Horne, 2004; Savant & McDaniel, 2004).

Fruitaric® acid (a composite of malic acid, tartaric acid and fumaric acid) a commercial acidulant from Isegen South Africa (Pty) Ltd. was included in the study. Although the ratio of the different acids in this mixture was revealed to the researcher as proprietary information, no other physical properties of the acidulant i.e. melting point, pKa value, solubility in water was made known. If known, these factors could also have been included in the multivariate analyses to assist in understanding the behaviour of this acidulant among the different applications.

To investigate the effect of different acidulants on sourness perception previous studies have compared the sourness intensity of acidulants at equal pH and titratable acidity values (Norris *et al.,* 1986; Noble *et al.,* 1986; CoSeteng *et al.,* 1989; Sowalsky & Noble, 1998). Noble *et al.* (1986) stated that one of the difficulties in determining the sourness intensity of acidulants is “isolating” a single variable. The pH and titratable acidity of the final product are influenced by the concentration of the acidulant added and therefore the pH and titratable acidity are co-varying factors. Sowalsky & Noble (1998) stated that the sourness intensity is independently influenced by acidulant concentration, pH and anion species of the acidulant. The results from this study showed that pH or the titratable acidity of the solution on its own was insufficient to predict the perceived sourness intensity of the final product and therefore do not agree with the hypothesis of Shallenberger (1996). Pangborn (1963) also found no significant correlations between pH, titratable acidity and sourness intensity of acidulants. To determine the individual effects of pH or titratable acidity on the sourness intensity the samples should have been adjusted to have either equal pH or titratable acidity values. This would have been useful to determine if only the concentration of the acidulant used in the sports drink were responsible for the sourness intensity without the possible interaction of pH or titratable acidity differences, i.e. to test only one variable at a time.

During the sensory panel screening phase of the study the best estimate detection threshold (BET) value for sour taste of the individual panellists was determined. The results showed that 8 out of the 10 panellists were able to detect the citric acid solution at the lowest concentration (0.0037% w/v citric acid) indicating that the concentration range selected was not sensitive enough to determine their real threshold values for citric acid. However, the aim of using the BET test was to determine if the panel was sensitive enough to evaluate the sourness intensity of acidulants at the concentrations used during the study and not to determine threshold values and was therefore an adequate screening method.

The trained sensory panel compared the sourness of malic, tartaric, fumaric and Fruitaric® acids to citric acid using LMS. Each set consisted of citric acid (0.2%) and another acidulant, either at equal weight or equal sour concentration. Citric acid was used as a reference. It would have been useful to include a control set in the evaluation consisting of the reference and a blind citric acid sample to investigate panellists' performance. One would expect to find no significant differences in the sourness intensity of the two citric acid samples using LMS. The sourness intensities of the acidulants in application were only compared with citric acid and the relative sourness of all possible acidulants, e.g. the sourness intensity of malic vs. tartaric acid, was not compared. Although the equal sour concentrations used in this study were equally sour to citric acid, differences in sourness intensities between malic and tartaric acids could exist. If the sourness intensity of the acidulants were compared using a different method (i.e. rating on a line scale) it would have been easier to interpret the relative sourness intensity of the acidulants as individual ratings and not only as compared to citric acid.

The results from this study confirmed that lower quantities of malic, tartaric, fumaric and Fruitaric® acids could be added to the *Lemon & Lime* flavoured sports drink to elicit an equal sour taste perception than citric acid. This may potentially have economic advantages. These equal sour concentrations seemed to be flavour specific, as the same concentrations of tartaric and Fruitaric® acids were found to be significantly less sour, while malic and fumaric acid were equally sour to citric acid when compared in the *Grape* flavoured sports drink.

Added at equal weight concentrations fumaric and Fruitaric® acids were found to be significantly more sour than citric acid in water and both sports drink flavours, while tartaric acid was significantly more sour than citric acid in water and *Lemon & Lime* flavoured sports drink. Contrary to the results from previous studies (Fabian & Blum, 1943; Buechenstein & Ough, 1979; CoSeteng *et al.,* 1989 and Noble *et al.,* 1986) no differences were found between the sourness intensity of malic and citric acid in all three applications.

Chemical properties were reported to clarify the differences in sourness intensities of acidulants at equal weight concentrations. The influences of molecular weight (CoSeteng *et al.,* 1989), pKa values, chemical structure (Moskowitz, 1971; CoSeteng *et al.,* 1989; Siebert, 1999; Johanningsmeier *et al.,* 2005) were also investigated. These properties were found to be sufficient to explain some differences in sourness intensity for some of the acidulants but not all. Although malic acid has a lower molecular weight, a higher pKa value and one less carboxylic group than citric acid, these acids did not differ significantly in sourness perception at equal weight concentrations in all three applications.

The results from the LMS showed that the equal sour concentrations, as determined earlier, were not confirmed in the *Grape* flavoured sports drink. The sourness intensity of tartaric and Fruitaric® acid *Grape* flavoured sports drinks was less intense than the citric acid sports drink. The equal sour concentrations for tartaric and Fruitaric® acids needed to be adjusted for it to be considered equal sour. The equal sour concentrations used during the determination of the temporal sourness intensity of the acidulants were therefore not equal sour in the *Grape* flavoured sports drink. The results from the TI study also showed that the flavoured sports drinks with added citric acid was found to have a higher total sour impact compared to tartaric and Fruitaric® acid. Ideally adjustment and further testing of the concentrations used should have followed. However, the results were only analysed after the TI study was completed and therefore this insight was only gained retrospectively. The lesson learnt: analyse data as you progress.

The LMS method confirmed the equal sour concentrations to be correct in water. Acidulants do not only affect sourness but potentially also other taste qualities (Bonnans & Noble, 1993; Pelletier *et al.*, 2004; Savant & McDaniel (2004). The inclusion of other taste/flavour qualities such as bitterness, sweetness, overall flavour and fruitiness when comparing the flavoured sports drinks could also have been useful to compare with other results. ^{o}Brix were measured and Brix/acid ratios were calculated in the flavoured sports drinks. Potter & Hotchkiss (1995) stated that the higher the Brix/acid ratio the sweeter and less sour is the beverage. The inclusion of sweetness intensity evaluation (using LMS) and temporal sweetness perception would have been useful to confirm this claim also in a sports drink application.

During the TI evaluation the sourness intensity of the different samples was recorded for 80 seconds; the results showed that some of the panellists still perceived sourness at 80 seconds. The time period used to evaluate the sourness of the acidulants was therefore not long enough and this influenced some of the parameters analysed e.g. duration of the perceived sourness as recorded by the individual panellists. During the TI training sessions, the time given for recording the sourness should have been long enough to make sure that all the panellists perceived no sourness at the end. The time needed for all the panellists to reach no more sourness (baseline) should rather have been used during the final TI evaluations.

A 2% citric acid in water solution served as a reference and its maximum intensity was selected by consensus to be 75% on the line scale. The significant panellist effect for all TI parameters in both water and flavoured sports drinks indicated that the different panellists used the scale differently. Although trained to score the reference sample as 75% on the line scale, the scores was between 33% and 100%, with an average of 71%. Although the average maximum intensity (71%) was close to 75%, the variation in results showed that the panel needed further training, even on the reference sample, before the commencement of the actual TI evaluations.

It was decided to include only the *Lemon & Lime* flavoured sports drink in the repeated exposure consumer test as the equal sour concentrations were confirmed in the *Lemon & Lime* sports drink but not in the *Grape* sports drink. The consumers evaluated five sports drinks (each sports drink contained one of the five acidulants included in this study) and rank the five samples in order from 1 to 5 where 1 represented "Like it the most" and 5 represented "Like it the least". Using a ranking test to determine consumer preferences for the five sports drink samples forced the consumers to make a choice regarding preference for each samples as the differences in the sensory profiles in the sports drinks with the different acidulants were presumably relatively small. No ties were allowed, so the consumers were not allowed to indicate that they preferred samples equally. One of the disadvantages of using a ranking test is that it does not provide information on the relative size of the differences in consumer preferences for the different samples. The inclusion of both a rating and ranking test, using two different sets of samples, (as in the study by Luckow *et al.,* 2005) in the consumer evaluations would have been useful. This would have forced the consumers to indicate their preferences, but also provide information on the size of differences in acceptability of the different sports drink samples.

For the home use exposure the consumers were divided in six experimental groups. Five groups received one of the sports drink samples to consume at home. The last group served as the control group and received no sports drinks to evaluate at home and was only asked to attend the next laboratory session. The intention was to have at least 25 consumers per group. The final numbers for the six experimental groups were smaller (n=19 – 24) than what is normally used for consumer tests. Hough, Wakeling, Mucci, Chambers, Gallardo, Alves (2006) listed consumer studies where a wide range of number of consumers was used (10 – 581 consumers). Stone & Sidel (2004) suggested 25 – 50 consumers for a laboratory consumer test. Recent studies that included repeated exposure testing used smaller number of consumers in subgroups. Luckow *et al.* (2005) used 19 consumers in a subgroup, while Chung & Vickers (2007b) included 20 – 21 consumers in a subgroup. Statistical guidelines on sample size used in a consumer test usually concentrate on either comparing only two samples (Lawless & Heymann, 1998) or when using a scale (Stone & Sidel, 2004; Hough *et al,* 2006). Although not referring to specific numbers, Resurrecion (1998) stated that if products included in a consumer test vary a lot, a larger number of consumers are necessary to participate in the evaluation, while smaller panels can be used when the variability is small. The differences in the sensory profiles of the sports drinks were relatively small. Hough *et al.* (2006) presented estimations of number of consumers used in a trial for different values of Type I and Type II errors. The authors suggested to use an average RMSL (root mean square error divided by scale length) of 0.23 if the researcher does not have previous knowledge of an appropriate RMSL. If a total of 22 consumers were to be used in a subgroup and a difference in the mean rank (d) of 0.2 is sought, then the probability of Type I error is 5% and the probability of Type II error (β) is 20% resulting in the power of the test to be 80%. The final numbers of the subgroups were between 19 and 24 resulting in calculated power levels between 74 and 85%. If 35 persons were included in a subgroup, the power of the test would have increased to 95%. It is therefore recommended that a minimum of 35 persons be included in a consumer test where the probability of a type I error is reduced to 5% and where a relative large difference in means of acceptability ratings are required or expected.

The preference results of the different exposure groups were different at the start of the study (pre exposure). The citric acid, malic acid and the Fruitaric® acid groups did not significantly prefer any of the five sports drink samples pre exposure. The tartaric acid group initially significantly preferred sports drinks with tartaric acid, citric and Fruitaric® acid over the sports drink with malic acid. Pre exposure, the fumaric acid group significantly preferred sports drinks with citric acid, malic acid and tartaric acid to sports drink with Fruitaric® acid. This indicates that the six groups were not really totally comparable. One would have expected to make the same conclusion from the results of the six groups at the pre-exposure phase. The groups that were exposed to malic and fumaric acids had more males than females per group. The average ages of the consumers in the fumaric and Fruitaric® acid groups were slightly higher compared to the other groups. More care should have been taken to ensure that the demographic profiles of the different exposure groups were more similar. These differences in preference results make it more difficult to attribute the differences in preference results post exposure to only the repeated exposure and not to the differences between the groups. Although the subgroups from previous studies (Luckow *et al.,* 2005, Luckow *et al.,* 2006) were balanced for age and gender, the results also showed differences in initial preference ranking results in different subgroups.

Consumers were exposed to the sports drinks based on their experimental groups and were not allowed to choose which drink to consume at home. Although consumers were exposed to sports drinks in a controlled/systematic manner Zandstra *et al.* (2004) stated that repeated exposure studies of the same food product might result in different results when tested with or without a choice situation. If consumers were allowed to choose the drink that they would like to consume at home, it could have lead to different results. Meiselman (1996) recommended that sensory evaluation should be conducted in more naturalistic testing situations. Further research is needed to determine whether choice of familiar food products in a natural choice environment is enhanced by repeated exposure. Observing how the individual consumer's choices or preferences changed over the exposure period would have been useful to identify specific personality behaviour in changes in consumer preferences similar to the study by Chung & Vickers (2007b) that identified constant switchers, acquired likers, non-switchers and systematic switchers. Being repeatedly exposed to a product could be perceived as monotonous which may lead to boredom with the procedure of the methodology, which could lead to false temporary decrease of product acceptance/preference (Sulmont-Rossé, Chabanet, Issanchou & Köster, 2008).

The results from the consumer test showed that a clear shift in consumer preferences occurred after repeatedly consuming the sports drinks. The consumers (n=128) significantly preferred the sports drink with citric acid to the sports drinks with fumaric acid and Fruitaric® acid respectively at the start of the trial, while a significant preference for sports drinks with tartaric, fumaric, and Fruitaric® acids compared to the sports drink with citric acid were found post exposure. Consumer preferences post exposure could not have been predicted with a traditional consumer taste test at the start of the study. Similarly, in a study by Sulmont-Rossé *et al.* (2008), consumers were exposed to four unfamiliar fruit drinks over 24 trials. The results showed that the more unfamiliar the drinks were perceived to be, the more acceptability improved after repeatedly being exposed to the drink. The authors hypothesized that repeated exposure to unfamiliar food products, decreased the uncertainty a consumer may experienced about the product, which will increase long term acceptability of the product. The liking ratings for the drink with high arousal potential (novelty, complexity and taste intensity) and low sweetness intensity increased after repeated exposure, while the liking ratings for the drink with moderate arousal potential and higher sweetness were unaltered. Weijzen *et al.* (2008) exposed consumers (N=66) to three soups for 14 days and to four snack samples for 5 days (N=61). The samples differed in arousal potential (complexity and taste intensity). The results from both studies showed that the samples which were perceived to be high in complexity and intensity were more resistant to a decrease in acceptability ratings compared to the samples with lower complexity and taste intensity. Repeated exposure testing of sports drinks with different acidulants resulted in hedonic adjustment. The findings of this study surely challenge the validity of sensory evaluation test strategies that rely on single exposure testing to predict long term consumer preferences.

CHAPTER 6 : CONCLUSIONS & RECOMMENDATIONS

At both equal sour and equal weight concentrations, malic and tartaric acids were found to be more similar to citric acid compared to fumaric and Fruitaric® acids. The substitution of citric acid with either malic acid, tartaric acid, fumaric acid or combinations thereof at equal sour concentrations in a sports drink should be made with caution as the equal sour concentrations seems to be flavour specific. It seems to be possible to substitute citric acid with malic or Fruitaric® acid in both *Grape* and *Lemon & Lime* flavoured sports drinks. Tartaric or fumaric acid could be used to replace citric acid in *Lemon & Lime* flavoured sports drinks, but in order for the replacement of citric acid in the *Grape* sports drink, the concentrations need to be adjusted to ensure that it is equal sour. Using lower concentrations of malic, tartaric, fumaric or fruitaric acids in the sports drinks could lead to cost savings. Future studies should include more flavours to investigate specific flavour-acidulant interactions. More information on the profiles of the flavours will be needed to understand these interactions. Although the acidulants were found to be equal sour in water, some differences in sourness intensity were observed in the flavoured sports drink. Substitution of citric acids with other acidulants in other food models should also be investigated.

The results from this study rejected anecdotal reports that acidulants differ in their temporal sensory profiles. The temporal impact of the different acidulants was compared at equal sour concentrations. Future TI studies should investigate the effect of acidulants at equal weight concentrations to investigate if some of the acidulants will elicit a lingering effect or higher maximum sourness intensity. The acidulant concentration used in this study was selected on the basis of the concentration currently used in the sports drink. Future TI studies of acidulants at equal sour concentrations could also include different sourness levels/concentrations.

Consumers were exposed to the sports drinks based on their experimental groups and were not allowed to choose which drink to consume at home.

Future repeated exposure tests could also give consumers the opportunity to choose the samples they would like to consume during the home exposure period. The substitution of citric acid with a different acidulant in the sports drink at equal sour concentrations did not have a large effect on the sensory profiles of the final product. Future studies could include products with sensory profiles that are more different. There is no standard methodology yet to investigate the effect of repeated exposure on potential changes in consumer acceptability. More research should be done to compare different exposure time, quantities consumed and general experimental design. Additional research is needed to better understand changes in acceptability that could lead to hedonic adjustment.

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