Analysis of cherry flavour using gc-ms

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Cherry is one of the most important fruits globally and a non-climacteric stone fruit, mainly grown in temperate climate countries. The most important factors that contribute to the uniqueness of cherry include skin colour, sweetness (sugar content), sourness (organic acid content), fruit firmness, fruit weight and aroma. The compounds that contribute to the final aroma of cherry represent a very small portion, only 0. 01% – 0. 001% of the fruit fresh weight, but have a substantial impact on its quality (Zhang et al., 2007; Vavoura et al., 2015). Aroma is one of the most valuable attributes of cherries which may affect the consumer acceptance of the fruit and is a result of a complex mixture of chemical compounds, such as esters, alcohols, aldehydes, organic acids, ketones, terpenes, *etc.* (Valero and Serrano, 2010). According to the literature, most of the studies examined cherry fruit have used various techniques for extraction and analysis of the compounds. These methods include static and dynamic head space analysis, supercritical CO_2 extraction and solid-phase micro extraction (SPME) combined with gas chromatography-mass spectrometry (GC-MS) (Bernalte et al., 1999; Malaman et al., 2011; Vavoura et al., 2015). Scientists used these have conclude that SPME with GC-MS is the simplest, most rapid and effective method to analyse fruit volatiles (Zhang et al., 2007; Li et al., 2008; Vavoura et al., 2015). This review will examine the existing researches on the compounds present in cherry fruit that contribute to its flavour and on the methods that applied to obtain them.

Origin of cherry

Cherry fruit belongs to the Rosaceae family, which also includes other fruits such as peaches, apricots and plums. Cherries are available in many species, but two of them are selected for human consumption, the sweet cherry which is a direct descendant of the wild cherry *Prunus avium* and the sour cherry *Prunus cerasus* . Those two species differ largely in taste and thus they are considered to be separate species (Wen et al., 2014). A ripe cherry fruit has bright shiny pale to deep red or purple colour with very thin peel, but there are some cultivars that produce yellow fruit. The colour, aroma, taste and health properties of cherries have made them very popular and greatly appreciated. Sweet cherries are cultivated mainly for fresh consumption because they are highly perishable and have short fruiting seasons. Although, they are processed into jam, juice and wine (Revell, 2008; Wen et al., 2014).

Wild cherry is originated from Europe, Northwest Africa, Western Asia, from the British Isles south to Morocco and Tunisia and east to Southern Sweden, Poland, Ukraine, Caucasus and northern Iran (Revell, 2008).

Cherry flavour

Flavour is the sensation produced by a material taken in the mouth and perceived principally by the chemical senses of taste and smell. The sense of taste is detected by five basic tastes on the human tongue which are sweet, bitter, sour, salty and lately discovered umami taste (Taylor and Mottram, 1996). According toFisher and Scott (1997), the resulting flavour of fruit are a blend of the sweetness due to sugars such as glucose, fructose and sucrose and the sourness of organic acids, such as citric and malic acids.

However, it is the aroma of the different volatile components of fruits that allow us to distinguish among them. Flavour of each fruit is a complicated area, as every attribute is a result of specific interactions between various compounds present in fruit like sugars, phenolics, organic acids and more specialised flavour compounds including an extensive range of aroma volatiles (Tucker, 1993). The differences in the type and proportion of these compounds produced have an impact on the distinctive flavour and aroma of a particular fruit. The concentration of these constituents which included in cherries shows a fluctuation and this may be the source of flavour variations between the individual fruit and each cultivar (Bernalte et al., 1999).

Flavour compounds present in cherries can be complex but the majority of them are relatively simple molecules which are volatile and contribute to the fruit's odour and aroma. These two terms are usually misinterpreted and it is important to distinguish them in order to be fully understood. Odour is the smell of food before the consumption and is perceived orthonasally, whereas aroma is the smell of food during consumption in the mouth and is sensed retronasally (Revell, 2008). These compounds, as it was mentioned before, are analysed by SPME method coupled with GC-MS.

Volatile Analysis

Gas chromatography-mass spectrometry analysis demands extraction of the aroma volatiles from cherries to create a sample suitable for injection to the instrument. The most widely applied techniques for the extraction of volatiles are solvent extraction and solid phase micro extraction (SPME). The dominant factor that determines the selection of the type of solvent is the polarity of the volatiles. Therefore, it is apparent that polar volatiles require a polar solvent like methanol, while non-polar volatiles require organic solvent like hexane. Especially, asLi et al. (2008)underlines, a non-polar solvent is suitable for the key volatiles of cherry flavour. Furthermore, a known or quantified internal standard is absolutely necessary to enable quantification of the other compounds, as the area of different peaks from various volatiles in the cherry sample will be compared with the peak area of the known internal standard. As a result of this, the polar compounds such as acids and sugars end up in the water phase whereas the volatiles in the hexane layer. Centrifugation is crucial to separate the polar and non-polar compounds. After the application of centrifugation, the hexane layer which is formed in the top of the solution is removed and analysed GC-MS. GC-MS analysis uses only a small quantity (1μl) of the volatile sample which is injected into the instrument via a hot region which evaporate the liquid. The resulting gas including various volatiles is swept on the chromatographic column with the aid of a carrier gas (usually helium). The increasing temperature of the column provokes the compounds to leave the gum lining, where they are deposited initially, and enter the carrier gas flowing through the chromatographic column. The compounds with the lowest boiling point pass through the column first. This separates the aroma volatiles before they enter the ionisation and detection in the mass spectrometer (Revell, 2008).

Volatile Compounds

During the last decades, extensive research has been done on different cherry varieties from various countries in different periods of fruit development. A recent study found out a total of 18 compounds in cherry fruit classified into the groups: alcohols, aldehydes, ketones, hydrocarbons/terpenes and esters were identified and semi-quantified using 4-methyl-2-pentanone as the internal standard for the GC-MS analysis (Vavoura et al., 2015). Almost all these compounds have been previously identified in fresh sweet cherry fruit (Serradilla et al., 2012; Zhang et al., 2007; Bernalte et al., 1999; Mattheis et al., 1992; Girard and Kopp, 1998).

Many studies have shown that carbonyl compounds, specifically aldehydes, ketones and esters, are some of the most significant compounds of sweet cherry fruit aroma (Girard and Kopp, 1998; Mattheis et al., 1992; Zhang et al., 2007; Bernalte et al., 1999). Matsui (2006)has identified that 2-hexenal and hexanal, which are carbonyl compounds, give green leafy notes in the fresh cherry fruit and for this reason are known as " green leaf volatiles" with low perception threshold. The results fromVavoura *et al.* (2015)showed that 2-propanone was the most abundant volatile compound identified in all four cherry cultivars that they examined; Lapins, Canada giant, Ferovia and Skeena followed by 2-hexenal and acetaldehyde. The carbonyl compounds thatVavoura *et al.* (2015)identified were linear and aromatic and the most abundant was 2-propanone followed by 2-hexenal and acetaldehyde. Moreover, Vavoura *et al.* (2015)found that carbonyl compounds showed the most abundant signals present in sweet cherry aroma.

In contrast with these results, Serradilla et al. (2012)found that alcohols are the most abundant compounds present in sweet cherry, which include linear, aromatic and branched compounds. The most abundant among them was (*E*)-2-hexen-1-ol and also the main alcohol found in Picato type and Sweetheart sweet cherries in Spain. Furthermore, along with (*E*)-2-hexen-1-ol, hexanal and 2-hexanal are important compounds which are related with green notes and fresh green odours associated with vegetables and fruits. Girard and Kopp (1998)have also underlined that these compounds are predominant flavour volatiles in cherries. The only alcohols thatVavoura et al. (2015)identified were 2-Hexen-1-ol and benzyl alcohol present in the Skeena cultivar and thus they are used as a marker to distinguish this cherry cultivar from the others.

There are other minor components which contribute to the aroma profile of cherry cultivars such as esters (methyl-2-hydroxybenzoate), alkenes (2-methyl-1, 3-butadiene) and terpenes (D-limonene) (Vavoura et al., 2015; Serradilla et al., 2012). Although, studies on strawberry and kiwifruit showed that esters compounds were the important aromas of the fruits because they have low perception threshold and high aroma value of these compounds (Perez et al., 1996; Li et al., 2002).

According toVavoura *et al.* (2015), the most representative compounds in the Skeena cultivar were C_6 and aromatic compounds. Furthermore, in many studies the content of C_6 compounds and aromatic ones are the most representative class of compounds (Mattheis et al., 1992; Zhang et al., 2007; Sun et al., 2010).

Girard and Kopp (1998)studied 12 sweet cherry cultivars from the same orchard and identified 50 volatiles with the combination of two techniques dynamic headspace and gas chromatography, (E)-2-hexenol, benzaldehyde, hexanal and (*E*)-2-hexanal were predominant compounds which could be used to segregate commercial and new cherry selections into various subgroups. Similarly, Sun *et al.* (2010)conducted their study in order to determine the aroma-active compounds present in five sweet cherry cultivars from Yantai region in China. A total of 52 volatiles were identified, among these were hexanal, (*E*)-2-hexenal, 1-hexanol, (*E*)-2-hexen-1-ol, benzaldehyde, and benzyl alcohol. Also, they suggested that hexanal, (*E*)-2-hexenal, (Z)-3-hexenal, nonanal, benzaldehyde and geranylacetone are responsible for the green, orange, almond and floral notes of the cherry fruit (Sun et al., 2010). In a similar study, Zhang *et al.* (2007)using the same techniques identified 37 volatiles in sweet cherries in China. Especially, reported that hexanal, (*E*)-2-hexen-1-ol, (*E*)-2-hexenal, benzaldehyde, ethyl acetate and hexanoic acid ethyl ester were the characteristic aroma volatiles of sweet cherry fruit. Moreover, they examined the various developmental cherry periods and concluded that the optimal harvest time of sweet cherry was at the commercial stage (Zhang et al., 2007).

According toReineccius (2006), cherry flavour changes across its developmental stages as it cannot be identified in the primary stages of the fruit formation but grows during a brief ripening period. During this period, metabolism of the fruit changes to catabolism and hence the flavour development starts. This is obvious as carbohydrates, lipids and amino acids are enzymatically converted to simple sugars or acids and volatile compounds.

All the previous studied had focused on the volatiles compounds which are in a free form but the aroma of cherries might also come from non-volatile glycosidically bound precursors. These aroma precursors have been extensively examined in a wide range of fruits such as blackberries, mangos, pineapples, strawberries, kiwifruit, oranges and grapes (Fan et al., 2009;

Chyau et al., 2003; Garcia et al., 2011). As for the cherry, in a recent study, a total of 97 volatile compounds were reported. The groups of the chemicals compounds which were found, were alcohols, aldehydes, acids, esters, ketones, terpenes, norisoprenoids, furans, phenols and benzenes. The majority of these constituents have been previously identified, as it is mentioned before, in fresh sweet cherries. Of the 97 compounds, most of them were in a free form while 13 of them were glycosidically bound. In addition, 20 terpenoid compounds and 7 norisoprenoids were reported. One important thing that has to be taken into account is that many of these compounds such as citronellol, nerol, geraniol, γ-geraniol, (*E*)-isogeraniol, (*Z*)-isogeraniol, 1, 1, 6-trimethyl-1, 2-dihydronapthalene (TDN), (*E*)-1-(2, 3, 6-Trimethyl-phenyl)buta-1, 3-diene (TPB) are identified for the first time in cherries. In contrast to the free volatiles, which were predominantly aldehydes and alcohols, the bound volatile profiles were slightly different. The most abundant compounds were benzyl alcohol, geraniol and 2-phenyl-1-ethanol, followed by 3-methylbutanoic acid and 3-methyl-2-buten-1-ol. In terms of sensory evaluation, the free volatile compounds illustrated a fresh green, citrusy and floral aroma while the bound volatiles were odourless in the fresh fruit (Wen et al., 2014).

Conclusion

To sum up, extensive research has been done on identification of volatile compounds in cherry fruit but the techniques that have been applied to obtain and identify the volatiles are limited. Therefore, our research is intended to examine different cherry varieties both commercially available and from farmers. The methods that will take place for the extraction of the volatiles from the cherries are liquid-liquid extraction, solvent-assissted flavour evaporation technique or most commonly known as SAFE method and headspace solid phase microextraction as in the previously mentioned studies. The results from these techniques will then be identified by gas chromatography-mass spectrometry method (GC-MS) and gas chromatography-olfactometry analysis (GC-O). Then, a preliminary aroma reconstitution experiment will be conducted in order to be created a “ juice” that resembles the organoleptic properties of original cherry juice after a quantification of the concentrations of the identified predominant aroma compounds. This experiment have been previously achieved in other fruits, such as strawberries but not in cherries (Prat et al., 2014).

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