

# QUALITATIVE ANALYSIS OF THE EXTRACTIVES OF ORANGE WOOD

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**Abstract.** Orange production is the third largest agricultural activity in São Paulo state, Brazil, but unfortunately, the orange orchards there have been facing phytosanitary problems. As a result, orchards are being eradicated by burning which may not be the most appropriate way of dealing with this potentially useful biomass. A better approach might be to extract the valuable chemicals from the orange wood, but little information exists on the nature of the extractive components and their chemical potential. Accordingly, the aim of this study was to determine the qualitative chemical composition of orange wood extractives. Four solvent systems were used for this work (acetone, ethanol:toluene [1:2], dichloromethane, and water at two different temperatures), 221 different chemical components were extracted, and 33 components were identified in the extracts using gas chromatography coupled with mass spectrometry. The ethanol:toluene system extracted the most compounds (48.5% of the total) and the dichloromethane extracted the least (16.3% of the total). Based on a literature review of potential applications, 79% of the identified chemical components in the extractives have potential use in the chemical, pharmaceutical, and/or food processing area. This is important information for the future of orange wood utilization and warrants further studies related to the quantitative analysis of the compounds.

**Keywords:** Chemical compounds, extractives, gas chromatography, orange wood.

## INTRODUCTION

According to the Ministry of Agriculture in Brazil, the Brazilian citrus sector is responsible for 60% of the world's production of orange juice and is a lead exporter of the fruit. São Paulo, Brazil,

and Florida, the United States are the two largest processed orange producing regions in the world (FAO 2003). Furthermore, FAO (2014) indicates that Brazil had 680,324 ha of harvested area while the United States had 239,493 ha of harvested areas, both in the year 2014.

Orange cultivation is the third agricultural income of São Paulo State, accounting for 80% of the

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Brazilian production of this fruit (CitrusBr 2011). According to Porto et al (2013), the production area of the orange crop there is around 465,000 ha.

Unfortunately, because of the low market demand for orange juice over the last few years (Neves 2010), some orange producers have chosen to leave the fruit in the orchard which has caused irreversible damage to the trees because of phytosanitary problems. Eventually, this damage leads to the complete eradication of the orchards.

The main phytosanitary issue regarding orange trees is connected to “greening” (Huanglongbing), which is a disease caused by the bacterium *Candidatus liberibacter* spp. transmitted by the psyllid *Diaphorina citri* (Ministério da Agricultura, Pecuária e Abastecimento 2009). “Greening” affects the vascular system of the plant and is so prevalent among orange trees that it has been estimated that 13 million plants per year are destroyed because of it (Neves 2010). According to Porto (2013), crop eradication accounted for the loss of about 37,000 ha in 2013/2014.

Currently, infected orange biomass is burned as a means of disposing of and this is problematic. For example, Bortolan (2012) and Fundecitrus (2013) have highlighted the producers’ issues in obtaining the environmental authorization to burn the biomass. Specifically, according to these researchers, the producers need several documents justifying that the material needs to be burned. Other problems associated with combusting the biomass are that it is not the best approach with regard to environmental quality and value. One potential, noncombustion use of the wood would be for panel and board products (Orwa et al 2009), but it is not clear if the necessary permitting could be guaranteed or if the wood could be appropriately sanitized. Clearly, and in summary, what is needed is a convenient alternative to processing the biomass that, preferentially, can also yield profit.

One possibility for using the orange biomass in a noncombustive manner is to remove the useful chemicals from it before disposal. Such an approach could potentially allow for the recovery of value-added chemicals. For example, D-limonene

is a natural oil contained in the rinds and seeds of citrus fruits and it is obtained through a steam extraction unit (Citrosuco 2013). D-Limonene is already used as an insecticide, fertilizer, solvent, repellent, glue and an aroma, and recovering it from orange wood could potentially bring value to it.

In accordance with the above, the main objective of this work was to identify solvent extractable chemical components of orange wood. The wood (specifically, the trunks) was readily available from a local supplier and it represents the largest part of orange biomass on a weight basis. In an effort to maximize the type of chemicals extracted, several common and readily available solvent systems were examined including organics of varying polarities as well as water (polar) at two temperatures, 20 and 75°C.

The technique employed to analyze the solvent extracts was gas chromatography coupled with mass spectrometry (GC/MS) because it is highly specific and universal for the analysis of volatile, synthetic, and/or natural compounds (Ferreira 2011). It is also possible to obtain qualitative and quantitative information from GC/MS, (Dias 2008). However, only qualitative work was done in the study. Quantitative analysis using separate standards, as common for GC/MS, will be the subject of a future communication from this laboratory.

With the exception of the water solvent, the orange extracts were directly injected into the GC/MS. However, to examine the chemicals extracted from the orange wood by the water, solid-phase microextraction (SPME) sampling was used to avoid injecting water into the GC/MS which can cause damage to it and/or result in poor separations. SPME is based on the equilibrium partitioning of analytes present in the sample with a solid-phase fiber (Nicolli 2009). The SPME apparatus consists of a fused silica fiber coated with a thin layer of a polymer absorbent or an immobilized liquid (Spietelun et al 2013).

After the identification of the extracted orange components, they were evaluated for their potential use according to information found in the literature.

## MATERIALS AND METHODS

### Material

Orange trees (*Citrus sinensis*) destined for eradication were collected from plantations belonging to the Citrosuco company located in the region of Bebedouro, São Paulo State, Brazil. Samples discs between 10 and 5 cm in diameter were removed along the orange tree trunk, processed in a hammer mill and sieved to 40 mesh particle size. The wood possessed a lignin content of 22% and an average moisture of 10%. All samples were stored in closed plastic bags at room temperature (20°C) in the laboratory.

### Organic Solvent Extractions and Analysis

The wood samples were extracted in triplicate with acetone, dichloromethane, and ethanol:toluene (1:2) solvent systems following TAPPI (2007) standard T-204 cm-07. One gram of each sample was refluxed in a Soxhlet apparatus for 6 h.

After the extractions, the solutions were concentrated in a rotary evaporator at a temperature of 80°C and under vacuum. The initial solvent volume of 150 mL was reduced to 15 mL.

Analyses of these extracts were performed on an Agilent Technologies GC/MS. The instrument used a HP-5MS UI column which was 30 m × 0.25 mm and 0.25 mm in diameter. Helium was used as carrier gas. The temperature program of the oven was from 40 to 230°C with heating of 6°C/min for a total of 33.67 min per sample.

The extracts were injected into the GC/MS manually, and 3 µL volumes were utilized.

Chemicals were identified by comparing the mass spectra of the chromatographic peaks to those in the NIST 2012 database.

### Water Extractions and Analysis

To obtain the extractives that were soluble in water, 2 g samples, considering the MC, were used in triplicate for each treatment and extractions

were performed for 6 h with mild agitation. Two water temperatures were examined: 20 and 75°C. After the extractions, the sample was filtered through a qualitative Grade 1 filter paper.

Before GC/MS analysis, the SPME fiber was conditioned for 20 min at the GC/MS injector at 250°C. It was then cooled to room temperature and then placed into direct contact with 150 mL of the water solution in a 400-mL jar at room temperature. The SPME fiber was equilibrated for 2 h. After this time, the fiber was inserted into the injector of the GC/MS and maintained until the end of the analysis. The program used for the GC oven was a ramp of 40-230°C at a rate of 6°C/min.

## RESULTS AND DISCUSSION

### Extraction and Analysis

Considering all used solvents, a total of 221 chemical compounds were extracted from the orange wood, and a portion of them are shown in Table 1. It should be noted that not all of the 221 compounds could be readily identified with the NIST mass spectral database, so accordingly, only those compounds with NIST library hit (probability) factors greater than 63% are shown in Table 1. Given the complexity of the chromatograms (ie the number of compounds and the degree of coelution) and the signal-to-noise ratio of the mass spectral data, this hit factor represents the floor for a reasonable confidence of prediction. Note that the calculations were done using peak areas.

It is noteworthy, but not unexpected, that only components with boiling points lower than ca. 230°C were observed overall. This temperature was the final temperature used in the GC/MS program and thus, higher boiling point fractions were not observable. In addition, it should be pointed out that a few compounds in the chromatograms—compounds such as acetic acid, acetone, and the larger-chain carboxylic acids—might arise from the fragmentation of thermally labile compounds in the extracts. Further work on the extracts using a nonthermal analysis technique is necessary

Table 1. Chemical compounds identified by chromatography using different solvents.

Chemical compounds	Retention time	Solvents <sup>a</sup>									
		1		2		3		4		5	
		b	c	b	c	b	c	b	c	b	c
Acetone	1.61	—	—	—	—	—	—	82%	2X	85%	X
Methylene chloride	1.75	—	—	—	—	—	—	94%	X	—	—
Acetic acid	2.13	81%	57X	88%	X	—	—	76%	X	79%	X
2-Propanone, 1-chloro-	2.19	—	—	71%	X	—	—	—	—	—	—
Tetrahydrofuran	2.33	—	—	91%	X	—	—	79%	X	—	—
Hexanal	4.53	—	—	—	—	—	—	81%	X	79%	X
2-Pentanone, 4-hydroxy-	5.61	86%	X	—	—	—	—	—	—	—	—
2-Pentanone, 4-hydroxy-4-methyl	6.10	92%	X	—	—	—	—	—	—	—	—
Benzaldehyde	8.21	—	—	—	—	—	—	77%	X	81%	2X
Butanoic acid	8.92	—	—	—	—	—	—	71%	X	77%	X
Pentanoic acid	8.96	—	—	—	—	66%	X	—	—	—	—
Hexanoic acid	9.85	88%	X	—	—	—	—	—	—	—	—
1,1-Ethanediol, diacetate	10.47	64%	X	—	—	—	—	—	—	—	—
Glycerin	11.20	94%	X	—	—	—	—	—	—	—	—
Sorbic acid	12.11	74%	X	—	—	—	—	—	—	—	—
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.94	—	—	—	—	96%	X	—	—	—	—
2-Furanmethanamine, tetrahydro-	15.74	—	—	—	—	73%	X	—	—	—	—
Nonanoic acid	15.89	—	—	—	—	—	—	87%	2X	77%	X
2-Propanol, 1-(2-butoxy-1-methylethoxy)-	16.26	84%	X	—	—	—	—	—	—	—	—
Ether, hexyl isopropyl	18.00	—	—	—	—	—	—	86%	X	—	—
N,N'-Diacetylenediamine	18.01	—	—	—	—	—	—	—	—	75%	X
Methyleugenol	18.82	—	—	—	—	—	—	72%	X	63%	2X
Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	21.47	—	—	—	—	—	—	78%	X	76%	X
Azelaic acid	24.43	77%	X	—	—	—	—	—	—	—	—
Xanthoxylin	24.72	76%	X	—	—	—	—	70%	X	—	—
Isoelemicin	24.78	73%	X	—	—	—	—	—	—	—	—
Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	25.50	73%	3X	—	—	80%	X	—	—	—	—
4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	25.92	87%	2X	—	—	89%	X	—	—	—	—
Tetradecanoic acid	26.72	72%	X	—	—	—	—	—	—	—	—
Benzeneacetic acid, 1,1-dimethylethyl ester	27.29	63%	X	—	—	—	—	—	—	—	—
Pentadecanoic acid	28.45	82%	X	—	—	—	—	—	—	—	—
n-Hexadecanoic acid	29.47	68%	53X	65%	X	68%	16X	—	—	—	—
Scoparone	29.91	92%	2X	—	—	93%	X	—	—	—	—

<sup>a</sup> Solvents: 1-acetone, 2-dichloromethane, 3-ethanol:toluene(1:2), 4-water in 20°C, 5-water in 75°C. b—NIST confidence (hit) level, c—relative quantity. Note that because of the lack of calibration curves, only the data in the rows should be compared. Also, the water entries have not been adjusted for the fiber/solvent partitioning factor of the solid-phase microextraction (SPME) device so they should not be directly compared with those from the solvent extractions.

to determine the identities of these compounds and/or their parents, and this will be the subject of future work.

For all 221 compounds, 48.4% of them were extracted with the ethanol:toluene (1:2) solution, 33.5% with water at 75°C, 30.8% with water at 20°C, 25.3% with acetone, and 16.3% with dichloromethane. However, for the list of identified compounds shown in Table 1, 7% of them were extracted with the ethanol:toluene (1:2) solution, 27% with water at 75°C, 36% with

water at 20°C, 55% with acetone, and 4% with dichloromethane. The larger number of total compounds for the ethanol:toluene solution is because of the polarity combination of the two solvent components, where ethanol is polar and toluene is apolar.

Water, a polar solvent, was used to extract compounds with similar polarity. As can be seen in Table 1, there was very little overlap between the compounds extracted by the water solutions vs the other, lower-polarity organic solvents. It

should be pointed out, however, that SPME was used in the analysis of the extractions in which water was used as the solvent. In this way, it was possible to identify these higher-polarity compounds.

### Value-Added Use

The potential applications of the compounds identified in this study are shown in groups in Fig 1. These groups were created by examining the applications of the chemicals based on the published literature including Hodgman (1963) and databases as PubChem (2004) and The Good Scents Company Information System (2015).

The groups in Fig 1 consist of their use in chemistry, eg as organic synthesis agents or solvents (Chem and Chemical in Fig 1); food processing as flavoring, preservatives, or packaging (Food in Fig 1); and in pharmaceuticals as preservatives in cosmetics, aromas for perfumes, and components of medicines (Pharm and Pharmaceutical in Fig 1). Note that these compounds can be used in one or more applications.

As shown in Fig 1, 79% of the extracted components identified in Table 1 have potential applications, in which 18% of them have specific application in chemistry and 15% have both

chemical and pharmaceutical applications. As an example, Mackay et al (2009) explains the potential use of some of the identified chemical compounds such as acetic acid, furfural, and vanillin. In general, these uses could be considered an improvement over simply burning the biomass, which, of course, is a way of adding value to this material.

As shown in this work, the orange biomass has chemical potential beyond combusting it. However, technology will be required to extract and utilize the chemicals which may be expensive to develop and use. However, studies are advancing in this area to overcome these limitations, so the removal and use of these value-added compounds are a tangible possibility. Clearly, there is a need for further studies to explore the scaling-up of the extraction of the components as well as the economies of this potential avenue.

### CONCLUSIONS

In conclusion, using GC/MS and with the assistance of SPME, 221 chemical compounds were extracted from the orange wood, and 33 of them were identified with high confidence. Ethanol: toluene (1:2) solvent extracted the larger number of total compounds; however, acetone extracted the larger number of identified

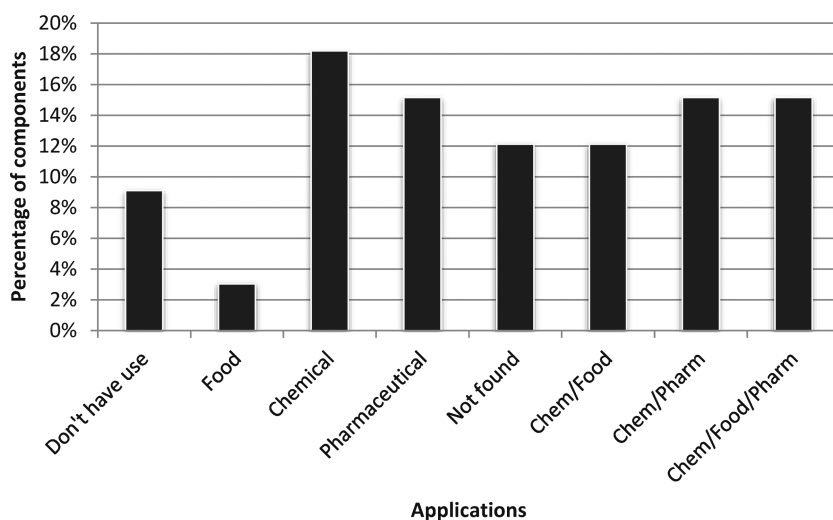


Figure 1. Classification of extractives according to the literature.

compounds. Water was used to extract more polar compounds than any of the organic solvents studied. Finally, 79% of the components have application in the chemical, pharmaceutical, and/or food processing areas according to the literature.

Future work in this area includes determining the identities of the unknown compounds and their value-added uses, if any. In addition, tests on the extracts and the compounds will be performed to examine their antioxidant and antimicrobial activities. Finally, future work will examine the utility of orange wood as a feedstock for pyrolysis.

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