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Ráice, Rui

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# Aroma characterization and retention after heat treatment and drying of fruits studied using extraction and GC-MS analysis

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RUI T. RAICE | DEPARTMENT OF FOOD TECHNOLOGY, ENGINEERING & NUTRITION  
DOCTORAL THESIS | LUND UNIVERSITY





# **Aroma characterisation and retention after heat treatment and drying of fruits, using extraction and GC-MS analysis**

Rui Ráice

2015



**LUND**  
UNIVERSITY

Doctoral Thesis

By due permission of the Faculty of Engineering at Lund University, Sweden,  
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*Faculty opponent*

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Abstract This study concerns the identification and characterisation of volatile components of fruits, and evaluation of the effect of heat treatment and drying on retention or loss of volatiles of fruits. The investigation included developing a procedure to extract volatile components from the fruit matrix, a purification step, separation, identification and quantification.  Initial experiments with <i>Vangueria infausta</i> L. showed that some components, especially sugars, degrade during heating in the GC analysis, producing furfural, hydroxyl methyl furfural (HMF) and other volatiles. These compounds are obtained together with the native aroma components of the fruit, making analysis difficult. We developed a procedure using a hydrophobic column that could retain the hydrophobic aroma components and eliminate sugars that could disrupt the analyses. The extract was analysed by GC. The volatile components found in pulp of <i>V. infausta</i> were primarily hexanoic acid, octanoic acid, ethyl hexanoate, ethyl octanoate, methyl hexanoate and methyl octanoate. Based on the odour activity values, it could be concluded that the odour of the fruit is mainly attributed to ethyl hexanoate and ethyl octanoate. (paper I).  Drying is often used to process and preserve food stuff but many food attributes including aromas which are important for palatability and consumer interest are affected by the process. Our research showed that the principal aroma components are well preserved during the initial phase of drying (down to about a relative water activity of 0.65). However the aromas are lost after more extensive drying. A possible explanation for volatilisation is the sugar crystallisation that occurs below a RH of around 0.70. (paper II).  Also we evaluated the effects of drying with or without blanching on volatiles of mango ( <i>Mangifera indica</i> L.). Fresh, blanched and dried mango samples were analysed (paper III). The fresh sample presented a very large number of peaks. The blanching was carried out in water at 70°C, 10 min and in a microwave at 90°C, 2 min. Blanching increased the levels of aroma components. Both blanching procedures had no dramatic effect on the impact of the blanching. Prolonged hot air drying ( $a_w=0.65$ ) reduced most of the aroma. $\alpha$ -pinene and 1-butanol were strongly affected due their volatility. Drying had no great effect on components with high boiling points, which displayed significant retention even after extensive dehydration. Ethyl butanoate was high retained despite its high volatility.  To evaluate a possible influence of sugar crystallisation on aroma retention a further study involved three model matrixes based on oils plus carbohydrates aqueous solution: I) pectin-sucrose-water-oils, II) pectin-microcrystalline cellulose-sucrose-water-oils and III) microcrystalline cellulose-sucrose-water-oils. The oil phase comprised the reference materials of the most powerful aroma components found in <i>V. infausta</i> (hexanoic acid, ethyl hexanoate and ethyl octanoate). The model mixture was dried at 80°C, 3 m/s for 60-420 min prior to GC analysis (Paper IV). The aroma components were preserved in all models throughout the drying process (until $a_w \approx 0.8$ ). So sugar crystallisation did not induce the loss of volatiles. However noticeable sugar crystallisation was observed in model II. We assume that the presence of pectin and microcrystalline cellulose increased the ability of the matrix to compact, as water activity decreased during drying. So sugar crystallisation is probably the reason for aroma retention within the matrix.  The results of the studies in this thesis illustrate what happens to volatiles during thermal processing of fruits. These results could help design a better strategy for aroma isolation and characterisation, and explain the aroma entrapment due to sugar crystallisation during drying of fruits. The results can also be used to design a strategy for sustainable utilisation of volatile components of fruits like <i>V. infausta</i> , one of the wild fruits to be included in a formulation of new industrial food products. However, more studies are needed in order to learn more about sustainable utilisation of various wild fruits growing in Mozambique and southern Africa.			
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# **Aroma characterisation and retention after heat treatment and drying of fruits, using extraction and GC-MS analysis**

Rui Ráice

2015



**LUND**  
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Universidade Eduardo Mondlane  
Maputo - Mozambique

Front cover photo: *Mangifera indica* fruit, pictured by Bishnu Sarangi.

Back cover photo: *Vangueria infausta* fruit, pictured by Rui Ráice during field work in Bobole (Maputo-Mozambique), March 2010.

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## Abstract

This study concerns the identification and characterisation of volatile components of fruits, and evaluation of the effect of heat treatment and drying on retention or loss of volatiles of fruits. The investigation included developing a procedure to extract volatile components from the fruit matrix, a purification step, separation, identification and quantification.

Initial experiments with *Vangueria infausta* L. showed that some components, especially sugars, degrade during heating in the GC analysis, producing furfural, hydroxyl methyl furfural (HMF) and other volatiles. These compounds are obtained together with the native aroma components of the fruit, making analysis difficult.

We developed a procedure using a hydrophobic column that could retain the hydrophobic aroma components and eliminate sugars that could disrupt the analyses. The extract was analysed by GC.

The volatile components found in pulp of *Vangueria infausta* were primarily hexanoic acid, octanoic acid, ethyl hexanoate, ethyl octanoate, methyl hexanoate and methyl octanoate. Based on the odour activity values, it could be concluded that the odour of the fruit is mainly attributed to ethyl hexanoate and ethyl octanoate (paper I).

Drying is often used to process and preserve food stuff but many food attributes including aromas which are important for palatability and consumer interest are affected by the process. Our research showed that the principal aroma components are well preserved during the initial phase of drying (down to about a relative water activity of 0.65). However the aromas are lost after more extensive drying. A possible explanation for volatilisation is the sugar crystallisation that occurs below a RH of around 0.70. (paper II).

Also we evaluated the effects of drying with or without blanching on volatiles of mango (*Mangifera indica* L.). Fresh, blanched and dried mango samples were analysed (paper III). The fresh sample presented a very large number of peaks. The blanching was carried out in water and microwave at 70°C during 10 minutes and at 90°C during 2 minutes. Blanching increased the levels of aroma components. Both blanching procedures had no dramatic effect on the impact of the blanching. Prolonged hot air drying ( $a_w=0.65$ ) reduced most of the aroma.  $\alpha$ -pinene



and 1-butanol were strongly affected due their volatility. Drying had no great effect on components with high boiling points, which displayed significant retention even after extensive dehydration. Ethyl butanoate was high retained despite its high volatility.

To evaluate a possible influence of sugar crystallisation on aroma retention a further study involved three model matrixes based on carbohydrates aqueous solution plus oils: I) pectin-sucrose-oils, II) pectin-microcrystalline cellulose-sucrose-oils and III) microcrystalline cellulose-sucrose-oils. The oil phase comprised the reference materials of the most powerful aroma components found in *Vangueria infausta* (hexanoic acid, ethyl hexanoate and ethyl octanoate). The model mixture was dried at 80°C, 3 m/s for 60-420 min prior to GC analysis (Paper IV). The aroma components were preserved in all models throughout the drying process (until  $a_w \approx 0.8$ ). So sugar crystallisation did not induce the loss of volatiles. However noticeable sugar crystallisation was observed in model II. We assume that the presence of pectin and microcrystalline cellulose increased the ability of the matrix to compact, as water activity decreased during drying. So sugar crystallisation is probably the reason for aroma retention within the matrix.

The results of the studies in this thesis illustrate what happens to volatiles during thermal processing of fruits. These results could help design a better strategy for aroma isolation and characterisation, and explain the aroma entrapment due to sugar crystallisation during drying of fruits. The results can also be used to design a strategy for sustainable utilisation of volatile components of fruits like *Vangueria infausta*, one of the wild fruits to be included in a formulation of new industrial food products. However, more studies are needed in order to learn more about sustainable utilisation of various wild fruits growing in Mozambique and southern Africa.

## Popular Scientific Summary

Fruits are a key complement to the diet of many people in the southern region of Africa, especially in rural areas. maphilwa (*Vangueria infausta* L.), maçanica (*Ziziphus mauritiana*), maçala (*Strychnos spinosa*), mapsincha (*Salacia kraussi*), cajú (*Anacardium occidentale* L.), mavungwa (*Landolphia kirki*) are some of the most commonly found fruits in sub-Saharan Africa, particularly in Mozambique, Botswana, Madagascar, South Africa, Zambia and Zimbabwe. They play an important role in the diet and gastronomy of the rural communities.

Several investigations of nutritional aspects have been carried out, and showed that the fruit is rich in dietary fibre and sugars and have a high micronutrient content in the form of minerals and vitamins. *Vangueria infausta* L. belongs to the family Rubiaceae. The common names are African medlar in english or maphilwa in ronga (one of local language in southern Mozambique). The fruit is usually harvested between February and April. It is brownish orange when ripe and is spherical in shape. The fruit is about 2-5 cm in diameter and contains 3-5 seeds. The fresh fruit is sweet and tastes like medlar (*Mespilus sp.*), although with some similarities to green apple and pineapple. The fruit can be eaten fresh, cooked or dried. It's used also to prepare juice, jam puddings and marmalade. We assume that the fruit may be useful and the taste and aroma profile attractive and appreciated. Information about identification of aroma on African medlar is limited.

The investigation included developing a procedure to extract volatile components from the fruit matrix, a purification step, separation, identification and quantification. The extraction procedure used solvents (ethanol, diethyl ether and pentane). Initial experiments showed that some components, especially sugars, are degraded during the heating in the Gas Chromatography (GC) analysis, producing furfural, hydroxyl methyl furfural (HMF) and other volatiles. These compounds are obtained together with the native aroma components of the fruit, making analysis difficult.

We developed a procedure using a hydrophobic column with a capability to retain the hydrophobic aroma components and wash out the hydrophilic components (sugars) using water. The aromas were released using a mixture of pentane and diethyl ether prior injection

into the GC. The aroma components were separated on the basis of their retention times, followed by identification through MS. The identification was verified using standards. Each peak was quantified, taking into account the peak areas of components relating to the internal standard.

The main aroma components identified in *Vangueria infausta* were hexanoic acid, octanoic acid, ethyl hexanoate, ethyl octanoate, methyl hexanoate and methyl octanoate. The esters (methyl hexanoate and ethyl octanoate) are the main contributors to the aroma of the fruit (Paper I).

The second aspect of this work was to evaluate the effect of drying upon aroma components of the fruits. Samples of *Vanueria infausta* pulp were convectively dried at 80°C, 3 m/s for up to 480 min. The results showed that the principal aroma components of pulp are well preserved during the initial phase of drying down to a relative water activity of about 0.65, but are lost after more extensive drying. This is due to the volatilisation induced by sugar crystallisation that is likely to occur below a relative humidity of around 0.70 during the drying process (Paper II).

The third study of this thesis aimed to evaluate the effect of blanching and drying on the aroma of mango (*Mangifera indica L.*). Three samples of mango (fresh, blanched and dried) were analysed (Paper III). The blanching was carried out in water at 70°C during 10 min and in a microwave at 90°C during 2 min. The most relevant aromas *Mangifera indica* are 1-butanol,  $\alpha$ -pinene, 3-carene, myrcene, limonene, terpinolene, and ethyl butanoate. The experiments show that the levels of aroma components are increased when the material is blanched while hot air drying reduced most of the aroma when the drying is prolonged below 0.65 in  $a_w$ . Also the study shows that water blanching, microwave blanching, long period/low temperature or short period/high temperature had no marked effect on the impact of the blanching.

Another goal of this thesis was to investigate the influence crystallisation of carbohydrates on retention or loss of aroma. (Paper IV). Three models were evaluated: I -pectin-sucrose-aromas; II -pectin-microcrystalline cellulose-sucrose-aromas and III -microcrystalline cellulose-sucrose-aromas. The aroma fraction was composed of the main aroma components identified in *Vangueria infausta*: hexanoic acid, ethyl hexanoate and ethyl octanoate. Each model was dried on over at 80°C, 3 m/s during 60-420 min. GC results showed considerable aroma retention in all models at least

starting when the  $a_w$  value reached 0.8. Model with pectin and microcrystalline cellulose rapidly exhibited low  $a_w$  values and more consistency. Our assumption is that the crystallisation of sugars could play a role on aroma retention during the drying of fruits.

The results from these studies show what happens to aroma during heat treatment of fruits. It is useful to understand the encapsulation of aroma due to sugar crystallisation during drying. The results can help design a better strategy for sustainable utilisation of aroma components of fruits, like the African medlar, one of the wild fruits now included in local industrial processing of new products. We believe that greater knowledge on volatiles can be useful in sustainable utilisation of wild fruits grown in Mozambique and southern Africa.

## Resumo Científico Popular

Frutos constituem um dos complementos na dieta da maior parte da população da região sub-Sahariana de África onde também uma grande parte de frutos indígenas são consumidos especialmente nas zonas rurais. maphilwa (*Vangueria infausta* L.), maçanica (*Ziziphus mauritiana*), maçala (*Strychnos spinosa*), mapsincha (*Salacia kraussi*), cajú (*Anacardium occidentale* L.), mavungwa (*Landolphia kirki*) são alguns dos frutos que se encontram destribuidos um pouco por toda a região subsahariana em especial Moçambique, Botswana, Madagáscar, África do Sul, Zâmbia e Zimbâbwe contribuindo um papel importante na dieta e gastronomia das comunidades rurais.

Muitas investigações feitas sobre aspectos nutricionais reportam que muitos frutos indígenas de Moçambique tais como a *Vangueria infausta* são ricos em fibras diéticas, açúcares e têm alto teor de micronutrientes (minerais e vitaminas). *Vangueria* pertence a família Rubiaceae. Os nomes vulgares são African medlar em inglês ou maphilwa (na língua ronga, sul de Moçambique). Normalmente amadurece entre Fevereiro a Abril. Quando maduro tem a coloração castanha-alaranjada. Tem em média 2-5 cm de diâmetro e contém cerca de 3-5 sementes. Quando fresco o fruto tem sabor doce, ácido e nalgumas vezes azedo e o cheiro semelhante ao da maçã silvestre (*Malus sp.*) ou da medlar (*Mespilus sp.*). A polpa pode ser consumida fresca, cozida ou seca. Também pode ser usada para preparar sumo, jam, pudim ou marmelada. O fruto pode também ser seco ao sol e guardado por mais tempo. Nos últimos tempos o fruto tem atraído muito interesse não só nas zonas rurais como também nas cidades para onde é trazido e comercializado.

A informação sobre a identificação de componentes aromáticos da maphilwa é escassa. Assumimos que o fruto pode ser útil tanto que o gosto e aroma são atraentes e apreciáveis. Daí decidimos investigar o perfil de aroma dos frutos indígenas de Moçambique, especialmente a maphilwa que é um dos frutos mais consumidos nas zonas rurais.

A investigação incluiu o desenvolvimento de procedimento para extracção de componentes voláteis dos frutos, purificação, separação, identificação e quantificação. A extracção foi feita usando solventes (ethanol, éter

dietílico e pentano). As primeiras experiências deste trabalho mostraram que alguns componentes do fruto especialmente açúcares eram degradados durante o aquecimento dentro da coluna de gas cromatógrafo (GC) e produziam compostos caramelizados. Esses voláteis eram obtidos juntamente com os aromas propriamente do fruto tornando a análise difícil. Desenvolvemos um procedimento usando uma coluna hidrofóbica com a capacidade de reter os componentes aromáticos hidrofóbicos e eliminar os hidrofílicos (açúcares) com água. Os aromas eram extraídos com mistura de pentano e éter dietílico e depois eram analisado no GC. No GC os componentes aromáticos eram separados em função de seus tempos de retenção seguido de identificação por MS. A identificação foi verificada com uso the materiais de referência. A quantificação de cada component foi feita considerando a relação entre as áreas do pico e do material de referência interna.

Os principais componentes aromáticos identificados na polpa da *Vangueria infausta* foram: ácido hexanoico, ácido octanoico, hexanoato de etil, octanoato de etil, hexanoato de metil e octanoato de metil. Os ésters (hexanoato de etil e octanoato de etil ) são os principais contribuintes do aroma da maphilwa. (Paper I)

O segundo aspecto deste trabalho foi de avaliar o efeito da secagem nos components aromaticos dos frutos. Amostras da polpa de maphilwa foram secas num forno a 80 °C, 3 m/s durante cerca de 480 min. Os reultados mostraram que os principais componentes aromáticos da polpa eram retidos quando a actividade da água na polpa era cerca de 0.65. Os mesmos eram perdidos depois de secagem prolongada devido a volatilização provocada pela cristalização de açúcares que se assume ocorrer abaixo da humidade relativa de cerca de 0.70 durante o processo de secagem. (Paper II).

O terceiro estudo desta Tese teve como objectivo avaliar o efeito de branqueamento e secagem nos aromas da manga (*Mangifera indica* L.), um fruto globalmente conhecido. Três amostras da manga (fresca, branqueada e seca) foram analisadas (Paper III). O branqueamento foi feito em água e no microondas a 70 °C durante 10 min e a 90 °C durante 2 min. As substâncias mais importantes na manga analisada são: 1-butanol,  $\alpha$ -pinina, 3-carina, mircina, limoneno, terpinolene e butanoato de etil. As experiencias monstraram que os níveis de aromas são acrescidos quando a amostra é branqueada enquanto que grande redução de voláteis foi

observada quando a secagem da manga é prolongada até cerca de 0.65 em  $a_w$ . Também o estudo mostrou que o modo como foi feito o branqueamento (em água ou em microondas a 70 °C, 10 min e a 90 °C, 2 min) não teve qualquer influência.

Outro objectivo desta tese foi de investigar a influência da cristalização de carboidratos na retenção ou perda de aromas (Paper IV). Três modelos foram avaliados: I) pectina-sacarose-água-aromas, II) pectina-microcristalinacelulose-sacarose-água-aromas e III) microcristalinacelulose-sacarose-água-aromas. A fracção aromática era composta por standards dos principais aroma componentes identificados na *Vangueria infausta* (ácido hexanoico, hexanoato de etil e octanoato de etil). A secagem de cada modelo foi feita no forno a 80 °C, 3 m/s durante 60-420 minutos. Os resultados de GC mostraram que, em todos os modelos, os componentes aromáticos eram preservados durante o processo de secagem pelo menos a partir de quando o valor da  $a_w$  atingisse 0.8. Modelo conendo pectin e MCC rapidamente hexibiu baixa  $a_w$ , e mais consistência. Assumimos que a cristalização pode ter grande implicação na retenção de aromas durante a secagem de frutos.

Os resultados obtidos desta tese mostram oque acontecem com aromas durante a secagem de frutos. Estes resultados são úteis para entender a encapsulação de aromas devido a cristalização de açúcares durante a secagem. Os resultados podem ajudar no desenho de melhor estratégia para a utilização sustentável dos componentes aromáticos dos frutos, tais como a maphilwa, um dos frutos indigenas com forte potencial para ser incluso no processamento industrial de alimentos novos. O “kno-how” tecnológico na área de voláteis pode impulsionar a utilização sustentável da diversidade de frutos indígenas que se desenvolvem em Mocambique.



## List of papers

**Paper I.** Raice, R. T., Sjöholm, I., Hong-lei, Wang and Bergenstahl, B. (2015). *Identification of volatile components isolated from Vangueria infausta (Africana medlar) by using GC-MS.* *Journal of Essential Oil Research*, 27 (1), 76-81.

**Paper II.** Raice, R. T., Chiau, E. F., Sjöholm, I. and Bergenstahl, B. (2015). *The loss of aroma components of the fruit of Vangueria infausta L. (African medlar) after convective drying.* *Drying Technology*, 2015, 33 (8), 887-895.

**Paper III.** Raice, R. T., Guiamba, I. Arhné, L., Svanberg, U. and Bergenstahl, B. (2015). *Effects of drying with or without blanching on volatiles in dried mango fruit (Mangifera indica L.)* Submitted.

**Paper IV.** Raice, R. T., Chiau, E. F., Sjöholm, I. and Bergenstahl, B. (2015). *Crystallization of sugars in a drying matrix containing soluble and insoluble fiber - a model to understand the role of fibres on sugar crystallization and aroma retention.* Manuscript.

## The author's contribution to the papers

**Paper I.** The author designed the study together with co-authors, performed the field work and lab work, analysed and evaluated the results in collaboration with co-authors. The author wrote the draft of the manuscript, the final version of which was written in collaboration with the co-authors.

**Paper II.** The author designed the study together with co-authors and performed the field work. The lab work was carried out in collaboration with the co-authors. Analysed and evaluated the results in collaboration with co-authors. Calculations in the appendix were carried out by the co-authors. The author wrote the draft of the manuscript, the final version of which was written in collaboration with the co-authors.

**Paper III.** The author designed the study together with the co-authors. The samples were prepared by the co-authors and the drying experiments were performed by the co-authors. The multilinear regression model was built by the co-authors. The author analysed and evaluated the results together with co-authors and wrote the draft of the manuscript, the final version of which was written in collaboration with the co-authors.

**Paper IV.** The author performed the field work, designed the study together with co-authors, performed the lab work together with co-authors, analysed and evaluated the results together with co-authors and wrote the draft of the manuscript, the final version of which was written in collaboration with the co-authors.

## **Related publications not include on this thesis**

Raice, R. T., Sjöholm, I., Da Cruz, J. F. and Bergenstahl, B. (2011). *Identification of volatile components isolated from indigenous fruits of Mozambique: Maphilwa (Vangueria infausta)*; *Procedia Food Science*, 1, 404-7.

Raice, R.T., Sjöholm, I., Francisco, J.C. and Bergenstahl, B. *Identification of volatile components isolated from indigenous fruits of Mozambique: Maphilwa (Vangueria infausta)*; 11<sup>th</sup> International Congress of Engineering and Food; Presented at ICEF11 (May 22-26, 2011 – Athens, Greece) as paper FMS058.

Raice, R.T., Francisco, J.C., Sjöholm, I. and Bergenstahl, B. *Characterization of indigenous fruits and study of aroma profile of indigenous fruits of Mozambique: Mapsinsha (Landolpia kraussi) and maphilwa (Vangueria infausta)*; Annual Meeting: Process-Structure-Function Relationships. Presented at EFFoST 2011 (9-11 November 2011, Berlin, German) as paper P3.65.

# Contents

1. Introduction .....	1
2. Objectives.....	2
2.1 General objective.....	2
2.2. Specific objectives.....	2
3. Background .....	3
3.1 Aroma compounds found in common fruits.....	3
4. Methods for identifying aroma .....	13
4.1 Gas chromatography.....	13
4.2 Mass spectrometry.....	14
5. Retention time .....	15
6. Threshold and odour activity value .....	16
7. Aroma isolation from plant material .....	17
7.1 Contamination by artefacts.....	17
7.2 Isolation of aroma based on volatility .....	18
7.2.1 Simultaneous distillation-solvent extraction .....	18
7.3 Isolation of aroma based on solubility.....	19
7.3.1 Solvent extraction.....	19
8. Experimental work for aroma analysis.....	20
8.1 General overview of analytical procedure (Paper I).....	20
8.1.1 Sample, collection and preparation (A).....	21
8.1.2 Primary extraction (B).....	21
8.1.3 Purification of the extract (C).....	23
8.1.3.1 Recovery of the internal standard.....	24
8.1.4 Separation of the aroma components (D).....	25
8.1.5 Identification of the aroma components (E). .....	26

8.1.6 Quantification of the aroma components (F).....	30
9. Results and discussion.....	30
9.1 Investigation of aroma components of <i>Vangueria infausta</i> L. ....	30
9.1.1 Variation of aroma components between collection events .....	32
9.1.2 Sensory analysis .....	33
10. Drying of <i>Vangueria infausta</i> (Paper II) .....	35
10.1 Aroma retention.....	35
11. Effects of drying with or without blanching on volatiles in dried mango fruit ( <i>Mangifera indica</i> L.) (Paper III) .....	38
11.3 Results and discussion.....	45
12. Design of model involving sugar, pectin and microcrystalline cellulose for better understanding of the loss or retention of aroma due the crystallisation of aqueous carbohydrate matrix (Paper IV).....	49
12.2 Model I (pectin, sucrose, water and oils ‘C <sub>1</sub> ’).....	52
12.3 Model II (pectin, microcrystalline cellulose, sucrose, water and oils ‘C <sub>2</sub> ’) .....	52
12.4 Model III (microcrystalline cellulose, sucrose, water and oils ‘C <sub>2</sub> ’).....	53
12.5 Results and discussion.....	53
13. Concluding remarks.....	59
Recommendations and further perspectives .....	60
Acknowledgments .....	61
References .....	63

# Abbreviations

IS	internal standard
HMF	hydroxyl methyl furfural
HdMF	hydroxyl dimethyl furfural
OAV	odour activity value
GC-MS	gas chromatography mass spectrometry
StpTMC <sub>18</sub>	hydrophobic column C <sub>18</sub>
FID	fused ionisation detector
HRGC	high resolution gas chromatography
LRGC	low-resolution
MCC	microcrystalline cellulose
DM	dry matter
RH	relative humidity
PPO	polyphenol oxidase
AAO	ascorbic acid oxidase
a <sub>w</sub>	water activity
X <sub>w</sub> , W <sub>c</sub>	water content
LOX	lipoxygenase
HPL	hydroperoxidelyase
ADH	alcohol dehydrogenase
NIST	National Institute of Standards and Technology

# 1. Introduction

Food preservation aimed at obtaining a desirable quality presents a major challenge. During food processing and the preservation of food, especially fruits, many attributes are affected, including volatile components that are important for palatability and consumer interest. Taste and smell are believed to be two crucial attributes used in assessing the quality and safety of foods. However, some concepts regarding volatility are still misunderstood. One issue susceptible to ambiguity is differentiation between flavour and aroma. Flavour is a combination of sensory inputs derived from taste or gustatory perception on the tongue that detects basic sensations such as sweet, bitter, salt, sour and umami, together with sensations from the mouth (pungency) and olfactory perception from the nose. Aroma is mostly attributed to the volatile molecules that are perceived by olfactory receptors in the nose.

The pleasant fragrance of fruit adds value in the evaluation and assessment of fruits, and plays an important role in gastronomy (Kahkonen, et al., 1999; Kwang-Geun and Shibamoto, 2002). Several studies have shown that the volatile chemicals present in leaves, flowers and fruits have also been widely used in aromatherapy since ancient times, suggesting that they impart some beneficial health effects (Kwang-Geun and Takayuki, 2002).

Rather than merely discussing the concepts and findings, our interest was in investigating the aroma components in selected fruits, such as *Vangueria infausta* L. and *Mangifera indica* L. *Vangueria infausta* is a wild fruit distributed over the countries in southern Africa, such as Mozambique, Botswana, Madagascar, South Africa, Zambia and Zimbabwe. The fruit plays an important role in the diet in rural areas (Laverdière and Mateke, 2002; Amorteifio and Maosase, 2006; Styger et al., 1999).

Studies of the nutritional aspects report that these fruits are rich in dietary fibre and sugars and have high content of micronutrients like mineral and vitamins, (Magaia, 2013; Amorteifio and Maosase, 2006). However, no reports were found about the identification and quantification of aroma components on *Vangueria infausta*. Only one study of characterisation of



volatile components of the closely related *Vangueria madagascariensis* has been found (Pino et al., 2004).

It has been reported that heat treatment induces alteration of several characteristics of food, particularly fruits. The aim of this work was to investigate the aroma profile of the fruits, particularly wild fruits of Mozambique like *Vangueria infausta*, one of the most consumed fruits especially in rural areas. Another aim was to evaluate the effect of heat treatment (blanching) and drying on volatile components of the selected fruits involved on this study.

## 2. Objectives

### 2.1 General objective

The general objective was to characterise the aroma composition of the fruits and to understand aroma retention or loss after heat treatment and drying of fruits, through extraction and GC analysis.

### 2.2. Specific objectives

Specific objectives were to:

- Develop a suitable method for aroma extraction from *Vangueria infausta*.
- Identify and characterise the aroma components of *Vangueria infausta*.
- Understand the aroma loss (or retention) phenomena during drying of *Vangueria infausta* and design a possible strategy to capture the most powerful aroma components of *Vangueria infausta*.
- Understand the influence of water or microwave blanching prior to drying on aroma composition of the fruits (*Mangifera indica* L.).
- Design a model based on aromas and aqueous carbohydrate solutions to improve understanding of the aroma loss (or retention) phenomena during drying of the fruits.

### 3. Background

*Vangueria infausta* L. is one of the wild fruits growing in southern Africa (Mozambique, South Africa, Botswana, Zimbabwe, Zambia, Namibia, and Swaziland). The fruit belongs to the family Rubiaceae. Its common names are African medlar in English or maphilwa in Ronga (local language in southern Mozambique). The fruit, usually harvested between February and April, is brownish orange when ripe and has a spherical shape. The size is about 2-5 cm in diameter and the fruit contains 3-5 seeds. The fresh fruit is sweet and tastes like green apple, rather like pineapple.

Rural populations eat the pulp fresh, cooked or dried. They also prepare juice, jam and puddings by adding water and sugar or cook it to make marmalade. The pulp can also be fermented to produce alcoholic drinks. Often the fruit is dried in the sun and then stored for a long time. The fruit has been attracting interest lately, not only in rural areas but also in the cities where it is very often bought as a commercial product.

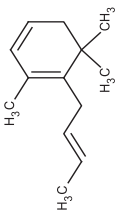
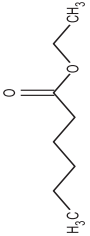
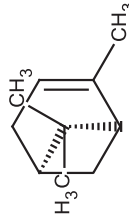
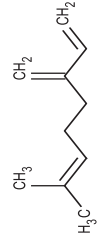
Some studies of the nutritional aspects have been carried out, and showed that *V. infausta* is rich in dietary fibre and sugars and has a high content of micronutrients such as minerals and vitamins (Magaia, 2013; Amorteifio and Maosase, 2006). Our assumption is that the fruit may be useful and the taste and aroma profile attractive and appreciated. However, we have found no reports about the identification and quantification of aroma components in *Vangueria infausta*. Only one study of the characterisation of volatile components of the closely related *Vangueria madagascariensis* has been found (Pino et al., 2004), so the aim of this work was to investigate the aroma profile of wild fruits of Mozambique, particularly *Vangueria infausta*, one of the most commonly consumed fruits, especially in rural areas.

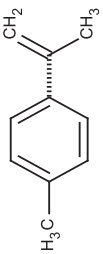
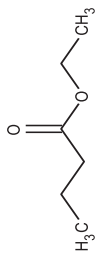
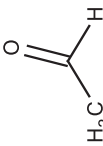
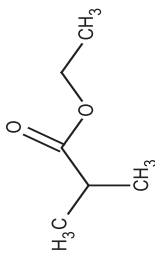

#### 3.1 Aroma compounds found in common fruits

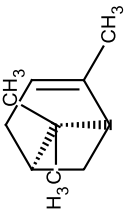

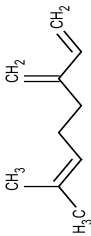
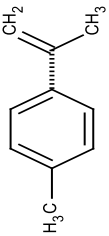
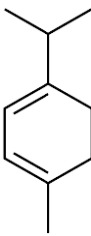
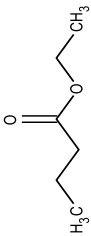
The aroma compounds present in the fruit mostly originate from a mother plant and biochemical processes like the development of enzymes during the maturation stage, and metabolic pathways during fruit development and storage (Cheetham, 2010; Wright, 2010 and Kaewtathip, et al., 2012).

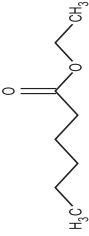
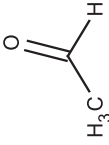
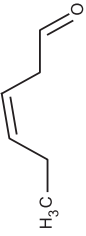
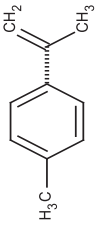
In Table 1, aromas from common fruits are listed as typical examples of common structures.

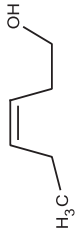
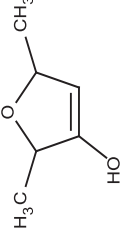
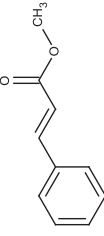
**Table 1.** Flavour compounds found in the most common fruits and in fruits investigated in our study.

Common fruit (Scientific name)	Main aroma compound	Chemical structure	bp (°C)**	log P **	Threshold (µg/l)	Lit. ref.
Apple ( <i>Malus domestica</i> Borkh)	β-damascenone		167	3.99*	10 <sup>b</sup>	(e)
Pear ( <i>Pyrus serotina</i> L.)	ethyl hexanoate		166	2.3	1, 0 <sup>a, b, c</sup>	(f)
Orange ( <i>Citrus sinensis</i> Osbeck)	α-pinene		155	4.37	6 <sup>b</sup>	(g)
	myrcene		167	4.58	15 <sup>b</sup>	

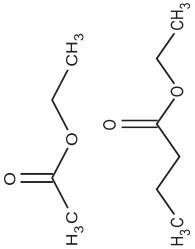
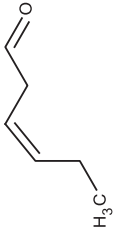
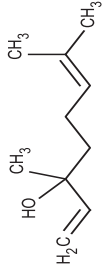
Lemon ( <i>Citrus Limon</i> L.)	limonene		177	4.45	1000 <sup>b</sup>	(h)
Mango ( <i>Mangifera indica</i> L.)	ethyl butyrate		120	1.85	66 <sup>b</sup>	(i)
	acetaldehyd e		20	0.6	17 <sup>b</sup>	
	ethyl 2- methyl propanoate		110	1.77	0.1 <sup>b</sup>	
Mango ( <i>Mangifera indica</i> L.)	1-butanol		118	0.81	500 <sup>b</sup>	(i)*

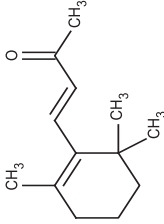
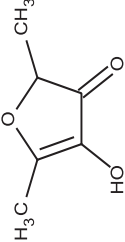
<b><math>\alpha</math>-pinene</b>		155	4.37	6 <sup>b</sup>
<b>3-carene</b>		168	2.80	770 <sup>b</sup>
<b>myrcene</b>		167	4.58	15 <sup>b</sup>
<b>limonene</b>		177	4.45	1000 <sup>b</sup>
<b>terpinolene</b>		174	3.16	330 <sup>b</sup>
<b>ethyl butanoate</b>		120	1.42*	1 <sup>b</sup>

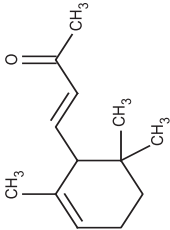
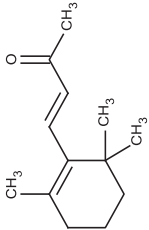
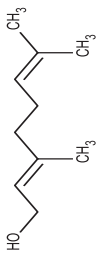
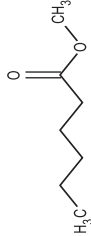
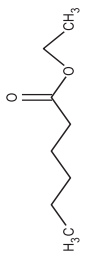
<p>Banana (<i>Musa sapientum</i> L.)</p>	<p>ethyl hexanoate</p>		<p>166</p>	<p>2.3</p>	<p>1, 0<sup>a, b, c</sup></p>	<p>(k)</p>
<p>Grapefruit (<i>Citrus paradisi Macfayden</i>)</p>	<p>acetaldehyd e</p>		<p>20</p>	<p>0.6</p>	<p>17<sup>b</sup></p>	<p>(l)</p>
<p>cis-3-hexenal</p>		<p>126</p>	<p>1.29*</p>	<p>17<sup>b</sup></p>	<p>1000<sup>b</sup></p>	<p>177</p>
<p>limonene</p>		<p>20</p>	<p>4.45</p>	<p>17<sup>b</sup></p>	<p>1000<sup>b</sup></p>	<p>177</p>



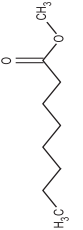

<p>Strawberry (<i>Fragaria x ananassa</i>)</p>	<p>cis-3-hexenol</p>		<p>-61</p>	<p>1.61*</p>	<p>70<sup>b</sup></p>	<p>(m)</p>
	<p>4-hydroxy-2,5-dimethylfuran-3-one</p>		<p>N/f</p>	<p>1.64*</p>	<p>0.03<sup>b</sup></p>	
	<p>methyl cinnamate</p>		<p>261</p>	<p>2.52*</p>	<p>191<sup>b</sup></p>	



Plum ( <i>Spondias mombins</i> L.)	ethyl acetate  ethyl butyrate	 <p>The image shows two chemical structures. The first is ethyl acetate, a short-chain ester with a methyl group on the carbonyl carbon and an ethyl group on the oxygen. The second is ethyl butyrate, a longer-chain ester with a butyl group on the carbonyl carbon and an ethyl group on the oxygen.</p>	77  120	0.86  1.85	5000 <sup>b</sup>	(n)
Guava ( <i>Psidium guajava</i> L.)	cis-3-hexenal	 <p>The image shows the chemical structure of cis-3-hexenal, a six-carbon chain with a double bond between carbons 2 and 3 in the cis configuration, and an aldehyde group at the end of the chain.</p>	126	1.29*	17 <sup>b</sup>	(o)
Apricot ( <i>Prunus armeniaca</i> L.)	linalool	 <p>The image shows the chemical structure of linalool, a ten-carbon chain with two double bonds (one terminal and one internal), a hydroxyl group, and two methyl groups.</p>	198	3.28	6 <sup>b</sup>	(p)

Peach ( <i>Prunus persica</i> L.)	β-ionone		126	3.28*	0.007 <sup>b</sup>	(q)
Pineapple ( <i>Ananas comosus</i> L.)	4-hydroxy-2,5-dimethyl-3-(2H)-furanone		N/f	0.21*	0.03 <sup>b</sup>	(r)

Raspberry ( <i>Rubus idaeus L.</i> )	$\alpha$ -ionone		N/f	3.32*	0.76 <sup>b</sup>	(s)
	$\beta$ -ionone		126	3.28*	0.007 <sup>b</sup>	
	geraniol (IS)		230	3.28	40 <sup>b</sup>	
	methyl hexanoate		150	2.65	70 <sup>b</sup>	(t)
	ethyl hexanoate		166	2.3	1,0 <sup>a, b, c</sup>	

African medlar ( <i>Vangueria infausta</i> L.)	hexanoic acid		206	1.98	3000 <sup>b</sup>
	octanoic acid		237	2.98	3000 <sup>b</sup>
	methyl octanoate		192	2.85 <sup>*</sup>	200 <sup>a, b</sup>
	ethyl octanoate		208	3.20 <sup>*</sup>	5 <sup>d</sup>

\*\* - The data is from Reaxys or has been estimated from Marvin Sketch Software (\*).

In table 1, the superscripted lower character indicates the source from which the date or threshold data was collected: <sup>a</sup> Odour threshold concentration in water (µg/l), from Monteiro-Calderón et al. (2010). <sup>b</sup> Odour threshold concentration in water (µg/l), from Pino and Mesa (2006). <sup>c</sup> Odour threshold concentration in water (µg/l), from Takeoka, et al. (1995). <sup>d</sup> Odour threshold concentration in wine model (µg/l), from Ferreira et al. (2000), Gómez et al. (2012), Wu et al. (23), Qian and Wang (2005). (e) Xu and Qian, (2007). (f) Takeoka et al., (1992). (g) Moshonas, and Shaw, (1994). (h) Moufida, (2003). (i) Pino and Mesa, (2006); Adedeji, et al., (1992). (j\*) From our study (Paper III). (k) Jordan, et al (2001). (l) Schieberle, (2001). (m) Vaughn, et al., (1993). (n) Sagrero-Nieves, (1992). (o) Steinhaus, et al., (2009). (p) Takeoka, et al., (1990). (q) Takeoka, et al., (1990). (r) Wu et al., (1991). (s) Larsen, (1991). (t) From our study (Papers I and II).

Table 1 shows that a majority of these aromas have boiling points in a range of 100 up to 200°C, and they also show a hydrophobic character signalised as high values of the log (P) in the range 0.21-4.58, the distribution coefficient between octanol and water. We assume that by taking into account these two parameters, and also by using non-polar extraction solvents, a GC procedure can be set within a certain temperature range, which may provide a reliable aroma profile. Several functional groups can comprise the aroma compounds, but terpenoids and esters are the most common classes of aroma compounds found in fruits.

## **4. Methods for identifying aroma**

Today numerous techniques are available for analytical purposes, but only very few of them are really suitable for flavour research. The extremely low quantities of aroma available for analysis ( $10^{-9}$  g or less), mostly obtained after long purification and concentration steps, makes the number of choices very limited with regard to the amount (about  $10^{-4}$  g) needed to perform analysis by any traditional identification techniques. GC-MS is the most useful technique for aroma component identification (Reineccius, 2010).

### **4.1 Gas chromatography**

Gas chromatography (GC) is a basic tool for analysing aroma. GC is used for separation in an analysis of substances susceptible to vaporisation without decomposition. The main characteristic of GC is a mobile phase, usually an inert gas (helium or nitrogen), and a stationary phase, often a microscopic layer comprised of polymer on an inert solid inside a column. The main stationary phases are cyanopropylsiloxane, octadecyl groups, and sulfoxyl groups. The boiling point of the substance and the

interaction between the substances being analysed with the stationary phase are key factors for separate elution of the component at specific times (retention times), which is later compared with similar reference materials. (Reineccius, 2010).

The analysis starts by using a micro-syringe to inject the sample into the GC column, where the mobile phase is continuously moving at a certain velocity. The substances are retained separately by adsorption, depending on the size and type of molecule, and are released from the column at different times (retention time). This process is monitored by a detector that determines not only the time when the substance leaves the column but also the amount of the substance through the peak area or intensity on the correspondent chromatogram. Commonly, the substances are qualitatively determined by the order in which they are released from the column (Poole, 2003).

## **4.2 Mass spectrometry**

Mass spectrometry (MS) is a second technique, and is used after the GC. While the GC is performed to separate the components, the MS is the mass-selective GC detector responsible for the identification of these components. Its ability to connect to the GC, its great sensitivity measured in the hundreds of pictograms, and also its great capability to provide more structural information make the MS the first choice for identifying volatility.

The MS is operated in selective ion-detection mode (quadrupole) to select ions at very short mass intervals. This allows separate quantification of two components simultaneously eluted from GC, for example isotope counterparts (Reineccius, 2010).

It should be emphasised that an MS detector is generally more sensitive than Flame Ionization Detection (FID) and this high ion selectivity is the key factor for achieving excellent results in the analysis of aroma extracts. Poor GC resolution or a limited sample may make the interpretation of MS

chromatogram difficult, but good extraction of flavour provides good MS results.

Mass spectrometer apparatus can be divided into two main groups: low-resolution (LR) and high-resolution (HR) instruments. The LR instruments provide sufficiently accurate mass measurements to the nearly whole mass unit and so can allow determination of elemental composition. While many elements or combinations of elements may give the same unit mass (isomers), LR only gives molecular mass. LRs were the most commonly used instruments a few years ago due to their easy operation and low cost compared to HR equipment.

The use of GC-MS is now widespread, and allows the design of more understandable spectral libraries and matching systems for quick and secure identification of flavour compounds.

## **5. Retention time**

Retention time is the time from when the compound is injected until its detection after passing the heated gas chromatography column. The boiling point of the substances to be separated and the affinity to the stationary phase within the column, play an important role in the retention time.

In this study the DB-225 column was used, which is a poly cyanopropyl phenyl dimethyl siloxane composed of 50% of cyanopropyl phenyl siloxane groups. This stationary phase is useful in GC due its high temperature stability, which makes it suitable for wide temperature ranges, chemical inertness and good film forming properties (Wiridena et al., 2001). The high thermal stability is beneficial for the experiment since we assume that most of the volatiles are substances with high boiling points. The large size of the cyanopropyl phenyl siloxane groups enables more cohesion with volatile compounds, which is crucial for selective separation as the temperature increases inside the column.

Specific conditions between experiments may vary, and factors such as flow rate of the carrier gas, length of the column, and gas pressures make

retention time values uncertain and make the absolute retention value an uninteresting measure. One way to overcome this limitation is to use relative indices (RI) or Kovat's indices (Takács, et al., 1971; Acree, 2001; Majlát, et al., 1974; Kondjoyan and Berdague, 1996). Relative retention indices represent the retention time of an unknown compound relative to an n-paraffin or ethyl esters series. Usually RI and its thermal sensitivity are directly dependent on the column polarity. It is assumed that an appropriate stationary phase column and a good range of temperature programs are the key factors for confident separation and identification of the compounds by comparing the observed RI with a similar compound from a spectral library (Wei-chuan et al., 1995). However the use of only RI is, in many cases, not sufficient for compound identification so additional data obtained from the peaks, such as mass spectrum, is needed (Wiridena, 2001, Reineccius, 2010). In this study, we used mainly the reference materials and MS data for preliminary identification.

## **6. Threshold and odour activity value**

An important aspect of aroma analysis is the intensity of the perception. However, it is difficult to evaluate the intensity of an odour sensation since the perceived intensity depends on time, and the character may depend on the intensity of the exposure. The most common approach is to measure the critical concentration when the perception can be recognised. This limit is known as a threshold that is defined as a minimum concentration value at which an aroma can be active and sensorially perceived (Plotto, et al., 2008; Grosh, 1994; Rothe and Thomas, 1963). The structure also plays a prominent role with regard to flavour threshold; for example, the 2-ethylhexyl 2-methyl-2-propenoate has a threshold of 0.02 µg/L in aqueous solution while the threshold of 2-ethylhexyl 2-propenoate is only 0.005 µg/L, a four-fold difference due to the slight complexity of the first compound (Gemert, 2011; Cheetham, 2010).

The ratio of concentration by threshold is called the odour activity value (OAV). It has been suggested that, any individual flavour component needs to be present at about ten times its threshold in order to have a significant flavouring effect (Preininger and Grosh, 2001). However, it is



necessary to take into account that the perception of the flavour attributes (quality, character and intensity) is dependent on prior evaluator experience (regional, cultural, age) for most basic aroma sensations.

The aromas perceived when sniffing the fruit are a combination of various compounds and cannot be attributed to a unique component (Munoz et al., 2007). Even at concentrations below the odour threshold, the aroma compounds may produce an aroma that is perceived as a result of perceptual synergy that gives the fruit a specific impact (Grosh, 2001, Gomez Garcia-Carpentero et al., 2011; Kaewtathip and Charoenrein, 2012; Lopez et al., 2003; Ferreira et al., 2001). Some studies have been conducted to determine the minimum concentration of flavour that could be perceived by the human olfactory sense. Large data collections are available. Table 1 above presents the threshold values of some fruits measured in aqueous solution.

## **7. Aroma isolation from plant material**

The aroma components may be found in all major parts of plants including fruits, roots and leaves. Various methods can be used to isolate the aroma compounds from the tissue to be analysed. The main principles of extraction are based on volatility or solubility of aroma compounds (Sides, et al., 2000, Wright, 2010). The close constraint is that water is also volatile. All suggested procedures represent compromises between different types of errors such as aroma losses, oxidation, thermal degradation, and contamination by artefacts, and they may be time-consuming (Sides, et al., 2000).

### **7.1 Contamination by artefacts**

When handling samples, special attention must be paid to ensuring extraction with the minimum amount of contamination possible from all sources. The use of lab gloves, for example, can prevent skin lipids from contaminating the sample. Use of GC-grade solvents and distilled water is

always recommended, and avoiding the use of greases and rubber-based materials. It should also be highlighted that the heating should be moderate and, preferably, not more than 60°C, because already at this temperature volatile material can be expected to evaporate from the sample. Another source of contamination due to heating is the risk of degradation of sugars to produce reaction products such as hydroxyl methyl furfural (HMF) and hydroxyl dimethyl furfural (HdMF) (Reineccius, 2010). However, heating may be necessary in order to inhibit enzymatic degradation, and to obtain an enzymatic release from various complex carbohydrate structures, as well as to disrupt the botanical microstructure.

## **7.2 Isolation of aroma based on volatility**

The aroma components are characterised by exhibiting sufficient vapour pressure to be detectable by the olfactory system. One way to isolate aroma substances is based on volatility. The most common techniques are distillation, and static and dynamic headspace. The distillation methods are the oldest techniques for isolating aroma from tissues.

### **7.2.1 Simultaneous distillation-solvent extraction**

This is one of the most useful techniques for isolating aromas. The substance being analysed is distilled, which is immediately followed by extraction with solvents using a coolant substance, often methanol, at 2°C or a water bath at 10°C, to prevent the temperature increasing. The solvent is then removed using a Vigreux column (Núñez, 1984). The major advantage of the method is the low risk of contamination by artefacts. A disadvantage is the risk of explosion, so temperature control is highly recommended.

## **7.3 Isolation of aroma based on solubility**

### **7.3.1 Solvent extraction**

This is another traditional procedure used in isolating aroma components through their solubility in secondary media. Most of the aroma components (Table 1) are hydrophobic and soluble in an organic phase. The extraction comprises a sequence of steps. The sample is placed in contact with the solvent for a certain time, the mixture shaken gently and left to rest. Phase separation is performed using an appropriate funnel, and possibly drying through the use of anhydrous salts ( $\text{Na}_2\text{SO}_4$ ). The concentrated aroma is obtained by using a stream of nitrogen or a vapour rotator device, or other purification procedures, in order to remove other components that may disturb the analyses. The disadvantages are the small quantity of the adsorbent available for volatile adsorption and low phase ratio. Finally, the concentrated extract is analysed by being injected into the GC apparatus (Reineccius, 2010). Apart from the presence of lipids, the main disadvantage of the method is the GC-grade solvent needed.

A common method is to use a solid-phase micro-extraction (SPME) for isolating aroma. SPME is a technique for qualitative analyses. An inert fibre, coated with an adsorbent material, usually activated carbon or coconut charcoal, is immersed in a sample solution or placed in contact with the headspace above the liquid or solid sample. The equilibrium is more effective in a headspace rather than in liquid, as no liquid can interfere with the diffusion of the analyte. The aroma components are adsorbed at the adsorbent material and then released and injected into the GC (Sides, et al., 2000, Pérez, et al., 2007, Reineccius, 2010). The technique offers solvent-free, rapid sampling, easy operation, good sensitivity and low cost. The reported disadvantages are that most coating fibres are type-mixed phase coatings that allow competitive absorption, thereby making difficult selectivity of the analytes. However experimental conditions like rigorous control of sampling method (immersed/headspace), sample pH, liquid/headspace volume, salt addition, sampling time and temperature; can

## 8. Experimental work for aroma analysis

### 8.1 General overview of analytical procedure (Paper I)

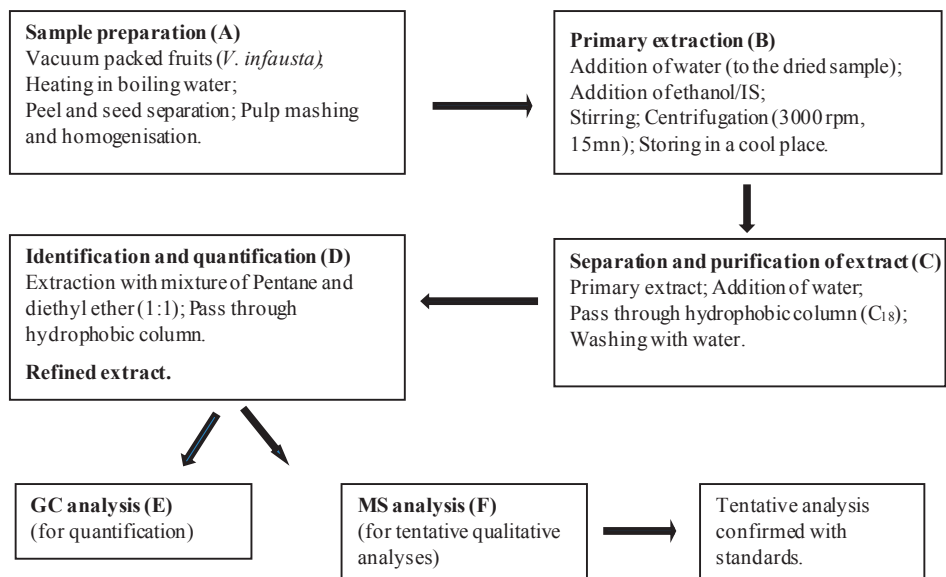


Figure 1. Purification of the extract (Paper I)

### **8.1.1 Sample, collection and preparation (A)**

Mature *Vangueria infausta* fruits were collected from Bobole and Marracuene (Maputo, Mozambique) during maturation stage, in March 2010-2012. Defect-free fruits were selected, washed, vacuum-packed, sealed in plastic polyethylene bags, and frozen at -20°C.

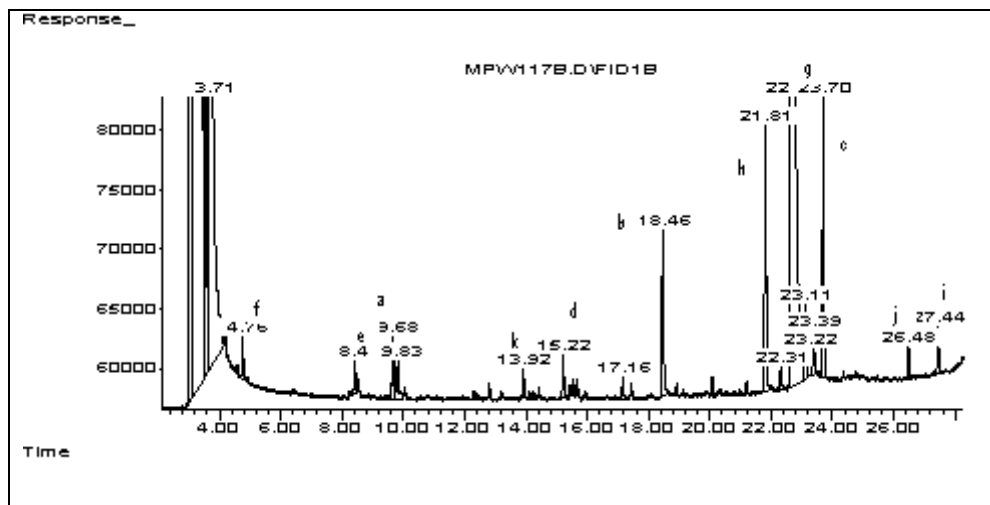
Before the experiment, the vacuum-packed samples were thawed in boiling water for 10 minutes in order to inactivate enzymes like lipoxygenase (LOX), hydroperoxidelyase (HPL) and alcohol dehydrogenase (ADH). The aim was to prevent degradation during the extraction that could cause uncertainty in the extraction process (Bai et al., 2011 and Baldwin et al., 2004). The fruits were allowed to cool. Peels and seeds were manually separated from the pulp. The pulp samples were disintegrated and homogenised by mashing with a clean spoon.

### **8.1.2 Primary extraction (B)**

The sample was macerated in a glass Erlenmeyer flask with 100 ml of ethanol containing internal standards of geraniol (2.3 mmol/l prepared in alcoholic medium). A small amount of distilled water was added to the dried sample to enable disintegration of the matrix, which improves extractability. The extraction was performed under continuous stirring using a magnetic stirrer for 72 hours to allow time for the extraction. The suspension was centrifuged at 3000 rpm for 15 min, after which the primary extract, the supernatant, was divided into three replicates of 10 ml.

Ethanol was the preferred solvent due to its hydrophilic character, which is assumed to allow better penetration into fruit tissue and effective extraction of aromas enclosed in the tissue structure (Parke & Birch, 1999; Hennell, 1826). Ethanol also denatures enzymes such as  $\beta$ -glycosidase, which may prevent degradation during extraction time that could have a negative effect on the results (Bai, et al., 2011). An internal standard (geraniol) was added to the sample together with the ethanol in the initial mixing, as the liquid phase could not be quantitatively separated from the suspension.

Direct analyses of primary extracts have been evaluated. The GC chromatogram showed many peaks representing products originating from sugar degradation reactions, mostly HMF and hydrocarbons (Figure 2). Pino and co-workers had found similar substances on *Vangueria madagascariensis* (Pino et al., 2004) and considered these to be the aroma of the fruit. In fact, these substances do not come from the fruit, and originate instead from sugars that are decomposed in the GC column or injector system. One study reports that sugars (glucose, fructose and sucrose), when extracted with a hydrocarbon phase (tetradecane and dodecane) and injected into the GC, result in the formation of HMF due to the degradation of sugars in the column (Cheetman, 2010). This inconsistency forced us to include a purification step prior to the GC analysis with the aim of eliminating the sugars.



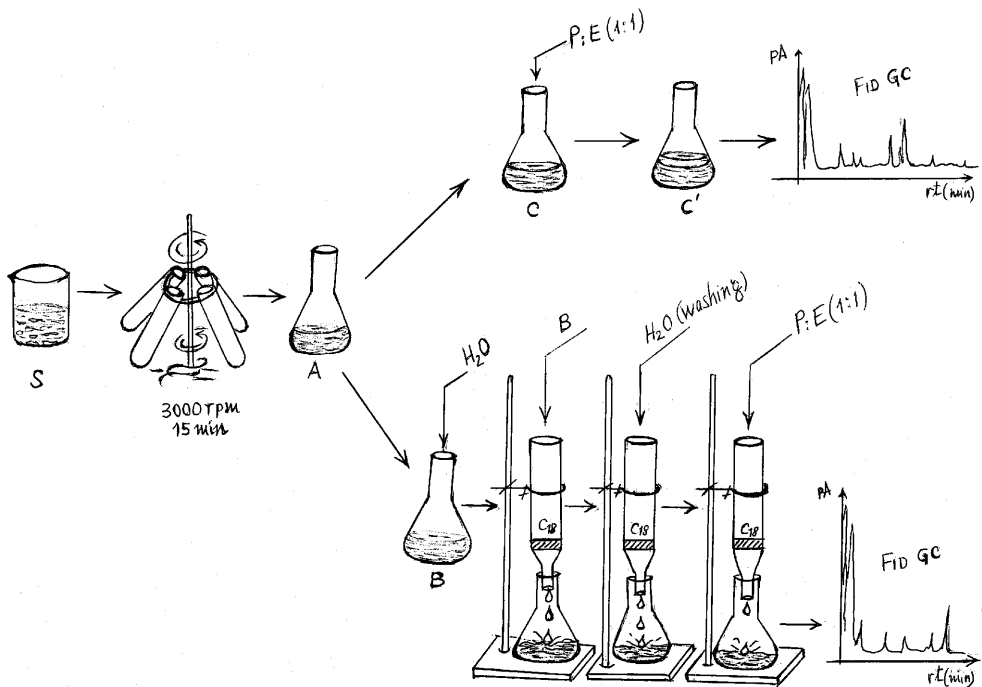
**Figure 2.** FID chromatogram showing aroma compounds found in the primary extract (before purification) of *Vangueria infausta*. The chromatogram shows peaks representing products of sugar degradation like furfural (a) and 5-hydroxymethylfurfural (c), which were later confirmed by MS analysis.

In addition to sugar degradation products, other products such as hydrocarbons (octane, undecane, tridecane), peroxides such as di-tert-butyl peroxide (DTBP) and 2,6-di-tert-butyl-4-methyl-phenol or butylated hydroxytoluene (BHT), and disulfite were found in unpurified extract, as

shown of Figure 5. These substances are probably impurities deriving from fingerprints, rubber tube polymerisation agents and stabilisers of the diethylether such as BHT, which is added as an antioxidant to control peroxide formation. The DTBP probably originates from the polyethylene bags (used as initiators for the polymerisation reaction). The sulphide probably also originates from the polymer synthesis.

### **8.1.3 Purification of the extract (C)**

The purification in the extraction phase is a crucial step in aroma analysis (Hattab, et al., 2007). The purification is intended to eliminate or minimise the influence of the artefacts in the analyses. Previous studies have reported that material such as silica, activated charcoal, coconut charcoal, siloxane, cyanopropylphenyl, diethylpolysiloxane are used for purification of aroma extracts (Lopez et al., 2003, Reineccius, 2010, Lopez et al., 1999, Gomez et al., 2011). In this work the objective of the purification step was mainly to remove saccharides from the extract to eliminate the formation of degradation products during the analyses. Here the purification was carried out using a hydrophobic C<sub>18</sub> column. The hydrophobic volatile compounds are assumed to adsorb during this step. We monitored the yield to optimise the polarity of the extraction liquid for the purification. The polarity of the solvent phase was increased by adding water prior to the purification (30 ml of pure water was added to 10 ml of primary extract). To ensure effective cleaning, the column was rinsed with 5 ml of water in order to eliminate remaining sugars and other low molecular-weight polar compounds. The aroma components were then extracted with a 10 ml mixture of pentane and diethyl ether (1:1) through desorption from the hydrophobic column C<sub>18</sub>. The column contains octadecyl unendcapped bonded silica (from Capitol Scientific Inc., Austin, Texas, USA). The elution was performed with a flow rate of 1 ml/min. The refined extract was collected, and introduced to GC analysis via a 2.0 ml vial.



**Figure 3.** Analysis of non-purified and purified extract of *Vangueria infausta*

### 8.1.3.1 Recovery of the internal standard

The efficiency in terms of recovery of internal standard was estimated by comparing the concentration of added geraniol with the extraction solvent with the geraniol concentration. The following expression was used to calculate the yield:

$$Yield = \frac{P_S}{P_{IS}} \cdot \frac{C_i}{C_{IS}} \cdot \frac{V_{s,centrifg} \cdot V_{s,extract}}{V_{IS,added}} \cdot 100 \quad (1)$$

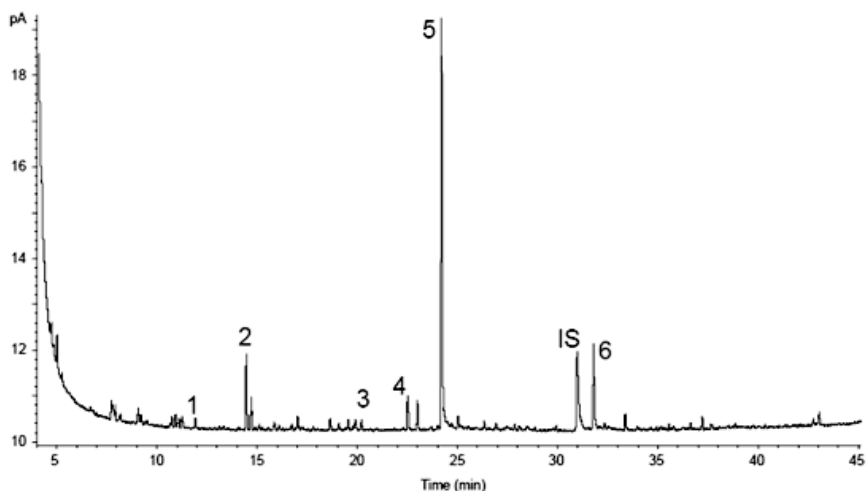
$P_S$  is the (pAs) peak area of sample;  $C_i$  ( $\mu\text{g/l}$ ) is the concentration of aroma reference;  $V_{s,centrifg}$  (ml) is the volume of sample after centrifugation;  $V_{s,extract}$  is the (ml) volume of sample after purification;  $P_{IS}$  (pAs) is the peak area of IS;  $C_{IS}$  ( $\mu\text{g/l}$ ) is the concentration of IS;  $V_{IS,added}$ , (ml) is the volume of IS added to the sample.



The yield was estimated to be between 70-90% in controlled experiments. A mixture containing about 5% of each reference material was dissolved in 50 ml of ethanol. One ml of this solution was diluted to 50 ml of ethanol, after which 1 ml of the solution was diluted separately in 25, 75, 100, 150 and 200 ml of ethanol. These solutions were injected into GC, after which the yield of each reference was calculated, taking into account the respective sensitivity (pA/Conc). The results were quite reproducible, close to about 90%; despite small deviations originating from uncontrolled factors (measurements, equipment external conditions) believed to occur during the analysis process.

### **8.1.4 Separation of the aroma components (D)**

GC analysis was carried out using an Agilent GC 6890 (G1530), Network GC system, serial US10322054, purchased from J&W Scientific, USA. It is a bench-top instrument equipped with a fused capillary column (J&W DB225, 30 m x 0.25 mm, coating thickness 0.25  $\mu\text{m}$ ) containing hydrophobic stationary phase of cyanopropylphenyl-diethylpolysiloxane (50%). The inlet volume was set on split mode at split ratio 20:1 and split flow 12:1. The chromatogram conditions were set as follows: injector and interface temperatures were 250°C; the column temperature was held at 50°C for 5 min, programmed at 3°C/min to 200°C and then held there for 3 min. Helium was used as carrier gas at a flow rate of 0.6 ml/min, 55.5 kPa. The hydrogen flow rate was 30 ml/min. The syringe size of the injector was 10  $\mu\text{l}$ , the volume sample of 2.0  $\mu\text{l}$  was injected three times, and n-hexane was used to wash five times. The FID chromatogram (Figure 4) revealed that the main components can be efficiently separated within 34 minutes. The retention times of the target components (in each replicate) were comparable with those for the reference material.

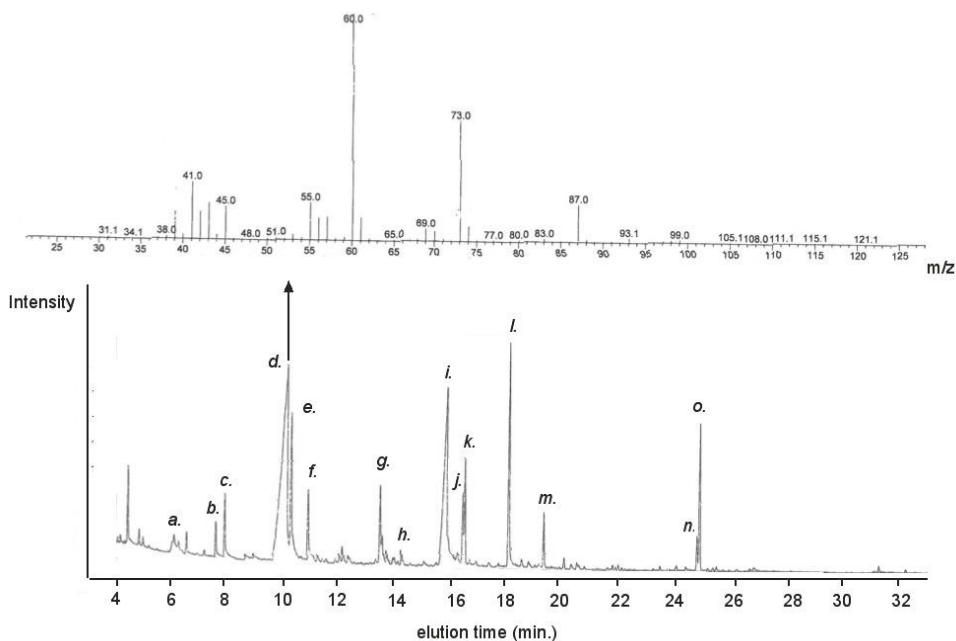


**Figure 4.** FID chromatogram of *Vangueria infausta* showing aroma compounds found in the cleaned extract (after purification with C<sub>18</sub>), using a DB-225 column (30 m x 0.25 mm x 0.25 μm film thickened, split ratio 20:1 and flow ratio 12:1): 1-methylhexanoate (Rt=12.03 min), 2-ethylhexanoate (Rt=14.54 min), 3-methyloctanoate (Rt=20.28 min), 4-ethyloctanoate (22.58 min), 5-hexanoic acid (Rt=24.30 min), 6-octanoic acid (Rt=31.85 min), IS-geraniol (Rt=31.02 min). The number, as well as the intensity, of the peaks is clearly much reduced, with none of them representing the typical saccharide decomposition products such as furfurals (HMF), and other impurities were observed.

### 8.1.5 Identification of the aroma components (E).

MS analysis was performed on an Agilent (Palo Alto CA, USA) 5972 mass selective detector together with an Agilent 6890 GC equipped with a HP-5MS capillary column (30 m x 0.25 mm i.d., SGE, Austin, TX, USA). The column was coupled in the electron ionisation (EI) mode at 70 eV. The components were tentatively identified by matching obtained spectra against the Wiley Spectral Library using the PBM logarithm. The identification was confirmed using pure standards. The retention times and the MS spectra were considered to be similar. The target components identified were: methyl hexanoate, ethyl hexanoate, methyl octanoate, ethyl octanoate, hexanoic acid and octanoic acid. To confirm this finding, GC-MS analyses using two different columns were carried out, one with high polarity Innowax, and one non-polar column HP-5. The GC-MS

results from these two columns were in a different order and showed that co-elution was not a major source of misinterpretation, and all peaks corresponded with reference compounds. Using highly polar columns results in poor resolution of fatty acids so, to avoid possible misinterpretations, further experiments with methanolysis conversion were performed on a DB-225 column from which the chromatogram presented the potential aroma component of the fruit. Figure 5 shows the fragment patterns for hexanoic acid, and Table 3 shows the concentration of the components.



**Figure 5.** GC-MS analysis of the extract from *Vangueria infausta* using HP-5 column. The peak at the retention time of 10 minutes has been selected for displaying the MS pattern. The fragmentation pattern of the peak suggests that it is hexanoic acid (d). Other target substances were *c*-methylhexanoate, *e*-ethylhexanoate, *h*-methyloctanoate, *i*-octanoic acid, *j*-ethyloctanoate, *l*-geraniol (IS). As stated above, other peaks representing impurities were also found among the target substances: *a*-octane, *b*-butanol, *f*-disulfide, *g*-undecane, *k*-dodecane, *m*-tridecane, *n*-DTBP, *o*-BHT.

The peaks obtained were tentatively identified using MS, by comparing the experimental results with figures in the MS database from NIST. Reference materials of the most relevant aromas found were also used to verify the identification through retention indices performed on an hp-

innowax column. Table 2 shows the mass fragments and the Kovat's retention indices (RI) of the target components, calculated using n-paraffin references (C<sub>12</sub>-C<sub>24</sub>).

**Table 2.** Retention index mass fragment patterns of most potent aroma compounds found in *Vangueria infausta*

Aroma Component	Experimental data (in an hp-innowax)		Literature (and ref.) RI (min)	MS fragments m/z
	Rt (min)	RI (min)		
Hexanoic acid	17.4	1850.6	1874 <sup>a</sup> , 1836 <sup>b,c</sup> , 1825 <sup>e</sup>	41, 35, 55, 60, 73, 87, 105, 116.
Octanoic acid	23.8	2173.1	2085 <sup>a</sup> , 2040 <sup>c</sup> , 2032 <sup>e</sup>	43, 55, 60, 73, 85, 101, 115, 124, 129, 144, 152.
Methyl hexanoate	6.7	1195.2	1173 <sup>e</sup>	39, 43, 55, 59, 74, 87, 99.
Ethyl hexanoate	7.7	1241.2	1251 <sup>a</sup> , 1181 <sup>b</sup> , 1238 <sup>d</sup> , 1224 <sup>e</sup>	43, 57, 71, 88, 99, 115, 127, 144.
Methyl octanoate	10.7	1489	N/A	43, 55, 74, 87, 101, 115, 127, 158.
Ethyl octanoate	11.7	1536.1	1454 <sup>a</sup> , 1424 <sup>e</sup>	41, 57, 73, 88, 101, 115, 127, 43, 157, 172.

The superscripted lower case letter indicates the reference from which the value has been taken: a: Qian, et al., 2005; b: Peter and Acree, 1998; c: Peter et al., 1998; d: Ferreira et al., 1998; e: Pino and Marbot, 2001.

## 8.1.6 Quantification of the aroma components (F)

The amount of each aroma compound was calculated relative to the dry matter (DM) of the samples, considering the signal intensities of the reference compounds. We assume that through this procedure we eliminate dilution errors and variations in the extraction yield caused by solvent entrapment. However, errors caused by unfavourable distribution equilibriums in the first and the second extraction respectively, cannot be fully compensated. The equations below were used for the aroma concentration calculation:

$$C_{aroma} = f \cdot \frac{P_{aroma, sample}}{P_{IS, sample}} \cdot \frac{m_{IS}}{m_{sample} \cdot X_{DM}} \quad [2]$$

$$f = \frac{P_{IS, reference}}{P_{aroma, reference}} \cdot \frac{m_{aroma, reference}}{m_{IS, reference}} \quad [3]$$

In equations 2 and 3:  $C_{aroma}$  ( $\mu\text{g/g}$ , DM) is the concentration of the aroma;  $f$  is the factor of proportionality of detector response;  $P_{aroma, sample}$  (pA) is the peak area of aroma measured in the sample;  $P_{IS, sample}$  (pA) is the peak area of internal standard measured in the sample;  $m_{IS}$  (g) is the mass of the internal standard added;  $m_{sample}$  (g) is the mass of the sample;  $X_{DM}$  is the mass fraction of the dry matter;  $P_{IS, reference}$  (pA) is the peak area of the internal standard in the reference;  $P_{aroma, reference}$  (pA) is the peak area of the aroma reference.

## 9. Results and discussion

### 9.1 Investigation of aroma components of *Vangueria infausta* L.

The aroma profile was investigated using solvent extraction followed by GC analyses as described above. The most important substances identified were esters (methyl hexanoate, methyl octanoate, ethyl hexanoate and ethyl octanoate) and fatty acids (hexanoic acid and octanoic acid). The results are shown in Table 3. It is clear that hexanoic acid and octanoic

acid are the most abundant aroma compounds in *Vangueria infausta*, while the methyl and ethyl esters sequentially appear in relatively low amounts. We should note that, paradoxically, the aroma perceived from the fruit comes from ethyl hexanoate and ethyl octanoate even when they present low concentrations. This fact can be understandable if we look at threshold values of these components. The fatty acids have highest threshold values (3000 µg/l) against not more than 5 µg/l for esters (Pino, 2006). Therefore the odour activity values exhibited by esters are higher than those exhibited by fatty acids, despite the high amount (Attaie, 2009; Guth, 1997).

**Table 3.** Concentration of potential aroma components calculated as function of dry matter and fresh weight. (Paper I)

Compound	Conc. (µg/g, DM)	Conc. (µg/g, fW)	Odour threshold (µg/g)	OAV	Odour Description (in this investigation)
Hexanoic acid	5600	1800	3 <sup>b</sup>	600	The odour is somewhat rancid or cheesy. Resembles of the smell of goats.
Octanoic acid	240	90	3 <sup>b</sup>	30	The odour is somewhat rancid or cheesy, but milder than hexanoic acid. Resembles of coconut milk or liquid.
Ethyl hexanoate	44	14	0.0001 <sup>b, c</sup>	10000	The odour is fruity. Resembles of banana.
Ethyl octanoate	13	4,2	0.005 <sup>d</sup>	800	The odour is somewhat fruity, with a slightly wine character. Resembles of the

					African medlar, <i>Vangueria infausta</i> .
Methyl hexanoate	15	4,9	0.07 <sup>b</sup>	70	The odour is sharp and fruity. Somewhat pear like.
Methyl octanoate	12	3,7	0.2 <sup>a, b</sup>	20	The odour is somewhat sharp and fruity.

The reference from which the threshold value has been taken is given in superscripted lower case letter: <sup>a</sup> – by Monteiro-Calderón et al., 2010; <sup>b</sup> - by Pino and Mesa, 2006; <sup>c</sup> - Takeoka, et al. 1995; <sup>d</sup> - by Gómez et al. 2012.

### 9.1.1 Variation of aroma components between collection events

A set of experiments involving samples collected over four consecutive years (2010-2013) were performed to evaluate the variability of the aroma profile in the material. The experiments were conducted in three replicates for the sample from each season and from each season-replicate; one extraction was performed, with three replicates for each. The target components were present in the samples from all seasons (Table 4). In general the variation of concentration is considerably small, but quite high values were found in the sample from 2012, probably due to the different level of maturation of sample from that season.

**Table 4.** Concentration ( $\mu\text{g/g}$ , DM) of the most important substances isolated from *Vangueria infausta* L. over four consecutive seasons.

Aroma component	Season (harvest year)				
	Concentration ( $\mu\text{g/g}$ , DM)				
	2010	2011	2012	2012a	2013
Hexanoic acid	4640	6000	5600	30700	4400
Octanoic acid	320	350	240	2000	300
Ethylhexanoate	48	43	44	440	44
Ethyl octanoate	24	20	13	230	20
Methylhexanoate	8	17	15	59	17
Methyloctanoate	9	9	12	66	11



Many factors, such as climatic changes between seasons, rains or drought, fertility, soil conditions, differences of the trees and locality where the sample has been collected, and especially the variation in maturity of the fruits, could cause these discrepancies, at least between seasons.

### **9.1.2 Sensory analysis**

A tentative aroma description of *Vangueria infausta* was carried out by sniffing the fruit and comparing this with the smell of a reference compound. The panel was comprised of ten people aged 23-60 years who were staff members at the Department of Food Technology at Lund University, and who had previously, in some way, experienced the sensory evaluation of food and beverages using qualitative descriptive analysis. Table 5 summarises the aroma description of the most pronounced volatile found in *Vangueria infausta*.

Roughly 90% of the panellists concluded that the *Vangueria infausta* has a smell comparable to ethyl hexanoate and/or ethyl octanoate. Findings from the literature review show that these compounds have a strong similarity with the fruity smell of banana, apple, floral and fresh odour descriptions (Francis and Netwon, 2005, Qian and Wang, 2005, Gomez et al, 2010). This non-uniform description can be easily understandable if we take into account the difficulty and weakness in our ability to describe what we recognise, and to select the proper smell among numerous flavours released at the same time from the fruit. Another reason is that the total intensity is a sum of the individual OAV of each component, regardless of the rest of the compounds presents in the sample (Laska and Hudson, 1992, Le Guen et al., 2000, Lopez et al., 2003).

**Table 5.** Smell description of aroma components identified in extracts from *Vangueria infausta*.

Fruit or aroma compound	Odour description	
	From the panellist	From the literature
<i>Vangueria infausta</i> fruit	The smell resembles that of a banana. The odour is somewhat fruity, with a slightly vinous character, somewhat like green apple and pineapple that is characteristic for the African medlar.	Not found
Methyl hexanoate	The smell is fruity. It's like pear.	Ethereal fruity (pineapple-apple) <sup>g</sup> .
Ethyl hexanoate	The smell resembles that of a banana. Very fruity.	Fruity <sup>a, c, d, e, g</sup> , banana <sup>d, e, g</sup> , <sup>h</sup> , green apple <sup>b, c, d, g, h</sup> , strawberry <sup>c</sup> , anise <sup>c</sup> , brandy <sup>d</sup> , apple peel <sup>a</sup> , winey <sup>g</sup> , wine <sup>g</sup> , apple <sup>g</sup> , pineapple notes <sup>g</sup> , strong <sup>g</sup> .
Methyl octanoate	The odour is somewhat sharp and fruity.	Strong, <sup>g</sup> winey-fruity <sup>g</sup> , orange-like <sup>g</sup> .
Ethyl octanoate	The smell is very close to floral character. Somewhat fruity.	Fruity <sup>a, b, d, e</sup> , floral <sup>c, d, e, i</sup> , sweat <sup>b</sup> , pineapple <sup>c, h</sup> , pear <sup>c, d</sup> , banana <sup>d</sup> , brandy <sup>d</sup> , fat <sup>a</sup> , soapy <sup>h</sup> .
Hexanoic acid	The smell is rancid or like cheese. Like goats smell.	Cheesy <sup>c, d, h</sup> , sweat <sup>a, b, f</sup> , rancid <sup>c, d, e</sup> , fatty <sup>d</sup> .
Octanoic acid	Resembles milk or coconut liquid. Like somewhat rancid/cheese.	Rancid oily <sup>h</sup> , sweat <sup>a, b, f</sup> , cheesy <sup>a, b, c, d</sup> , rancid <sup>c, d</sup> , fruity <sup>c, d</sup> , fatty <sup>c</sup> , sour <sup>e</sup> , goaty <sup>e</sup> , fatty <sup>c</sup> .

Superscripted lower case letter indicate the source in which the value has been taken: a: Francis et al. (2005), b: Gomez et al. (2012), c: Li-Hi et al. (2008), d: Wu et al. (2011), e: Qian et al. (2005), f: Ong, et al. (1998), g: Kaewtathip and Charoenrein (2012), h: Zea, et al. (2007), i-Munoz et al, (2007)

## 10. Drying of *Vangueria infausta* (Paper II)

An experiment was carried out to evaluate the aroma retention of *Vangueria infausta* during controlled drying of the volatile concentration. A sample of *Vangueria infausta* was dried in an isothermal convective dryer at 80°C at a velocity of 3 m/s, as described by Chiau (2013).

Thirty-five batches of homogenised pulp (5 g each) were packed into a mould, placed on a tray, and then five samples were removed after 60, 120, 180, 240, 300, 360, and 420 minutes of drying. The temperature profile was controlled with thermocouples (type K, 0.5 mm) connected to the HP Compaq S720 computer. Dry matter (DM) and water activity ( $a_w$ ) were measured during the course of the drying process as described in Paper II.

The decreasing water content and water activity was monitored as a function of drying time during the convective drying of *Vangueria infausta*. Samples dried for 240-300 minutes gave an average DM and water activity equal to  $0.20 \pm 0.03$  g/g, and dry basis equal to  $0.65 \pm 0.01$  respectively. The drying must be continued for a sufficiently long time to reach humidity below a water activity of 0.70 as there is a high risk of mould growth above that level (Tsotsas and Mujumdar, 2011, Jangam et al., 2008). However, at the same level of dryness, the product tends to become very hard, making direct consumption difficult. From the point of view of preservation of food, water activity in the range of 0.60-0.70 is assumed to be acceptable, because at this range the risk of microbial growth is low (Bonazzi, 2011).

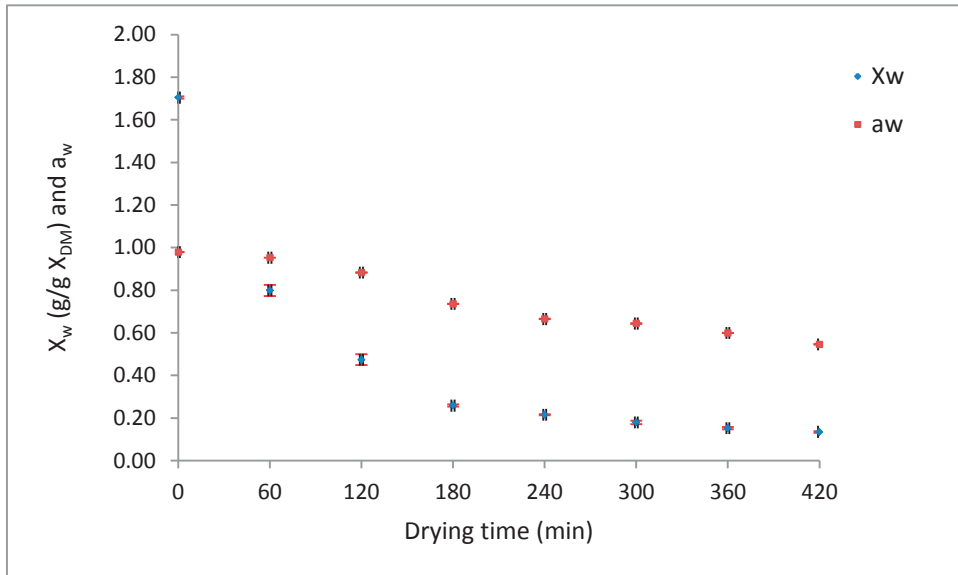
### 10.1 Aroma retention

The results in Figure 6 tell us that the water is removed quickly during the first 180 min of drying, followed by a slow drying period with a decreasing rate from 180 min to 420 min. Similar behaviour was also observed for the water activity parameter. Figure 7 shows that, initially, all aroma components (esters and fatty acids) are retained within the fruit matrix at least until 240 min of drying time (corresponding to water content of 0.20 g/g dry basis and a water activity of 0.65). At this time

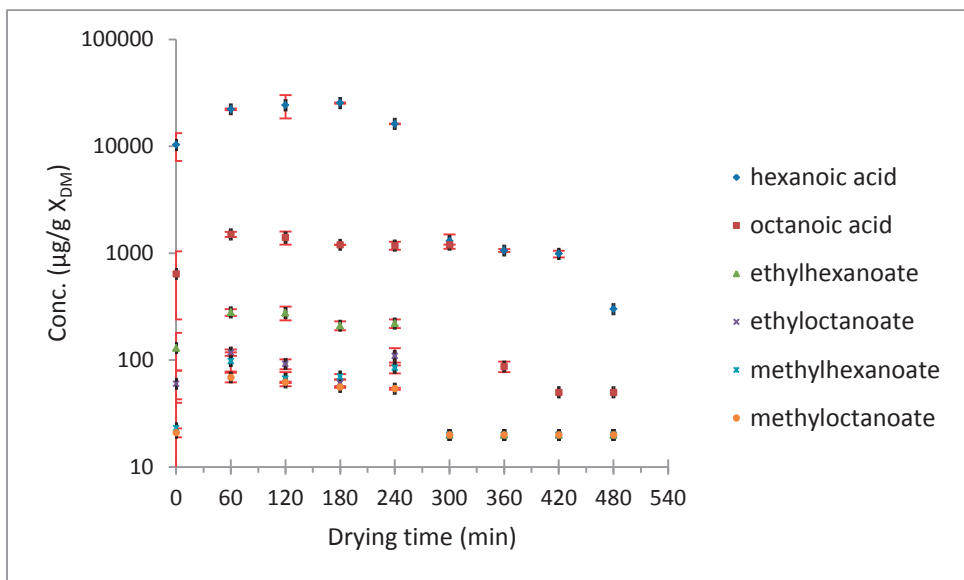
there is a dramatic release of aroma material. After 300 min only fatty acids remain within the fruit matrix. However, the main outcome is that we can remove almost 85% of water without any loss of aroma compounds. The experiment was repeated and the results were confirmed.

A possible reason for aroma loss could be the volatilisation of volatiles due to the increasing sample temperature during the drying process (Coumans, 1994). However, as can be observed in Figure 6, the aroma loss is more or less independent of the volatility (e.g. the boiling point) of the individual aroma components, and the temperature increase between 240 and 300 minutes is from 70°C to 75°C. Consequently, general volatility seems an unlikely explanation to the sudden aroma loss after 240 minutes of drying.

Another possible explanation is that sudden crystallisation takes place when the water content is around 10-15% leading to microstructural changes and releasing entrapped volatile components (Roos, 1995, Salmon, et al., 1996). Microscopy observations could not clearly support a dramatic crystallisation within the matrix, as there was a dominating presence of crystalline cellulose and hemicellulose in the material, making the observation non-conclusive. However our assumption that the loss of the key aroma compounds (esters) could be due to the sugar crystallisation phenomena is supported by the estimated phase boundary between the liquid state and the crystalline state for a complex sugar solution (Paper II).



**Figure 6.** Relationship between water content ( $X_w$ ) on dry basis, water activity ( $a_w$ ) and drying time during a convective-air drying of *Vangueria infausta* at 80°C, 3 m/s. The error bars presents the standard error of mean.



**Figure 7.** Concentration ( $\mu\text{g/g DM}$ ) of aroma components isolated from *Vangueria infausta* L. after isothermal convective drying up to 420 min. The open symbols represent detection limits of the aromas when not detected. The error bars show the mean standard error. The measurement was carried out in triplicate.

## **11. Effects of drying with or without blanching on volatiles in dried mango fruit (*Mangifera indica* L.) (Paper III)**

Mango fruit is popular, mainly due its pleasant flavour. The aroma composition of mango is very extensive, comprising different classes of compounds, but the most abundant compounds are terpenes hydrocarbons (3-carene, myrcene, limonene, pinene, terpinolene) and esters (ethyl butanoate, hexa and oct-alkyl esters). The variability in the aroma composition of the fruit differs from one variety to another, and is also dependent on cultivar, maturity stage, processing and method of isolation (Pino, 2005).

Previous studies have shown that volatile components are affected by various factors, particularly heat. It has been reported that heat treatment enhances the aroma in fruit due to release of aroma compounds from glucosides (such as eugenol, ethyl benzaldehyde, myrcenol, propyl 3-hydroxybutanoate, safranal) or terpene rearrangements (such as pinene and cineol) inside the fruit structure (Adedeji, et al., 1992; Poll, et al., 2006). Other reports state that heat treatment inactivates enzymes inside the fruit matrix, so they cannot inhibit the release of aroma compounds (Rodriguez-Lopez et al., 1999). The inactivation has to be controlled because the temperature optima of activation could be between 40 and 70°C for typical plant enzymes. Most aromas are susceptible to losses due to heating. To clarify our findings about aroma losses during drying of fruits, one more study was carried out to describe and attempt to evaluate the effect of heat treatment (blanching prior to drying) on volatiles. For this purpose we used mango. (Paper III)

The mango samples were obtained through collaboration with Chalmers University of Technology in Sweden. The samples were used in another study about inhibition of enzymatic activity, in particular PPO and AAO activity.

The study began with a literature survey to collect data about the most common aroma components in mango (*Mangifera indica* L.). The most expressive aroma compounds were 1-butanol,  $\alpha$ -pinene, 3-carene, myrcene, limonene, terpinolene, and ethyl butanoate. There is a hypothesis

in this study, that there are differences due to different type of heat treatment as well as due to different time temperature settings.

### **11.1 Blanching of *Mangifera indica* samples**


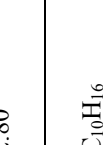





The samples were blanched in water at 70°C for 10 min and in water and microwave at 70°C during 10 minutes and at 90°C during 2 minutes, prior to drying in hot air circulation oven at 70°C. During this pre-treatment, DM and  $a_w$  were accurately measured using methods described in earlier parts of this thesis.

### **11.2 Analysis of volatiles of *Mangifera indica* L.**

The volatility was investigated again by GC methods, using similar procedures to those described elsewhere in this thesis. Geraniol was used as internal standard, at a concentration of 2.3 mmol/l prepared in alcoholic medium. All samples were subjected to a purification step involving centrifugation and elution through a hydrophobic column composed of desisopropyl atrazine 10% octadecil. The latter has the capability to retain only the hydrophobic volatile components and release the hydrocarbons molecules, essentially sugars, and other products generated during blanching treatment. Unlike the previous study, in this study the temperature program started from an initial temperature of 60°C, was kept constant for 3 min at 1°C/min, and then increased at a rate of 5°C/min to 200°C. All other GC parameters were as used on previous experiments.

This change to the temperature profile was made to improve the separation between components with a narrower range of boiling points. This enabled us to separate the key aroma substances identified through the literature study. Tables 6 and 7 show the physical properties of the target volatile components in mango, and the extraction procedures used for their isolation.

**Table 6.** Physical properties of the main volatiles components of mango (*Mangifera indica* L.)

Property	1-butanol	$\alpha$ -Pinene	3-Carene	Myrcene	limonene	$\alpha$ terpinolene	Ethyl butanoate
<b>MW</b> (g/mol)	74.12	136.23	136.24	136.23	136.23	136.23	116.16
<b>density</b> (g cm <sup>-3</sup> )	0.81	0.858	0.867	0.794	0.8411	0.8375	0.879
<b>bp</b> (°C <sup>(a)</sup> )	117.7	155	168 - 169	166-168	176	174-175	120-121
<b>Solubility</b> (g L <sup>-1</sup> )	73	Very low	N/A	N/A	N/A	N/A	Very low
<b>Log P</b> <sup>(b)</sup>	0.81	2.80	2.80	3.09	3.57	3.16	1.42
<b>Molecular formula</b>	C <sub>4</sub> H <sub>10</sub> O	C <sub>10</sub> H <sub>16</sub>	C <sub>10</sub> H <sub>16</sub>	C <sub>10</sub> H <sub>16</sub>	C <sub>10</sub> H <sub>16</sub>	C <sub>10</sub> H <sub>16</sub>	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>
<b>Chemical structure</b>							

<sup>(a)</sup> – Extracted from the literature using Reaxys, version 1.7.8; Elsevier; 2012.

<sup>(b)</sup> – Estimated using Marvin Sketch, version 5.7.0; Budapest, Hungary; 2011.



**Table 7.** Extraction methodology and amount of the target components identified in *Mangifera indica* L.

Solvent and extraction method	Extraction solvent and Hansen parameters	Aroma component identified	Conc. (µg/g FM)	Literature reference
Extract. Solv. - Pentane Simultaneous distillation extraction (SDE).	Pentane	butanol	N/D	(a)
	D	H	28	
	P	Δ	11	
	14	0	6	
		limonene	16	
		α-terpinolene	136	
		ethylbutanoate	N/D	
Sample was thawed Extract. Solv. - Pentane, IS - 2-octanol. SDE	Pentane	butanol	N/D	(b)
	D	H	5	
	P	Δ	554	
	14	0	N/D	
		limonene	16	
		terpinene	29	
		ethylbutanoate	N/D	
Fresh and processed sample Extract. Solv. - Pentane IS – camphor. SDE	Pentane	butanol	N/D	(c)
	D	H	0.01	
	P	Δ	0.40	
	14	0	0.02	
		limonene	0.01	
		α-Terpinolene	N/D	
		ethylbutanoate	N/D	
Extract. Solv. - Isopentane	Isopentane			
	D	H		
	P	Δ		

	14	0	0	0	40	
Mango	Diethyl ether					
Extract. Solv. - Diethyl ether	D	P	H	Δ		
IS - methyl nonanoate	13	3	6	34		
SDE,	1-butanol α-pinene 3-carene myrcene limonene α-Terpinolene ethylbutanoate					
Sample pulp	butanol α-pinene 3-carene myrcene limonene α-terpinolene ethylbutanoate					
Extract. Solv. - Diethyl ether	D	P	H	Δ		
IS - methyl undecanoate,	13	3	6	34		
SDE	butanol α-pinene 3-carene myrcene limonene α-terpinolene ethylbutanoate					
Extract. Solv. - 2-methylbutane	2-methyl butane					
Double distillation	D	P	H	Δ		
	13	3	6	34		
	butanol α-pinene 3-carene myrcene limonene α-terpinolene ethylbutanoate					
Tissue was ground,	butanol α-pinene 3-carene myrcene limonene α-terpinolene ethylbutanoate					
Extract. Solv. - dichloromethane	D	P	H	Δ		
IS - nonyl acetate.	17	6	4	32		
SED	butanol α-pinene 3-carene myrcene limonene α-terpinolene ethylbutanoate					

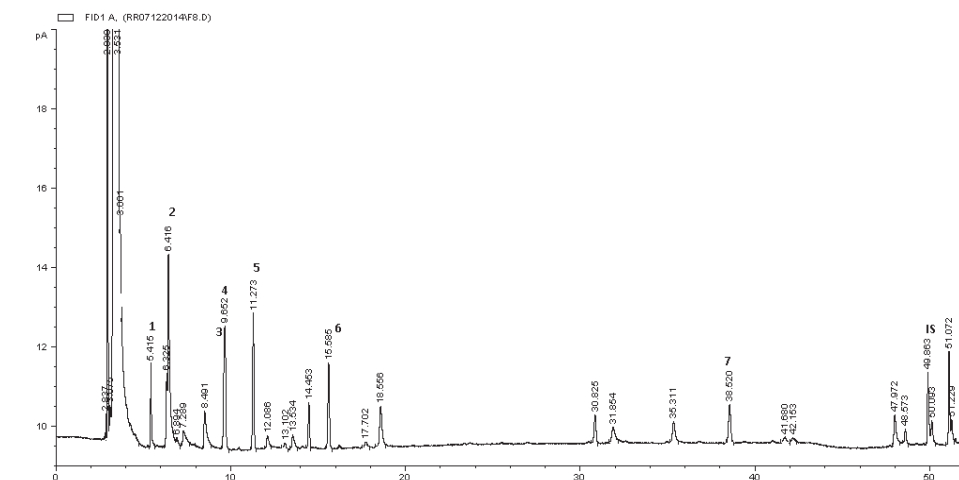
Sample was ground Extract. Solv. - dichloromethane IS - nonyl acetate SED	Dichloromethane			butanol $\alpha$ -pinene 3-carene myrcene limonene $\alpha$ -terpinolene ethylbutanoate	N/D 25 2046 853 311 N/D N/D	(h)
	D	P	H			
	17	6	4			
			32			
Tissue was ground. Extract. Solv. -dichloromethane IS - methylphenyl acetate.	Dichloromethane			butanol $\alpha$ -pinene 3-carene myrcene limonene $\alpha$ -terpinolene ethylbutanoate	N/D 38 2.0 119 12 2 N/D	(i)
	D	P	H			
	17	6	4			
			32			
Ethanol	Ethanol					
	D	P	H			
	16	9	19			
Sucrose	Sucrose					
	D	P	H			
	22	26	29			
			-			

(a) Macleod, 1984; (b) Olle, 1997; (c) Torres, 2007; (d) Macleod, 1988; (e) Chidley, 2009b; (f) Pandit, 2009; (g) Pandit and Kulkarni, 2009; (h) Pino, 2005; (i) Marbot, 2005  
D- dispersion; P- polarity; H- hydrogen bonding;  $\Delta$ - distance between solvent and sucrose  
(\*) - Partly extrapolated; (\*\*)- From Hansen, C. M., 2000.

Available reports show a very large variation in the amount of volatile compounds identified in mango (Table 7). This variability could mostly be caused by differences in extraction procedure and by the solvent used for extraction. Some of the researchers did not include, for example, the purification step, which is very important for elimination of saccharides and other water soluble interfering substances that can affect the final results of the analysis. The table 8 shows also how the solvent could interact with the material. The Hansen parameter is a thermodynamic parameter that gives the interactions between the molecules distributed over three parameters (dispersion, polar and hydrogen bonding) (Hansen 2000). To evaluate the interaction we monitor the distance between the interaction material and the solvent in three dimensions. As the material that we are extracting from is a sugar rich matrix we use sucrose as the comparing material. The distance ( $\Delta$ ) is a measure on how well the solvent may interact with the matrix and thereby possibly a measure of expected extraction efficiency. Pentane is thus expected to be too hydrophobic to penetrate efficiently. Diethyl ether somewhat better and, possibly, dichloromethane significantly better. We expect that ethanol is the providing a good penetration ability. Our results and methodology are reasonable.

## 11.3 Results and discussion

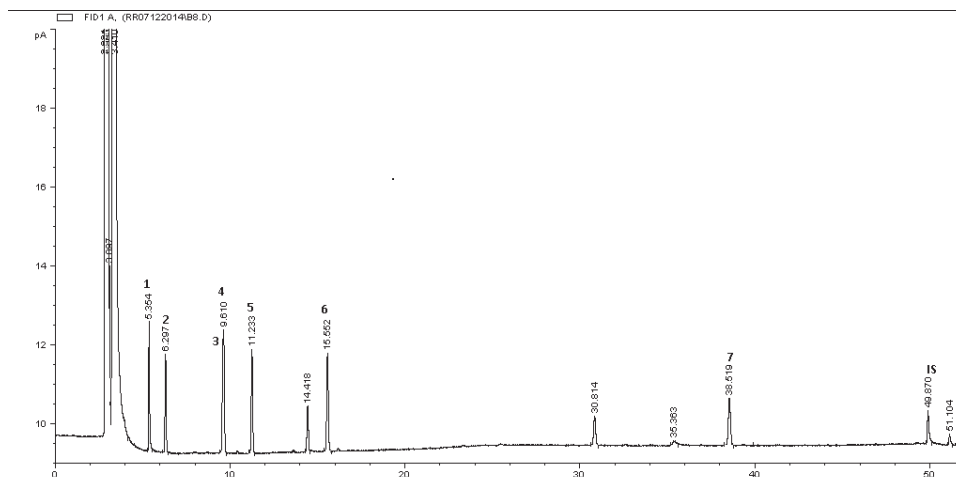
The aroma components showed different profiles depending on how the sample was treated. The frozen fresh sample showed numerous peaks among the relevant volatiles identified through the reference materials. These peaks could indicate the presence of compounds that are believed to come from degradation products and also as results of enzymatic reactions within the fresh mango matrix (Figure 8). The main aroma components were 1-butanol,  $\alpha$ -pinene, 3-carene, myrcene, limonene, terpinolene, and ethyl butanoate. Pinene (3000-6000  $\mu\text{g/g}$  FM), 1-butanol (1600-2000  $\mu\text{g/g}$ , FM), and ethyl butanoate (2400-3400  $\mu\text{g/g}$ , FM) are the most abundant volatiles in *Mangifera indica* L. fruit.



**Figure 8.** FID GC chromatograms showing the volatile components identified, as well as unidentified peaks obtained in frozen fresh sample of mango: 1: 1-butanol; 2:  $\alpha$ -pinene; 3: 3-carene; 4: myrcene; 5: limonene; 6: terpinolene; 7: ethyl butanoate; IS: geraniol.

The levels of aroma are enhanced if the sample is blanched. Fewer peaks were obtained from the frozen blanched samples (Figure 9), but all components appear at high amounts, up to 1100-2000  $\mu\text{g/g}$ , DM. The reason for volatile enhancement is not clear, although previous studies suggest that the glycosidic linkages formed between monoterpenes due to

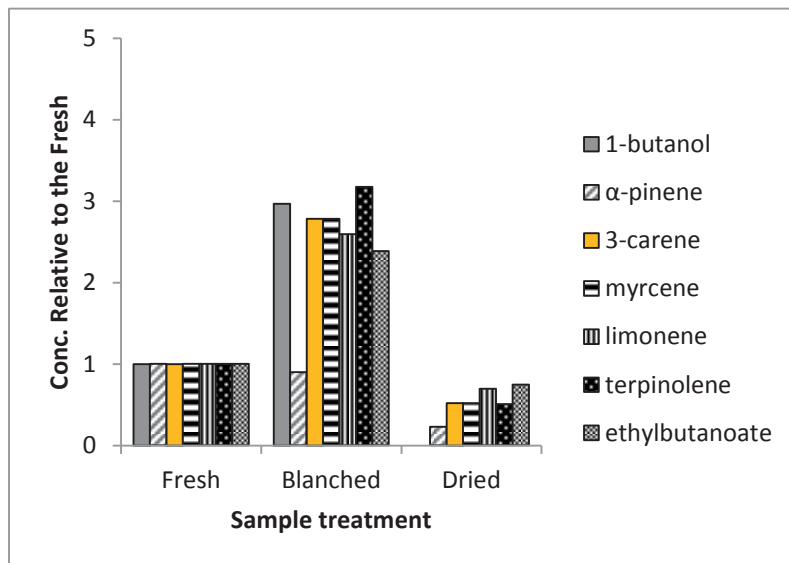
the hydrolysing mechanism are disrupted by heating, producing free aroma volatiles (Olle, et al., 1998).



**Figure 9.** Examples of FID-GC chromatograms showing the volatile components identified, as well as unidentified peaks in frozen blanched mango sample. 1: 1-butanol; 2:  $\alpha$ -pinene; 3: 3-carene; 4: myrcene; 5: limonene; 6: terpinolene; 7: ethyl butanoate; IS: geraniol.

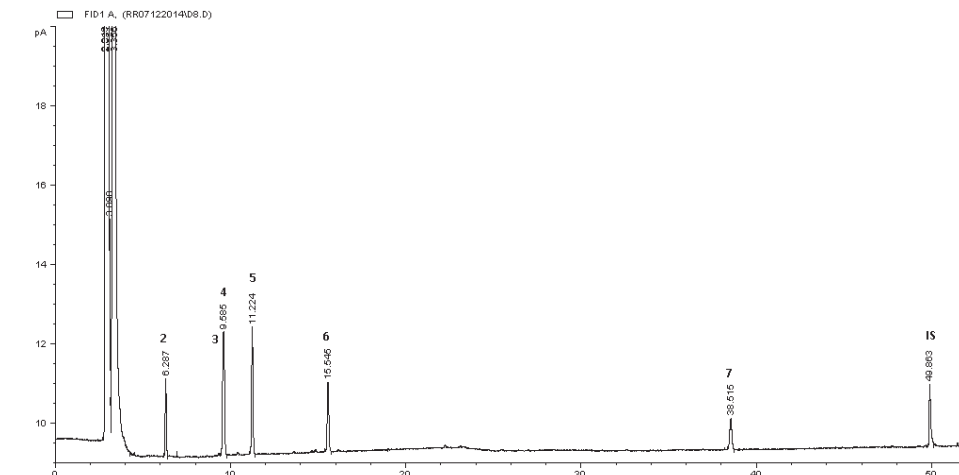
A similar effect was shown by both samples blanched either in water at 70°C during 10 min or in a microwave at 90°C during 2 min. The effect of blanching is clearly visible.

Figure 10 shows the changes in the aroma concentration in the blanched mango sample.



**Figure 10.** Aroma components of mango identified in frozen fresh, frozen blanched and frozen dried samples. The blanching was performed in water and microwave at 70°C during 10 minutes and at 90°C during 2 minutes.

Another outcome is shown after hot air drying of the heat treated samples of mango. The drying was carried out immediately after the blanching. The results show a strong reduction in most volatiles previously present in blanched samples. Others, such as 1-butanol (bp =118°C) and  $\alpha$ -pinene (bp =155°C), were strongly affected by heating. The actual level of drying (final water concentration) determines the loss. The boiling point of the aroma plays a role in the sensitivity. In this study, the esters, similar to what has previously been reported regarding aroma loss during convective drying; seem to be preserved, even after a long period of drying. Ethyl butanoate remains the major component in both blanched and dried samples of *Mangifera indica*. Figure 11 shows the aroma components presents after drying of mango fruit.



**Figure 11.** FID GC chromatograms showing the volatile components identified in dried mango sample: 2:  $\alpha$ -pinene; 3: 3-carene; 4: myrcene; 5: limonene; 6: terpinolene; 7: ethyl butanoate.

Another evaluation examined the relationship between water activity after drying and ratio between the concentration after blanching and after drying. Most aromas are lost during the drying, when water activity is in the range 0.6-0.7. Similar behaviour has been reported for *Vangueria infausta* which lost most of its volatiles at  $a_w$ , approximately equal to 0.6, after prolonged convective drying, although volatility was well preserved down to 0.65 (Raice, at al., 2015).



## **12. Design of model involving sugar, pectin and microcrystalline cellulose for better understanding of the loss or retention of aroma due the crystallisation of aqueous carbohydrate matrix (Paper IV).**

### **12.1 Background**

Retaining desirable quality in food has been a major challenge to producers since ancient times. During food processing and preservation of food, especially fruits, many attributes are affected, including volatile components that are of key importance and may determine the acceptability of the food. Consequently, aroma components must be preserved, not only for sensory quality purposes but also to conceal undesirable flavours released by the product itself or as consequence of processing.

The relative volatility of volatile components are believed to play a significant role on loss or retention of aroma. Volatile components of high molecular weight are better retained than those with lower molecular weight (Goubet et al., 1998; Rosenberg et al., 1990). Sugar crystallisation promotes the loss of volatile compounds entrapped in dried carbohydrate systems (Islesias, 1978; Goubet, 1998). Carbohydrates like maltodextrins, gum arabic and modified starch has been suggested as materials to be used to preserve the volatiles (Shahidi and Han, 1993; Dziezak, 1988). The mechanism of aroma retention or loss due to crystallisation of carbohydrates has been suggested (Roos, 1995; Deborah et al., 1996; Goubet at al., 1998; Rosenberg et al., 1990). In our previous study we discussed the possibility of crystallisation of carbohydrates, mainly sugars, being the reason for loss of aroma after drying of *Vangueria infausta* L. a tropical fruit growing in Mozambique and neighbouring countries (Raice et al., 2015).

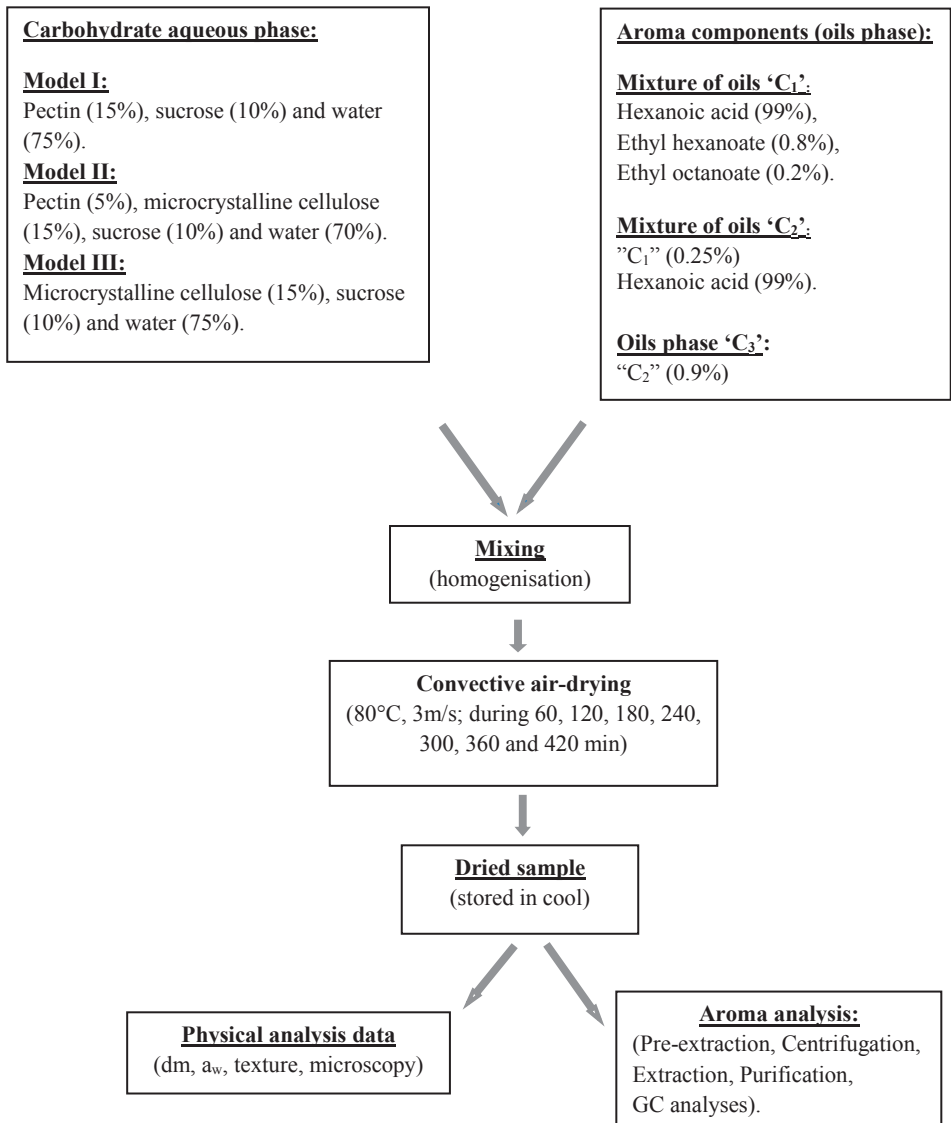
Studies have shown encapsulation to be a useful procedure for ensuring preservation of the aroma components in an amorphous glassy carbohydrate matrix, which protects the material from further changes during storage (Madene et al., 2006; Baristain et al., 2002; Bhandari and

Hartel, 2005). Low water activity achieved by the drying process leads to low mobility in the glassy state, and reduces crystallisation of sugars. (Bell, 1994; Jaya et al, 2004; Onwulata, 1995).

Our previous work showed that the aroma components of *Vangueria infausta* were retained after isothermal convective drying (80°C, 3 m/s), at least until water activity reached about 0.6 (Raice et al., 2015). This could be due to the sugar crystallisation that also takes place during the same range of drying time i.e. when  $a_w$  is about 0.6. However this assumption needed to be verified using a model where the sugars are allowed to crystallize as well as a system where the sugars are prevented from crystallizing. We created two systems. One was a non-crystallizing system obtained by adding a soluble fibre, pectin, to the system. A second system, a crystallizing system, was obtained by adding an insoluble model fibre, microcrystalline cellulose (MCC).

Pectin is mostly present in the cell walls of vegetables and fruits (apple pomace (1.5-2.5%) and orange peel (3.5-5.5%)) (Beli, et al., 1997). The main function of pectin is to bind the cellulose fibrils of the cellwalls together and to provide the plant tissue with mechanical strength. The pectin backbone is composed of  $\alpha$  (1-4) D galacturonic acid chains in which most carboxylic groups are methoxyesters. In the human gastrointestinal tract, pectin binds to a cholesterol and slows glucose absorption by trapping carbohydrates. This is why pectin can be regarded as a soluble dietary fibre. Microcrystalline cellulose (MCC) has been also used in studies. MCC is an insoluble natural polymer comprising glucose units linked by  $\beta$  (1-4) glycosidic bonds. Its high binding ability allows it to be used in various ways as a bulking agent in food production. Water soluble and insoluble fibres have also found in *Vangueria infausta* (Amarteifio and Maosase, 2006; Saka and Msonthi, 1994; Magaia et al., 2013ab).

The following diagram (Figure 12) shows the general procedure in the study.



**Figure 12.** Diagram showing preparation and analysis of models to improve understanding of the loss or retention of aroma due the crystallisation of aqueous carbohydrate matrix. The model solutions were prepared in two steps to reduce measurement error.

The models were prepared as follow:

## **12.2 Model I (pectin, sucrose, water and oils ‘C<sub>1</sub>’)**

Sucrose (10%) was added to pectin (15%) and mixed with water (75%). The mixture was homogenised using a spoon and warmed in a water bath at 80-85°C for 2 hours to allow better dissolution and homogenisation of the components. The viscous mixture was then kept in a fridge overnight. A mixture was made of volatile components (‘C<sub>1</sub>’) comprising hexanoic acid (99%), ethyl hexanoate (0.8%) and ethyl octanoate (0.2%). ‘C<sub>1</sub>’ (0.25%) was then transferred to an aqueous phase, and mixed vigorously using a spoon until completely homogenised. The beaker containing the sample was covered with aluminium foil and stored in a fridge until the drying experiment carried out within two days.

## **12.3 Model II (pectin, microcrystalline cellulose, sucrose, water and oils ‘C<sub>2</sub>’)**

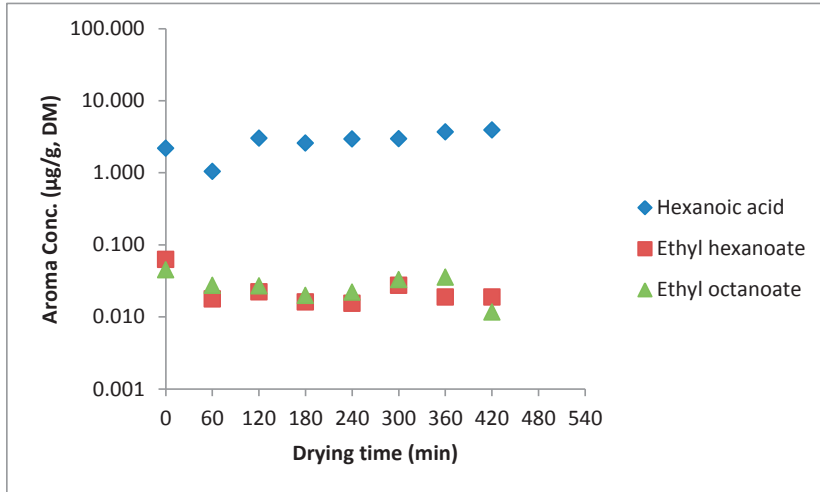
The aqueous phase comprised pectin (5%), microcrystalline cellulose (15%), sucrose (10%), water (70%) and oils. The mixture was homogenised using a spoon and warmed in a water bath at 80-85°C for 2 hours for better dissolution and homogenisation of the components. The viscous mixture was kept in a fridge overnight. Another mixture of volatile components (‘C<sub>1</sub>’) was prepared, comprising hexanoic acid (99%), ethyl hexanoate (0.8%) and ethyl octanoate (0.2%). In order to dilute the mixture of oils, ‘C<sub>1</sub>’ (0.25%) was added to hexanoic acid (99%) and, from this diluted mixture (‘C<sub>2</sub>’), 0.25% g was transferred to the aqueous phase. The mixture was well mixed using a spoon until completely homogenised. The beaker containing the sample was covered with aluminium foil and stored in a fridge until the drying experiment was carried out within 48 hours. The drying of each model and the extraction of volatile was carried out using similar procedures to those described previously (Papers II & III).

## 12.4 Model III (microcrystalline cellulose, sucrose, water and oils ‘C<sub>2</sub>’)

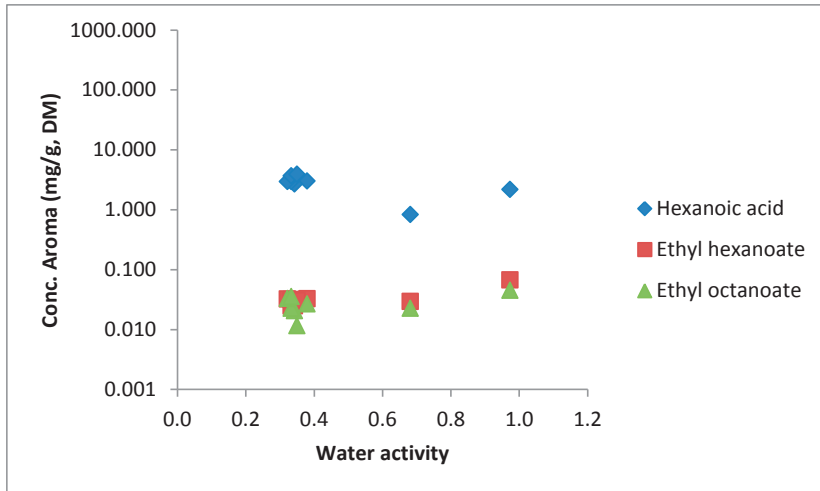
The aqueous phase comprised microcrystalline cellulose (15%), sucrose (10%), water (75%) and oils. The mixture was homogenised using a spoon and warmed in a water bath at 80-85°C for 2 hours for better dissolution of the components and further homogenisation. The viscous mixture was then kept in a fridge overnight. Another mixture was prepared of volatile components (‘C<sub>1</sub>’), comprising hexanoic acid (99%), ethyl hexanoate (0.8%) and ethyl octanoate (0.2%). In order to dilute the mixture of oils, ‘C<sub>1</sub>’ (0.25%) was added to hexanoic acid (99%) and, from that diluted mixture (‘C<sub>2</sub>’), 0.25% was transferred to the aqueous phase and mixed vigorously using a spoon until completely homogenised. The beaker containing the sample was covered with aluminium foil and stored in a fridge until the drying experiment carried out within 48 hours.

## 12.5 Results and discussion

Model aromas (I -pectin, sucrose, water and oils, ‘C<sub>1</sub>’; II -pectin, microcrystalline cellulose (MCC), sucrose, water and oils, ‘C<sub>2</sub>’ and III -MCC, sucrose, water and oils, ‘C<sub>2</sub>’) were dried using a convective oven at 80°C, 3m/s. During the drying, the water content, water activity and dry matter decreases with drying time in all models. Loss of aroma during drying may be caused volatilisation of the entrapped aroma. Crystallisation would squeeze non-crystallising material (salts, proteins, carbohydrates, lipids and aromas) into quite concentrated domains with poor entrapment capacity. Growing crystals may open closed biological structures, thereby contributing to aroma loss. This study showed that, in the aqueous pectin-sucrose mixture (model I), aromas were not lost when drying time was increased apart from a slight reduction during the first hour of drying (Figure 13a). A similar pattern is shown for water activity (Figure 13b), where the aroma components were mostly preserved, even when  $a_w$  value reached 0.4.



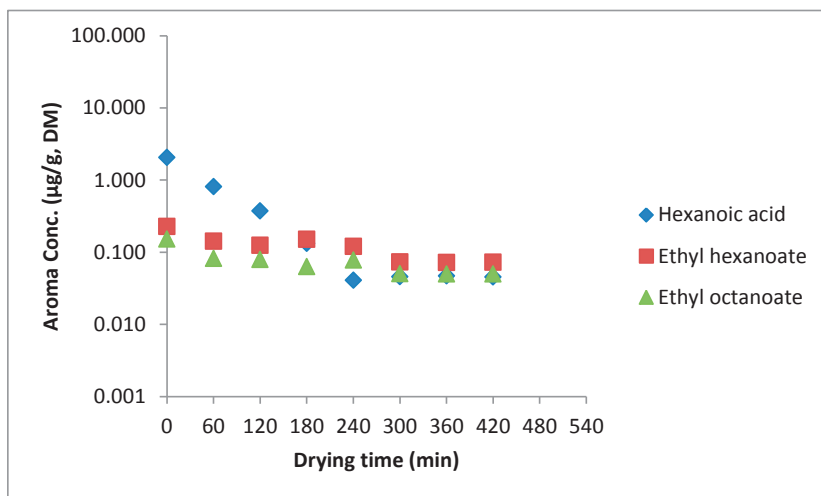
a)



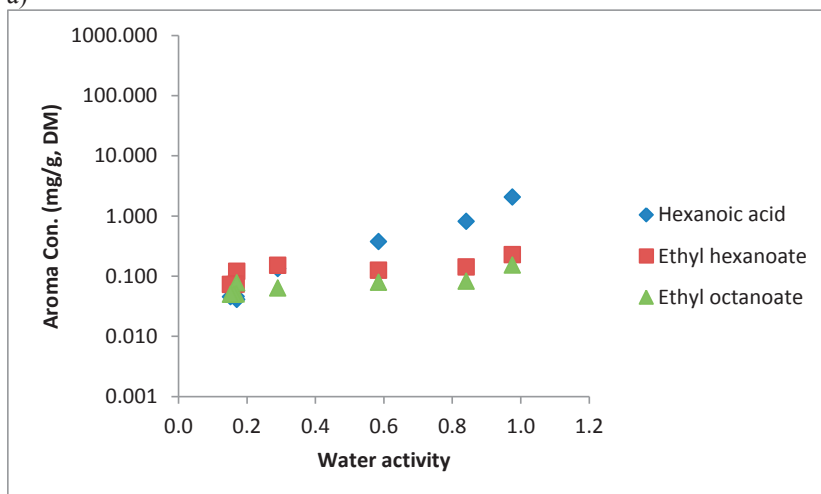
b)

**Figure 13.** Model sample composed of pectin, sucrose, water and oils: (a) aroma retention shown as a function of time; (b) aroma retention shown as a function of  $a_w$ .

In model II the combined presence of pectin and MCC decrease the rate of absorption, and the volatiles are drastically reduced during first 3-4 hours of drying. After 240 min the volatiles remain unchanged, even when the drying is prolonged (Figure 14a). Similar information is shown in Figure 14b, where the aroma components are reduced when the  $a_w$  value decreases from 0.97 to 0.6. Below this range the volatiles are constantly preserved. This could be due to the highly crystalline character of the matrix, which somehow protects the aroma components from loss.



a)

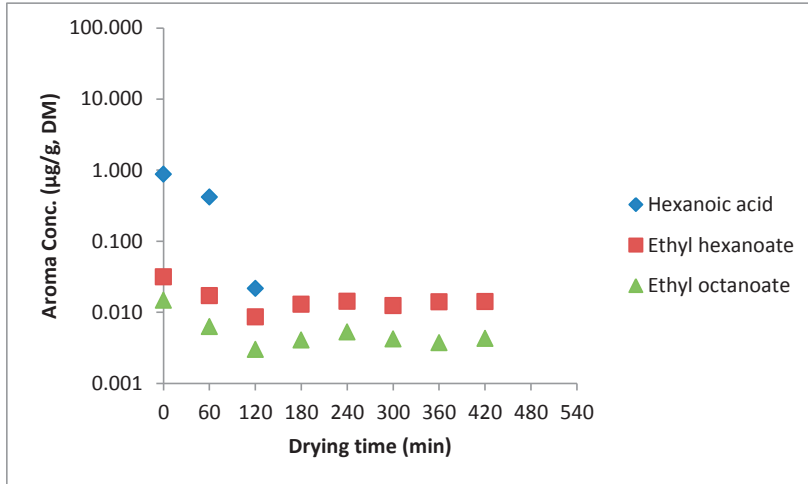


b)

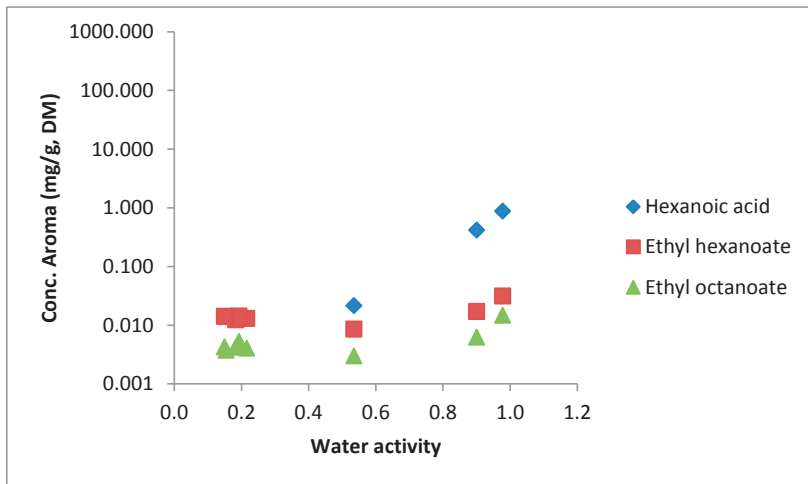
**Figure 14.** Model sample comprising MCC, pectin, sucrose, water and oils: (a) aroma retention shown as a function of time; (b) aroma retention shown as a function of  $a_w$ .

The incorporation of MCC in model III induced a slight delay in rate of water absorption, so rate of reduction of aroma level was slow during the first two hours of drying. After that time, the volatiles did not show any noticeable reduction for the rest of process (Figure 15a). The volatile quickly decreased in range when  $a_w$  was in the range 0.98 to 0.8. Outside this interval, no changes were observed down to  $a_w$  of around 0.3 (Figure 15b). Here we can assume that the system starts in an amorphous state and then gradually changes to rubber and glassy states, so the diffusion of low molecular material gradually decreases until  $a_w$  reaches about 0.8. Our interpretation is that, below this level, the matrix-forming material solidifies as a glassy solid that could give a good encapsulation of the volatiles. If the matrix crystallises, the space between the crystals may act as canals that promote the release of the volatiles. This is indicated by the decreasing rate at the start (Figure 15b). In parallel, as crystallisation takes place, a slight pellicle (encapsulation) forms at the surface, protecting the volatile from loss. We assumed a constant level of volatile during the rest of drying.





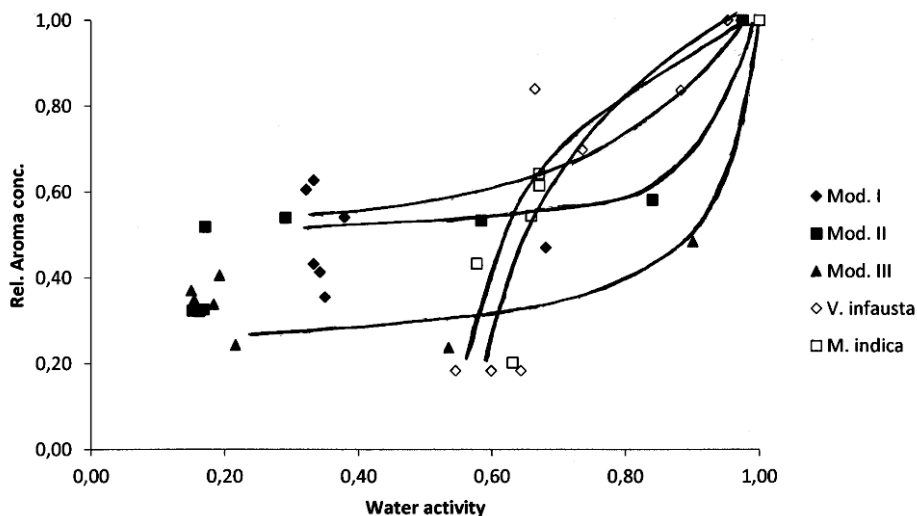
a)



b)

**Figure 15.** Model sample comprising MCC, sucrose, water and oils: (a) aroma retention shown as a function of time; (b) aroma retention shown as a function of  $a_w$ .

Finally, to attempt any overview about the study we summarized the findings accomplished to different materials studied in this work. Figure 16 shows the aroma retention behavior of all material studied on this work.



**Figure 16.** Aroma retention behavior in fruits (*Vangueria infausta* and *Mangifera indica*) and matrixes of aroma models (I, II and III).

The fruits (*Vangueria infausta* and *Mangifera indica*) have similar behavior and are quite comparable. However it is important to control the drying. It is recommended to not dry below 0.70 in water activity to avoid considerable loss of aroma components. Other outcomes can be considered to the three models here investigated. Model III (pectin-MCC-sucrose-water-aromas) releases aroma in very early stage when water activity is in range of 1.0-0.80. Down this interval the aroma are preserved specially at range of  $a_w=0.8-0.7$ . The model I (pectin-sucrose-water-aromas), retain the volatiles until very low water activity rough 0.3. This model contain very high amount of water soluble fibers. It is unrealistic. The model II is very simple to reflect the complexity of fruit material. The combination (pectin-MCC-sucrose-water-aromas) seems realistic but not reflects the cellular structure that may be a critical difference.

### 13. Concluding remarks

Extraction of aroma from plant source has to be effective and purification step by using hydrophobic column C<sub>18</sub>, is essential for obtaining a material without contamination. The volatile components found in pulp from *Vangueria infausta* are hexanoic acid, octanoic acid, ethyl hexanoate, ethyl octanoate, methyl hexanoate, and methyl octanoate. The odour of *V. infausta* mainly originates from ethyl hexanoate and ethyl octanoate. In other hand the most relevant aromas of mango fruit (*Mangifera indica* L.) are 1-butanol,  $\alpha$ -pinene, 3-carene, myrcene, limonene, terpinolene, and ethyl butanoate. In both studied fruits the aroma components are well preserved during the drying process, down to about a relative water activity of 0.65, but considerable loss is observed after more extensive drying probably due to volatilisation through sugar crystallisation which occurs below a relative humidity of around 0.70. The levels of aroma components are enhanced if the material is blanched. Water blanching, microwave blanching, long period of low temperature or short period of high temperature short time made little difference to the impact of blanching. The study shows that the levels of aroma components are enhanced if the material is blanched. Nevertheless water blanching, microwave blanching, long period of low temperature or short period of high temperature short time made little difference to the impact of blanching. Low boiling point aromas are very sensitive to drying in comparison with high-boiling components that shows significant retention.

The models composed by carbohydrates (pectin, MCC, sucrose) and aromas allow fast and high degree of crystallisation of the carbohydrate matrix leading to aroma retention beginning when  $a_w$  is below 0.8. However, extensive drying results in aroma loss.

The results achieved from this work illustrate what happens to aroma during heat treatment of fruits, and are useful in understanding the encapsulation of aroma due to sugar crystallisation during the drying. The results can help to design a better strategy for sustainable utilisation of aroma components of fruits, particularly *Vangueria infausta*, one of the wild fruits used in industrial processing of new products. We believe that technical knowledge about volatility can help promote sustainable utilisation of wild fruits grows in Mozambique and southern Africa.

## **Recommendations and further perspectives**

Through this study we observed strong increasing of aroma levels after blanching. Thus there may be possibilities to optimize the blanching process in order to release more aromas from fruit matrixes.

In other hand the further work could involve investigating the role of the particular structure of the fruit matrix in aroma retention. The models involving aqueous carbohydrate solution, such as soluble and insoluble fibers, closely represent a primitive model of a fruit tissue and seem interesting for encapsulation of volatiles in a fruit matrix.

We believe that encapsulation of the aroma may increase the benefit of both wild and farmed fruits. It may improve appreciation of fruits in local gastronomy. Therefore it is interesting to use the results of this investigation as basis for further studies on this matter involving other indigenous fruits as well to improve aroma retention and encapsulation and to design a sustainable utilization of the diversity of wild fruits growing in Mozambique. The dream is to find ways to incorporate the indigenous fruits as ingredients and to design of new lines of product formulations.

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# Paper I



## Characterization of volatile components extracted from *Vangueria infausta* (African medlar) by using GC–MS

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Volatile compounds isolated from the African medlar (*Vangueria infausta* L.) were identified by gas chromatography and gas chromatography–mass spectrometry. The volatile components identified in the extract were: hexanoic acid (5600 µg/g, dry matter, DM), octanoic acid (240 µg/g, DM), methyl hexanoate (15 µg/g, DM), ethyl hexanoate (44 µg/g, DM), methyl octanoate (12 µg/g, DM) and ethyl octanoate (13 µg/g, DM). The principal aroma from the *V. infausta* fruit originates from ethyl hexanoate and ethyl octanoate with a minor contribution from methyl hexanoate and methyl octanoate.

**Keywords:** *Vangueria infausta*; volatile components; GC–MS; aroma profile

### 1. Introduction

Fruits are an essential part of a healthy and appetizing diet. They are considered rich sources of essential dietary micronutrients, and they have also been recognized as important sources of phytochemicals, as mentioned by Asgari et al. (1) and Pérez-Silva et al. (2). The fruit part of the diet of southern Africa is dominated by common farmed fruits, but a wide range of more or less wild indigenous fruits is also consumed. A few studies relating to the nutritional aspects of indigenous fruits of Mozambique (e.g. *Vangueria infausta*, *Landolpia kirki*, *Andonsonia digitata*, *Ziziphus mauritiana*, *Strychnos spinosa*, *Salacia kraussi*, *Anacardium occidentale*) have shown that most of these fruits have high levels of micronutrients like minerals and vitamins, as described by Styger et al. (3), Amarteifio and Maosase (4), Magaia (5) and Osman (6).

This study focuses on the fruit of the African medlar (*V. infausta* L., family Rubiaceae). In Mozambique, *V. infausta* is commonly called maphilwa (Ronga language) or wild medlar (South African English), and African medlar is used as the English name in eastern Africa. It is brown-orange when ripe and has an orange parenchyma tissue. The fruit is about 2–5 cm in diameter and contains three to five seeds. The fresh fruit is sweet, sour, somewhat bitter and slightly astringent, and the aroma is reminiscent of the common medlar (*Mespilus* sp.) or the wild apple (*Malus* sp.). The pulp can be eaten fresh or cooked. The pulp is used to prepare juice, jam,

marmalade and puddings by adding water and sugar, or cooked to make a kind of porridge. The fresh fruit can also be dried in the sun and then stored for a long time. In the southern part of Africa, the fruit is usually harvested from February to April.

Fruits in general contain volatile compounds as described by Pino et al. (7), Monteiro-Calderón et al. (8) and Licciardello et al. (9). The volatile chemicals present in natural leaves, flowers and fruits have been widely used in aromatherapy since ancient times, suggesting that they impart some beneficial health effect in addition to their pleasant odor. This helps to add value in the evaluation and assessment of the fruits, and has played an important role in gastronomy since ancient times (10). However, there have been few studies on the identification and quantification of the aroma components in *V. infausta*. Pino and co-workers (7) characterized the volatile components of the closely related *Vangueria madagascariensis*. They found sixty-two substances, including 2- and 3-furfural (15 µg/g), hexadecanoic acid and tetradecanoic acid (around 5 µg/g), shorter fatty acids (hexanoic, heptanoic and octanoic acids, around 6 µg/g), fatty acid methyl and ethyl esters (methyl hexanoate 4µg/g), other aldehydes (benzaldehyde, phenylacetaldehyde), alcohols (butanol, furfuryl alcohol, etc., 2 µg/g) and terpenoids (limonene 2µg/g). The aim of this work was to identify the aroma profile of *V. infausta* and thereby determine the chemical components responsible for the fruitiness.

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## 2. Material and methods

### 2.1 Chemicals

The solvents (ethanol, pentane, diethyl ether) and the reference compounds (hexanoic acid, octanoic acid, methyl hexanoate, ethyl hexanoate, methyl octanoate and ethyl octanoate) were purchased from VWR International (Radnor, Pennsylvania, USA). Geraniol was used as the internal standard (IS), which was of chromatographic purity, acquired from Merck (Darmstadt, Germany). All chemical solvents were of analytical grade while the standards were reagents of technical grade. Distilled and deionized water was obtained from a Milli-Q ionic exchange and carbon filter apparatus (Millipore, Bedford, MA, SA). The gases for gas chromatography (GC; helium, nitrogen and oxygen) were of 99.9% purity (from L'Air Liquide S.A, Paris, France).

### 2.2 Raw material

Ripe fruits of *V. infausta* were collected in Bobole, a rural village located approximately 30 km north of Maputo city in Mozambique during the first week of March 2012. The fruits were collected from different trees located in the same area within the radio of approximately 50 m. The fruit were kept in plastic baskets and transported to the laboratory in the Chemistry Department (Eduardo Mondlane University). Only fruits without damage on the surface and of a full-grown size, approximately 4 cm in diameter, were selected. The fruits were washed with tap water and packed in vacuum bags (fifteen to twenty units in each bag), sealed and frozen at  $-20^{\circ}\text{C}$  for two weeks. Then, the fruits were transported to Lund University (Sweden) using a thermo bag in order to keep the sample frozen during transport.

### 2.3 Sample preparation

Before the experiment, two packed vacuum bags (about thirty-five fruits in total) were thawed at room temperature and then boiled in water for 10 minutes to deactivate the enzymes such as lipoxygenase (LOX), hydroperoxidase (HPL) and alcohol dehydrogenase (ADH). This to suppress the production of  $\text{C}_6$  volatiles, as observed by Bai et al. (11). The fruits were allowed to cool, peeled using a clean knife and the seeds were manually separated from the pulp. The pulp were mashed and homogenized by stirring with a clean spoon. The pulp was packed in vacuum bags.

## 3. Extraction of volatiles

The volatiles were isolated using liquid/liquid extraction. The selection of extraction solvents was made to ensure the release of volatiles trapped in the structure,

using a semipolar solvent with good abilities to penetrate the tissue. Ethanol (96%) was chosen for this purpose.

Forty grams of fruit homogenate (moisture content equal to 68%, 33.4° Brix) was initially mixed with 150 mL of ethanol (with geraniol,  $2.3 \times 10^{-3}$  mol/L, as internal standard) and stirred for 48 hours to produce a suspension of fruit particles in an aqueous-ethanol liquid phase. A similar procedure was carried out by Hattab et al. (12) and Sides et al. (13). The suspension was centrifuged at 3000 rpm, for 15 minutes, at  $18^{\circ}\text{C}$  on an AllegraX-15R (Beckman Coulter Inc., Brea, CA, USA). The supernatant was used as the raw extract; 20 mL of raw extract was cleaned from the polar components (e.g. saccharides) using a hydrophobic column containing a bonded hydrophobic phase of 10% desisopropyl atrazine (Bakerbond  $\text{C}_{18}$ , Capitol Scientific Inc., Austin, TX, USA). The extraction liquid was diluted with water (ratio 3:1) to ensure a high affinity of non-polar components to the carrier phase. The diluted extraction liquid was allowed to pass through the column followed by a washing step with 10 mL water. Thereafter, the trapped material in the column was eluted with 25 mL of a 1:1 mixture of pentane and diethyl ether. The experiment was conducted in triplicate.

## 4. GC analyses

The aroma extract was analyzed using GC. The GC apparatus was an Agilent Technologies 6890N (G1530), Network GC system, serial number US10322054, purchased from J&W Scientific (Folsom, CA, USA). The equipment had a hydrophobic DB-225 column (length 30.0 m, diameter 250.0  $\mu\text{m}$ ; film thickness 0.25  $\mu\text{m}$ ), containing hydrophobic stationary phase of cyanopropylphenyl-diethylpolysiloxane (50%). The oven temperature program started from an initial temperature of  $50^{\circ}\text{C}$ , was kept constant for 3 minutes and then increased at a rate of  $3^{\circ}\text{C}/\text{minute}$  to  $200^{\circ}\text{C}$ , over 54 minutes. The injector and detector temperatures were  $250^{\circ}\text{C}$ . Helium was used as the carrier gas, at a flow rate of 0.6 mL/minute, pressure 55.2 kPa. The inlet split ratio was 20:1 and the split flow was 12 mL/minute. Hydrogen flow was at 30 mL/minute. The injector had a syringe size of 10  $\mu\text{L}$ , volume sample of 2.0  $\mu\text{L}$ , was injected three times, and *n*-hexane was used to wash five times.

The GC-MS analysis carried out on an coupled Agilent (Palo Alto CA, USA) 5972 mass selective detector, together with an Agilent 6890 GC equipped with a HP-5MS or Innnowax capillary column (30 m  $\times$  0.25 mm i.d., SGE, Austin, TX, USA). The oven temperature was programmed from  $50^{\circ}\text{C}$ , held constant for 3 minutes, increased by  $5^{\circ}\text{C}/\text{minute}$  to  $230^{\circ}\text{C}$ , then held constant for 10 minutes. The MS data was

compared with mass spectra of the fragment pattern from the MS databank from the NIST (National Institute of Standards and Technology) library. This chromatographic method has been applied in previous works by Glasl et al. (14), Janes et al. (15), Reineccius (16) and Kaewtathip and Charoenrein (17). The amount of each aroma compound was calculated in relation to the dry matter (DM) of the sample, taking into account the signal intensities of the reference compounds. The retention index (RI) of each target compound is calculated using *n*-paraffin references (C<sub>12</sub>–C<sub>24</sub>) on a PH-88 column (30 m × 0.25 mm i.d., and 0.20 μm film thickness; J&W Scientific, Agilent Technologies).

The amount of each aroma compound was calculated in relation to the DM of the sample, taking into account the signal intensities of the reference compounds. Equations (1) and (2) were used for the aroma concentration calculations:

$$C_{aroma} = f \cdot \frac{P_{aroma,sample}}{P_{IS,sample}} \cdot \frac{m_{IS}}{m_{sample} \phi_{DM}} \quad (1)$$

$$f = \frac{P_{IS,reference}}{P_{aroma,reference}} \cdot \frac{m_{aroma,reference}}{m_{IS,reference}} \quad (2)$$

where  $C_{aroma}$  is the concentration of the aroma;  $f$  is the factor of proportionality;  $P_{aroma,sample}$  is the peak area of aroma measured in the sample;  $P_{IS,sample}$  is the peak area of internal standard measured in the sample;  $m_{IS}$  is the mass of internal standard added;  $m_{sample}$  is the mass of sample;  $\phi_{DM}$  is the fraction of dry matter;  $P_{IS,reference}$  is the peak area of the internal standard in the reference; and  $P_{aroma,reference}$  is the peak area of the aroma reference.

## 5. Results and discussion

The GC analyses of the raw extract of *V. infausta* produced results dominated by a wide range of peaks, of which more than one was identified as different furfurals by MS, as mentioned by Raice et al. (18), in agreement with the study of aroma profile of *V. madagascariensis* by Pino et al. (7). However, further experiments in which a comparable extract of an aqueous sugar solution (glucose, fructose and sucrose) was injected showed that the furfurals were caused by the presence of the sugars in the injected extraction phase, and that the different furfurals were probably decomposition products when the sample were injected into the column in the oven. Similar degradation has been observed by Cheetham (19).

A cleaning procedure using a hydrophobic column as described in the experimental section was introduced to eliminate the sugars from the extract. The yield of the internal standard was measured to about 80% of the complete extraction and cleaning procedure.

Figure 1 shows a GC chromatogram of the extract after the cleaning procedure. The number as well as intensity of the peaks is much reduced, and none of them represents typical saccharide decomposition products such as furfurals.

The peaks obtained were reanalyzed for tentative identification using GC–MS. Two different columns were used to control eventual co-elution. One column was a high-polar Innowax and one a non-polar HP-5. The GC results from these two columns gave different orders between peaks and some of the peaks fused together when the polar column were used. As the use of polar column may result in poor resolution of fatty acids, another experiment with methanolysis conversion

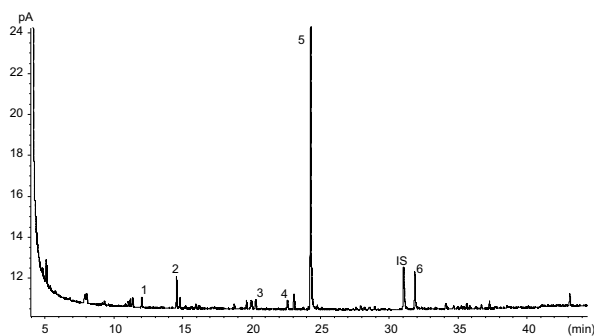


Figure 1. Flame ionization detector (FID) chromatogram of analysis of the *Vangueria infausta* extract, as obtained using a DB-225 column: 1-methylhexanoate (Rt=12.02 minutes), 2-ethylhexanoate (Rt=14.55 minutes), 3-methyloctanoate (Rt=20.29 minutes), 4-ethyloctanoate (22.59 minutes), 5-hexanoic acid (Rt=24.29 minutes), IS-geraniol (Rt=31.03 minutes) and 6-octanoic acid (Rt=31.86 minutes).

was performed on a polar HP-5 column. The reference materials of the target aromas were used in order to verify the identification. All tentative identifications were confirmed with reference compounds (MS spectra and retention times).

The mass spectrum of hexanoic acid showed intensive rearrangement fragments at  $m/z$  60, which, in combination with ion at  $m/z$  73, suggested an aliphatic carboxylic acid structure. The tentative molecular ions at  $m/z$  116 and 99 ( $M-OH$ )<sup>+</sup> in the spectrum indicated a saturated C<sub>6</sub> acid, which was finally confirmed as *n*-hexanoic acid by comparing the retention times and mass spectra with the reference compound under both polar (Innowax) and non-polar (HP-5) columns. A similar fragmentation pattern was observed in the spectrum of the second most abundant homolog octanoic acid, in which a tentative molecular ion at  $m/z$  144 implied two CH<sub>2</sub> units longer than hexanoic acid and the compound was finally identified as saturated *n*-octanoic acid.

The methyl hexanoate and methyl octanoate showed typical mass spectra of the methyl esters of corresponding hexanoic and octanoic acid, characterized by the combination of the McLafferty rearrangement ions at  $m/z$  74 and 87, as well as the ( $M-OCH_3$ )<sup>+</sup> fragment at  $m/z$  99 and 127, and the molecular ions at  $m/z$  130 and 158, respectively, whereas components ethyl hexanoate and ethyl octanoate presented the mass spectra of ethyl esters of hexanoic and octanoic acid, characterized by the ion pairs at  $m/z$  88 and 101, the ( $M-OCH_2CH_3$ )<sup>+</sup> fragment at  $m/z$  99 and 127, and the molecular ion at  $m/z$  144 and 172, respectively. All these esters were further confirmed by the synthetic references.

The final outcome of the identification is shown in Figure 1. Disulfides, hydrocarbons (octane, undecane, tridecane), BHT (butylated hydroxytoluene) and DTBP (di-tert-butylperoxide) were also found. BHT is included in the diethyl ether as an antioxidant to

control peroxide formation. The DTBP probably originates from the polyethylene bags (used as initiators for the polymerization reaction). The sulfide probably also originates from the polymer synthesis. The remaining substances were considered a potential aroma.

The peaks were quantified using the intensity relative to the intensity of the IS peak and compared with the ratio of intensity between reference materials and the IS. The major constituents were hexanoic acid (5600 µg/g, DM), octanoic acid (240 µg/g, DM), methyl hexanoate (15 µg/g, DM), ethyl hexanoate (44 µg/g, DM), methyl octanoate (12 µg/g, DM) and ethyl octanoate (13 µg/g, DM). The description of aroma and the quantitative data is listed in Table 1. Most of the identified components were reported in the previous study on *V. madagascariensis* by Pino et al. (7). For example, hexanoic acid, octanoic acid and their corresponding methyl esters have been found. However, Pino and co-workers did not find the ethyl esters identified in this study.

The impact of an aroma for the perception of a fruit is a concentration issue. The odor activity was estimated to evaluate the impact of the individual compounds for the actual aroma profile of the fruit. The odor activity value (OAV) is the ratio between the concentration and the threshold value of the aroma compound, as described by Pino and Mesa (20), Guadagni et al. (21) and Wright (22). According to Cheetham (19), the probability of a compound's odor being detected is expected to be greater with increasing OAV. In this study, the OAV of each compound was estimated from the threshold values measured in aqueous solutions, found in the literature, according with previous studies by Moreno et al. (23), Poisson and Schieberle (24), and Charoenrein et al. (25).

The results show that the ethyl and methyl esters, in particular ethyl hexanoate, are expected to be the

Table 1. Concentration related to the dry matter (DM), fresh weight (fW), odor threshold values and odor activity values (OAVs) and odor description of the potential aroma components found in the extract of the *Vangueria infausta*.

	Concentration (µg/g, DM)	Concentration (µg/g, fW)	Odor threshold (µg/g)	OAV	Odor description (in this investigation)
Hexanoic acid	5600	1800	3 <sup>b</sup>	600	The odor is somewhat rancid or cheesy. Resembles the smell of goats
Octanoic acid	240	90	3 <sup>b</sup>	30	The odor is somewhat rancid or cheesy, but milder than hexanoic acid. Resembles coconut milk or liquid
Ethyl hexanoate	44	14	0.001 <sup>b,c</sup>	10000	The odor is fruity. Resembles banana.
Ethyl octanoate	13	4.2	0.005 <sup>d</sup>	800	The odor is somewhat fruity, with a slightly wine character. Resembles the African medlar, <i>V. infausta</i>
Methyl hexanoate	15	4.9	0.07 <sup>b</sup>	70	The odor is sharp and fruity. Somewhat pear like
Methyl octanoate	12	3.7	0.2 <sup>a,b</sup>	20	The odor is somewhat sharp and fruity

Notes: The concentrations are given with a two-digit precision, the threshold value and OAV with one-digit precision. The reference from which the threshold value has been taken is given in superscripted lower case letter: <sup>a</sup>Monteiro-Calderón et al. (8); <sup>b</sup>Pino and Mesa (20); <sup>c</sup>Takeoka et al. (31); <sup>d</sup>Gómez García-Carpintero et al. (28).

most powerful contributors of aroma in the fruits despite the low concentration of these esters. This is because the threshold values are comparatively low and the OVA consequently high. The short-chained fatty acids have a comparable weak odor as observed by Elss et al. (26) and Attaie (27) and this explains the low OAV exhibited by hexanoic and octanoic acids despite the high concentration.

To verify the evaluation of the contribution of each component to the flavor of the *V. infausta* fruits, the flavor of each individual component was compared. The results were found to be in somewhat agreement with similar descriptions in previous studies by Kaewtathip and Charoenrein (17), Gómez Garcia-Carpintero et al. (28), Li et al. (29) and Zea et al. (30). The evaluation was made by sniffing freshly cut pieces of the fruit as well as the pure compounds. The aroma presented with this fruit predominantly originates from esters, which gives a characteristic fruity flavor with a slight element of banana, green apple and pineapple, which is characteristic of the *V. infausta* (African medlar).

## 6. Conclusion

The principal aroma in the *V. infausta* fruit is originates from ethyl hexanoate and ethyl octanoate with a minor contribution from methyl hexanoate and methyl octanoate.

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# Paper II



## The Loss of Aroma Components of the Fruit of *Vangueria infausta* L. (African Medlar) After Convective Drying

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The loss of aroma components after drying the fruit of *Vangueria infausta* L. was studied by means of convective air drying and gas chromatography techniques. The samples were dried at 80°C, with an air flow of 3 m/s at different drying times in the range of 60 up to 420 min. Aroma composition, dry matter, and water activity were measured for each sample throughout the drying time. The study shows that the targeted aroma components present in the fresh fruit sample identified in our previous work (hexanoic acid, methyl hexanoate, methyl octanoate, octanoic acid, ethyl hexanoate, and ethyl octanoate) are retained in the matrix for at least 240 min of drying. Samples dried for 300 min showed decreased amounts of volatiles and practically no aromas were found after 420 min. It is hypothesized that the results are explained as a consequence of the crystallization of sugars during the drying process. The suggested explanation is supported by a prediction of the water activity that is critical for obtaining crystallization of a mixture of sugars corresponding to the fruit.

**Keywords** Aroma; Crystallization; *Vangueria infausta* L.; Volatiles; Water activity

### INTRODUCTION

Drying is an ancient technique used for the preservation of fruits. A critical aspect is that of ensuring the extent to which desirable properties of the fruit are maintained during processing. It is believed that drying is a process that is particularly likely to cause quality loss.<sup>[1]</sup> Among these quality attributes, one of the most challenging is that of the aroma.<sup>[1,2]</sup> Different types of drying, such as spray drying and freeze drying, have been used to study aroma retention using model systems consisting of aqueous carbohydrate solutions containing small amounts of aroma components.<sup>[3–5]</sup>

In several studies, the mass transfer within the drying material matrix is described as a diffusion process.<sup>[6–8]</sup> However, one particular issue is that of the role of the

matrix forming the continuous structure of the dried food. The continuous structure in most dried fruits is mainly composed of different carbohydrates. The carbohydrates may, after drying, be present in a crystalline or in a glassy state and it has been observed that the flavor retention is much higher when encapsulated in a continuous structure such as a glassy state than if the structure is in a polycrystalline state.<sup>[7]</sup> It has also been suggested that low mobility in the glassy state reduces chemical reactions as well as mass transfer.<sup>[9]</sup> The decrease of the water activity ( $a_w$ ) value achieved by the drying process plays an important role in the physical state of the encapsulation matrix.<sup>[10–12]</sup> In this work, fruit of the *Vangueria infausta* L. was used to study the effect of the drying process on aroma components.

*Vangueria infausta* L. (African medlar) is an underutilized wild fruit growing in southern Africa. *Vangueria infausta* L. belongs to the family Rubiaceae. It is commonly called African medlar in English or maphilwa in Ronga (the local language in southern Mozambique). The fruit is usually harvested from February to April. It is brownish orange when ripe and has a spherical shape. The size is about 2–5 cm in diameter and the fruit contains 3–5 seeds. The fresh fruit is sweet with an apple- to pineapple-like aroma. The rural population eats the pulp fresh, cooked, or dried. They also prepare juice, jam, and puddings by adding water and sugar or they prepare marmalade. The fruit is often dried in the sun and then stored for a long time.<sup>[13]</sup> Lately, it has attracted an increasing interest as a commercial fruit in the cities. A few investigations of the nutritional aspects have been carried out showing that the fruit is rich in dietary fiber and sugars and that it has a high content of micronutrients such as minerals and vitamins.<sup>[14–16]</sup> In our previous work, we investigated the aroma profile of the *Vangueria infausta* L. and it was found that it is mainly composed of hexanoic acid, octanoic acid, methyl hexanoate, methyl octanoate, ethyl hexanoate, and ethyl octanoate.<sup>[17]</sup> A commercial use demands rapid and effective preservation, which motivates studies of the drying properties. The fruit has an attractive flavor. The

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volatiles of the fruit are rich in short fatty acids and ethyl and methyl esters of the same acids, making it gastronomically interesting to combine with wine and cheese, assuming the flavor matching theory.<sup>[18,19]</sup> The objective of this study is to describe and understand the retention of the aroma of *Vangueria infausta* L. during a convective drying process.

## MATERIALS AND METHODS

### Fruit Samples and Drying

Ripe fruits of the *Vangueria infausta* L. were collected in Bobole, Maputo, Mozambique, during the harvest season of 2012. Mature fruits, free from defects (fruit without mechanical or insect damages on the surface), were selected and washed, sealed in polyethylene plastics bags, and frozen at  $-20^{\circ}\text{C}$ . Before use, the fruit was thawed at room temperature for 24 hours. After thawing, the fruit was blanched in boiling water for five minutes to stop enzymatic activity. Peels and seeds were manually separated from the pulp using a knife. The fruit pieces were disintegrated and homogenized using a domestic puree sieve. The puree was distributed into molds and was then ready to dry.

Preliminary convective drying tests were performed in advance to select the best drying parameters to dry the puree. The drying air temperatures at 40, 60, and  $80^{\circ}\text{C}$  and air velocity 1, 3, and 4 m/s were investigated to dry samples until constant weight. The drying temperature of  $80^{\circ}\text{C}$  in combination with air velocity of 3 m/s resulted in a reasonable drying time, hardness of the final product, and a safe water activity.<sup>[20,21]</sup>

Forty samples of *Vangueria infausta* pulp with 5 gr of pulp in each, were filled into spherical molds (5 mm thickness, 28 mm in diameter), purposely designed for this experiment, and were dried in a convective oven set at  $80^{\circ}\text{C}$ , 3 m/s. The drying procedure has been described previously.<sup>[20]</sup> The experiment was carried out as follows: Five samples were used as not dried (fresh) pulp and 35 samples were placed on a tray from which five samples were removed at time intervals of 60, 120, 180, 240, 300, 360, and 420 minutes of drying. The temperature profile was followed using (type K, 0.5 mm) thermocouples connected to a HP Compaq S720 computer. Water content ( $X_w$ ) and water activity ( $a_w$ ) were monitored after each drying time throughout the drying process. The water content was obtained using a vacuum oven (Forma Scientific, Marietta, OH, USA), at  $70^{\circ}\text{C}$  for 24 hours. The  $a_w$  was measured at  $20^{\circ}\text{C}$  using an AquaLab water activity meter (model S3TE, Decagon Devices, Pullman, WA, USA) calibrated using saturated salt solutions (NaCl:  $a_w = 0.760$  M and KCl:  $a_w = 0.984$ ). The soluble solid was determined at  $20^{\circ}\text{C}$  using a digital sucrose refractometer with an accuracy of  $\pm 0.2\%$  Brix (modell HI9681, Woonsocket, RI, USA).

### Chemicals

The solvents (ethanol, pentane, diethyl ether) and the reference compounds (hexanoic acid, octanoic acid, methyl hexanoate, ethyl hexanoate, methyl octanoate, and ethyl octanoate) were purchased from VWR International (Radnor, PA, USA). Geraniol (internal standard) was also of chromatographic purity, acquired from Merck (Kenilworth, NJ, USA). All chemical solvents were of analytical grade while standards were of reagent or technical grades. Deionized and purified water was obtained from a Milli-Q ionic exchange and carbon filter apparatus (Millipore, Bedford, MA, USA). The gases for GC (helium, nitrogen and oxygen) were of 99.9% purity (from L'Air Liquide S.A., Paris, France).

### Extraction of Aroma from the Dried *Vangueria infausta* Pulp

The extraction and GC analysis follows the methodology described in our previous study.<sup>[17]</sup> A similar procedure was also used by Hattab and others.<sup>[22-24]</sup>

The volatiles were isolated using solvent extraction. A semipolar solvent (96% ethanol) with assumed good ability to penetrate the fruit tissue was selected as extraction solvent to release volatiles entrapped in the structure.

A total of 40 g of homogenate fruit pulp (moisture content equal to 68%, 33.4° Brix) was initially mixed with geraniol, 150 ml (2.3 mmol/l) as internal standard. The suspension of fruit particles in an aqueous-ethanol liquid phase was stirred for 48 hours to allow for complete extraction. Dried samples were wetted before extraction. A dry sample of 2 g was taken and rehydrated using 30 mL of distilled water and left to equilibrate overnight, then 40 ml of extraction solvent was added for extraction. The obtained suspension was centrifuged at 3000 rpm during 15 min at  $18^{\circ}\text{C}$  (AllegraX-15R, from Beckman Coulter Inc., Brea, CA, USA). The supernatant was used as a raw extract and was divided into three replicates of 10 ml each. The polarity of the raw extract was increased by adding 30 ml of water (to obtain about 21% of ethanol) to allow for an efficient separation of hydrophobic aroma components from hydrophilic sugars using a hydrophobic column. The hydrophobic column had a bonded hydrophobic phase of desisopropyl atrazine 10% (Bakerbond speTM Octadecyl ( $\text{C}_{18}$ ) from Capitol Scientific Inc., Austin, TX, USA).<sup>[25]</sup> Thereafter, the volatile entrapped material was recovered from the column by eluting with 15 ml of a non-polar solvent mixture (pentane and diethyl ether (1:1)), forming a uniform liquid phase with good solubilizing capacity for non-polar volatiles.<sup>[26]</sup> The experiment was conducted in triplicate. The efficiency in terms of recovery of internal standard was estimated by comparing the concentration of added geraniol with the extraction solvent against the geraniol concentration. The yield was estimated

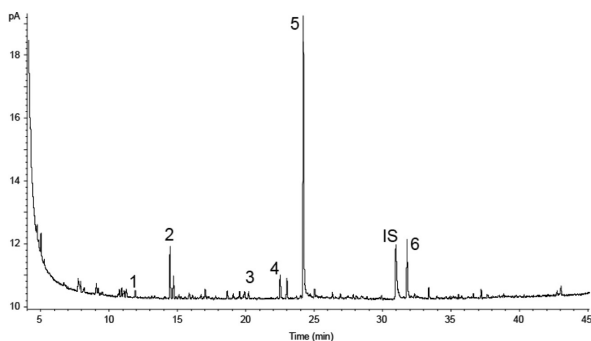


FIG. 1. FID chromatogram for *Vangueria infausta* sample as obtained using a DB-225 column: 1—methyl hexanoate (Rt = 11.91 min), 2—ethyl hexanoate (Rt = 14.45 min), 3—methyl octanoate (Rt = 20.21 min), 4—ethyl octanoate (22.58 min), 5—hexanoic acid (Rt = 24.21 min), 6—octanoic acid (Rt = 31.79 min), IS—geraniol (Rt = 30.95 min).

to be between 70–90% in controlled experiments. The following equation was used to calculate the yield:

$$\text{Yield} = \frac{P_S}{P_{IS}} \cdot \frac{C_i}{C_{IS}} \cdot \frac{V_{S,\text{centrifug}} \cdot V_{S,\text{extract}}}{V_{IS,\text{added}}} \cdot 100 \quad (1)$$

$P_S$ , (pAs) peak area of sample;  $C_i$ , ( $\mu\text{g}/\text{l}$ ) concentration of aroma reference;  $V_{S,\text{centrifug}}$ , (ml) volume of sample after centrifugation;  $V_{S,\text{extract}}$ , (ml) volume of sample after purification;  $P_{IS}$ , (pAs) peak area of IS;  $C_{IS}$ , ( $\mu\text{g}/\text{l}$ ) concentration of IS;  $V_{IS,\text{added}}$ , (ml) volume of IS added to the sample.

The reference material (hexanoic acid, octanoic acid, methyl hexanoate, methyl octanoate, ethyl hexanoate, and ethyl octanoate) were standards prepared at concentration of 0.2% in ethanol each, injected apart and used for peak comparison (in terms of retention time) with the one from the studied sample. The comparison was also based on retention index and mass spectral database (Agilent). This work was reported in our previous study about identification and characterization of volatiles isolated from *Vangueria infausta*.<sup>[17]</sup>

### GC and GC-MS Analysis

The aroma extract was analyzed using gas chromatography (GC). The GC apparatus was of model 6890N (G1530) from Agilent Technologies, Network GC system, serial US10322054, purchased from J&W Scientific, USA. A hydrophobic DB-225 column (length 30.0 m, diameter 250.0  $\mu\text{m}$ , film thickness 0.25  $\mu\text{m}$ ), containing a hydrophobic stationary phase of cyanopropylphenyl-diethylpolysiloxane (50%), was used. The oven temperature program started from an initial temperature of 50°C, was kept constant for 3 min, and then increased at a rate of 3°C/min to 200°C over 54 min. The injector and detector temperatures were

250°C. Helium was used as carrier gas at a flow rate of 0.6 ml/min, pressure 55.2 kPa, and split ratio of 20:1 flowing at 12 mL/min. Hydrogen flow was at 30 ml/min. The injector had a syringe size of 10.0  $\mu\text{l}$  and a sample volume of 2.0  $\mu\text{l}$ . The samples were injected with three repeated strokes and the syringe was rinsed five times with n-hexane between the samples.

The peaks were preliminarily identified using GC-MS followed by a confirmation using reference standards as described by Raice.<sup>[17,27]</sup> An example of a GC chromatogram is shown in Fig. 1.

The amount of each aroma compound was calculated by relating the peak area of the substance to the peak area of the reference compounds and correcting for the signal strength of the identified compounds (Equations (2) and (3)). The concentration was calculated relative to the dry matter (mDM) of the sample:

$$C_{\text{aroma}} = f \cdot \frac{P_{\text{aroma, sample}}}{P_{\text{IS, sample}}} \cdot \frac{m_{\text{IS}}}{m_{\text{sample}} \cdot X_{\text{DM}}} \quad (2)$$

$$f = \frac{P_{\text{IS, reference}}}{P_{\text{aroma, reference}}} \cdot \frac{m_{\text{aroma, reference}}}{m_{\text{IS, reference}}} \quad (3)$$

$C_{\text{aroma}}$ , ( $\mu\text{g}/\text{g DM}$ ) concentration of the aroma;  $f$  is the response factor;  $P_{\text{aroma, sample}}$  (pAs), peak area of aroma measured in the sample;  $P_{\text{IS, sample}}$  (pAs), peak area of internal standard measured in the sample;  $m_{\text{IS}}$  (g), mass of internal standard added during the extraction;  $m_{\text{sample}}$  (g), mass of sample;  $X_{\text{DM}}$ , mass fraction of dry matter;  $P_{\text{IS, reference}}$  (pAs), peak area of the internal standard in the reference;  $P_{\text{aroma, reference}}$  (pAs), peak area of the aroma in the reference.

TABLE 1  
Retention index mass fragment patterns of most potent aroma compounds found in *Vangueria infausta* L.

Aroma component	Experimental data (in a hp-innowax)		Literature (and ref.) RI (min)	MS fragments m/z
	Rt (min)	RI (min)		
Hexanoic acid	17.376	1850.6	1874 <sup>a</sup> , 1836 <sup>b,c</sup> , 1825 <sup>e</sup>	41, 35, 55, 60, 73, 87, 105, 116.
Octanoic acid	23.796	2173.1	2085 <sup>a</sup> , 2040 <sup>e</sup> , 2032 <sup>e</sup>	43, 55, 60, 73, 85, 101, 115, 124, 129, 144, 152.
Methyl hexanoate	6.743	1195.2	1173 <sup>c</sup>	39, 43, 55, 59, 74, 87, 99.
Ethyl octanoate	7.686	1241.2	1251 <sup>a</sup> , 1181 <sup>b</sup> , 1238 <sup>d</sup> , 1224 <sup>e</sup>	43, 57, 71, 88, 99, 115, 127, 144.
Methyl octanoate	10.724	1489	N/A	43, 55, 74, 87, 101, 115, 127, 158.
Ethyl hexanoate	11.659	1536.1	1454 <sup>d</sup> , 1424 <sup>e</sup>	41, 57, 73, 88, 101, 115, 127, 143, 157, 172.

The superscripted lower case letter indicates the reference from which the value has been taken: a- [28]; b- [29]; c- [30]; d-[31]; e-[32].

The peaks obtained were tentatively identified using MS by comparison of the experimental results with figures in the MS database from NIST. Also, reference materials of most relevant aromas found were used to verify the identification through retention indices performed on a hp-innowax column. Table 1 shows the mass fragments and the Kovat's retention indices (RI) of the target components calculated using n-paraffin references (C<sub>12</sub>-C<sub>24</sub>).

#### Microstructural Observations

Microstructural observations were made in a microscope equipped with a polarizing device and a hot-stage device. Samples were removed from the dryer (during a separate drying event) while preserving the temperature (+2 to -10°C relative to the drying temperature) during the transfer to the hot stage of the microscope. The sample was disintegrated using a spatula and tweezers and immersed into coconut oil on the microscopic slide. The observations were performed within 10 minutes after the sample was removed from the dryer.

#### Statistical Analysis

All analyses were conducted in triplicate for extraction. The results reported are the average of these three replicates. The statistical analyses were carried out using Microsoft Office Excel 2010. Analysis of variance (ANOVA) was type general linear model (one way) procedure.

## RESULTS

#### Drying

The drying of *Vangueria infausta* L. pulp was performed using a convective oven at 80°C, 3 m/s. Figure 2 shows the decreasing water content, water activity, and dry matter as a function of drying time during the drying. The samples display a constant drying rate during the first hour, followed by a decaying drying rate. Samples dried for 240-300 minutes give an average water content of

0.20 ± 0.03 g/g and water activity equal to 0.65 ± 0.01. This value is assumed to be acceptable from the point of view of food preservation, as the risk of microbial growth is low for  $a_w$  values in the range of 0.6-0.7.<sup>[21,33,34]</sup>

#### Aroma Analysis

The investigation of aroma profiles was performed using solvent extraction followed by GC analyses. The target components identified were esters (methyl hexanoate, methyl octanoate, ethyl hexanoate, and ethyl octanoate) and acids (hexanoic acid and octanoic acid). The results are shown in Table 2. We can note that the fresh fruit is characterized by high amounts of short fatty acids and of the ethyl and methyl esters of the same fatty acids. The dried fruit has a low level of both short fatty acids and of their esters. This table shows that the fruit aromas are

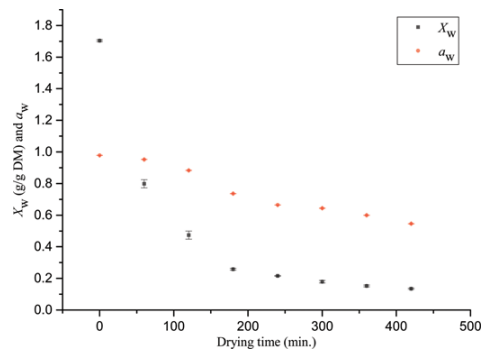


FIG. 2. Relationship between water content ( $X_w$ ) on dry basis, water activity ( $a_w$ ), and drying time during a convective air-drying of *Vangueria infausta* at 80°C, 3 m/s. The error bars presents the standard error of mean.

TABLE 2

Concentration ( $\mu\text{g/g DM}$ ) of aroma components isolated from *Vangueria infausta* L. after isothermal convective drying up to 420 min; the measurement was carried out in triplicate

Drying time (min)	Conc. ( $\mu\text{g/g dm}$ )					
	Hexanoic acid	Octanoic acid	Ethyl hexanoate	Ethyl octanoate	Methyl hexanoate	Methyl octanoate
0	10000 $\pm$ 3000	640 $\pm$ 400	130 $\pm$ 50	60 $\pm$ 20	20 $\pm$ 20	21 $\pm$ 2
60	22200 $\pm$ 400	1500 $\pm$ 80	280 $\pm$ 20	120 $\pm$ 8	100 $\pm$ 20	69 $\pm$ 7
120	24000 $\pm$ 6000	1400 $\pm$ 200	280 $\pm$ 40	90 $\pm$ 10	70 $\pm$ 60	62 $\pm$ 1
180	25400 $\pm$ 400	1200 $\pm$ 4	210 $\pm$ 20	60 $\pm$ 5	70 $\pm$ 4	56 $\pm$ 1
240	16200 $\pm$ 80	1200 $\pm$ 100	220 $\pm$ 20	110 $\pm$ 20	90 $\pm$ 10	54 $\pm$ 1
300	1300 $\pm$ 200	1200 $\pm$ 1	<20*	<20	<20	<20
360	1060 $\pm$ 30	90 $\pm$ 10	<20	<20	<20	<20
420	990 $\pm$ 70	<50*	<20	<20	<20	<20
480	<300*	<50	<20	<20	<20	<20

(<...\*indicate the detection limit of the component).

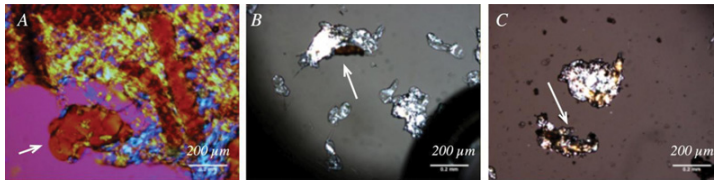


FIG. 3. Changes in microstructure during drying of *Vangueria infausta*. The photos are taken using a 5 $\times$  objective and polarizing light. The temperature is kept close to the sample temperature of the sample: (A) Sample before drying; (B) after 60 minutes; (C) after 360 minutes. The arrows point on visible cells showing the shrinking with drying.

well retained during the first part of the drying. We are actually able to remove 90% of the water without any loss of fruit aroma. However, a dramatic change in the concentration of aroma is observed after about 240 minutes of drying. This loss of aroma retention in the fruit can be considered a major loss of quality and it occurs just when we have reached the target level in water activity ( $a_w = 0.60\text{--}0.70$ ) and is therefore a major challenge for the development of high-quality dried fruit products of *Vangueria infausta*.<sup>[35]</sup>

Microscopic observations were carried out to evaluate eventual changes in microstructural properties. From the images in Fig. 3 it is possible to observe that the morphology drastically changes during the initial drying process. The cells changed from rounded into shrunk and wrinkled structures within the first hour. Intense birefringence was observed during the complete drying process due to the presence of crystalline fiber structures (initially), and later by the presence of both fiber structures and saccharide crystals. However, it was not possible to clearly identify the critical drying time for the crystallization of the saccharides in the fruit.

## DISCUSSION

Loss of aroma during drying may be caused by thermal degradation or by volatilization.<sup>[5]</sup> Because of the low drying temperature (80°C) and the late occurrence in the process, the loss of aroma components in this experiment most likely cannot be attributed to thermal degradation reactions. Therefore, the most tangible explanation is that the loss is due to volatilization of the entrapped aroma. From Table 2, it is clear that this is a sudden event, occurring after 240 minutes of drying, most likely due to a rapid change in the microstructure.

A possible process is a rapid crystallization of sugars also present in the fruit. Crystallization would squeeze non-crystallizing material (potassium salts, proteins, gums, other carbohydrates, lipids, and aromas) into quite concentrated domains with poor entrapment capacity. Growing crystals can possibly also open closed biological structures, thereby contributing to aroma loss. Our intention with the microstructural investigation was to prove this explanation. However, it was not possible to obtain sufficiently clear evidence from these observations. We thus selected

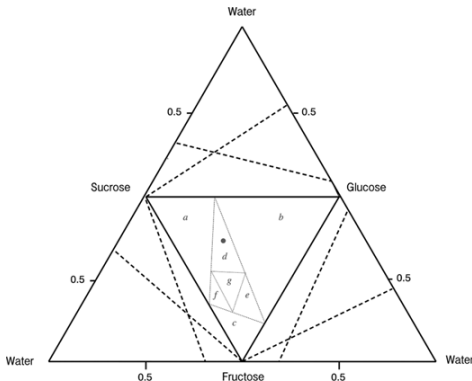


FIG. 4. Simplified phase diagram of sucrose-glucose-water, sucrose-fructose-water, and fructose-glucose-water at 30°C. The inner triangle represents the composition of the precipitated solid phase from a quaternary water-sucrose-glucose-fructose solution of a given sugar composition. The first precipitate is in area *a*, sucrose; *b*, glucose hydrate; *c*, fructose; *d*, sucrose and glucose hydrate; *e*, glucose hydrate and fructose; *f*, sucrose and fructose; *g*, sucrose, glucose hydrate, and fructose. The dot marks the composition of the saccharides in the *Vangueria infausta*. Redrawn after data from Pancoast and Junk<sup>[38]</sup> and Bubnik and Kadlec.<sup>[39]</sup>

a different strategy in order to analyze the possibilities of a microstructural change.

We assume that the system is a suspension of insoluble fibers in an aqueous saccharide solution. This simplistic

model of the fruit pulp can be compared with compositional data from Magaia.<sup>[36]</sup> The remaining water during the drying process is distributed between the fiber component and the aqueous solution phase. Assuming equilibrium between both these phases, we estimate the availability of water for keeping the saccharides dissolved from the water activity. The solubility of the saccharides composition of the *Vangueria infausta* (50% sucrose, 25% glucose, and 25% fructose<sup>[36]</sup>) is obtained by a comparison with the phase diagrams of sucrose, fructose, and glucose<sup>[37–39]</sup>, as redrawn in Fig. 4. By interpolating in the almost fully linear phase diagram and by using the solubility of sucrose, fructose, and glucose,<sup>[38,39]</sup> we could observe that the sucrose and glucose hydrates are the saccharides that are expected to crystallize first during the drying process. However, when the temperature has risen, the glucose hydrate dissolves and the solubility of glucose increases more rapidly than the solubility of sucrose, leading to an expansion of area *a* in Fig. 4. Thus, it can be expected that pure sucrose will be the first saccharide solid that will precipitate during the drying process.

The water availability in the saccharide solution phase is shown by the water activity. The water activity at the solubility limit of sucrose in the sucrose-fructose-glucose solution can be compared with the water activities during the drying process and can be used to estimate the likelihood for crystallization of the sucrose during the drying process.

The activity of water in the binary solutions of saccharides is used to obtain an estimation of the solubility and activities of the saccharides.<sup>[38,39]</sup> An extrapolation from binary systems into a quaternary water-sucrose-glucose-fructose system

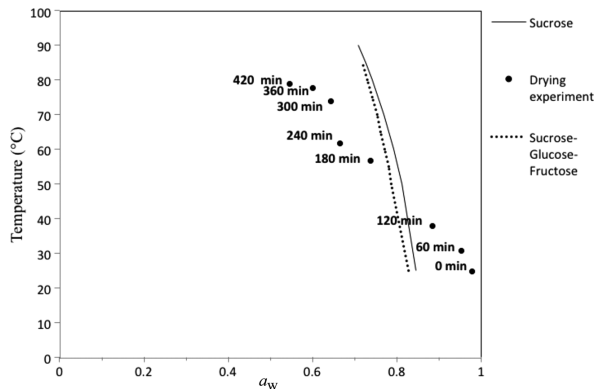


FIG. 5. Relationship between the water activity and sample temperature during the drying process of fruit pulp from *Vangueria infausta* (filled dots). The result is compared with the water activity at the phase boundary for crystallization of sucrose in water (using the  $a_w$  model by Starzak and Mahtlouthi<sup>[42]</sup> and solubility data from Bubnik and Kadlec<sup>[39]</sup>) and the water activity at the phase boundary for crystallization of sucrose from a solution of 50% sucrose, 25% fructose, and 25% glucose as estimated in the Appendix.



is used to estimate the water activity by numerically integrating the Gibbs-Duhem equations as described in the Appendix. The result of this estimation is a curve providing the water activity at the sucrose crystallization limit as a function of temperature. In Fig. 5, the water activity and temperature at the crystallization limit, as obtained from the procedure in the Appendix, are compared with the drying trajectory from our experiment (water activity and sample temperature during the drying process). As a reference, the water activity<sup>[38]</sup> and temperature at the phase boundary<sup>[39]</sup> of the binary sucrose-water system are included.

Figure 5 shows that the water activity initially is high (0.97) and that the temperature is low. After 60 minutes of drying,  $a_w$  starts to decrease and the sample temperature to increase. No crystallization can be expected yet. Gradually, during the progress of the drying process, the water activity decreases. When the drying trajectory passes the solubility curve, we may expect the system to crystallize. In Fig. 5, the data of the experimental drying trajectory is presented by the "dots in bold."

The results indicate that it is likely that sucrose crystallizes after about 120–180 minutes of drying and it is expected that crystallization may lead to loss of aroma during the drying process.<sup>[40]</sup> However, one may always speculate if the crystallization occurs or if the system remains super-cooled. It is possible that the presence of crystalline cellulose may act as a nucleation site and reduce super-cooling. It is also possible that there are concentration gradients that may initiate crystallization. On the other hand, it is also possible that soluble fibers may repress the crystallization, as this fraction is high.<sup>[12,41]</sup> The further evaluation of these effects is difficult, as the specific chemical nature (pectin, galactanes), as well as the solubilization at a molecular level (most likely low) of the fiber, is not known.

The alternative hypothesis is that the volatilization of the aromas is due primarily to the increasing sample temperature. During the progress of the drying, the temperature of the sample is increasing, as shown in Fig. 5 it reaches about 60°C at the moment of the sudden aroma loss at 240 minutes (Table 2). However, the different aromas correspond to quite different volatilities while the volatilization involves all components equally. Hence, although there is a volatilization contribution due to the increasing sample temperature, the results suggest that the main explanation for the sudden character of the loss event is due to a possible crystallization and microstructural change.

## CONCLUSIONS

This work shows the effect of convective drying on the aroma retention of fruit pulp from *Vangueria infausta* when subjected to isothermal drying at 80°C. The aroma level is well preserved during the first part of the drying.

After a removal of 90% of the water, corresponding to a water activity of 0.65, a loss of aromas has been observed. It seems likely that the crystallization of sucrose and the increasing object temperature are the most critical factors for the loss of volatile retention. A method to approximately predict crystallization during drying from sugar composition, water activity, and temperature is presented.

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## APPENDIX

### Estimation of the Water Activity at the Solubility Limit of Sucrose in the Ternary Mixture of Sucrose, Fructose, and Glucose Using the Gibbs-Duhem Equation

The Gibbs-Duhem Equation was used to estimate the water activity at the solubility limit of the sucrose in the quaternary mixture of water, sucrose, fructose and glucose. The starting point was the solubility limit in the binary systems.<sup>[39]</sup> At the solubility limit, the aqueous solution is in equilibrium with one pure solid phase, which is considered the reference condition of the sugars. The activities of the sugars in the binary solutions as a function of mole fraction water were obtained by a numerical integration of Eq. (2) using a recursion approximation (3) as suggested by Hempel et al.<sup>[41]</sup>:

$$x_s \cdot d \ln a_s = -x_w \cdot d \ln a_w \quad (1)$$

$$\ln a_s(x_w) = - \int_{\ln a_w(x_w)}^{\ln a_w(x_w)} \frac{x_w}{x_s} \cdot d \ln a_w \quad (2)$$

$$\ln a_{s,j+1} = \ln a_{s,j} + \frac{(\ln a_{w,j+1} - \ln a_{w,j}) \cdot (x_{w,j} + x_{w,j+1})}{x_{s,j} + x_{s,j+1}} \quad (3)$$

The water activities for sucrose at different temperatures were estimated using the equation suggested by Starzak and Mahtlouthi,<sup>[42]</sup> while water activities as a function of concentration at 25°C for glucose and fructose were obtained using the approximation by Miyawaki et al.<sup>[43]</sup>

By using the numerical integration of Eq. (3), the activity and the activity coefficients of sucrose were obtained as a function of concentration and temperature. For fructose and glucose, the activity and activity coefficients were only obtained at 25°C, and we thus assumed they were less temperature-dependent.

$$\sum_j^n x_{s_j} \cdot d \ln a_{s_j} = -x_w \cdot d \ln a_w \quad (4)$$

$$\ln a_w(x_w) = - \int \frac{\sum_j^n d \ln a_{s_j}(x_{s_j})}{\sum_j^n d \ln a_{s_j}(0)} \cdot \frac{\sum_j^n x_{s_j}}{x_w} \cdot \sum_j^n d \ln a_{s_j}(x_{s_j}) \quad (5)$$

$$\ln a_{w,i+1} = \ln a_{w,i} + \frac{(\sum \ln a_{s_j,i+1} - \sum \ln a_{s_j,i}) \cdot (\sum x_{s_j,i} + \sum x_{s_j,i+1})}{x_{w,i} + x_{w,i+1}} \quad (6)$$

$$a_{s_j} = \gamma_{s_j} \cdot x_{s_j} \text{ where } \gamma_{s_j} \sim \gamma_s(x_w) \quad (7)$$

The activity coefficients of the sugars in the mixture are assumed to be approximately equal to the activity coefficient in the corresponding binary mixture with the same mole fraction water, as suggested by Walstra.<sup>[44]</sup> Thus, the activity coefficient of the saccharides in the complex ternary mixture (0.5 sucrose, 0.25 glucose, and 0.25 fructose (mass fraction)) was obtained by using the approximation that the activity coefficient is determined by the mole fraction water and thereby can be obtained from the binary data obtained when Eq. (2) was solved as described in Eq. (7). The water activities are obtained by integrating Eq. (5) using the recursion procedure in Eq. (6) until the activity of 1 is obtained. The results are summarized in Table 3.

The approximations used in this estimation are obviously rather rough. However, having compared them with scattered experimental observations, we think the results are not too far from what would be obtained with stricter assumptions.

TABLE 3

The results of the numerical procedures aimed to obtain an approximate value of the sucrose and total solute concentrations and water activities when crystallization may be induced in a sucrose-glucose-fructose system. The composition of the dry matter is a mass fraction of 0.5 for sucrose, 0.25 for glucose and fructose, respectively. *C* is solubility in mass fraction, *X* solubility in mole fraction, and *a<sub>w</sub>* the predicted water activity

T	C <sub>sucrose</sub>	C <sub>solutes</sub>	C <sub>water</sub>	X <sub>sucrose</sub>	X <sub>solute</sub>	X <sub>water</sub>	a <sub>w</sub>
25	0.319	0.637	0.363	0.040	0.119	0.881	0.828
50	0.344	0.687	0.313	0.048	0.145	0.856	0.787
55	0.349	0.697	0.303	0.050	0.151	0.850	0.780
60	0.355	0.710	0.290	0.053	0.159	0.842	0.771
65	0.362	0.724	0.276	0.056	0.168	0.833	0.762
70	0.367	0.735	0.265	0.059	0.176	0.825	0.753
75	0.374	0.748	0.252	0.062	0.186	0.815	0.742
80	0.380	0.761	0.239	0.066	0.197	0.804	0.729
85	0.385	0.771	0.229	0.069	0.206	0.795	0.719



# Paper III



# Effects of drying with or without blanching on composition of volatiles in dried mango fruit (*Mangifera indica* L.)

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## Abstract

The aim of this study was to evaluate the effect of pretreatment method water and microwave blanching on the volatile compounds composition in mango (*Mangifera indica* L.). Blanching in water or microwave at 70 °C or 90 °C during 10 min or 2 min, respectively prior hot air drying at 70 °C. Gas Chromatography was conducted to determine the concentration of the aroma components. The principal substances to be followed in this study were selected based on literature: 1-butanol,  $\alpha$ -pinene, 3-carene, myrcene, limonene, terpinolene and ethyl butanoate.

The results showed an increased intensity of aroma after blanching. Method of blanching had less impact. Convective drying induced a considerable reduction of most of the aroma components particularly when the sample was dried below 0.65 in water activity. The reduction depends on the boiling points of the volatiles as well as on the polarity.

**Keyword:** Mango, blanching, microwave, volatile, aroma, retention.

## 1. Introduction

Mango is one of the most popular fruits in the world, mainly because of its nutritional value and pleasant flavour (Chiumarelli, Ferrari, Sarantópoulos and Hubinger, 2011). In food and beverage industry, aroma has become one of the most valuable attributes, not only to ensure the consumer acceptance but also to evaluate the food quality (Ruse, et al., 2012; Cano, Hernandez, and De Ancos,, 1997). However, it is well known that heat treatment has significant impact on heat sensitive components like ascorbic acid through degradation and volatiles through evaporation (Varming, Andersen and Poll, 2006; Sakho, Crouzet, and Seck, 1985; Vieira, Teixeira., and Silva, 2000). Available reports also show that inactivation of enzyme like lipoxygenase (LOX), hydroperoxidelyase (HPL) and alcohol dehydrogenase (ADH) is important because they may contribute aroma degradation (Pino, Mesa, Munoz, Martia and Marbot, 2005; Larsen, Poll, and Olsen, 1992). Cano and coworkers reported that the presence of peroxidase (POD) and polyphenoloxidase (PPO) activities inhibits the development of volatiles in several fruits and induce the loss of color due to the degradation of anthocyanins (Takeoka, Buttery, Teranishi, Flath and Guntert, 1991; Bai, Baldwin, Imahori, Kostenyuk, Burns and Brecht, 2011). However, some study indicates that in a majority of fruits when submitted to stress factor, such as UV light, osmotic stress and blanching, volatile compounds were rather developed then lost (Pino, 2012). It has been observed by Poll et al. that blanching may enhance the level of aroma in fruits. Studies on blanched apples and blackcurrant samples showed about 40% increasing of volatile components, especially of ethyl butanoate, butanol, terpineole and  $\alpha$ -pinene (Poll, Nielsen, Varming and Petersen, 2006). But, in a study on strawberry it was observed that neither water blanching nor microwave blanching increased the level of volatiles of the fruits (Escriche, Chiralt, Moreno and Serra, 2000; Zabetakis, and Holden, 1997).



Blanching is usually performed at 80-100 °C during 5-10 minutes using water, steam or using microwave heating (Takeoka, Dao, Rodriguez and Patterson, 2008). Blanching is aimed to significantly reduce microbiological activity and to reduce the enzyme activity (Escriche, Chiralt, Moreno and Serra, 2000; Hadi, Zhang, Wu, Zhou and Tao, 2013; Olle, Baumes, Bayonove, Lozano, Sznaper and Brillouet, 1998). In other hand, blanching process can also bring undesirable effects and losses of heat sensitive components such as vitamin C, carotenoids and volatile compounds (Pino, Mesa, 2006). This processing step is usually used prior to freezing or drying. High temperatures or long drying times in conventional air drying can also lead to further loss of flavour, color and nutrients (Varming, Andersen and Poll, 2004; Ansari and Ali, 2004).

Several studies about the volatile composition of mango either fresh or samples or after various pretreatments. The different authors found quite long lists of components, but only a few substances were more abundant than others. The different authors also used different extraction procedures with very different results. Majority of the authors appointed the ethyl butanoate as the most abundant aroma compound in mango (Buttery, Seifert, Guadagni and Ling, 1971; Pandit, Chidley, Kulkarni, Pujari, Giri and Gupta, 2009; Olle, Baumes, Bayonove, Lozano, Sznaper, and Brillouet, 1997; Macleod, Macleod and Snyder, 1988; Torres, Talens, Carot, Chiralt and Escriche, 2007; Rawson, Patras, Tiwari, Noci, Koutchma, Brunton, 2011; Rodriguez-Lopez., et al., 1999). Pino and coworkers reported 1-butanol,  $\alpha$ -pinene, myrcene, limonene and 3-carene as some of the most abundant volatiles in mango (Bai, Baldwin, Imahori, Kostenyuk, Burns and Brecht, 2011; Buttery, Seifert, Guadagni and Ling, 1971; Drouzas, Tsami, Saravacos, 1999; Baldwin, 2004; Beyers and Thomas, 1979). Other studies mention also the terpinolene as another expressive contributor to the mango aroma (Escriche, Chiralt, Moreno and Serra, 2000; Pandit, Chidley, Kulkarni, Pujari, Giri and Gupta, 2009; Sian and Ishak, 1991). Thus, based on data from literature we selected 7 substances with high odor activity as characteristic aromas and thereby identified as the target substances to be followed in our study. The substances were as follow: 1-butanol, alpha-pinene, 3-carene, myrcene, limonene, terpinolene, ethyl butanoate.

The aim of this work was to evaluate the effect of water and microwave blanching prior hot air drying on composition of volatiles in mango fruit (*Mangifera indica L.*).

## 2. Materials and methods

The reference materials pinene, 3-carene, myrcene, limonene, terpinolene, ethylbutanoate and 1-butanol were of analytical grade purchased from VWR International (Radnor, Pennsylvania, USA). Distilled water was obtained from a Milli-Q ionic exchange and carbon filter apparatus (Millipore, Bedford MA, USA). The solvents (ethanol, pentane and diethyl ether) and geraniol (internal standard) were of chromatographic purity from Merck, Darmstadt, Germany. The gases for GC were of 99.9% purity, from L'Air Liquide S.A, Paris, France.

## 3. Sample preparation for blanching and drying

Fresh, medium ripe mango (*Mangifera indica L.*) of osteen variety, purchased in a local market and stored at 8-10 °C, was sliced into 2 cm diameter and 0.5 cm thick pieces, and the samples were subjected to water or microwave blanching processes followed by hot air drying at 70 °C. The average moisture content and water activity of the samples was  $0.84 \pm 0.02$  g/g mango and  $0.983 \pm 0.002$ , respectively.

### 3.1 Blanching and drying processes

Screening tests were performed with 200 g of mango slices to obtain the experimental set up giving the target combination of time-temperature: 2 min at 90 °C (High Temperature Short Time - HTST) or for 10 min at 70 °C (Low Temperature Long Time - LTLT) and similar temperature histories for water and microwave blanching. The central point temperature of three mango cylinders was recorded every second during the heating processes using optic fiber (microwave) or thin-wire copper-constantan thermocouples (water) previously calibrated and connected to a data log.

Water blanching was carried out by immersion of a plastic strainer containing 200 g of sliced mango into thermostatic water bath Julabo Shake Temp SW23, with a 10000 mL volume of water at constant temperature (70 °C or 90 °C) for a corresponding blanching time (10 or 2 min, respectively).

Microwave blanching was performed using a domestic MW oven (Panasonic NE-C1453). The power generator for this unit operates at a frequency of 2450 MHz. The 200 g of sliced mango were placed over a net into the oven. A power of 800 W was applied for MW blanching at 90 °C for 2 min, while variable power was applied for the MW blanching at 70 °C for 10 min that is 1350 W for 1 min and 420 W for 9 min.

At the set-point temperature of 90 °C the mango samples reached a final temperature of 89 °C, while at the set point temperature of 70 °C, the reached final temperature was 70 °C, for both blanching methods at correspondent conditions.

After blanching the mango was cooled in iced water for 5 min to stop the residual enzymatic activity, weighed and air dried at 70 °C using an air circulation oven (Elektro Helios Garomat) with air velocity of 1 m/s 70 °C up to a water activity of approximately 0.6. The time required was determined in screening tests and validated by determination of water content and water activity of mango after drying. The average drying time was  $1.40 \pm 0.00$  for untreated samples,  $1.12 \pm 0.04$  h for microwave blanched mango and  $1.32 \pm 0.08$  h, for water blanched samples.

### **3.1.1 Analyses**

Water content was measured using AOAC Method 934.06 (AOAC, 2000). which involves drying the samples in a 80 °C vacuum oven at 100 mmHg until a constant weight is reached. Water activity was measured with a water activity meter at 24 °C (AquaLab Series 3 – Decagon).

### **3.2 Sample preparation and extraction of volatiles**

Volatiles were extracted from fresh, water and microwave blanched samples and dried mango. Humid samples were extracted as they were while dried samples were first rehydrated. About 2.3 g of dried sample was preliminarily hydrated with 10 ml of distilled water during 15 min. Then the sample was extracted with 40 ml of ethanol containing internal standard (geraniol, 2.3 mmol/l). The extraction was performed during 72 hours to allow for an effective penetration of ethanol. The suspension was centrifuged at 3000 rpm, during 15 min (AllegraX-15R, from Beckman Coulter Inc., Brea, California, USA). 20 ml of the obtained clear but yellow supernatant was collected and 20 ml of water was added to increase its polarity. The solution was then allowed to pass through a hydrophobic column containing bonded phase of desisopropyl atrazine 10% octadecyl (Scientific Inc., Austin, Texas, USA), which aimed to retain the hydrophobic volatile components and separate them from more hydrophilic components, essentially sugars, which may interfere on further analyses by formation of degradation products during the GC analyses. The column was cleaned with 5 ml of water to remove the reminiscent carbohydrates. Thereafter the column was rinsed with 15 ml of a mixture of pentane and diethyl ether (1:1) to release the aroma components. The amount of obtained extract was recorded. The cleaned, slightly yellow, extract was introduced in a 1.5 ml vial, crimped and taken to GC

analysis. The efficiency in terms of recovery of internal standard was estimated by comparing the concentration of added geraniol with the extraction solvent against the geraniol concentration. The total yield was estimated to be between 70-90% in controlled experiments.

### **3.2.1. GC analysis**

The aroma extract was analyzed using Gas Chromatography (GC) equipped with flame ionization detector. The GC apparatus was model Agilent Technologies 6890N (G1530), Network GC system, serial US10322054, purchased from J&W Scientific, USA. Samples were analyzed on a hydrophobic DB-225 column (30.0 m x 0.250 mm inner diameter, cross-linked cyanopropylphenyl-diethylpolysiloxane (50%), 0.25  $\mu\text{m}$  film thickness). Injector and detector temperature was 250°C. The oven temperature program started from an initial temperature of 60°C, was kept constant for 3 min at 1°C/min, and then increased at a rate of 5°C /min to 200°C. Helium was used as carrier gas and column flow rate was of 0.6 ml/min, pressure 55.2 kPa, and split ratio of 20:1 flowing at 12 ml/min. Hydrogen flow at 30 ml/min. The injector had a syringe size of 10.0 $\mu\text{l}$ , volume sample of 2.0  $\mu\text{l}$ , split less, was injected three times, and n-hexane was used to wash five times. The GC data was compared with that one from reference materials.

## **4. Results and discussion**

All the identified target substances were found in fresh samples: 1-butanol,  $\alpha$ -pinene, 3-carene, myrcene, limonene, terpinolene and ethyl butanoate. The identification is made by comparing the GC chromatograms of the sample with GC chromatograms of reference materials. The other peaks observed were not identified

The results given as concentrations of the fresh frozen samples are given in Table 1. The concentrations of the target substances vary between 300 and 800  $\mu\text{g/g}$  fresh matter.

The concentration of odor components varies strongly in the literature between 1 to 1000  $\mu\text{g/g}$  fresh matter (Vieira, Teixeira and Silva, 2000; Buttery, Seifert, Guadagni and Ling, 1971; Pandit, Chidley, Kulkarni, Pujari, Giri and Gupta, 2009; Olle, Baumes, Bayonove, Lozano, Sznaper, and Brillouet, 1997; Macleod, Macleod and

Snyder, 1988). The results in our study are in a comparable range with that found in the literature (table1), when using a comparable liquid extraction methodology.

Table 1. Concentration of selected aroma component in fresh-frozen samples of the *Mangifera indica* L. fruit. The error on the averages shows the standard deviation between the independent replicates of 5 different but comparable samples. The precision used is two value digits.

Aroma compound		Conc. ( $\mu\text{g/g}$ , FM; $\mu\text{g/g}$ DM)						literature
		F1	F2	F3	F4	F5	Avrg	
1-butanol	( $\mu\text{g/g}$ , FM)	330	320	280	300	260	300 $\pm$ 30	N/A
	( $\mu\text{g/g}$ , DM)	1800	2200	1600	1700	2100	1900 $\pm$ 260	
$\alpha$ -pinene	( $\mu\text{g/g}$ , FM)	830	620	590	900	770	740 $\pm$ 130	30-140 <sup>a</sup>
	( $\mu\text{g/g}$ , DM)	4500	4200	3400	5000	6200	4700 $\pm$ 1000	
3-carene	( $\mu\text{g/g}$ , FM)	250	200	240	250	250	240 $\pm$ 22	240-2050 <sup>b,a</sup>
	( $\mu\text{g/g}$ , DM)	1400	1400	1400	1400	2000	1500 $\pm$ 270	
myrcene	( $\mu\text{g/g}$ , FM)	250	200	240	240	240	230 $\pm$ 19	120-850 <sup>a</sup>
	( $\mu\text{g/g}$ , DM)	1300	1400	1400	1300	1900	1500 $\pm$ 260	
limonene	( $\mu\text{g/g}$ , FM)	250	240	300	350	280	280 $\pm$ 44	16-300 <sup>c, a</sup>
	( $\mu\text{g/g}$ , DM)	1400	1600	1700	1900	2200	1800 $\pm$ 300	
terpinolene	( $\mu\text{g/g}$ , FM)	290	240	310	290	250	280 $\pm$ 30	3-29 <sup>b</sup>
	( $\mu\text{g/g}$ , DM)	1600	1600	1800	1600	2000	1700 $\pm$ 200	
ethylbutanoate	( $\mu\text{g/g}$ , FM)	450	400	530	460	430	460 $\pm$ 48	N/A
	( $\mu\text{g/g}$ , DM)	2400	2800	3100	2600	3400	2900 $\pm$ 400	

Obs: The superscripted lower case letter indicates the reference from which the value has been taken: a – (Buttery, Seifert, Guadagni and Ling, 1971; b- Pandit, Chidley, Kulkarni, Pujari, Giri and Gupta, 2009; c- Olle, Baumes, Bayonove, Lozano, Sznaper, and Brillouet, 1997; F(1-5)- fresh sample; N/A - Not available.

Table 2. Concentration of aroma component identified on blanched samples of the *Mangifera indica* L. fruit. The precision used is two value digits.

Aroma compound	Conc. ( $\mu\text{g/g}$ , FM; $\mu\text{g/g}$ DM)				
		MW <sub>B2,70</sub> *	W <sub>B3,70</sub> **	MW <sub>B4,90</sub>	W <sub>B5,90</sub>
1-butanol	( $\mu\text{g/g}$ , FM)	730	400	850	740
	( $\mu\text{g/g}$ , DM)	5300	3500	6600	6800
$\alpha$ -pinene	( $\mu\text{g/g}$ , FM)	460	460	550	520
	( $\mu\text{g/g}$ , DM)	3400	4000	4300	4800
3-carene	( $\mu\text{g/g}$ , FM)	590	480	460	550
	( $\mu\text{g/g}$ , DM)	4300	4200	3500	5100
myrcene	( $\mu\text{g/g}$ , FM)	580	470	450	530
	( $\mu\text{g/g}$ , DM)	4200	4100	3440	4900
limonene	( $\mu\text{g/g}$ , FM)	730	510	520	600
	( $\mu\text{g/g}$ , DM)	5300	4500	4000	5700
terpinolene	( $\mu\text{g/g}$ , FM)	600	740	670	710
	( $\mu\text{g/g}$ , DM)	4300	6500	5200	6600
ethylbutanoate	( $\mu\text{g/g}$ , FM)	670	780	1120	830
	( $\mu\text{g/g}$ , DM)	4800	6900	8700	7700

\*MW<sub>B,70</sub> – microwave blanching at 70<sup>o</sup>C; \*\*W<sub>B,70</sub> – water blanching at 70<sup>o</sup>C;

B(1-5) –blanched samples.

Table 3. Concentration of aroma component identified on dried samples of the *Mangifera indica* L. fruit. The precision used is two value digits.

Aroma compound	Conc. ( $\mu\text{g/g}$ , FM; $\mu\text{g/g}$ DM)					
	D1	D2	D3	D4	D5	
1-butanol	( $\mu\text{g/g}$ , FM)	< 30	< 30	< 30	< 30	< 30
	( $\mu\text{g/g}$ , DM)	< 30	< 30	< 30	< 30	< 30
$\alpha$ -pinene	( $\mu\text{g/g}$ , FM)	580	< 30	860	620	1500
	( $\mu\text{g/g}$ , DM)	700	< 200	1040	760	1800
3-carene	( $\mu\text{g/g}$ , FM)	580	100	900	660	1050
	( $\mu\text{g/g}$ , DM)	700	120	1100	820	1300
myrcene	( $\mu\text{g/g}$ , FM)	560	100	870	640	1020
	( $\mu\text{g/g}$ , DM)	670	110	1060	790	1300
limonene	( $\mu\text{g/g}$ , FM)	600	< 20	1100	900	1640
	( $\mu\text{g/g}$ , DM)	720	< 20	1340	1100	2000
terpinolene	( $\mu\text{g/g}$ , FM)	540	< 30	780	670	960
	( $\mu\text{g/g}$ , DM)	650	< 30	950	800	1200
ethylbutanoate	( $\mu\text{g/g}$ , FM)	1010	2500	1450	1800	2100
	( $\mu\text{g/g}$ , DM)	1200	2900	1800	1270	3700

Obs: \* (< ...) indicate the detection limit of the component; D(1-5) –dried sample

The blanching has been performed in water (W) and in a microwave (MW) at 70°C for 10 minutes and at 90°C during 2 minutes respectively.

Figure 1 shows a GC chromatogram from the extract of a fresh-frozen sample (a) compared to a chromatogram from a blanched-frozen sample (b). The difference is obvious. The fresh sample has a wide range of components while the blanched sample has a few components at comparable high concentration. Figure 2 shows marked changes of the relative aroma concentration after blanching of mango's sample. The results show that the concentration of the characteristic aroma compounds increases

with the exception of  $\alpha$ -pinene that remains constant. There is an increase of about 100 to 300% after blanching. The results are summarized in Table 2.

The results were evaluated using multiple linear regression (MLR) using the assumption that the blanching and different variations of blanching can be treated as independent and additive effects. The analyses used the partial F-test as described in (Montgomery and Runger, 2010). The variance was assumed being constant throughout the experiment. Each analysis included 9 samples resulting in of 4 degrees of freedom. The evaluation was performed at each volatile analysed. The model is formalised as:

$$y = \beta_0 + \beta_1 \cdot x_1 + \beta_2 \cdot x_2 + \beta_3 \cdot x_3$$

where  $y$  is the concentration of the volatility in the sample (mg/g dry matter) and  $\beta_0$  is the concentration of the untreated sample (fresh, frozen) (in mg/g dry matter),  $\beta_1$  is the impact of blanching (change of concentration in mg/g dry matter),  $\beta_2$  is the impact of blanching temperature and time (change of concentration in mg/g dry matter) and  $\beta_3$  is the impact of blanching type (change of concentration in mg/g dry matter).  $x_1$  is 0 if the sample is not blanched and 1 if the sample is blanched,  $x_2$  is 0 if the sample is blanched at 70°C and 10 minutes and 1 if the sample is blanched at 90°C and 2 minutes,  $x_3$  is 0 if the sample is blanched in water and 1 if the sample is blanched using microwaves. The result of the analyses is shown in Table 4.

Clearly, with exception of  $\alpha$ -pinene, the effect of blanching is well noticeable on all volatiles. High temperature, short time increases the concentration of butanol and ethyl butanoate while the other aroma components do not display significant differences depending on the procedures. The method of heating does not display large variation in this system. The only observation is that MW heating leads to a somewhat lower increase of terpinolene compared to heating in water.



Table 4. Multilinear regression model of volatiles in mango and impact of blanching temperatures and methodology on the results. All data is given as  $\mu\text{g/g}$  dry matter.

Aroma component	Concentration in fresh frozen sample.	Effect of blanching.	Effect of blanching temperature	Effect of microwave instead of water blanching
	$\beta_1$	$\beta_2$	$\beta_3$	$\beta_4$
1-butanol	1900	2900**	2300**	(800)
pinene	4700	(-1300)	(900)	(-600)
carene	1500	2400**	(100)	(-700)
myrcene	1500	2300**	(100)	(-700)
limonene	1800	2800**	(0)	(-400)
terinolene	1700	2800**	(400)	-1800**
ethylbutanoate	2900	2700*	2300*	(-600)

\* 95% significance and \*\* 99% significance, ( ) marks insignificant values

It has previously been reported that heat treatment both may enhance as well as decrease the aroma in fruit (Poll, Nielsen, Varming and Petersen, 2006; Rodriguez-Lopez, et al., 1999). There are several attempts to explain the reason of increasing of aroma components after blanching process. Some authors suggest that enzymes may contribute to the release of aromas bound by hydrolyses of glycosidic bonds (Pino, Mesa, 2006). Heating is activating enzymes, partly as they are more active at higher temperatures and partly because of disruption of internal structures inside the cell (particularly the vacuole) as well as within the tissue. However when we reach the denaturation temperature we may expect a reduction of enzymatically catalyzed processes as well (Torres, Talens, Carot, Chiralt and Escriche, 2007; Reineccius, 2010; Raice, Sjöholm, Wang and Bergenståhl, 2014).

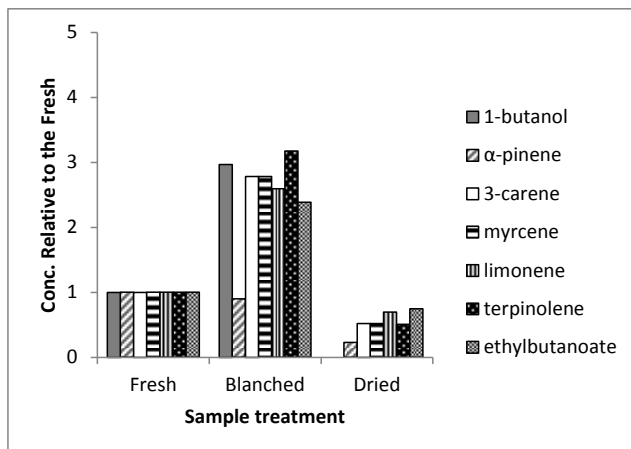


Fig. 2. Aroma components of mango identified in fresh, blanched and dried samples. The blanching was performed in W and in a MW both at 70 and 90°C during 10 and 2 minutes respectively.

However the scenario is drastically changing after hot drying of the samples. The figure shows clearly that the drying is decreasing majority of the volatiles previously presents in fresh-frozen or blanched-frozen sample.

The results (the relative concentration to after blanching, based on dry matter) as a function of water activity after drying are shown in Figure 3. It is clear that the extent of drying determines the loss. Majority of aromas are lost during the drying when water activity is below 0.65. Similar results has been reported for *Vangueria infausta* that lost majority of its volatile components after drying below  $a_w$  of 0.65, after prolonged convective drying (Raice, Chiau, Sjöholm and Bergenståhl, 2015).

The boiling point of the aroma (table 5) has a role for the volatility. Aroma components like 1-butanol (bp =118 °C) and  $\alpha$ -pinene (bp =155 °C) are very sensitive to evaporation and, simply, disappeared from the matrix. Components with a comparable high boiling point are retained to an higher extent. However the esters components constitute an exception seems to be more retained when compared with other components with a comparable volatility. The ethyl butanoate for example, still remain being the major component even after hot air drying processes. One possibility is that the retention of the ethyl butanoate could be due to its comparable high polarity (log P around 1.4) that may lead to more cohesion with the hydrophilic stationary matrix.

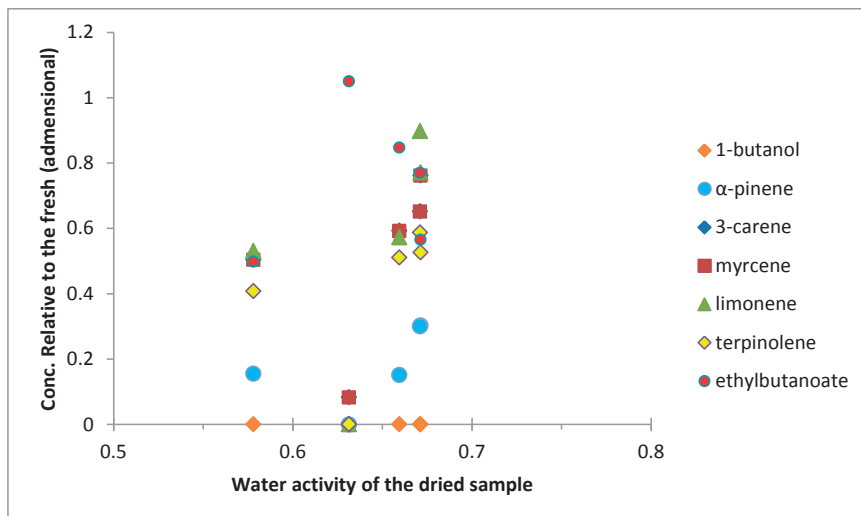


Figure 3. Relationship between water activity after drying and ratio between the concentration after blanching and after drying.

## 5. Conclusions

Blanching and drying has been evaluated upon the aroma development and retention of mango (*Mangifera indica* L.).

The levels of aroma components are increased if we allow the material to be blanched.

Neither water blanching and microwave blanching nor long time low temperature or high temperature short time made any dramatic difference on the impact of the blanching.

Hot air drying induced drastic reduction most of the aroma if the drying was prolonged below 0.65 in water activity.  $\alpha$ -pinene and 1-butanol were strongly affected due its volatility. On other hand the drying did not bring great effect on high boiling components that display significant retention even after extensive dehydration.

Unexpected high retention was observed for ethyl butanoate.

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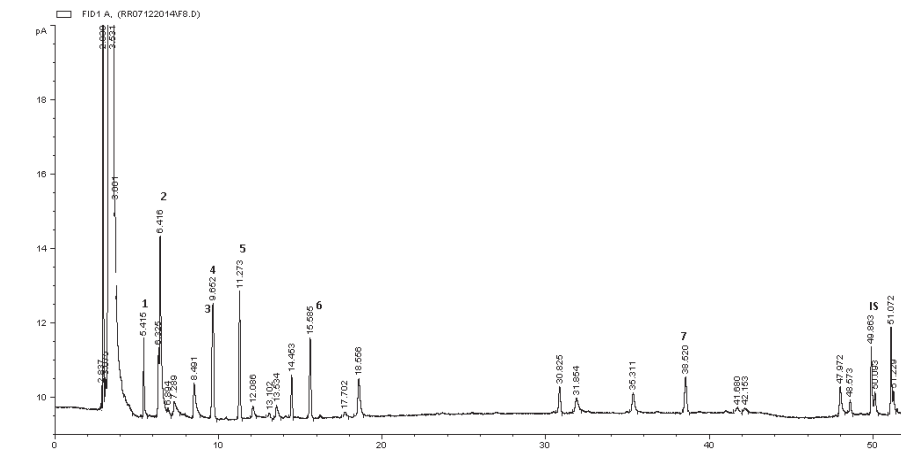
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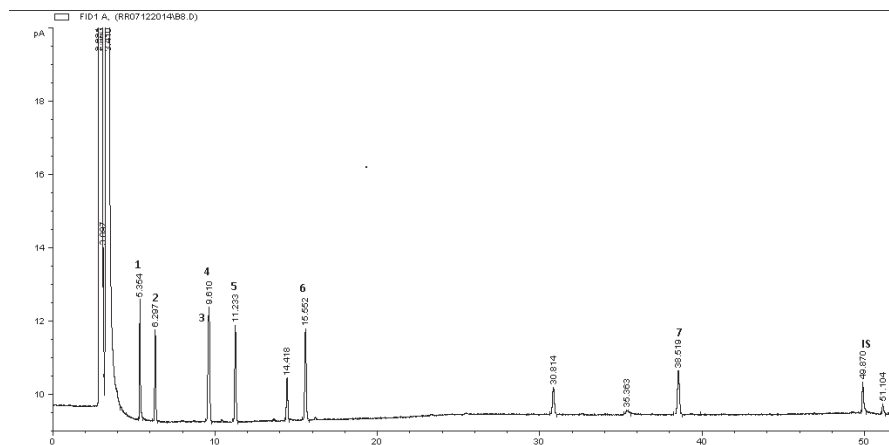
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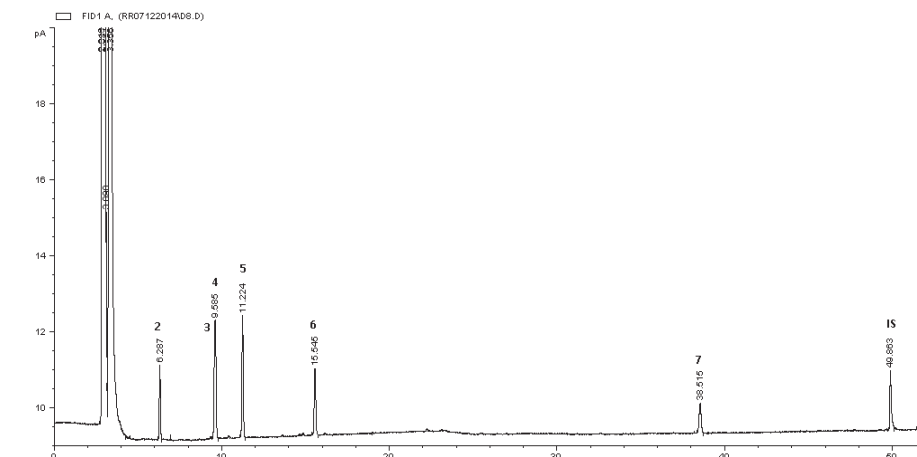
# Appendix



(a)



(b)



(c)

Figure 1. Examples of FID-GC chromatograms presenting the volatile components identified as well as unidentified peaks in mango samples obtained after three different pretreatment: (a) – frozen fresh sample; (b) – frozen blanched sample; (c) – frozen dried sample. The numbers are presenting the identified components: 1- 1-butanol; 2-  $\alpha$ -pinene; 3- 3-carene; 4- myrcene; 5- limonene; 6- terpinolene; 7- ethyl butanoate; IS- geraniol.

Table 5. Boiling points of the target substances identified on after blanching and after dried of mango (*Mangifera indica* L.).

Arom compound	1-butanol	$\alpha$ -pinene	3-carene	myrcene	limonene	terpinolene	ethylbutanoate
bp. (°C)*	118	155	168	167	176	174	121

\* Data obtained from (Reaxys, version 1.7.8; Elsevier; 2012).

# Paper IV



# **Crystallization of sugars in a drying matrix containing soluble and insoluble fiber - a model to understand the role of fibres on sugar crystallization and aroma retention**

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## Abstract

The study concerns the aroma retention inside of dried carbohydrates matrix due sugars crystallization. It has been conducted through a convective air drying and GC methods. Initially were designed three models based on aqueous solution of carbohydrates namely: A) pectin and sucrose, B) pectin, microcrystalline cellulose and sucrose and C) microcrystalline cellulose and sucrose. The reference materials of the most powerful aroma components of *Vangueria infausta* L. (hexanoic acid, ethyl hexanoate and ethyl octanoate) were added to each model. Thereafter each mixture (model) was heated in a water bath at 85°C, homogenized and kept in a cool place. The mixture was dried on an isothermal convective air-dryer at 80°C, 3 m/s during 60-420 min sampling after each time interval of 60 minutes to control physical properties (water activity and dry matter) prior to analysis of the aroma profile. GC analysis showed that experimental models composed by pectin, sugars, MCC, and aromas can predict the retention or loss of aroma components in an aqueous carbohydrate solution. Volatiles are well retained in systems with a high content of water soluble fibers while water insoluble fibers had a less powerful ability to contribute to aroma retention. There is a difference between different aroma components in their tendency to be lost during drying. The hexanoic acid tended to be less retained than esters with the same volatility. The models confirms that crystallization of sugars could partly contribute to the aroma loss although also models with extensive sugar crystallization still were able to preserve about 30% of the original ester content.

**Keyword:** *Vangueria infausta* L., pectin, microcrystalline cellulose, polysaccharides, volatile, crystallization.

## Introduction

The quality of processed fruits is based mainly in sensory and nutritional value. Therefore the appearance and characteristic flavor, aroma composition and concentration are important parameters to take into account during quality evaluation of the food (Bonazzi, 2011). It is well known that during food processing and preservation of food, especially fruits, many these attributes are lost or fading to an extent that it may reduce the acceptability of the fruits (Varming and Poll 2004).

It has been reported that high molar mass, high polarity reduce the relative volatility of aroma components plays a significant role on the extent of aroma loss or retention (Goubet et al., 1998, Rosenberg et al., 1990). Thus, there is a need to preserve the aroma components during processing. Carbohydrates like maltodextrins, gum arabic and modified starch among others, are suggested as encapsulating materials able to protect the volatiles from loss during processing (Shahidi and Han, 1993, Dziezak, 1988). It has been suggested that loss of aroma retention may be due crystallization of matrix forming carbohydrates (Roos, 1995, Deborah et al., 1996, Goubet et al., 1998, Rosenberg et al., 1990, Islesias and Chirife, 1978; Goubet et al., 1998). Some studies show that an amorphous glassy carbohydrate matrix may protect the material from further changes during storage time (Madene et al., 2006; Baristain et al., 2002; Bhandari and Hartel, 2005). Particularly, low water activity achieved by the drying process result in low mobility in the glassy state and prevent crystallization of sugars and protect the entrapped volatile compounds from evaporating (Bell and Hageman, 1994; Onwulata and Holoinger, 1995; Beristain et al., 2002; Jaya et al, 2004).

In a previous study of aroma loss during drying of the *Vangueria infausta* L. a tropical fruit growing in Mozambique and neighbor countries (Raice et al., 2014, 2015); It was shown that the aromas were well preserved during the initial phase of the drying (down to a water activity of 0.6) while the loss was extensive in the later phase (below a water activity of about 0.6). Based on these results, a hypothesis suggesting that the crystallization of saccharides is being one of the main determining factors of aroma loss. However, it has been observed that this fruit is quite rich in water soluble and insoluble fibers (Amarteifio and Maosase, 2006, Saka and Msonthi, 1994, Magaia et al., 2013ab). Thus it can be assumed that the crystallization of the sugars is delayed by the presence of the water soluble as well as the insoluble fibre fractions. To evaluate this hypothesis a model of a soluble fibre (modeled by pectin) and an insoluble fibre (modeled by microcrystalline cellulose (MCC)) together with sugars were allowed to form an encapsulating matrix of an aroma emulsion.

## **Material**

Pectin type LM-102 AS (low methoxylated) was obtained from CP Kelco through Caldic, Malmö, Sweden. Ethyl hexanoate, ethyl octanoate and hexanoic acid were of analytical grade purchased from VWR International (Radnor, Pennsylvania, USA). Microcrystalline cellulose was acquired from Kebo Lab (Stockholm, Sweden), commercial sugar (from local market, Lund). Purified water was obtained using a Milli-Q ionic exchange and carbon filter apparatus (Millipore, Bedford MA, USA). The solvents (ethanol, pentane and diethyl ether) and geraniol (internal standard) were of chromatographic purity from Merck, Darmstadt, Germany. The gases for GC were of 99.9% purity, from L'Air Liquide S.A, Paris, France.

## **Sample preparation**

Pectin, sugar and microcrystalline cellulose were mixed with distilled water to form a homogeneous system, afterwards 0.25 % of a mixture of aroma components (oil phase) were added to the three different models (“A”, “B” and “C”). The mixture of volatile (oil phase) was composed by hexanoic acid (98.8%), ethyl hexanoate (0.9 %) and ethyl octanoate (0.3 %). The oil phase was obtained by dilutions in two steps to reduce measuring errors as described below. Each model was firstly dried on a convective air-dryer at 80 °C, 3m/s prior to aroma analysis. The carbohydrate models were prepared as follow:

### **Model “A” (sucrose, pectin, water and oils.**

Sucrose (40 g) was added to of pectin (60 g) and mixed with 300 ml of water. The mixture was homogenized using a spoon and warmed in a water bath at 80-85 °C during 2 hours to allow a homogeneous system to be formed. The viscous mixture was thereafter kept in a fridge overnight. An oil phase consisting of a mixture of the major volatiles was prepared by mixing hexanoic acid (9.9 g), ethyl hexanoate (0.09 g) and ethyl octanoate (0.03 g). 1 g of oil phase was added to aqueous phase, mixed vigorously by spoon until the system appears homogeneous. The beaker containing the sample was covered by alumina foil and stored in the fridge until the drying experiment was performed within next two days.



## **Model “B” (sucrose, pectin, MCC, water and oils**

To form the aqueous phase 30 g of sucrose was added to 10 g of pectin plus 60 g of MCC and mixed with 120 ml of distilled water. The mixture was homogenized using a spoon and warmed in a water bath at 80-85 °C during 2 hours to allow a better dissolution and homogenization of the components. Then the viscous mixture was kept in a fridge overnight. At the same time, was prepared the mixture of oil phase composed of hexanoic acid (8.9 g), ethyl hexanoate (0.8 g) and ethyl octanoate (0.2 g). In order to dilute the mixture of oils, 1 g of the oil phase was added to 9 g of hexanoic acid. From this diluted mixture 0.9 g was transferred to the aqueous phase. The mixture was well mixed using a spoon until complete homogenization. The beaker containing the sample was covered by alumina foil and stored on fridge until the drying experiment held within next 48 hours.

## **Model “C” (sucrose, microcrystalline cellulose (MCC), water and oils.**

To form the aqueous phase 40 g of sucrose was added to 60 g of MCC and mixed with 120 ml of distilled water. The mixture was homogenized using a spoon and warmed in a water bath at 80-85 °C during 2 hours to allow a better dissolution of the components and further homogenization. The viscous mixture was thereafter kept in a fridge overnight. An oil phase was prepared by mixing volatiles composed of hexanoic acid (8.9 g), ethyl hexanoate (0.8 g) and ethyl octanoate (0.2 g). In order to further dilute the mixture of oils, 1 g of the oil was added to 9 g of hexanoic acid. From this diluted oil phase, 0.9 g was taken and added to the aqueous phase and mixed vigorously by spoon until it appeared homogeneous. The beaker containing the sample was covered by alumina foil and stored on fridge until the drying experiment held within next 48 hours.

## **Drying**

Seven samples with five replicates on each were weighed, introduced into a cylindrical mold and left to dry on a convective air-dryer at 80 °C, velocity of 3 m/s during 60, 120, 180, 240, 300, 360 and 420 minutes. Five samples were removed on each time interval. The temperature profile was controlled by using thermocouples (type K, 0.5 mm) connected to the hp Compaq S720 computer. Microscopy structure, dry matter (DM), water activity ( $a_w$ ) and texture were monitored after each drying time along of

the drying process in order to see eventual changes. The microscopy observation was done using an Olympus BX50, imaging source, 5x objective and polarizing light (from Shinjuku, Tokyo, Japan). DM was performed on a vacuum oven (Forma Scientific, Marietta, OH, USA), at 70 °C for 20 hours and then each sample was weighed on a Mettler AE160 analytical balance. The water activity ( $a_w$ ) was measured at 20 °C using AquaLab water activity meter S3TE, supplied by Decan Devices, Inc (Pullman, Washington, USA) calibrated using standard salt solutions (NaCl:  $a_w = 0.760$  M and KCl:  $a_w = 0.984$ ) standard deviation of  $a_w = \pm 0.003$ . (from Decagon Devices, Inc, Pullman, Washington, USA).

## **Microscopy observations**

Dried samples were observed on a microscope adjusted with polarized and not polarized light (Olympus BX50, imaging source, 5x objective and polarizing light purchased from Shinjuku, Tokyo, Japan). The sugars crystals are present early on the sample dried for 120 minutes and continuously become more pronounced as the drying time increases.

## **Extraction and evaluation of aroma**

Distilled water (20 ml) was added to a dried sample, mashed using a spatula and kept in repose during 30 minutes. Then 40 ml of the internal standard (geraniol, 2.3 mmol/l) was added and the mixture stirred during forty-eight hours to perform first step of extraction. The suspension of particles in aqueous ethanol solution was centrifuged at 3000 rpm, 15 min, 18 °C on an AllegraX-15R, from Beckman Coulter Inc., Brea, California, USA. The supernatant was used to extract the aroma components. The extraction was performed on triplicate of 10 ml each. Prior extraction the polarity of supernatant was increased by addition of 20 ml of water and then cleaned from saccharides (polar agents) using any hydrophobic column containing bonded phase of desisopropyl atrazine 10% (octadecyl (C<sub>18</sub>) purchased from Scientific Inc., Austin, Texas, USA). The column was cleaned with 10 ml of water to remove remaining saccharides before second step of extraction. The second step of extraction was carried out by washing the column with a non-polar mixture of pentane and diethyl ether (1:1 (V/V)) which removed the aroma components in same way as described on previous works by Pino and others (Pino, 2006; Raice, 2014).

## GC analysis

The aroma extract was analyzed using Gas Chromatography (GC) equipped with flame ionization detector. The GC apparatus was model Agilent Technologies 6890N (G1530), Network GC system, serial US10322054, purchased from J&W Scientific, USA. Sample was analyzed on a hydrophobic DB-225 column (30.0 m x 0.250 mm inner diameter, cross-linked cyanopropylphenyl-diethylpolysiloxane (50%), 0.25  $\mu\text{m}$  film thickness). Injector and detector temperature was 250°C. The oven temperature program started from an initial temperature of 50°C, was kept constant for 3 min, and then increased at a rate of 3°C /min to 200°C, over 54 min. Helium was used as carrier gas and column flow rate was of 0.6 ml/min, pressure 55.2 kPa, and split ratio of 20:1 flowing at 12 ml/min. Hydrogen flow at 30 ml/min. The injector had a syringe size of 10.0 $\mu\text{l}$ , volume sample of 2.0  $\mu\text{l}$ , split less, was injected three times, and n-hexane was used to wash five times. The experimental GC data was compared with the data from standards.

## 11.4 Results and discussion

The drying of the models of aroma encapsulating systems (A- sucrose, pectin, water and aroma; B- sucrose, pectin, microcrystalline cellulose (MCC), water and aroma oil and C- sucrose, MCC, water and aroma) was performed using a convective oven at 80°C, 3m/s. The main aroma components were analyzed.

The soluble fibre system (model A) aqueous pectin-sucrose mixture the aromas are not lost when the drying time is increased although a slight reduction during the first hours of drying is observed (fig. 1a-c). The insoluble fibre, MCC, model C display a significantly decreasing concentration of aroma during first two hours of drying. In particular, the amount of hexanoic acid is lost within 120 minutes. Thereafter the volatiles do not show a further reduction for the rest of process (Fig. 2a). On model B the combined presence of soluble and insoluble fibre (pectin and MCC) the aroma loss proceeds under 300 minutes and the extent is less than in the insoluble fibre situation. (Fig. 3a).

During the drying the water content, water activity decreases as the drying time expands in all models. The loss of aroma during drying is assumed to primarily be caused by volatilization of the entrapped aroma (Coumans, et al., 1994, Bonazzi, et al., 2011, Varming, et al., 2004) partly driven by the crystallization of sugars often present in the fruit. Crystallization would squeeze non-crystallizing material (salts, proteins, carbohydrates, lipids and aromas) into quite concentrated domains with poor entrapment capacity (Roos, 1995). Growing crystals can possibly also open closed

biological structures, thereby contributing to aroma loss. As the crystallization of the sugars is a consequence of increasing sugar activity, that is related to reduced water activity, the thermodynamic driving force for sugar crystallization can be compared by following the aroma loss as a function of water activity (fig. 2a-c). We may here observe that the entrapment in the system dominated by the water soluble fiber, pectin, we observe a very strong encapsulation down to very low water activities, with both the water soluble and insoluble fibers present the encapsulation is powerful but we are still able to retain aromas down to values around 30% of the original concentrations for the esters while the acid is lost up to 90% when we reach very low water activities (a relative humidity of 0.2). In the system

In the model with only the water insoluble fiber present ( C ) we notice a complete loss of the hexanoic acid at a water activity of 0.6 while the esters only are retained to about 20-30% of the original level.

From the appearance it is clear that the model with soluble fiber do not crystallize, while the model with the insoluble fiber crystallizes quite strongly. The hexanoic acid is lost completely and thereby follows the expectations. However, the esters, behave different and are retained to a much larger extent. A possibility is that this is a consequence of different volatility. Here the volatility is defined as the tendency of a substance to vaporize (pass from liquid phase to vapor) when its vapor pressure is in equilibrium with atmospheric pressure at given temperature.

In figure 3 the volatility as a function of temperature is given for the different aroma components. According with the figure 3, the hexanoic acid has about similar volatility as the two esters and so that different volatility could not be the reason of loss. But, as suggested by Goubet (Goubet et al., 1998), the size of molecules may have an influence on diffusion of the compound from a matrix. For a small molecules like the hexanoic acid  $M_w=116$  g/mol (if compared with ethyl hexanoate,  $M_w=144$  g/mol and ethyl octanoate,  $M_w=172$  g/mol) can possibly more easily diffuse throughout through the structure and escape from the matrix leading to loss of the aroma components. Other possible explanation could be that the high polarity of the hexanoic acid makes it less retained in comparison with the ethyl hexanoate and ethyl octanoate that has relatively low polarity.

If the results of the models are compared with real fruits it may be observed that the fruits tend to retain the aroma better down to a relative water activity of about 0.65 while the models lost about 50% of their aroma content (with the exception of the extreme model A). Below the relative humidity of 0.65 the retention of the fruits were much lower than what we observed in the models suggesting that there are aspects of the plant tissues that were not included in the model. It can be the cell structure and it can be lipid material present in the cells that may contribute to differences in the properties.

## **Conclusions**

Experimental models composed by pectin, sugars, MCC, and aromas can predict the retention or loss of aroma components in an aqueous carbohydrate solution.

Volatiles are well retained in systems with a high content of water soluble fibers while water insoluble fibers had a less powerful ability to contribute to aroma retention.

There is a difference between different aroma components in their tendency to be lost during drying. The hexanoic acid tended to be less retained than esters with the same volatility.

The models confirms that crystallization of sugars could partly contribute to the aroma loss although also models with extensive sugar crystallization still were able to preserve about 30% of the original ester content.

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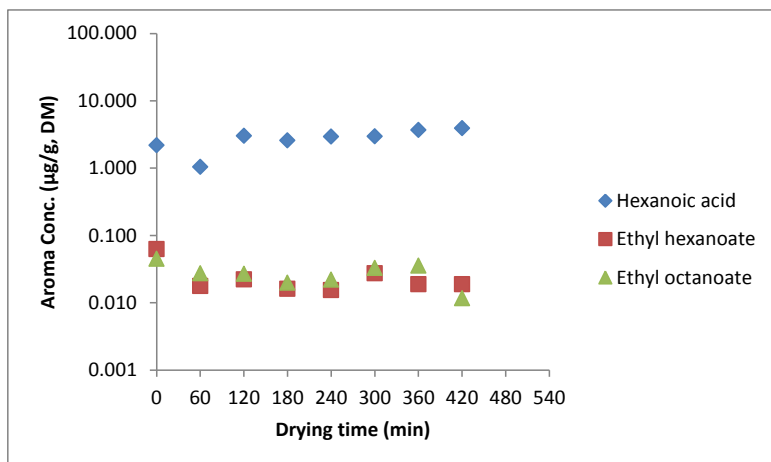
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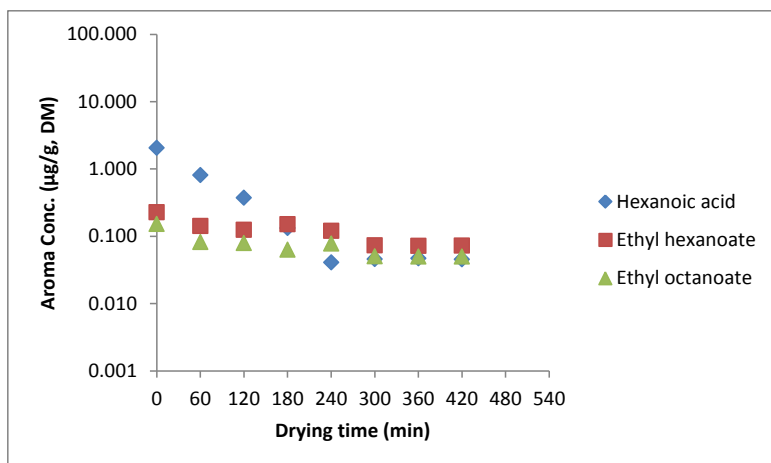
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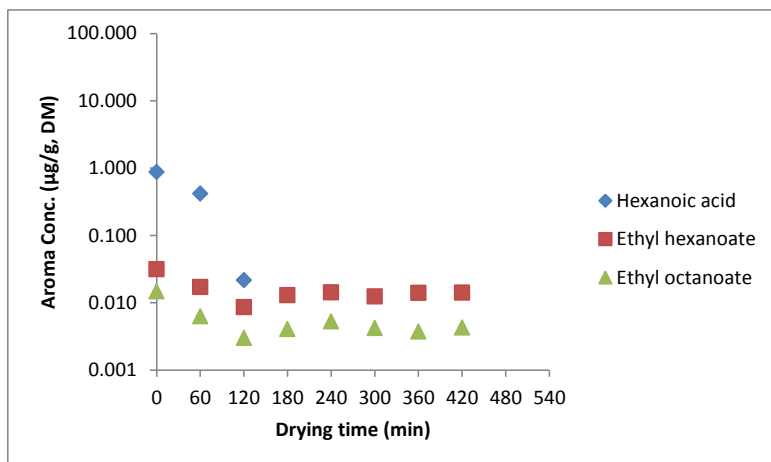




1a)

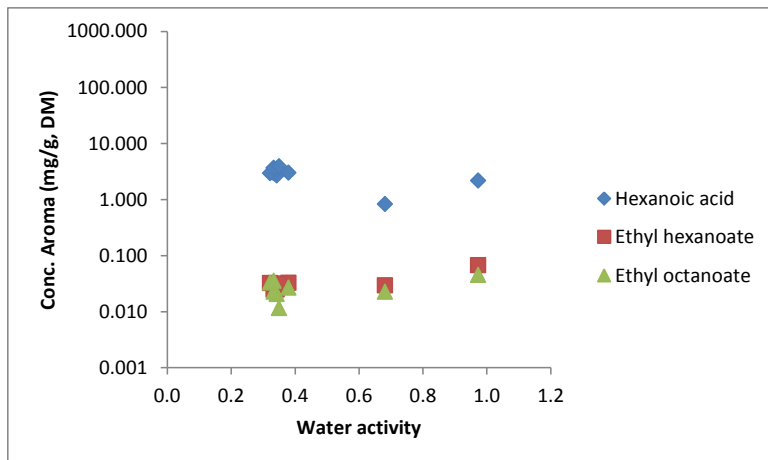


1b)

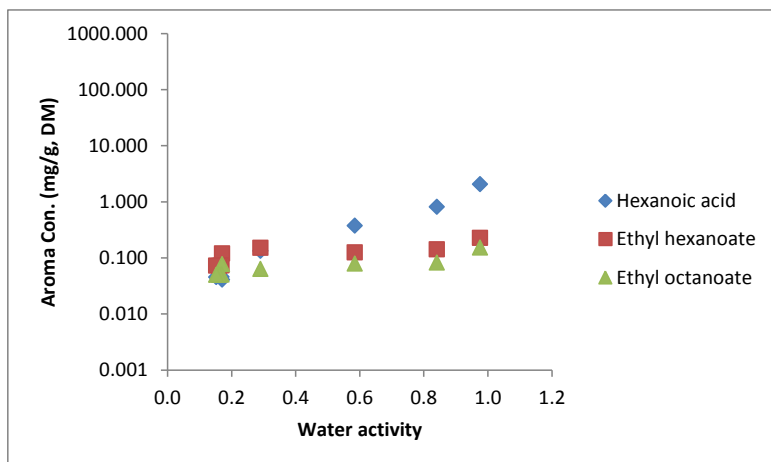


1c)

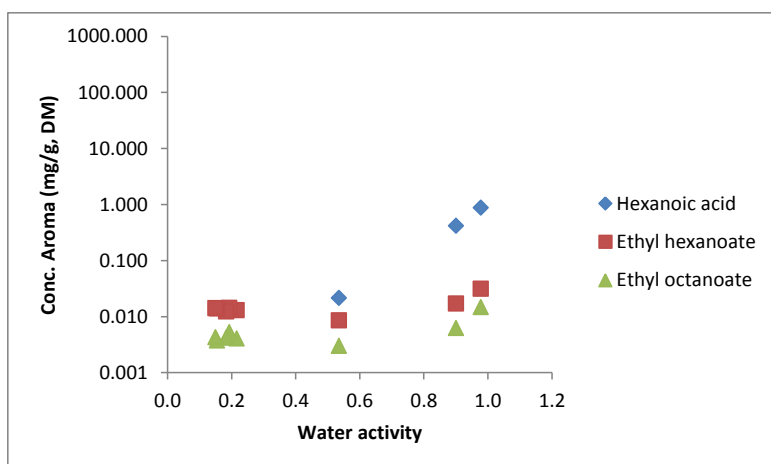
Fig. 1. aroma retention shown as a function of time; (1a) – Model sample composed by pectin, sucrose, water and aromas; (1b) – Model sample composed by pectin, MCC, sucrose, water and aromas; (1c) – Model sample composed by MCC, sucrose, water and aromas.



2a)



2b)



2c)

Fig. 2. aroma retention shown as a function of  $a_w$ .; (2a) – Model sample composed by pectin, sucrose, water and aromas; (2b) – Model sample composed by pectin, MCC, sucrose, water and aromas; (2c) – Model sample composed by MCC, sucrose, water and aromas.

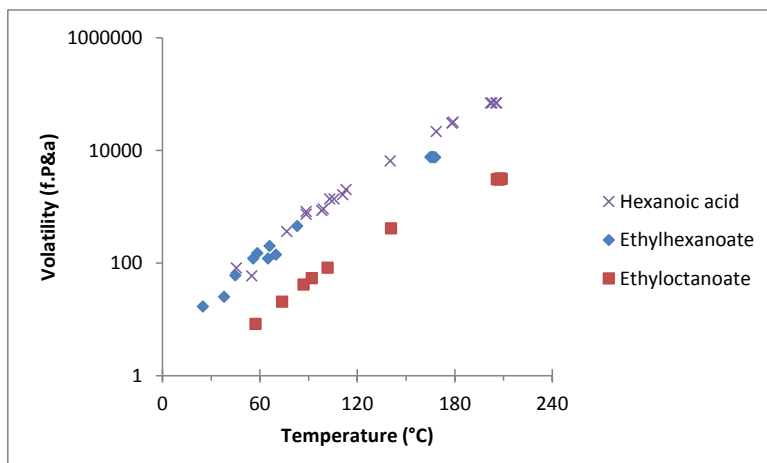


Fig. 3. A prediction of volatility of the aroma components presents in models A, B and C. The data set for Pressure and T has been collected from Reaxys. The activity was obtained from our experiment.



Fruits are a key complement to the diet of many people in the southern region of Africa. They are source of dietary fibre and sugars and have a high micronutrient content (minerals and vitamins). Also fruits contain aromas which are important for palatability and consumer interest for the food. African medlar (*Vangueria infausta* L.) is one of the most commonly found fruits in south eastern Africa, particularly in Mozambique. It is play an important role in the diet and gastronomy of the rural communities. The common names are African medlar



(in English) or maphilwa in ronga (one of the local languages in southern Mozambique). The fresh fruit is sweet and tastes like medlar (*Mespilus sp.*), although with some similarities to green apple and pineapple. The fruit can be eaten fresh, cooked or dried. It's used also to prepare juice, puddings and marmalade. The taste and aroma profile of African medlar are attractive and appreciated.

This study concerns the identification and characterisation of aroma components of fruits, and evaluation of the effect of heat treatment and drying on loss of aromas of fruits. The investigation included developing a procedure to extract aroma components from the fruit, a purification, separation, identification and quantification. The results in this thesis could help design a better strategy for aroma isolation, characterisation and explain the aroma entrapment. The study can be used to design a strategy for sustainable utilisation of wild fruits growing in Mozambique.

