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Title: Analytical and Sensory Evaluation of Hop Varieties

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The German hop Hallertauer, hallertauer is highly valued because of its "noble aroma", but it has a very low yield when cultivated in the U.S. Two new crosses of Hallertauer, U.S.D.A. 21455 and 21459, have high yields and promising aroma characteristics.

In order to predict sensory properties of beers as a function of the varieties of hop used in brewing, a more complete sensory and chemical characterization of hop oils is necessary.

The aims of this study were to compare the aroma profiles of the new crosses against the German variety by determining their most important odor active compounds, and correlating the sensory attributes evaluated by a descriptive sensory panel (DSP) with the odor intensities detected during the gas chromatograph (GC) effluent

detected during the gas chromatograph (GC) effluent sniffing. Oxygenated fractions were spiked into spring water and evaluated by the DSP. The same samples were injected into the GC and the effluents were evaluated quantitatively and qualitatively by four subjects using a special data collection device. Samples were then analyzed by mass spectrometry (MS). There were no significant differences among the three varieties based upon the DSP results and the "aromagrams" obtained during the sniffing of the GC effluents. Important odor active peaks were associated with humulene oxidation products. A number of statistical correlations existed between the sensory attributes and the odor active peaks. In summary, the new varieties are potential contributors to "noble aroma". Trials with beers brewed with these hops are underway in order to establish their contribution to beer flavor.

Analytical and Sensory Evaluation of Hop Varieties

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TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF TABLES (continued)

ANALYTICAL AND SENSORY EVALUATION OF HOP VARIETIES

INTRODUCTION

The addition of hops to beer goes back to the Germans in the 12th century, but the "hoppy" aroma character only became popular in the 19th century. Historically, hops are used in brewing to add bitterness to the finished beer, as well as a unique and distinctive "hoppy" aroma.

While the brewing value of hops can be easily defined as a function of their level of alpha acids, the compounds responsible for beer bitterness, opinions differ greatly on the value of hop constituents responsible for the beer hoppy aroma. One of the problems in the definition of the value of hop aroma compounds is that most of the components of the hop oil, mainly terpene and sesquiterpene hydrocarbons, are scarcely found in beer and are not considered responsible for hoppy aroma in beer.

It has been demonstrated that the chemical composition of hops depends on varietal, seasonal and climatic conditions. Studies on hop oils have shown marked differences among varieties (Lam et al., 1986a; Peacock et al., 1980, 1981b; Buttery and Ling, 1967; Likens and Nickerson, 1967; Peppard et al., 1989) demonstrating that

certain components or ratios of components of hop oil are highly specific for individual varieties.

Many brewers consider the major contributors to beer flavor to be the alpha acids; however, some breweries import the traditional low alpha acid European varieties, Hallertauer mittelfrüher, Hersbrucker, and Tettnanger, for their "noble" aroma character. Traditional European noble aroma hops are low in both yield and alpha acids. No references were found in the literature describing what noble aroma is. Generally it is associated with spicy, herbal characters in beer, but it has different meaning for each brewer.

For several years hop research in the United States has been oriented toward finding a substitute for the traditional noble aroma hop. Hop breeders are conducting extensive research looking for varieties with high yields, noble aroma and disease resistance. In 1983 at the Oregon Agricultural Experiment Station, a tetraploid Hallertauer was crossed to nine aroma type male hops. Five selections were identified in 1985 as having aroma properties similar to their parent Hallertauer mittelfrüher. Two of these selections (U.S.D.A. 21455 and 21459) were particularly close in characteristics to Hallertauer. These two varieties are genetically seedless triploids, early to medium early in maturity with an indicated yield potential at least twice that of their female parent (Haunold and

Nickerson, 1987).

Brewers have expressed the desire to have a method for characterizing hop varieties in order to select varieties that impart the desired sensory attribute to the beer before conducting large scale brewing trials. Many systems of hop identification, based on botanical description, have been established. In all cases variation within-plants and among-plants is substantial, a factor that frequently leads to overlapping of varietal traits.

Since the advent and utilization of gas chromatography, much has been learned regarding the composition of hop oil. The oils of different varieties displayed good varietal uniformity under an array of environmental circumstances (Likens and Nickerson, 1967). This forms an adequate basis upon which to build a reliable system of varietal identification based on oil analysis. As a result of progress in analytical techniques our knowledge of hop oil and beer has advanced tremendously. But it is still necessary to study their relationship to sensory analysis, as this is the ultimate criterion by which the overall sensory quality of the product may be judged. For this reason, a sensory/instrumental correlation approach is extremely important to this problem.

Some attempts have been made to correlate sensory

attributes with chemical composition of beer. Some of them include sniffing of GC effluent in order to determine which compounds account for the most noticeable character of the product (Fors and Nordlov, 1987; Fukuoka and Kowaka, 1983), while others involve determination of thresholds of the many compounds identified (Meilgaard, 1975a,b; Clapperton et al., 1978). Others deal with the use of sophisticated statistical techniques such as multivariate analysis in order to relate the chemical measurements with sensory measurements. In most cases the correlation was done between the sensory attributes and the area of peaks in the chromatogram. But it was not reported if those peaks were odor active or not. Here is where the sniffing of the GC effluents play the most important role.

In dealing with GC effluent sniffing, Acree et al., 1984, developed a technique named CHARM where the odor activity of a series of sample dilutions is determined. In that sense CHARM is founded in the measurement of the relative gas phase detection thresholds of individual chemicals. But as stated by Pangborn (1981), it is necessary to remember that thresholds and odor potency are not synonymous. The information collected is valid probably at threshold levels but does not necessarily reflect the behavior of the compounds in the real solution.

This study is concerned with the chemical and sensory characterization of the hop oil (only the oxygenated fraction) of the new experimental varieties (U.S.D.A 21455 and 21459) and a noble aroma hop, Hallertauer halleratuer. Only the oxygenated fraction was studied because it has been stated that the compounds responsible for the hoppy aroma in beer are oxidized products of the hydrocarbons that constitute 90 % of the hop oil, and these oxidized compounds are commonly found in beer (Howard and Stevens, 1959; Nickerson and Likens, 1966; Tressl et al., 1978a,b; Peacock et al., 1980; Peacock and Deinzer, 1981a; Lam et al., 1986a).

The objectives of this study are:

1- To characterize quantitatively and qualitatively sensory properties of the oxygenated fraction of the new experimental varieties (U.S.D.A. 21455 and 21459) and a noble aroma hop (Hallertauer, hallertauer) by Descriptive Sensory Panel (DSP) analysis.

2- To characterize quantitatively and qualitatively the sensory properties of each one of the odor active peaks sniffed through the Gas Chromatograph (GC) effluent.

3- To identify chemically each one of the odor active compounds detected during the GC effluent sniffing, using GC- Mass Spectrometric (GC-MS)

analysis.

4- To correlate the descriptive sensory data with the GC effluent sniffing data in order to determine which compounds are responsible for the sensory attributes of the samples as a whole.

5- To differentiate chemically and sensorially the hop varieties analyzed by comparing the chemical and sensory profiles determined during the evaluation of the samples by a DSP and GC-MS.

6- To study individual sensory responses to the same stimuli.

7- To establish the advantages, disadvantages and limitations of the GC sniffing technique.

LITERATURE REVIEW

A- HOP CHEMISTRY

1- Hop and hop oil chemistry

The chemical composition of hops has been reported as follows (Verzele, 1986) :

1- α-Acids: humulone, cohumulone, adhumulone, prehumulone, posthumulone.

2- ß-Acids: lupulone, colupulone, adlupulone, prelupulone, postlupulone.

3- Essential oil

4- Polyphenols, oils and fatty acids, wax and steroids, proteins, cellulose, water, chlorophyll, pectins.

The first important report on hops came from the second half of the 19th century where the hop bitter compounds were divided into a lead precipitable α-acids fraction and another fraction then named the ß-acids. These fractions hardened or resinified on standing in contact with air (oxidative crosslinking of the double bonds). Rigby and Bethune (1952 and 1953) showed that the α-acids are mixtures of homologues and analogues and they named the three major ones, cohumulone, humulone, and

adhumulone. They are isomerized in the wort to produce the so called iso-α-acids and iso-ß-acids which are much more soluble in wort and beer and are responsible for the bitterness in beer. The chemistry of the resins or α and ß acids has now largely been elucidated and the understanding of this chemistry has lead to tangible benefits for hop growers and brewers in terms of availability of new high α-acids varieties and the ability to control beer bitterness.

Despite a great deal of effort over the past 20 years, there is confusion about the effect of hop oil on beer flavor even though a great deal is known about its chemical composition. The composition of hop oil has been studied extensively. Contributions can be noted from Rigby and Bethune (1952), Howard and Slater (1958), Buttery et al. (1964), Peacock and Deinzer (1981a), Peacock et al. (1981b, 1989), Lam et al. (1986a), Moir (1988). Tressl et al. (1978 a,b, 1983), has probably contributed most to this topic. More than 200 compounds have been reported, while capillary GC has revealed up to 400 peaks in a chromatogram.

The percentage of hop oil in hops depends upon variety, conditions of growth, time of picking, aerial oxidation, age and storage conditions. Its determination is important for the brewer in order to adjust the hop addition to wort. Howard (1970) reviewed advantages and

disadvantages of some of the methodology used to measure the amount of hop oil and proposed the use of a different method.

Sharpe et al. (1981) in his review reported that hop oil consists of: a- hydrocarbon components; b- oxygenated components; and c- sulphur-containing components. The proportions of these compounds, especially hydrocarbons and oxygenated components vary according to the variety and age.

a- Hydrocarbons make up the bulk of the oil, but they are the most volatile and most ready to oxidize and polymerize of all the compounds in hop oil. They are also the least soluble in water, wort and beer. Only trace amounts of hydrocarbons are found in beer. The hydrocarbons constitute 80 to 90 % of the hop oil. They can be classified into three distinct groups: aliphatic, monoterpenes and sesquiterpenes. The most abundant monoterpene is the acyclic terpene myrcene.

b- The oxygenated fraction of the hop oil represents a small percentage of the oil (usually 20 to 30 %) and is an extremely complex mixture of alcohols, aldehydes, acids, ketones, epoxides and esters.

Howard and Slater (1957) and Jahnsen (1962) reported a clean separation of hop oil into hydrocarbon and oxygenated fractions. The latter fractionated the oxygenated fraction into alcohol, ester, and carbonyl

compounds. To have an idea of the complexity of this fraction it can be said that more than 50 alcohols have been reported in hop oil. These may be subdivided into three groups: terpene, sesquiterpene and aliphatic/aromatic alcohols. The most abundant of the terpene alcohols is linalool. Among the sesquiterpene alcohols are humulenol I, humulenol II, humulol, epi-cubenol, and nerolidol. Some of the aliphatic /aromatic alcohols include 2-methyl-propanol, and 3-methyl-1-butanol.

Several epoxides have been reported to be present in hop oil such as the humulene and caryophyllene oxidation products. The presence of 12 humulene diepoxides has been reported by Peacock et al. (1989) and Lam et al. (1987).

Tressl et al. (1983) reported the presence of tricyclic sesquiterpenes (including alcohols and epoxides) in hops which can be derivated from bicyclogermacrene, selinadienes, selinenes, and germacrene B. Some of these compounds were reported for the first time in hops.

Among the acids it has been reported that only a few free acids are present in hop oil. Some of them are reported to be oxidation products of hop resin constituents.

A large number of ketones have been reported to be present in hop oil (Jahnsen, 1962; Guadagni, 1966b; Tressl, 1978a). Many straight chain, branched chain,

unsaturated, and cyclic ketones were found although the position of the branching of the unsaturated ketones has not always been specified.

Esters are considered the most important of all the components owing to their contribution to hop aroma and flavor. These esters are either straight or branched chain saturated or unsaturated methyl esters of the alcohols.

In addition to the above mentioned compounds there is a group of miscellaneous oxygenated components that do not easily fit into the groups discussed. Some of them include damascenone, ß-ionone, and karahana ether.

In studying the flavoring effects of the hydrocarbon and oxygenated fractions of hop oil, Howard and Stevens (1959) spiked some of the fractions into an unhopped beer and into water. They concluded that the oxygenated compounds has a more marked effect upon flavor than the hydrocarbons. They reported a taste threshold of 0.3 ppm in beer and 0.1 ppm in water for the oxygenated fraction. Buttery et al. (1965), studied the volatile oxygenated constituents in four different varieties of hops and determined the thresholds of the different fractions showing that the oxygenated fraction has the lower olfactory threshold in water compared with the hydrocarbon fraction that account for 90 % of hop oil. The same results were stated by and Guadagni et al. (1966b).

To determine the survival quantities of the hydrocarbon and oxygenated fractions of hop oil in beer, Likens and Nickerson (1964) collected steam volatile, pentane soluble concentrates from wort (after and before hopping), fermented wort and beer using an apparatus that combined steam distillation and liquid-liquid extraction. Each one of the concentrates was fractionated into hydrocarbon and oxygenated compounds. They showed that more oxygenated compounds than hydrocarbons are in the wort after normal kettle boiling even though hydrocarbons occur in the oil at a concentration 10 times higher than the esters. Trans-esterification of methyl-4-decenoate and methyl-4,8- decadienoate was reported to occur during the brewing process.

Due to the apparent important role of the oxygenated components, our study was concerned mainly with the oxygenated fraction of the three varieties of hop oil.

The third group of compounds found in hop oil is the sulphur-containing compounds. A number of organic-sulphur compounds have been detected in hops. It is well established that organic sulphur compounds as a group is one of the most flavor active (Buttery et al., 1965; Guadagni et al., 1966b; Seaton et al., 1981). They impart undesirable sulphury, cooked vegetable and musty flavors to beer. The polysulphides, dimethyl trisulphide and dimethyltetrasulphide are the most flavor active compounds

detected in hop oil and have flavor thresholds of 0.1 and 0.2 ppb respectively. Most of these compounds result from the chemical reaction of the SO_2 used to spray hop and hop oil constituents.

Moir (1987) proposed that humulone, one of the resin acids, is one of the few simple building blocks from which nearly all the compounds found in hop oil are constructed.

2- Hop oil extraction

Isolation of hop oil by steam distillation at 100 °C has been reported since the early 19th century. Most investigators used some early designs such as that described by Wright and Connery (1951). This was later modified by Howard and Slater (1957). In this method the oil is steam distillated and collected in a trap where the insoluble oil goes to the top and the separated water return to the still. The main disadvantages of these methods are length of operation, (3-6 hs.), need for special equipment, thermal degradation, and incomplete recovery of compounds that are more water soluble and less volatile (Howard, 1970).

Another distillation technique has been devised whereby hop oil can be isolated from hops using low temperature steam (25 °C) (Pickett et al., 1977; Laws et al., 1977). In this technique, milled hops are placed in a

column and the hops are extracted by a current of low temperature steam produced by applying a high vacuum to the system. The distillate, a mixture of hop oil and water, is condensed at a low temperature. The disadvantage of this technique is that the water emulsion is unstable in storage and can not be analyzed directly without further separation.

Extraction using carbon dioxide (CO_2), liquid or at a supercritical state, has been reported by Laws et al. (1977). The solubility of many compounds in CO_2 shows it is as non-polar as hexane or pentane. This method is highly appropriate for extraction of hop oil that will be used during the brewing process because CO_2 is tasteless, colorless, easily removed, and non-flammable. Commercially, the big disadvantage of this methodology is the cost.

Lam et al. (1986b) more recently reported a hop oil extraction procedure which is based on the selective adsorption of the bittering components on alumina premixed with a potassium hydroxide solution, while the essential oil is eluted. It has the advantage that it can recover compounds that are less volatile and more water soluble. It is rapid, can be easily standardized, and only requires 10 g of hop in contrast with the 200 g required for steam distillation.

Oil for investigational purposes may either be isolated by the distillation methods described or by using headspace vapor analysis (De Metz and Verzele, 1968).

3- Effect of aging on hops

Tressl et al. (1978a), stated that most of the hop compounds found in beer are derived from major components by chemical or biochemical reactions during processing and storage of hops. They studied the chemical changes occurred during aging of Spalter hops after a storage time of 3 years. One hundred and forty four compounds were reported. Hop oil content decreased with aging. Aged hops presented an intensive odor of isovaleric and caproic acid, compounds known to produce off notes in beers. Hydrocarbon terpenes and sesquiterpenes decreased while the oxygenated fraction increased from 12 to 93 %. Hydrocarbons are transformed into hydrophilic components that may be easily transferred to beer.

Peacock and Deinzer (1981a), studied the changes in oil composition after one year of storage for 6 different varieties of hops, including American and European. Their findings agree with the results reported by Tressl et al. (1978a). They found that the varieties in which α-acids decrease most also show the most humulene oxidation. Thresholds of some compounds are reported.

Foster and Nickerson (1985) studied the effect of aging in 20 different varieties. They reported, in agreement with Tressl and Peacock's results, that aged hops showed losses of α-acids, hop oil, and myrcene in all varieties. They developed what is called "hoppiness potential value" (sigma). Using this sigma value, 20 different hop varieties were put into four hop categories based on changes in total hoppiness with respect to hop aging. They concluded that in order to get the best sensory properties in beer, the hops must be briefly to moderately aged, depending on the variety.

Similar results were reported by Lam et al. (1986a). They studied the effect of aging in two American varieties at two different aging stages. Beers with these hops were brewed in order to determine their contribution to beer flavor. They found that moderate aging of fresh hops prior to brewing is necessary to maximize the level of various aroma compounds. Excessive aging leads to a significant loss of these compounds, the loss being dependent on the variety. Sensory profiles of the beers were presented. They also suggested that beers with a high content of humulene oxidation products presented a herbal/spicy character.

4- Varietal differences

Likens and Nickerson (1965) indicated that certain components or ratios of components are highly specific for individual varieties. They reported seven compounds that were characteristic from domestically grown hops. They were considered good varietal indexes because their proportions were not affected by the maturity of the hops. Later, the same authors (Likens and Nickerson, 1967) reported that the essential oil of several varieties of hops displayed good varietal uniformity of composition under several environmental conditions. They concluded that hop oil composition could be used as a reliable criterion for a system of varietal identification. The same results were reported by Buttery and Ling (1967).

Tressl et al. (1983) found 15 new tricyclic sesquiterpenes in Hersbrucker and Huller Bitterer hops. These compounds were not found in any other hop variety. Tressl's results confirmed, once more, the fact that certain compounds or ratio of compounds are unique to each variety.

Stenroos and Siebert (1984) were able to classify essential oils from 148 hop samples grown in America and Europe using pattern recognition and multivariate analysis techniques. They indicated which peaks in the chromatogram were characteristic of varieties and of growing regions.

No sensory information was provided.

Analysis of hop oil composition is not sufficient to identify all hop varieties. Ratios of α and ß acid components have been also important in the varietal characterization of hops. Nickerson et al. (1986) established that the cohumulone ratio (the relative proportion of the cohumulone in the α-acids) is a varietal characteristic. They stated that this ratio can complement other data used to characterize varieties.

Similar conclusions were reported by Wackerbauer and Balzer (1988). They reported that the cohumulone ratio and the colupulone ratio are better varietal characteristics because they do not depend on the time of picking and remain constant when hop extract, powder or pellets are produced; whereas, the quotient of α-to-ß-acids has a much larger range of variation for the individual varieties and is affected by aging.

Much more work has been reported regarding the sensory and chemical characteristics that different hop varieties impart to the beer. Peacock et al. (1980) reported sensory differences between beers brewed with American and European hops. He reported the presence and thresholds of different hop derived compounds in each beer.

Peacock et al. (1981b) analyzed four beers by GC-MS for hop derived compounds: two American beers brewed with

Cascade and Cluster, respectively and two with a mixture of European hops. They reported that beers brewed with the American varieties presented floral aroma while those brewed with European hops had a more spicy character. They reported that linalool, geraniol and geranyl iso-butyrate were responsible for the floral characteristics of the beers and developed a floral index in order to predict the floral characteristics as a function of the compound concentration and relative thresholds.

Irwin (1989) studied the contribution of four different hop varieties (Hallertauer, Hersbrucker, Washington Cluster, and British Columbia Brewers Gold) to lager beers. He stated that each hop variety contributed a small number of unique flavor compounds to the beers. He also reported that more than half of the hop derived compounds isolated from beer were not detected in the hops and were thus formed during the brewing process. He reported a taste threshold of approximately 2 ppm for humulol and a mixture of epoxides, respectively.

Murakami et al. (1989), studied the effect of three different hop varieties on the beer volatile constituents by determining the composition of the headspace using a very sophisticated headspace method. They found unique peaks in each variety. They complemented their analytical work with sensory characterization of the beer presenting a flavor profile for each beer.

Although most of the 300 components of hop oil disappear during the boiling process and fermentation, some of them do survive in the finished product. All of the examples mentioned earlier confirm the dependence of beer flavor characteristics on the hop variety used.

5- Noble aroma hops

Native European hops, and particularly cultivars that arose from these indigenous hops, are known in the trade as "noble" aroma hops. They impart a characteristic aroma or flavor to beer. The best known are the German cultivars Hallertauer mittelfruher and Tettnanger and the Czechoslovakian Saazer. All are relatively low in α-acids and particularly low in cohumulone. In addition, the proportion of α-acids to ß-acids are nearly equal. Rigby (1972) suggested that such a quality profile is characteristic of all noble aroma hops. The essential oil of such hops, compared to other cultivars, is low in myrcene but high in humulene. Peacock et al. (1980) found that oxidation and hydration products of humulene make up a major part of the hop derived compounds found in beer, and they are of major importance for the characteristic noble aroma which some hop varieties impart to beer. Taste thresholds of few compounds were reported. Humulene epoxides have recently been shown to be among the major

aroma constituents of hops in finished beer (Lam et al., 1986a). The earliest mention of humulene oxidation products in beer is by Shimazu et al. (1974) where they found humuladienone in beer. Tressl et al. (1978b) also reported humuladienone in beer as well as humulenol II and humulol and epoxide I in a German beer. Lam and Deinzer (1987) reported the presence of five humulene diepoxides in American beer but found only two isomers in European beer. They reported that a mixture of diepoxides showed similar, but stronger aroma than the monoepoxides, and they gave a moderate to strong bitter taste but no thresholds or descriptors were reported. Although the authors mentioned above suspected that oxidation products of the α-humulene imparted a noble hop aroma/flavor to beer, other workers discussed their contribution to beer flavor. Fukuoka and Kowaka (1983) reported that none of the oxygenated humulenes such as epoxide I, II, humulenol II, or humulol proved to be contributors to the herbal hoppy flavor of beer based on their data on tasting synthesized compounds. They fractionated the hop oil until they found a fraction with strong herbal character, and then sniffed the GC effluent in order to determine which compound was the one responsible for the herbal character. Results from the GC-sniffing and GC-MS analysis of this herbal fraction, revealed that the compounds which impart herbal flavor to beer are certain unidentified

sesquiterpenoids that were also found in beer. Humulene epoxides I,II,III, humulenol II, humulol and caryophyllene oxides were synthesized and tested by a panel. Humulene epoxide I and humulol were described as having hay-like character. Humulene epoxide II was described as moldy; humulenol II as sagebrush-like, and caryophyllene oxide as cosmetic or menthol-like. This is probably the only paper reviewed that reported sensory properties and GC effluent sniffing of hop oil fractions. It is evident that these authors relate noble aroma with herbal character.

Irwin (1989) reported that of most of the humulene oxidation products found in beer, none have been demonstrated to have hoppy flavor. In addition, they are present in concentrations lower than their thresholds; therefore their contribution to the beer aroma/flavor is limited. Thresholds for a mixture of monoepoxides I,II, and III as well for humulenol and humulol were reported. In this case the author looked for "hoppy" compounds , but he does not define what "hoppy" means to him.

It is evident that the disagreement on the definition of noble aroma arose from the lack of a good descriptive sensory study on the hop oils of different varieties and their consequent contribution to beer flavor.

In looking for varieties with similar characteristics to the German varieties, high yields, and

resistance to diseases, Haunold and Nickerson (1987) reported the development of new crosses that have good potential noble aroma characteristics. In 1983 a tetraploid Hallertauer was crossed to nine aroma type male hops. The males were selected from known European aroma hops. Five selection crosses were identified in 1985 as having aroma properties similar to their parent Hallertauer. In this thesis, two of these five selections were chosen to be characterized and their sensory profiles compared against that of Hallertauer.

B- SENSORY EVALUATION

1- Individual responses to the same stimuli

High variability among subjects has been widely reported when determining thresholds. Amoore et al. (1968) and Amoore (1980) reported that olfactory thresholds are noted for their extreme variability among subjects. He reported that 96% of the population have sensitivities between 16 and 1/16 times of the normal. A considerably larger range has been reported by Brown et al. (1968) and Yoshida (1984). Punter (1983) and Stevens and Cain (1987) reported that some odorants exhibited much higher variability than others in the determination of their

thresholds. For example they found that the range of variation for D-limonene was much higher than the range of variation for iso-amyl butyrate. The variability in suprathreshold odor strength among compounds was studied by Stevens et al. (1988). They determined the psychophysical functions relating suprathreshold odor strength to concentration for pyridine, PEMEC, and butanol. They found that the exponents of these functions are very small and they are different for each compound. That means that it takes a huge increase in gaseous concentration to bring about an appreciable increase in the strength of an odor. For example a 76 fold ppm increase would cause a 2 fold increase in the odor strength for pyridine. It has not been established why these huge differences occur among compounds. It constitutes a major puzzle of olfactory psychophysics. This fact suggest caution in making a sensory-instrumental correlation.

Stevens et al. (1988) also studied the hypothesis that people vary greatly in sensitivity. This could be overall sensitivity to all compounds or specific sensitivity to a particular odorant. They stated that olfactory sensitivity could fluctuate from day to day, or hour to hour, or moment to moment. In their study they reported that variability for a given subject is comparable to that reported across subjects (Punter, 1983;

Stevens and Cain, 1987).

Goldstein (1989) reviewed the problems in dealing with odor quality. He said that it is difficult to describe odors because of the lack of a specific language for odor quality. Cain (1977) stated that at least three factors impede odor identification: 1- sluggish formation of an association between odors and names, 2- failure to retrieve the name in spite of a well formed association, and 3- inherent confusability of the stimuli. Later (Cain, 1979), he stated that with appropriate help in encoding and retrieval, people can identify as many substances as they can discriminate, and for which they posses well learned names.

In studying associations of odor-names, Engen (1987), also concluded that association between odors and their names is weak. In his experiment he found that once a subject has an expectation of what the name of an odor might be, that name will largely control the sensation. In contrast, the presentation of an odor, before a name is available, does not have a similar effect on the choice of names. In fact, the unavailability of a verbal response often leaves the subjects in the "tip of the nose state" (Lawless and Engen, 1977), unable to recall a name even though the odor is familiar. His conclusions, as well as Cain's findings, suggest extensive training in naming the odors before evaluating of the GC effluents, especially

since it is a dynamic experiment (the odor is there for a fraction of a second and then is gone).

Engen (1987) also studied human accuracy in identifying common odorants and what their responses were when they misidentified the odors. He found that people tend to categorize odors, but not with semantically cohesive general nouns. They do it in terms of similarity of odor, similarity of context or kind of object in which odors may be perceived.

Anosmia can also contribute to descriptor variability. O'Connell et al. (1989) defined anosmics as those who have a good sense of smell, but who lack the ability to perceive a particular odorant. They also reported that in addition to the pronounced shift in thresholds, anosmics often provided altered quality judgement when presented with liminal concentration of the product for which they are anosmic. That means that a single compound can elicit multiple intensity and quality reports from different subjects. They concluded that apparent anosmia to one odorant may influence sensitivity to odorants in the same odor class, or to odorants in another quality class.

The above mentioned facts justify why it is necessary to perform the evaluation of GC effluents using as many judges as possible.

2- GC-effluent sniffing

The Flame Ionization Detector (FID) chromatogram obtained when a mixture of compounds is separated by a GC column is not indicative of the aroma contribution of each compound.

Guadagni et al. (1966a) reported sniffing of GC effluents to identify which peaks were responsible for apple character in apple juice. After assessment of the effluent's odors, the most important peaks were collected, and thresholds and odor units were determined in order to establish the relative odor contribution of those peaks. They calculated the change in area of those peaks necessary to produce a difference in the sensory response when evaluating the juice.

Odor unit is defined as the ratio of the compound concentration in the aroma extract to its odor threshold. This concept is based on past studies which indicate that certain mixtures of odors are additive at the threshold level. This ratio merely gives the number of odor units that a specific component or fraction contributes to the total odor of a mixture, but it says nothing about the odor quality nor does it imply anything about the relationship between stimulus concentration and intensity of the sensation above threshold.

Guadagni proposed that in order for the GC data to have sensory meaning, it is necessary to: 1) determine which peaks contribute to the characteristic product aroma; 2) determine the relative importance of each peak; 3) find some way of relating the physical measurements to subjective responses; and 4) establish a mathematical relationship between the two types of measurements. When all these requirements are met, we can be reasonably sure that objective measurements will provide useful information for quality-control purposes. GC-effluent sniffing is the first step in obtaining these goals. Flath et al. (1967), Spencer et al.(1978), Seaton et al.(1981), Fukuoka and Kowaka (1983), and Fors and Nordlov (1987) reported sniffing of GC effluents, but in all cases only descriptors for the effluents are given. No odor quantification was reported, and only one dilution stage was evaluated.

Pangborn (1981), in agreement with Guadagni, added that GC data can be supplemented by sensory measurement in determining what no instrument can approximate, the relative pleasantness of individual compounds or of mixtures.

Acree et al. (1984) developed a technique named CHARM that formalizes the process of sniffing gas chromatographic effluent. This is a bioassay to determine the odor activity of each compound in the mixture by

sniffing the GC effluents through a series of dilutions. This technique has been used to determine odor activity of compounds in apple juice (Acree et al., 1984), grape juice (Braell et al., 1986), crackers (Yong et al., 1989), wines (Acree et al., 1985) and to determine variability in thresholds (Marin et al., 1988). The basic equipment used in the CHARM technique was described by Acree et al. (1976). In CHARM, the odor importance of the compounds is determined by adding the areas of the sensory responses detected by the subjects during each dilution. The responses in this case consist in a signal when the subject starts perceiving the odor and another signal when the subject finishes perceiving the odor in each one of the dilutions. Subjects do not evaluate the intensity of the odor. Usually the sample is diluted several times until it reaches the concentration in the original product extracted. In that sense CHARM is founded in the measurement of the relative thresholds of individual chemicals.

A similar technique, called Dynamic Gas Dilution Olfactometry or Aroma Extract Dilution Analysis, has been developed by Ulrich and Grosch (1987) and Schieberle and Grosch (1988). They defined the D-value as the highest dilution at which a substance is still smelled. Thus the undiluted sample has a D-value of 1. They plotted this D-value against the retention index in order to obtain an

aromagram. Schieberle et al. (1990) stated that the results obtained by CHARM and Dynamic Gas Dilution Olfactometry are directly proportional to the aroma values of the compounds also called odor units by Guadagni et al. (1966a,b).

The main objection to the sniffing of the GC effluents is that the information provided may be proportional to their thresholds, but it does not tell anything about interactions among compounds or the relative contribution of each peak at the same dilution stage as they are in the original product (Pangborn, 1981).

C- SENSORY-INSTRUMENTAL CORRELATION

1- Requirements and limitations

Sensory measurements are time consuming, subject to error due to the variability of human judgement, and require extensive statistical analyses to interpret the data. Instrumental analyses are more reproducible and sometimes less expensive, but they are of little importance unless they correlate with sensory judgment. In flavor research, the most widely-used analytical method for separating volatile compounds is GC. Wick (1965)

studied the chemical and sensory aspects of identification of odor constituents in foods and concluded that both the human nose and the GC detector vary widely in their sensitivity to different molecular species and to different compounds of the same chemical type. Furthermore, there was not a complete agreement between the GC and human nasal appraisals; the GC is a separator whose responses are linear with concentration whereas the human olfactory system is an integrator whose responses are often a power function with concentration. For that reason it is not recommended to correlate sensory responses with the area of the peaks calculated by the GC detectors. Noble (1975) pointed out that much of the flavor research purporting to correlate instrumental and sensory data is flawed by the use of inappropriate sensory techniques. The most common malpractice is to calculate linear correlations between instrumental data (unidimensional and usually linear with concentration) with hedonic sensory responses (multidimensional and usually parabolic functions).

Guadagni (1968) and Dravnieks (1976) reported useful guidelines for correlating sensory with instrumental data. Important requirements for making either statistical or non-statistical comparisons include: 1) the test samples must be identical for both measurements; 2) the test samples must cover a sufficiently wide range of the

variability under observation; 3) there must be sufficient replication of both measurements; 4) it is necessary to calibrate sensitivity and reproducibility of both measurements; 5) the same judges must participate in all sensory replications within a test; and 6) the same person should administer both tests.

2- Multiple correlation analysis

In a beef flavor study, Persson and von Sydow (1973), obtained sensory ratings of the intensity of 15 odor qualities of canned beef. Volatiles from the samples were then chromatographed. The GC peak heights were regressed against the sensory ratings, using up to four different peaks in any equation. Although it is assumed that sensory response is a non-linear function of stimulus concentration, the authors found that the height of the peak directly, or its log or power transformation, resulted in equally predictable and interpretable results.

Spencer et al. (1978), investigated the contribution of volatiles to the odor of clingstone peaches by exit port-sniffing, descriptive flavor analysis, and GC-MS analysis of fresh and canned samples of Haldford peaches plus nine other peach varieties. They correlated each one of the sensory attributes with the areas of the peaks determined by GC using stepwise regression analyses. Peaks

coming through the GC exit port were evaluated for descriptors but not for odor intensity.

Pino (1982) correlated the GC peaks from orange volatiles with hedonic responses from a sensory panel. He proposed several models to relate the sensory response with the concentration.

Paule and Powers (1989), using similar techniques as Spencer et al. (1968), examined aromatic and non-aromatic rices by using a trained sensory panel, GC-effluent sniffing, and GC-MS analysis. The areas of the peaks possessing the aromatic principles were correlated with the desirable aroma terms using stepwise regression analysis. In order to determine which descriptors discriminated the most among samples, stepwise discriminant analysis was applied.

3- Stepwise Discriminant Analysis (SDA)

Powers and Keith (1968) described the use of the SDA for classifying roasted coffee and potato chips, but no correlations with sensory terms were presented. Dravniecks et al. (1973) examined some corn samples which were classified into sour, musty and good categories by sensory evaluation. Discriminant functions were then developed using the peak areas which correctly assigned the corn samples to the appropriate category.

Brown and Clapperton (1978) used SDA to examine sensory and instrumental data on 46 different brands of ale from five brewing companies. Eighty of the ales were correctly assigned to brewing companies from data on four sensory and five instrumental parameters. The instrumental parameters included color, pH, dextrin, iso-amyl alcohol and others. The most important discriminators were iso-amyl alcohol and caprylic flavor.

4- Others

In studying the correlation of instrumental and flavor data for the property of fruitiness, Winell et al. (1979) used Discriminant Analysis and Pattern Recognition on sensory data and GC chemical response. They found five esters to be efficient discriminators indicating a causal relationship to fruitiness. No assessment of odor importance was done.

Moll et al. (1981) conducted Principal Component Analysis (PCA) and Factorial Discriminant Analysis on sensory and physicochemical data obtained on beers, malt, and barley in order to find a relationship between the different tasting parameters and to separate them in groups of beer of good, average, and poor quality. They found that the varietal factor had a considerable influence in the discrimination of good and poor beers.

Peppard et al. (1989) studied the sensory properties of beer brewed with different varieties of hop and correlated the sensory attributes with the GC peaks using Partial Least Squares Regression Analysis and Canonical Correlations. The latter failed because the variables were highly correlated. The former showed that European hop flavor was correlated with two isomeric linalool oxides, while the spicy flavor was related to several hop oil oxidation products. Again, the correlation was made without knowing if these compounds were odor active or not.

MATERIALS AND METHODS

A- EXTRACTION, FRACTIONATION, AND GENERAL CHEMICAL ANALYSES

The following hop varieties were analyzed:

a- U.S.D.A 21455

b- U.S.D.A 21459

c- Hallertauer, hallertauer (named Hallertauer in the rest of the text).

All the hop varieties were from the 1987 crop. They were acquired at John I. Haas, Inc., Yakima, Washington. The American varieties were grown in Washington. Hallertauer was an imported hop from Germany.

Approximately 250 g of pelleted hops were placed in a 12 L flask containing 5 L of 0.01 M sodium phosphate, pH=6.0, buffer and distilled for 6 hours using a Wright and Connery oil trap. The amount of oil collected was measured volumetrically (Wright and Connery, 1951).

Chemical analyses of the samples were conducted as follows:

1- Alpha and Beta acids by the ASBC method (American Society of Brewing Chemistry Methods of Analysis, HOPS-6A, 7th. edition, 1976).

The percentages of α and ß acids were determined using the following formulas:

α-acids, % = d (-51.56 A_{355} + 73.79 A_{325} - 19.07 A_{275})

β-acids, % = d (55.57 A_{355} - 47.59 A_{325} + 5.10 A_{275})

in which "d" is the dilution factor and A_{355}, A_{325}, and A_{275} are respective wavelengths at which dilutions are measured.

2- Alpha Acids composition according to the method of Verzele et al. (1980).

3- Hop Storage Index (HSI) according to Nickerson and Likens (1979). HSI is defined as the ratio of absorbance at 275 nm over the absorbance at 325 nm of an alkaline methanol solution of the non polar solvent extract of hops.

$$HSI = A_{275} / A_{325}$$

Hop oil (0.1 ml) was then fractionated into Oxygenated and Hydrocarbon compounds using a 5 g of Silica Gel (Activity II, 14% H_2O, Merck 7734) column of 100 X 5.0 mm (Nickerson and Likens, 1966). Hydrocarbons were eluted with 10 ml of pentane and the oxygenated fraction was eluted with 10 ml of diethyl ether. Samples were concentrated in a Vigreux column (Fig. 1).

Hop oil extraction, fractionation and general chemical analyses were conducted by Gail Nickerson at Agricultural Chemistry Department, Oregon State University.

B-DESCRIPTIVE SENSORY PANEL

1- Sample preparation

The oxygenated fraction, obtained as mentioned above, was processed as follows:

1- Ether was completely evaporated and 60 μl of the sample was dissolved in 160 μl of 95% ethanol (Food Grade).

2- From this solution, 2.5 μl were spiked into 50 ml of spring water into each 12 oz amber glass. The glasses were covered by an aluminum foil cup, sealed with Parafilm (American Can Company, Greenwich, CT), and left standing at room temperature for half an hour in order for the headspace to reach equilibrium.

2- Training procedure

A panel of 10 subjects was used to evaluate the hop oil oxygenated fractions spiked in water. Panelists were all non-paid volunteers, students or faculty of Oregon State University (OSU).

Panelists were trained over a period of three weeks to evaluate aroma intensity using a sixteen point scale with intensity rated 0="none", 3="slight", 7="moderate",

11="large", and 15="extreme". For each sample, panelists scored aroma attributes by matching or extrapolating between the overall intensity of references standards. These reference standards are listed in Table 1.

In order to describe the samples qualitatively, panelists developed their own terms and after a panel consensus, 11 attributes were selected to be evaluated: overall intensity, soapy, herbal, woody, grassy, citrus, sweet, fruity, artificial fruit, floral and vitamin "B". Vitamin B was included in the ANOVA because it was detected by half of the panelists. It was encoded in the "other" category in the ballot. Reference standards were provided in each session. The ballot used and the reference standards are listed in Fig. 2 and Table 2, respectively.

3- Experimental design and sample presentation

A Completely Randomized Balanced Block design was used with three replications. In each session, all three samples were presented to the panelists. In order to minimize the bias for order presentation, samples were randomized in each replication. Panelists seated in individual booths and were instructed to evaluate the samples from left to right. Samples were evaluated at approximately 70 ^{o}F.

4- Statistical analyses

Panelists were first individually evaluated for consistency of their responses. One panelist was removed because his observations resulted in high standard deviations in almost all the attributes tested.

Individual ANOVA for each attribute were conducted using PC.SAS (SAS Institute, Inc. Cary., N.C.). Since each panelist replicated three times, the replications could be used to construct a three way ANOVA (factors = panelists (P), replications (R) and treatments (T). Panelists and replications were treated as random sources of variation. Therefore the appropriate F tests were conducted as follows:

$$F(\text{Treatments}) = \frac{MS(T) + MS(PxTxR)}{MS(Pxt) + MS(RxT)}$$

$$F(\text{Replications}) = \frac{MS(R)}{MS(PxR)}$$

$$F(\text{Panelists}) = \frac{MS(P)}{MS(PxR)}$$

When the treatment source of variation was significant, means responses for each treatment were analyzed using Fisher's Least Significant Difference Test (LSD) ($p \leq 0.05$). The denominator of the type 1 F test for treatment effect was used as the MS(error) in the LSD computation (Schultz, 1946; Anderson and Bancroft, 1952).

The degrees of freedom for the F-Test and LSD computation were calculated according to the formula given in Anderson and Bancroft (1952).

Panelists were treated as random because descriptive panelists are randomly drawn from a population of panelists with varying sensitivities and susceptibility to different response behaviors irrespective of training level (Lundahl and McDaniel, 1988).

The Panelist x Treatment, Panelist x Replication and Treatment x Replication interactions were tested for significance using the MS(PxTxR) as a denominator in the F test.

When the interaction panelist by treatment was significant, means scores for each panelist were graphed against treatments in order to determine which panelist was responsible for the interaction and validate the significance of the treatment effect.

In order to visualize in space the differences among the samples and the intercorrelation among attributes, samples were analyzed using Principal Component Analysis (PCA) (Johnson and Wichern, 1988).

In order to see which sensory attributes contribute more to the differentiation of the samples, Stepwise Discriminant Analysis (SDA) (Afifi and Azen, 1979) was conducted on the data obtained by the whole panel during the descriptive work and on the data obtained by the three

panelists that participated in both experiments. The p value to introduce, keep or take one variable out of the model was set at $p \leq 0.15$.

C- GAS CHROMATOGRAPH (GC) EFFLUENT SNIFFING

1- Equipment description and GC conditions

The equipment used (Fig. 3) consisted of a GC to volatilize and separate components of a mixture and a human detector (Acree et al. 1984).

The Gas Chromatograph used in this study was a Hewlett Packard 5890 equipped with a 0.54 mm ID by 30 m fused silica column coated (0.25 μ) with Supelcowax 10 (Supelco, Inc., Bellefonte, PA). Operating parameters for the GC were:

1- Injector port temperature : 200 °C

2- Detector temperature : 250 °C

3- Split ratio : 16:1

4- Temperature program: 80 °C for 5 min.,rate A = 5 °C/min., final temperature A = 155 °C, rate B = 4 °C/min., final temperature B = 240 °C.

5- Carrier Gas : Helium at a linear velocity of 25 cm/sec.

The sniffer consisted of a 60 cm by 1 cm (i.d.)

glass tube, coated with silicone (Sylon CT, Supelco, Inc., Bellefonte, PA) to avoid the adsorption of compounds on the walls.

The GC was modified so that the column could be moved from the Flame Ionization Detector (FID) to another FID base detector without the flame. The latter is not destructive and allows the compounds to be directed to the subject. The glass sniffer sits on top of this detector.

The GC effluent then is mixed with humidified air at a flow of 11 liters/min. The air, coming from a compressed air tank, is filtered through a charcoal filter and directed to a water bath at 25 °C. The relative humidity of the air, measured by the difference in temperature between wet and dry bulb thermometer, was 60 % at the end of the sniffer.

In order to preserve resolution, the air flow was adjusted as the sum of the linear velocity of the Helium carrier gas plus makeup gas coming from column.

A time-intensity (TI) device consisting of a cursor with an attached scale (0="none", 7="moderate" and 15="extreme"), and a computer using a special data collection software was attached close to the GC. This equipment was used to record the sensory intensity of the GC effluent.

It should be mentioned that even though the GC equipment used is similar to that used by Acree et al.

(1984), the data collection procedure and the information collected is completely different.

2- Sample preparation

Ether was completely removed from the oxygenated fraction obtained as in **A**, and the fraction redissolved in pentane. An amount of 0.5 μl of $C_{14}H_{30}$ was added as a Internal Standard (IS) and the volume adjusted up to 1 ml.

Because the proportion of oxygenated fraction was different in each variety (Table 3), the volumes injected were adjusted so that the same amount of each one was delivered through the sniffer to the subjects.

Using the FID a series of normal hydrocarbons were chromatographed under the same operating conditions as the stimulus samples. Retention data for the hydrocarbon runs were used to convert stimulus retention times into Kovàts' Indexes (KI_{20M})(Kovàts, 1958).

3- Training procedure

Four female subjects, volunteers from OSU, were selected for this study. Three of them belonged to the Descriptive Sensory Panel that took part in this study.

In order to familiarize subjects with the sensory properties of the compounds to be evaluated, some of the

compounds present in the samples were evaluated spiked in spring water. Fifty microliters of each compound were spiked in 50 ml of spring water. The compounds evaluated and the descriptors assigned are listed in Table 4.

In order to familiarize the subjects with the TI device, different concentrations of linalool (2, 4, 8 μl in 500 μl of pentane) were injected and the TI responses were registered.

In order to familiarize the subjects with the samples to be evaluated, each sample was sniffed at least two times in a practice run.

4- Test procedure

Each sample was evaluated by each subject on four consecutive days. Each sniffing session took approximately 35 minutes.

Prior to sniffing, subjects were presented with standards in order to help them to describe the effluent. Standards used are listed in Table 5.

Subjects were instructed to breathe normally while sniffing the GC effluent and to make 2 responses to each odor perceived:

1- Move the TI cursor according to the intensity perceived.

2- Select and report a quality descriptor for that

odor.

The odor intensity responses were registered by the computer while the odor quality descriptors were relayed verbally to the experimenter.

5- Data analysis

The sensory responses collected in the computer consisted of a set of points that formed a peak with a maximum intensity value at the correspondent time. The times registered were coincident with the retention times of the peaks coming through the GC column.

Times and intensities of peaks detected at least 50 % of the time for each subject were averaged. With these new values a graph of time vs. intensity was constructed. These graphs are called "aromagrams" (Figs. 4, 5, 6, and 7).

Then, the time and the intensity of those peaks that were detected at least for three of the four subjects were averaged again and a "consensus" aromagram was obtained for each variety evaluated (Fig. 8).

Assessments by panelists were analyzed per peak by one way ANOVA (Factor = Treatments). Because no panelist effect was considered, the F value for testing treatment significance was calculated using the Mean Square Error as a denominator.

6- Odor assessment

Three solutions, having approximately the same concentration of each one of the compounds in pentane as in the sample, were prepared.

Solution A contained:

a- Humulene monoepoxide I

b- Humulene diepoxide II

c- Humulene diepoxide III

d- Humulene diepoxides A and B

Humulene, caryophyllene oxidation products and geranyl iso-butyrate were synthesized at the Agricultural Chemistry Dept., Oregon State University.

Solution B contained:

a- 2-decanone (98%, Aldrich Chemical CO., Milwaukee, WI)

b- 2-undecanone (98%, Fluka-Chemika Biochemika, Ronkonkoma, NY)

c- 2-dodecanone (95%, Chem Service, Inc., PA)

d- 2-tridecanone (99%, Aldrich Chemical CO., Milwaukee, WI)

e- 2-tetradecanone (97%, Pfaltz and Bauer, Inc. Waterbury, CT)

f- 2-pentadecanone (95%, Pfaltz and Bauer, Inc., Waterbury, CT)

g- Geraniol (98%, Aldrich Chemical Co., Milwaukee, WI)

h- Caryophyllene oxide

Solution C contained:

a- Linalool (97%, Aldrich Chemical Co., Milwaukee,WI)

b- Geranyl iso-butyrate (99%, Aldrich)

The amounts injected were adjusted so that the peak areas for each compound were similar to those for the same compounds in the samples. Three of the subjects that participated in this study evaluated the GC effluents and described them in the same way as the samples were evaluated.

D- GC-MS ANALYSIS

1- Sample preparation

The samples analyzed were the same as those used for the GC - Effluent Sniffing procedure.

2- GC-MS equipment description and conditions

Mass spectral data were acquired on a Finigan 4023 quadrupole mass spectrometer operated in the electro impact mode. The operating conditions were the following:

1- Resolution : 1000 or unit mass

2- Ion source temperature : 145 °C

3- Electron energy : 60 eV

4- Transfer line temperature : 260 °C

Data were acquired and stored on disks for later retrieval.

The GC used was a Varian 3400 with a 0.32 mm ID by 60 m fused silica column coated (0.25 μ) with Supelcowax 10 (Supelco, Inc, Bellefonte, PA). This phase is functionally equivalent to Carbowax 20M.

The operating conditions were as follows:

1- Injector port temperature : 250 °C

2- Split ratio: 50:1

3- Temperature program : 60 °C for 5 min, 5 °C/min up to 155 °C, 4 °C/min up to 240 °C.

3- Data analysis

Peak identification was only made on odor active regions of the chromatogram.

As in aid to identification of compounds, mass spectral data were compared with reference spectra from the National Bureau of Standards, and with a collection of reference spectra compiled in the Agricultural Chemistry Laboratory at Oregon State University. Off-line file searching made use of an IBM AT, using a combined Kovàts'

index and mass spectral file for aroma compounds that was created under Borland's PARADOX database program. The Wiley optical disk(CD-ROM) library was also used as an aid in the identification of some of the compounds. The latter identifications were made by Dr. Leonard Libbey at the Department of Food Science and Technology, Oregon State University.

E- SENSORY-INSTRUMENTAL CORRELATION

1- Statistical analysis

Data collected for the Descriptive sensory panel and GC-effluent sniffing were correlated using the following analyses:

1- A two sample t-test was performed for each one of the attributes using, as group 1, data obtained for the whole panel and, as group 2, the data obtained for the three common panelists. This procedure was conducted in order to see if the opinion of the three subjects that performed the GC-sniffing differed from the opinion of the whole panel. Because both groups of observations were not independent, t-values were calculated from comparison of the means for a group of three panelists and a group of six panelists. Results were then extrapolated to the comparison with the whole group (nine panelists) (Johnson

and Wichern, 1988, p.59).

2- A correlation matrix among each sensory attribute score (averaged over the three replications for the three common panelists in the descriptive sensory panel) versus the odor intensity assigned for each subject to each one of the GC peaks (averaged over those peaks that were detected at least 50 % of the time) was conducted. Those peaks that were correlated with the sensory attributes with a p value ≤ 0.15, were selected as independent variables for the next step.

3- A stepwise regression analysis was conducted for each sensory attribute determined by the three common panelists during the evaluation of the descriptive sensory panel, on each of the GC peaks that presented high correlation coefficients with the sensory attribute predicted. The procedure used a significance value of $p < 0.150$ in order to introduce, keep, or to take one variable out of the model.

All the correlation analyses were performed using PC.SAS (SAS Institute,Inc.,Cary, N.C.).

RESULTS AND DISCUSSION

A-CHEMICAL COMPOSITION OF HOPS

This study was concerned mainly with the characterization of the essential oil, but it has to be considered that α, and ß-acids play a very important role in the varietal differentiation of hops.

As can be seen in Table 3, Hallertauer contains more α-acids than the triploids. U.S.D.A. 21455 has the highest content of ß-acids. Usually, their content diminishes as hops age (Foster and Nickerson, 1985).

Determination of the percentage of cohumulone in the α-acid fraction of hops is important (Rigby 1972), since high cohumulone content, such as above 50 %, is somehow negatively correlated with hop quality. Rigby also suggested that high levels of isocohumulone (the isomerized product of cohumulone) are responsible for harsh, unpleasant bitterness in beer and poor aroma quality. All hop varieties associated with noble aroma, such as Hallertauer, have a cohumulone ratio of 25 % or less (Meilgaard, 1960). The cohumulone ratio, calculated as the percentage of α-acid, has long been recognized as a varietal characteristic although there is a disagreement on whether or not environmental conditions can influence

it (Meilgaard, 1960). Nickerson et al. (1986), stated that beers brewed with hops with low cohumulone content have somewhat greater foam stability. In that sense it seems that U.S.D.A. 21455 has a cohumulone content that fits the characteristic of the noble aroma hop. On the other hand, U.S.D.A. 21459 has a very high content of cohumulone making it less favorable for brewing.

The Hop Storage Index (HSI) (Nickerson and Likens, 1979) is an indicator of the aging stage of the hops. As can be seen in Table 3, Hallertauer has the lowest HSI, meaning that it is less aged than the other two varieties. It has been reported (Foster and Nickerson, 1985) that during the aging process, the amounts of essential oil, α and ß-acids and myrcene decrease. On the other hand, the amounts of humulene and myrcene oxidation products increase. From the data in Table 3, it can be concluded that the amount of oil was initially higher in U.S.D.A. 21455 than in the other two varieties because its content is still higher in spite of the aging. It has to be considered that the initial oil content depends on the extent of pollination and time of harvesting.

Hydrocarbons make up the bulk of the essential oil, but they are the most volatile, and also the most ready to oxidize and polymerize of all compounds in hop oil. They are also the least soluble in water, worried and beer. Only trace amounts of hydrocarbons are found in beer. On

the other hand, oxygenated derivatives of those hydrocarbons, which make up 20 to 30 % of hop oil, are found in beer and have lower thresholds (Likens and Nickerson, 1964; Howard and Stevens, 1959; Guadagni et al., 1966b). As it can be seen in Table 3, the proportion of the oxygenated fraction is much higher in the triploids than in Hallertauer. This fact can be closely related to the aging stage of these hops. For that reason the volumes injected into the GC were adjusted so that the amount delivered for each variety was the same.

A more complete chemical description of the oxygenated fraction is given in the Results and Discussion of the Mass Spectrometric Analysis.

B- DESCRIPTIVE SENSORY PANEL

Even though there are several reports indicating hop varietal differences based on the chemical composition of hops (Likens and Nickerson, 1967; Buttery and Ling, 1967; Hull, 1989), no previous reports were found in the literature regarding sensory characterization of hop varieties. Much has been done to establish the sensory attributes of the beers brewed with different hop varieties (Peppard et al., 1989; Peacock et al., 1981b; Murakami et al., 1989), but no information is presented

about the hop oil itself or particularly the oxygenated fraction. When the oxygenated fractions were presented to the DSP, eleven descriptors were developed and evaluated.

Three different analyses were applied to the data collected in this experiment :

1- Analysis of variance

2- Principal component analysis

3- Stepwise discriminant analysis

1- Analysis of Variance (ANOVA)

Table 6 contains F-values and significance levels for each source of variation. The PAN effect was statistically significant for all the attributes except for vitamin B and fruity. That means that summed over treatments and replications, judges differ significantly in their scores, indicating the use of different parts of the scale. (O'Mahony, 1986).

The interaction PANxTRT was only significant for the attribute vitamin B ($p \leq 0.01$). The trend of the mean for the vitamin B attribute, summed over replications, varied with the judges for the varieties analyzed. It can be explained (Fig. 9) because only panelists 1,3,4,5,6,and 9 perceived this attribute in Hallertauer, but the rest of the panelists did not.

The TRT effect was only statistically significant at the 0.001 level for the vitamin B attribute. Hallertauer presents a mean value of 2.4 while the other two varieties present a mean equal to zero (Table 7). That indicates that the oxygenated fractions of the three varieties are very similar except for this attribute.

2- Principal Component Analysis (PCA)

A method of showing a pattern in descriptive ratings is by use of PCA (Johnson and Wichern, 1988). By this technique the first principal component (PC1) is extracted which accounts for the maximum variability in the data. A second principal component (PC2) is then derived which is orthogonal (uncorrelated) with the first and this explains the maximum variability in the remaining variance. The process is repeated until "p" principal components have been extracted, where "p" is the number of original variables in the analysis (attributes evaluated in this case).

Each principal component is a linear combination of the original variables. That linear combination maximizes the variance in that direction.

Fig. 10 shows PCA graphs of the DSP data for the three hop varieties analyzed. The contribution of each attribute to each principal component is represented by

vectors. The length of the vector is proportional to its relative importance in explaining the variability among the samples. In PCA, those variables which are very short vectors can generally be ignored. Those vectors that are in close proximity are highly correlated while those in opposite direction are negatively correlated. Perpendicular vectors are independent.

As it can be seen in Fig. 10, PC1 accounts for approximately 26 % of the contributed variation, PC2 accounts for 19 % of the contributed variance, PC3 accounts for 15 % of the remaining variance and PC4 accounts for 11 % of the variance. In total the 4 PC's account for 71.6 % of the total variance.

In Fig. 10 it is possible to see that Hallertauer is relatively apart from U.S.D.A. 21459 and 21455. Fruity and vitamin B attributes are positively correlated. Herbal is negatively correlated with artificial fruit and sweet, woody and grassy are negatively correlated with floral and soapy. Soapy, overall intensity, floral and sweet are positively correlated. Vitamin B and fruity seem to be independent of artificial fruit, soapy and floral.

Table 8 lists the weights of each attribute on each principal component. PC1, weighted mainly by soapy, floral, overall intensity, and artificial fruit doesn't separate the samples very well. Hallertauer has the lowest scores in PC1, indicating that it has less of these

attributes.

PC2 separates the samples better. Hallertauer presents high scores in PC2, indicating that it has more herbal, citrus and vitamin B character.

PC3 separates the samples based on grassy, vitamin B and fruity, indicating that Hallertauer is the richest in these attributes.

PC4 separates the samples based mainly on vitamin B and fruity with Hallertauer being the richest in these attributes.

The other two varieties present similar scores in all the PC's indicating that they are very similar in the attributes analyzed.

3- Stepwise Discriminant Analysis (SDA)

Stepwise discriminant analysis (Afifi and Azen, 1979) is a procedure where variables most effective at classifying samples are selected one by one according to the contribution that each variable makes to the resolution of sample differences. The first variable chosen was the one with the highest F value. Table 9 a,b contains the partial R squares for each step.

When SDA was applied to the DSP data it was possible to conclude that vitamin B, soapy and woody are the main discriminator variables among the three varieties (Table

9a) The mean scores for soapy and woody are highest for U.S.D.A. 21455 and 21459 and the score for vitamin B is highest for Hallertauer (Table 7).

When SDA analysis is applied to the DSP data, considering only the data for the three panelists that participated in the GC effluent sniffing experiment, it is possible to conclude that again, vitamin B and soapy are the main discriminators, but now floral, instead of woody is the third most important discriminator (Table 9b).

C- GC-EFFLUENT SNIFFING

1- Individual responses to the same stimuli

Even though the objective of this study was mainly the understanding of the product rather than its observers, there are a lot of factors related to them that can not be ignored because of the nature of the technique used.

It is also important to state that, although this study is not involved with the determination of thresholds for each compound, but with the determination of its' relative odor importance at one dilution stage, all the statements that other authors have made regarding variability and determination of thresholds can be applied to this study.

In Tables 10, 11, and 12 are displayed the odor active peaks that were detected by each one of the four subjects that participated in this study. A tentative chemical compound is reported in the region where the odor is detected. It does not mean that this compound is responsible for the odor perceived. This point is discussed later.

From these tables it is possible to observe the high variability in descriptors and sensitivity among subjects. Subject 1, having an average of 31.1 detected peaks (Table 13), seemed to be the most sensitive. Subject 2 with an average of 13.4 odor active peaks detected, was the least sensitive of all four. Subjects 3 and 4 detected an average of 27.7 and 23.0 peaks, respectively.

The high variability among subjects is not a new issue. Amoore et al. (1968) and Amoore (1980) reported that olfactory thresholds are notable for their extreme variability among subjects. He reported that 96% of the population have sensitivities between 16 and 1/16 times of the normal. A considerably large range has been reported by Brown and Clapperton (1968) and Yoshida (1984).

Even though subject 1 seemed to be the most sensitive of all of the subjects, she was less or not sensitive to some of the higher molecular weight sesquiterpenoids such as the humulene monoepoxides or diepoxides (Tables 10, 11, and 12). On the other hand,

subject 2, with the least overall sensitivity, detected these compounds in two of the samples (Tables 11 and 12). Even though this subject seemed to be anosmic for linalool, she was able to detect odors that no one else could in a particular variety, such as the one at 19:48 min (1685) in Table 12 or at 41:84 min (2593) in Table 12 or 34:05 min (2266) in Table 10. This last peak was detected in all the other varieties by this subject. Subject 1 detected it in some of the varieties (Tables 11 and 12).

Subject 3 was the most sensitive to the high molecular weight sesquiterpenoids such as the humulene monoepoxides or diepoxides. An odor was always perceived for her in the region around KI_{20M} = 2353 to 2410 in Table 12 (noted by the I sign). The same trend is observed in this region in Tables 10 and 11).

Subject 4 detected an odor at 39:81 min (2580) in two of the varieties when all other subjects did not (Tables 11 and 12).

The observation mentioned above are in agreement with findings reported in the literature. Punter (1983) and Stevens and Cain (1987) reported that some odorants exhibited much higher variability than others in the determination of their thresholds. For example they found that the range for D-limonene was much higher than the range for iso-amyl butyrate. It has not been establish why

these huge differences occur among compounds. It constitutes a major puzzle of olfactory psychophysics. Stevens et al. (1988) stated that people do vary greatly in sensitivity. This could be overall sensitivity to all compounds or a specific sensitivity to a particular odorant. They also stated that olfactory sensitivity could fluctuate from day to day or even hour to hour or moment to moment. In their study they reported that variability for a given subject is comparable to that reported across subjects (Punter, 1983, Stevens and Cain, 1987). Day by day variability was also observed in our study. In days where subjects were stressed or tired they failed to detect some odors or could not describe the effluent. This and the fact that a subject can miss a peak when breathing out, justifies why each subject sniffed the same sample on 4 consecutive days.

Another aspect observed is the variability in descriptor terms assigned to each odor. As it can be seen in Tables 10, 11, and 12, those descriptors in bold are the most frequently assigned to an odor for each subject. There were cases, such as that at retention time 20:16 min, KI_{20M} 1712 (Table 10), in which different descriptors were reported for subject 1 in each replicate. In that case, all of them are presented in boldface. For some compounds there was good agreement among and within the subjects (retention time 26:70 min, KI_{20M} 1964 in Table 10)

for the descriptors, for others compounds no agreement was found. In the case of linalool, which was used to train subjects in the use of the intensity scale, the humulene monoepoxides, and geraniol, the homogeneity in descriptors can be attributed to the previous exposure and discussion of the sensory characteristics of these compounds before starting the evaluation of the GC effluents (Table 4). As has been stated by Cain (1977), at least three factors impede odor identification: 1- sluggish formation of association between odors and names, 2- failure to retrieve the name in spite of a well formed association and 3- inherent confusability of the stimuli. Cain (1979) also stated that with appropriate help in encoding and retrieval, people can identify as many substances as they can discriminate and for which they posses well learned names. For that reason it is recommend to familiarize the subjects with the odors that they will perceive during the evaluation of the samples. Some of the subjects exhibited what is called "tip of the nose" (Lawless and Engen, 1977). They could recognize the odor but were not able to name it. In those cases the subject was provided with terms developed by the other subjects. In many cases (peak at 26:70 min, KI_{20M}= 1964 in Table 10), they were able to categorize odors in general but they named them differently.

Goldstein (1989), stated that a problem in dealing with quality is that it is difficult to describe odors because of the lack of a specific language for odor quality. It is reported that odor quality is affected by physical and chemical properties of the compound such as chemical reactivity and electrical charges of elements that make up a molecule. Especially for smaller molecules making a small change in just one molecular group can cause large changes in odor.

Another fact that can explain the descriptor variability is stated by O'Connell et al. (1989). Here, anosmics are defined as those who have a good sense of smell but who lack the ability to perceive a particular odorant. They also reported that in addition to the pronounced shift in thresholds, anosmics often provided an altered quality judgement when presented with a liminal concentration of the product for which they are anosmic. That means that a single compound can elicit multiple intensity and quality reports from different subjects.

This fact was also observed in our study. It is possible that the descriptors assigned to some of the peaks that have low odor intensity, such as peaks C and D in U.S.D.A. 21459, are not correct because of the reasons stated above. It is very possible that the same phenomena causes the disagreement with the descriptors within each subject.

2- Consensus

Results from the GC effluent sniffing show that varieties U.S.D.A 21455 and U.S.D.A. 21459 have the highest number of peaks and total area in the aromagrams (Table 14). In Hallertauer the odor intensities of the peaks associated with humulene oxidation products are smaller than in the other two varieties (Table 15, and 16). This difference can be attributed to the much lower HSI of Hallertauer (Table 3).

a- Peaks that are common to three varieties

Nine of the peaks are present in all three varieties (Table 15) exhibiting aroma intensities of over 5 on the intensity scale. There were no significant differences ($p \geq 0.05$) among the intensities of these common peaks for the three varieties.

Peak 1, identified as 9-methyl-decan-2-one, has been reported as a hop oil component by Tressl et al. (1978a) and Meilgaard and Peppard (1986). From our study it is suspected to have a very low threshold since it can be detected when the amount present is 100 times smaller than linalool (assuming equal FID sensitivity for each compound). No threshold data or descriptor was found in the literature for this compound.

Peak 2, linalool, is present in high concentrations and it has been reported to have a very low threshold. For that reason it is a good contributor to the aroma. Meilgaard (1975a) reported a taste threshold in beer of 80 ppb and described it as having an aniseed and terpenoid odor. Guadagni et al. (1966b) reported a taste threshold of 6 ppb in water. It has been described as a floral/green by Fukuoka and Kowaka (1983). Peacock et al. (1981b) reported a taste threshold of 27 ppb in beer and it is considered one of the compounds responsible for the floral character of beer.

Peaks 3 and 4 could be associated with 2-undecanone and 2-dodecanone. When a mixture of the two ketones was sniffed by the same subjects that performed the evaluation of the samples, odor was not detected for 2-undecanone even at a concentration 1.5 times higher than that of the sample. It is possible to conclude that peak 3 is associated with a compound other than 2-undecanone. The compound 2-dodecanone was described as having a vinyl, fresh vegetative, and citrus character. The reported taste threshold for 2-dodecanone is half that of the 2-undecanone, but it is found in the samples in very low concentrations making it possible that this is not the compound responsible for the odor detected.

Meilgaard (1975a) reported the taste threshold of these compounds in beers as being, 0.4 ppm for 2-

undecanone, and 0.25 ppm for 2-dodecanone. They were described as having a varnish like character.

Peak 5 was possibly associated with citral B (geranial). When citral was sniffed trough the GC at the same concentration as in the sample, citral B was described as a having a citrus character and a very low odor intensity. Because of the odor characteristic and the coincident KI_{20M} calculated for the standard, it is possible to assign citral B as the compound responsible for the odor detected in that region. Meilgaard (1975a) reported a taste threshold of 0.15 ppm for the mixture of citral isomers and described it as lemon.

Peak 7 might be associated with an unidentified compound similar to geraniol.

Peak 8 had a high intensity in all the samples but it could not be identified.

Peaks 12 and 17 are discussed in the humulene oxidation products section.

b- Peaks that are common to two varieties

Peak 13 is common to Hallertauer and U.S.D.A. 21455. It could be associated with δ-cadinol which was tentatively identified (Table 16). There were no significant differences between the intensities of these peaks in either variety ($p \geq 0.05$). This compound was

previously reported by Tressl et al. (1978a), but no sensory reports were found in the literature.

Varieties U.S.D.A. 21455 and 21459 share 5 peaks, all of them of high intensity (Table 16), and they were not significantly different ($p \geq 0.05$) in either essential oil. Most of these peaks are associated with the humulene and caryophyllene oxidation products that are absent or at lower concentration in Hallertauer. All of these peaks are discussed later.

c- Unique peaks in each variety

Each variety presents a few unique odor active peaks (Tables 17, 18, and 19). U.S.D.A. 21455 had 4 unique peaks (peaks 6, 14, 15 and 18) most of them of low odor intensity. Peak 6 was suspected to be 2-tridecanone or geranyl iso-butyrate. When these two compounds were sniffed by the same subjects at the same concentration as in the sample, 2-tridecanone was described as being floral/vinyl/citrus for some of the subjects. No odor was detected by any of the subjects for geranyl iso-butyrate, even at concentrations much higher than in the sample. Based on the descriptors assigned to the standard and KI_{20M} proximity, it seems that 2-tridecanone was detected only by two subjects and it is not a consensus peak (Tables 10, and 11). The taste threshold reported for geranyl iso-

butyrate was reported to be 450 ppb in beer (16 times higher than linalool for the same author, Peacock et al. (1981b). Guadagni et al. (1966b) reported a taste threshold of 13 ppb in water. The amount of this compound in these samples is not enough to make a significant contribution to the aroma. For these reasons it is possible to conclude that the odor detected is due to a compound not identified here.

Peak 14 can be associated with α-cadinol or torreyol. α-Cadinol was reported in hop oil by Tressl et al. (1978a). Neither of these compounds were confirmed because of the non availability of standards.

U.S.D.A. 21459 had 4 unique peaks, all of them with odor intensities lower than or equal to 5. Peak C is possibly associated with spathulenol or humulol. No standards were available to confirm these compounds or to assess their odor characteristics. Humulol has been reported as being produced during fermentation or boiling in water and also during the aging of hops (Peacock and Deinzer, 1981a). It is expected to be found in higher concentrations in beer. Lam et al. (1986a) have found smaller amounts of humulol in American beers than in European beers. This compound had been described by Fukuoka and Kowaka (1983) as having a hay like odor. The taste threshold reported in beer is > 2 ppm (Irwin, 1989).

Peak E of U.S.D.A. 21459 could be associated with some of the humulenol isomers. Irwin (1989) isolated a mixture of humulenols. Peacock et al. (1980) reported humulenol II in beer at a concentration of 250-500 ppb. They speculated that humulenol II could be a hop flavor contributor and determined the threshold to be 500 ppb, but again the compound used was a mixture of humulenol I and II so the odor and the threshold of each isomer is not known. Peacock and Deinzer (1981a), later reported a taste threshold of 2500 ppb for humulenol II in beer and 100 ppb for humuladienone in beer.

Hallertauer had probably three unique peaks. Peak 4 had a KI_{20M} higher than in the other varieties but the descriptors assigned seem to be similar. The variability could be due to a different duration of the odor in this sample, or it could be a different compound. Peak A was a unique peak with an odor intensity higher than 5, described as tobacco, floral or prunes. Because of the similarities in KI_{20M} it could be associated with tridec-?-en-2-one or with geraniol. No standard was available for tridec-?-en-2-one and geraniol was not considered a consensus peak because it was detected by only two subjects (Table 10, 11, and 12).

Because of the similarities in descriptors it is possible that in Hallertauer the compound responsible for peak 7 is in higher concentration than in the other

samples causing a continuous odor in this region. It is also possible that some other geraniol derivative in that region is responsible for the odor.

d- Caryophyllene and Humulene oxides

Oxidation products of humulene, one of the major sesquiterpene hydrocarbons in hop oil, have been the focus of much attention in recent years as a possible source of the traditional noble hop aroma. That assumption was made indirectly without sensory confirmation: hops with higher concentrations of humulene oxidation products were those considered noble hops for the beer industry.

When a mixture of the three monoepoxides composed mainly by monoepoxide II (70%), was sniffed by the same panelists that evaluated the samples at concentrations similar to those found in the sample, all the subjects detected three peaks, all of them of high intensity and described as musty/floral/spicy. Because the descriptors assigned to these peaks in the sample were the same as those in the standards, and the KI_{20M} were also the same, we can conclude that peaks 10,11 and 12 are due to humulene monoepoxide I, II, and III, respectively. It was also possible to observe that monoepoxide III probably has a much lower threshold than monoepoxide I since it was possible to detect it even at concentrations 2 times

smaller than humulene monoepoxide I. Now the question is to determine if it has a threshold lower than monoepoxide II. Another aspect that needs to be mentioned to end up the discussion about these compounds is how these compounds behave through a dilution. It is important to determine if they are major aroma contributors when they are in solution such as that evaluated by the DSP. Another aspect will be to investigate if they survive the brewing process so that they can be responsible for the noble aroma in beer. The relative contributions of these compounds to the aroma of the hop oil and finished beer is still questioned (Meilgaard, 1986; Fukuoka and Kowaka, 1983; Irwin, 1989; Peacock et al., 1980, 1981a,b, 1989). These products are formed by oxidation of humulene during hop storage and fermentation of beer (Peacock and Deinzer, 1981a). Tressl et al. (1978b) reported large amounts of humulol and humulene monoepoxide I in beer and lesser amounts of humulene monoepoxide II. Peacock et al. (1980); Peppard et al. (1989); Lam et al. (1986a) have found smaller amounts of humulene monoepoxide I in American beers and noted that beers brewed with the noble aroma hops have more of these compounds, especially humulenol II. On the other hand, Fukuoka and Kowaka (1983) claimed to have demonstrated that several known humulene derivatives are relatively unimportant in relation to hop character. They were able to point out two unidentified

sesquiterpenoids which they believed to be important contributors to the herbal character of hops. Irwin (1989) suggested that because these products do not have a hoppy character and their thresholds in beer are high, they probably do not contribute to the hoppy character in beer. Peacock and Deinzer (1981a) stated that the main oxidation product formed during storage is humulene monoepoxide II, and that humulol and humulenol II are the main products formed during the fermentation. They estimated the taste threshold in beer for a synthetic mixture of humulene monoepoxide II and III, mainly monoepoxide II, as being 450 ppb. They also reported a taste threshold in water of 10 ppb for humulene epoxide I.

Irwin (1989) reported a taste threshold of approximately 2 ppm in beer for a mixture of humulene monoepoxides. No data exist in the literature regarding the threshold of the humulene epoxides individually.

Peak 9 is associated with caryophyllene oxide. When the purified compound was sniffed through the GC by the same subjects that have participated in the whole experiment, it was described as a having a very spicy/floral character. The odor was described as being similar to that of the humulene epoxides but not equal for the majority of the subjects. Because it was described in the same way during the sample evaluation, it is present in high concentration, and its KI_{20M} is similar, it is

possible to conclude that this compound is responsible for the odor detected in that region. No threshold information was found in the literature. Some authors reported that this compound probably does not survive the brewing process because it has not been found in beer (Peacock et al., 1980; Tressl et al., (1978b); Peppard et al., (1989).

Peak 18, as well as 17, are possibly associated with humulene diepoxides. The descriptors assigned to the odors in this region were similar to those assigned to the humulene monoepoxides. This fact was also stated by Lam and Deinzer (1987). But in that case it was not possible to assign a particular odor to each of the isomers because they could not isolate the diepoxides from the mixture. It has been reported that there are 12 possible isomers, depending on the location and relative configuration between the two epoxide rings (Lam and Deinzer, 1987; Peacock et al., 1989). These compounds were reported as being related to beer brewed with noble aroma varieties but no sensory information is available.

When panelists sniffed a mixture of diepoxide A and B, no odor was detected even at concentrations 25 times higher than that found in the sample. Having in mind that different enantiomeric forms and isomers can have different odors (Pickenhagen, 1989), it is possible that the odor perceived in this region is due to one of those 12 isomers mentioned above and not to diepoxide A or B.

3- Non-consensus peaks

Those odor active peaks that were detected at most by two of the subjects are considered in this study as non-consensus peaks. The identity of some of them was confirmed using the appropriate standards. Those include the compounds, 2-decanone, 2-tridecanone, 2-pentadecanone, and geraniol.

The compound 2-decanone was detected by subjects 3 and 4 only in Hallertauer where it is present in higher concentration. The standard was described as having a floral and rancid character.

The compounds 2-tridecanone and 2-pentadecanone were described as having a vinyl, fresh vegetative, and citrus character when sniffed by the same subjects that evaluated the samples. Because of the descriptors assigned it seems that 2-tridecanone was detected by only subjects 1 and 3 (Tables 10 and 11). For that reason 2- tridecanone was not the compound responsible for peak 6.

Meilgaard (1975a) has reported the taste threshold of these compounds in beers as being, 0.25 ppm for 2-decanone and 0.1 ppm for 2-tridecanone. All of the ketones were described as having a varnish-like character except for 2-decanone which was described as flowery and sweetish. He reported that the threshold of the methyl ketones decrease as the number of carbons increase. It is

believed that 2-pentadecanone was detected only by subjects 1 and 3 (Tables 10 and 12), because they assigned the same descriptor to this peak as they did to the standard.

Geraniol was described by some of the subjects as having a floral/citrus character when it was sniffed at the same concentration as in the sample. From Tables 10 and 11, it is possible to see that based on KI_{20M} proximity and odor descriptor, geraniol was detected only by subjects 1 and 4 (Table 10), or by subjects 1 and 2 (Table 11). For this reason it is not considered the compound responsible for the odor detected in the region of peak A. Geraniol has a reported taste threshold of 36 ppb in beer (Peacock et al., 1981b), and it is considered to be one of the compounds responsible for the floral character of beer.

There are some compounds such as: methyl-4-decenoate (KI_{20M}= 1643) or methyl-4,8-decadienoate (KI_{20M} = 1711) in Tables 10, 11, and 12, for which no standards were available in order to confirm their odor descriptors. Both esters seem to be in higher concentrations in Hallertauer. Esters constitute the largest single group of hop oil components and they are considered by some workers to be the most important (Meilgaard and Peppard, 1986; Guadagni et al., 1966b). These two compounds were reported for the first time by Nickerson and Likens (1966) in the

oxygenated fraction of hopped worried. Guadagni et al. (1966b) reported a threshold in water of 3 ppb and 10 ppb, respectively, indicating that these compounds are odor active. They weren't consensus peaks because of their low concentration, or because the subjects that performed the evaluation weren't sensitive to these compounds.

Other compounds include a non-identified sesquiterpene epoxide (KI_{20M}= 1927), and epicubenol (KI_{20M}= 2109).

4- Methodology discussion

a- Advantages of the GC-effluent sniffing

One of the most important features of this technique is the possibility of detecting odor active compounds in regions where the FID is not sensitive because of the presence of a compound that the detector can not recognize, or there is not enough compound to produce a good signal in the detector. A comparison of the FID and nose responses for each variety is shown in Figs. 11, 12, and 13, respectively. In Tables 20, 21, and 22 the descriptors for each odor active peak for each variety are listed along with the concentrations of the possible chemical compounds associated. As can be seen, the peak associated with 9-methyl-decan-2-one (peak 1 in the

consensus aromagrams) is very tiny in the FID, but has a relatively high odor intensity. It is possible that the threshold of this compound is very low if the sensitivity of the FID is high enough for this compound.

The opposite case is when there are high FID response but no sensory response. That is the case of peaks around retention time 32 to 35 min in Figs. 11, 12, and 13. Sometimes both responses are coincident such as in case of peaks 2, 10, 11, 12, and 13 in Fig.13.

These observations, along with the low exponents of the psychophysical functions, strongly suggest that in order to perform a sensory-instrumental correlation it is better to consider the odor intensities or the areas of the peaks in the aromagram as an instrumental response, instead of the area of the actual FID peak.

b- Disadvantages of the GC-effluent sniffing

Compounds that have a very close elution time and are present in high concentrations, can produce overlapping of odor so that the characteristics of the odor perceived may not correspond at any one of those peaks. That is the case of peaks 16, 17, and 18 in the consensus aromagrams (Fig. 13). The absence of the individual standards as well the uncertainty in the identification of these compounds, made it very difficult

to confirm the odor nature of these peaks. That is not the case for peaks 11, 12, and 13 in the consensus aromagrams where the sniffing of the individual standards confirmed the odor.

Another case is when the compounds are not resolved by the column. In those cases it is very difficult to assess an odor nature to a chemical compound.

These facts suggest that at this point it is very difficult to assign a chemical compound to each peak in each aromagram. In this study only a few peaks were confirmed by sniffing individual standards. Most of the compounds assigned to each odor and reported in the tables, are those with retention time or Kovàts' indexes closest to the retention time or Kovàts' indexes assigned to the odor active peak.

Further fractionation, concentration, and re-sniffing of these fractions is necessary. Use of columns coated with different phases as well as different temperature programs are important also in order to improve the resolution.

Taking into account the extreme variability in sensitivity reported for each subject (Tables 10, 11, and 12) it is very important to have as many subjects as possible evaluating the effluents. For that purpose and also to evaluate several dilutions of the sample, better software needs to be developed.

It has to be considered also that there is an error source when decoding the MS analysis results. Even though the same column was used for both analyses the KI_{20M} tend to be slightly different (Peppard and Ramus, 1988), and also the resolution of the sample is different. That produces uncertainty when chemical compounds are assigned to each odor peak. In order to overcome this problem it is necessary to use as many standards as possible.

What is not known, and is probably a function of each compound, is what happens with the odor intensity and its duration during the dilution process. It is possible that some compounds have high odor potency but as the sample is diluted one odor may disappear more quickly than other. In other words they have very different psychophysical functions (Pangborn, 1981). Therefore, the results obtained from the sniffing of the chromatographic effluents are not absolute and they need to be complemented by studying the behavior of the compounds in solution. In that sense the technique used in this study will give the correct information at the dilution stage that it was evaluated at, but it is possible that at other concentrations of the sample the odor intensity ratios may be different.

D- MASS SPECTROMETRIC ANALYSIS

Figure 14 represents the FID chromatograms for the three oxygenated fractions analyzed. Only the odor active portion is shown here. A chromatogram for U.S.D.A. 21455 with the identified peaks labeled is shown in Fig.15. The names and concentrations of these compounds are listed in Table 23 (numbers in Fig. 15 are coincident with numbers in Table 23). Those in bold were tentatively identified.

Considering the chemical complexity of this fraction, the identification of compounds was done mainly in the regions where odors were detected. It is important to mention that no standards were available for the majority of the sesquiterpene alcohols and they seemed to be odor active in this study. No standards were available for the confirmation of 9-methyl-decan-2-one, humulol, humulenol II, methyl-4-decenoate, methyl-4,8-decadienoate and farnesol H, but there was very good agreement in the results of the search in the three libraries and data base programs used.

It should mentioned that no sulphur compounds were detected in these samples, but some of the odors were described as having sulphury characteristics such as the one perceived at 32:80 min (2213) in U.S.D.A. 21455 (Table 12). It is suspected that these compounds are probably at a concentration not detected by the instrument.

Peak 17 in FID chromatogram (Fig. 15) was identified by the MS as being 2-pentadecanone, but when standards were used to confirm its identity, 2-pentadecanone eluted directly before humulene monoepoxide I. Peak 17 remains unidentified, being probably a different methyl ketone, possibly branched or there may have been an elution inversion due to the MS conditions.

In order to improve the identification of compounds it will be necessary to enrich the trace constituents by preparative GLC and to synthesize as many as compounds as possible since there are few standards available through the chemical companies.

E-SENSORY-INSTRUMENTAL CORRELATION ANALYSIS

1- Results

As stated by Noble (1975), those attributes that discriminate the most among samples are the most important to correlate because they are related to a flavor change.

Results from the SDA analysis (Table 9a) showed that vitamin B, soapy and woody were the most important discriminators for the data from the whole DSP.

Thus vitamin B, the main discriminator among varieties, is positively correlated (Table 24) with peak A

in Hallertauer, and it is negatively correlated with peaks 9, 10, 11 and 19 in the consensus aromagrams. It was not possible to identify peak A but it was described as having a tobacco, apple character. Peaks 9, 10, 11 and 19 are caryophyllene and three humulene oxidation products, respectively. All of them have been described as musty/floral/spicy. When stepwise regression was applied to these variables, only peak A is included in the equation. That is because peak A is negatively correlated with peaks 9, 10, 11 and 19 (Table 25). These observations confirm the results suspected from the GC effluent sniffing results: the lower amount of caryophyllene and humulene oxidation products, as well as the presence of a unique peak with a high intensity, may contribute to the vitamin B character of the Hallertauer oxygenated fraction. The equation obtained has a significant F value ($p \leq 0.001$), a high correlation coefficient (0.834), and a good Cp value (1.4) (Table 26).

Unfortunately, the next important discriminator, soapy, can not be extremely well correlated. It is correlated with peaks 14 and 3 in the consensus aromagrams. Peak 14 is a unique peak in U.S.D.A. 21455 but it is present at a very low intensity. Peak 3 was described as floral and it is not an extremely intense peak (Table 20, 21 and 22). Even though the equation has a significant F value ($p \leq 0.05$) and a relatively high

correlation coefficient, the Cp value is extremely high (102) when it should be around 3. This indicates that the equation is not well modeled statistically.

The third most important discriminator for the whole DSP, woody, is related to linalool. The descriptors assigned to linalool were floral/fruity/citrus and not woody. Linalool has been described as woody when evaluated in beer by Meilgaard, M. (1975a). The Cp value for this equation indicates the statistical validity of the equation.

The last most important discriminator for the data for the three common panelists was floral. Again, the equation obtained is statistically valid, but it may not have any sensory significance since this character is associated with peak E, which is very small and unique to U.S.D.A. 21459.

Another equation that may have sensory significance was the one that associates fruity with peaks 7 and 4 in the consensus aromagrams. Peak 7 was described as having prune character, and seems to be associated with some geraniol derivative. It has high intensity in all of the samples (Tables 20, 21 and 22) and is one of the most intense in Hallertauer. This variety had a high score for PC3 that is weighted mainly by fruity, so it is possible that peak 7 is a good contributor to the fruity character of the sample. Peak 4 was described as rancid and it was

not high in intensity. It was not identified.

It is interesting to note that the attribute grassy is associated negatively with peaks 12 and 16 (Table 24). These peaks were identified as a humulene monoepoxide III and a humulenol isomer, respectively (Tables 21 and 22). Peak 16 is absent in Hallertauer. Hallertauer again had a high score in PC3, which is weighted also for grassy (Fig.10).

Another interesting correlation is that of citrus with peaks 5 and 8 in the consensus aromagrams. Peak 5 was described as having a citrus, minty, and spicy character. This peak was associated with citral B. Peak 8 was not identified but everybody agreed that it was floral. From Tables 27 and 28 it is possible to see that citrus and herbal are correlated. It is possible that peak 5 contributes to this correlation because it is a common peak.

2- Requirements and limitations

Because of software and people constraints it was not possible for all the members of the DSP to evaluate the GC effluents. Just three subjects were available for the GC effluent sniffing experiment. The fourth subject that sniffed the GC-effluent did not belong to the DSP, but her presence was necessary to establish the consensus

aromagram. Thus the scores that these three subjects assigned to each attribute in the DSP, and the scores that those subjects assigned to the odor active peaks sniffed trough the GC were taken into account in the correlation.

Even though the correlations were derived from the data of the three subjects that participated in both experiments, the results can be easily applied to the whole sensory panel results. This is possible because:

1- Results from a t-test show that the mean scores over treatments, replications and panelists do not differ significantly ($p \geq 0.05$) for all the attributes except for citrus, where the three panelists scored higher than the rest of the panel ($p \geq 0.001$) (Table 29). The F values for each source of variation and the attribute means for the three panelists are listed in Tables 30 and 31, respectively. The treatment means are graphically presented in Fig.16 in order to examine the panelist by treatment interaction.

2- Results from the SDA show that the main discriminators listed in order of importance for the whole DSP were vitamin B, soapy and woody. The main discriminators for the three panelists were vitamin B, soapy and floral (Table 9b). Thus, only the third discriminator (that one that accounts for the least discrimination) is different.

The objective of the correlation analysis was to improve on the usual approach to GC-Sensory Correlation by using the odor intensity of the active peaks and not the FID areas.

Sensory rating is time consuming, subject to error due to the variability of human judgement and requires extensive statistical analysis to interpret the data. Instrumental analyses are more reproducible and sometimes less expensive, but they are of little importance unless they correlate with sensory judgment. Most of the flavor research purporting to correlate instrumental and sensory data is flawed by the fact that the correlations are made between the sensory data and the FID area of the peaks, without knowing if the peaks are odor active not. The approach taken in this study was to determine first the relative odor importance of each peak and then correlate these values with the sensory scores from the DSP.

Some of the pitfalls of the correlations established here can be stated as:

a- In order to assess most of the correlations a model system containing the compounds of interest has to be prepared in order to study the behavior of these compounds in solution. It is not known if the relative odor importance of the compounds at the dilution that the whole sample was evaluated by the DSP is the same as that established during the sniffing of the chromatographic

effluent.

b- Another aspect that needs to be studied is how these compounds interact with each other. When they are together do their odor intensities have an additive or suppressive effect? (Meilgaard, 1975b and Elizondo and Meilgaard, 1971).

c- Statistically it would be better to correlate both sets of data using some of the multivariate techniques now available such as Canonical Correlation or Factor Analysis because they take into account all the intercorrelations among the parameters evaluated. However, the number of observations in this study was too small to apply these methodologies.

d- Sample profiles were very similar among the three samples. Only one attribute can be considered a good discriminator. That was one of the reasons for which a p value ≤ 0.150 was established as a limit in order to enter or reject a variable in the correlation model.

For all the reasons stated, it is important to assume that the results obtained here can be orientative but they may not reflect cause and effect.

CONCLUSIONS

1- The new hop crosses are good potential noble aroma contributors to beer because their aroma profiles are very similar to that of Hallertauer. This conclusion was reached based upon results from the DSP and sniffing of the GC effluents.

2- The small differences found among the varieties can be attributed to the higher oxidation stage of the triploids, meaning there were higher amounts of caryophyllene and humulene oxidation products in these varieties. Since these products were shown to be the most odor active compounds in the triploids, it is possible that they make an important contribution to the overall aroma profile of these samples and cause the differences found.

3- Because of its' lower cohumulone ratio, U.S.D.A. 21455 seems to fit the noble hop chemical characteristics better than U.S.D.A. 21459.

4- Since correlation equations were in agreement with the results obtained during the analysis of the DSP data using PCA, and aromagrams, it is concluded that by considering the odor

intensities or the areas of the peaks detected by the nose instead of the areas of the peaks detected by the FID, more meaningful equations can be obtained.

5- The methodology developed here to characterize these varieties, once optimized, will provide both good chemical and sensory descriptions of the samples, and yield better tools to correlate sensory with chemical properties.

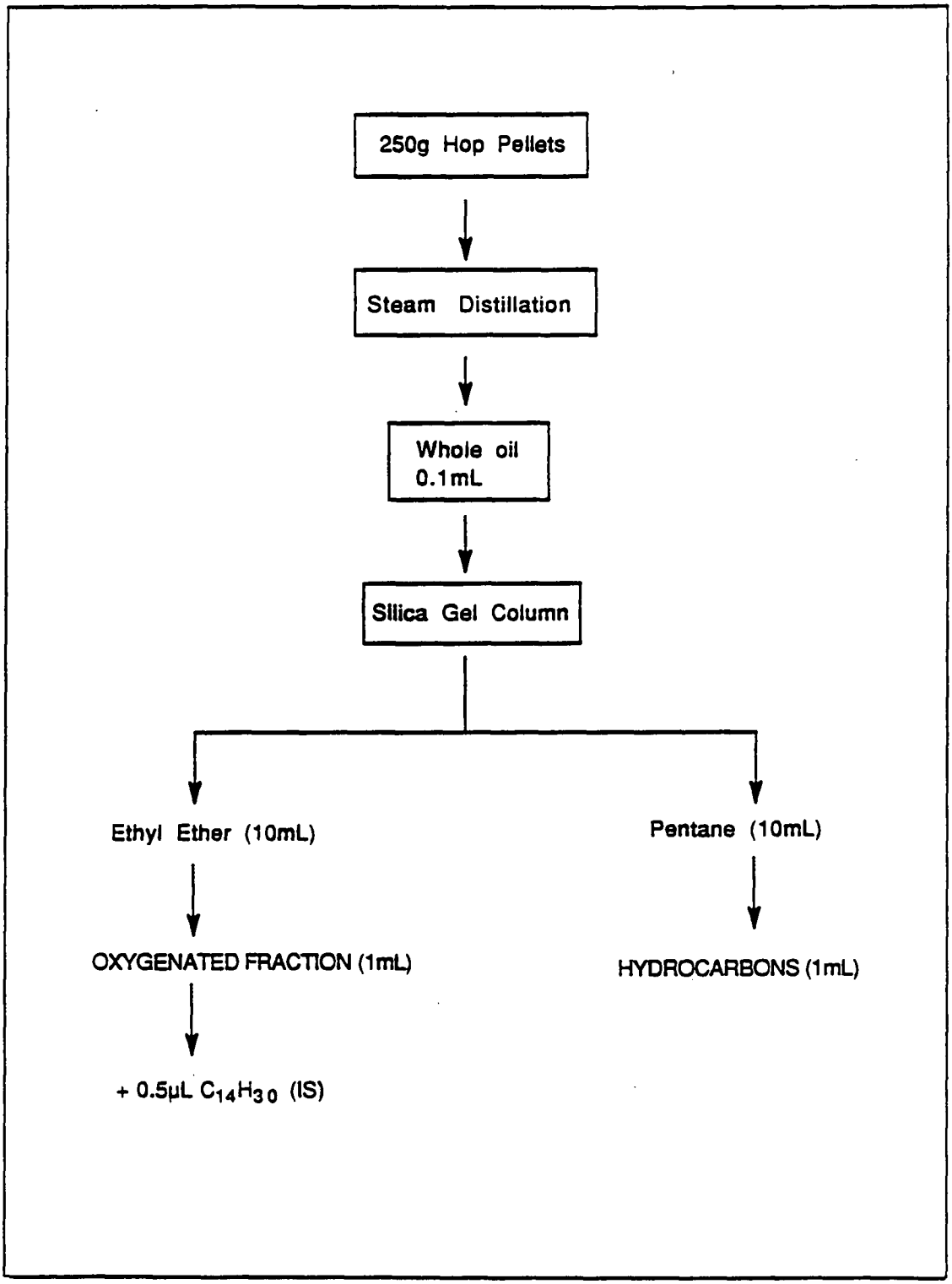

Fig.1 - Extraction and fractionation of hop oil.

DESCRIPTIVE BEER PANEL 1989

NAME: _____ Trial Ballot #1

DATE: _____

SCORING PROCEDURE: Please rate Overall Intensity first. Aroma descriptors under Overall Intensity must be scored equal to or less than Overall Intensity.

Rate the samples using the 15 point intensity scale given below.

0 = none
1 = just detectable
2
3 = slight (*oil*)
4
5 = slight to moderate
6
7 = moderate (*"orange" in orange drink*)
8
9 = moderate to large
10
11 = large (*"grape" in grape juice*)
12
13 = large to extreme
14
15 = extreme (*"cinnamon" in Big Red Gum*)

	SAMPLE #	SAMPLE #	SAMPLE #
	_____	_____	_____
AROMA			
Overall Intensity	_____	_____	_____
Soapy/Pungent	_____	_____	_____
Herbal	_____	_____	_____
Woody	_____	_____	_____
Grassy	_____	_____	_____
Citrus	_____	_____	_____
Sweet	_____	_____	_____
Fruity	_____	_____	_____
Artificial Fruit	_____	_____	_____
Floral	_____	_____	_____

COMMENTS:

Fig.2 - Ballot used for the aroma evaluation of hop oil oxygenated fractions.

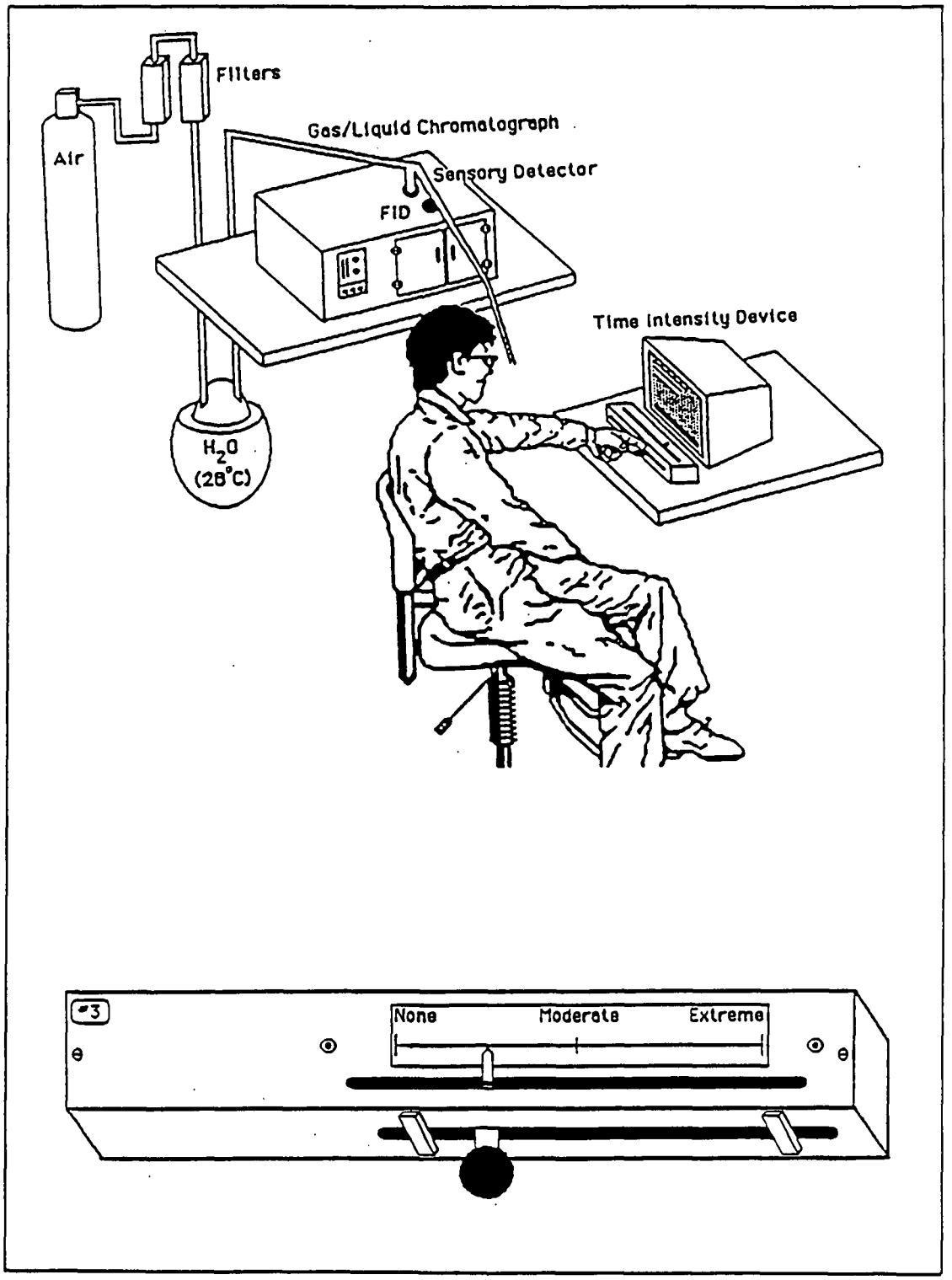

Fig.3 - Equipment used to sniff and evaluate the GC-effluents.

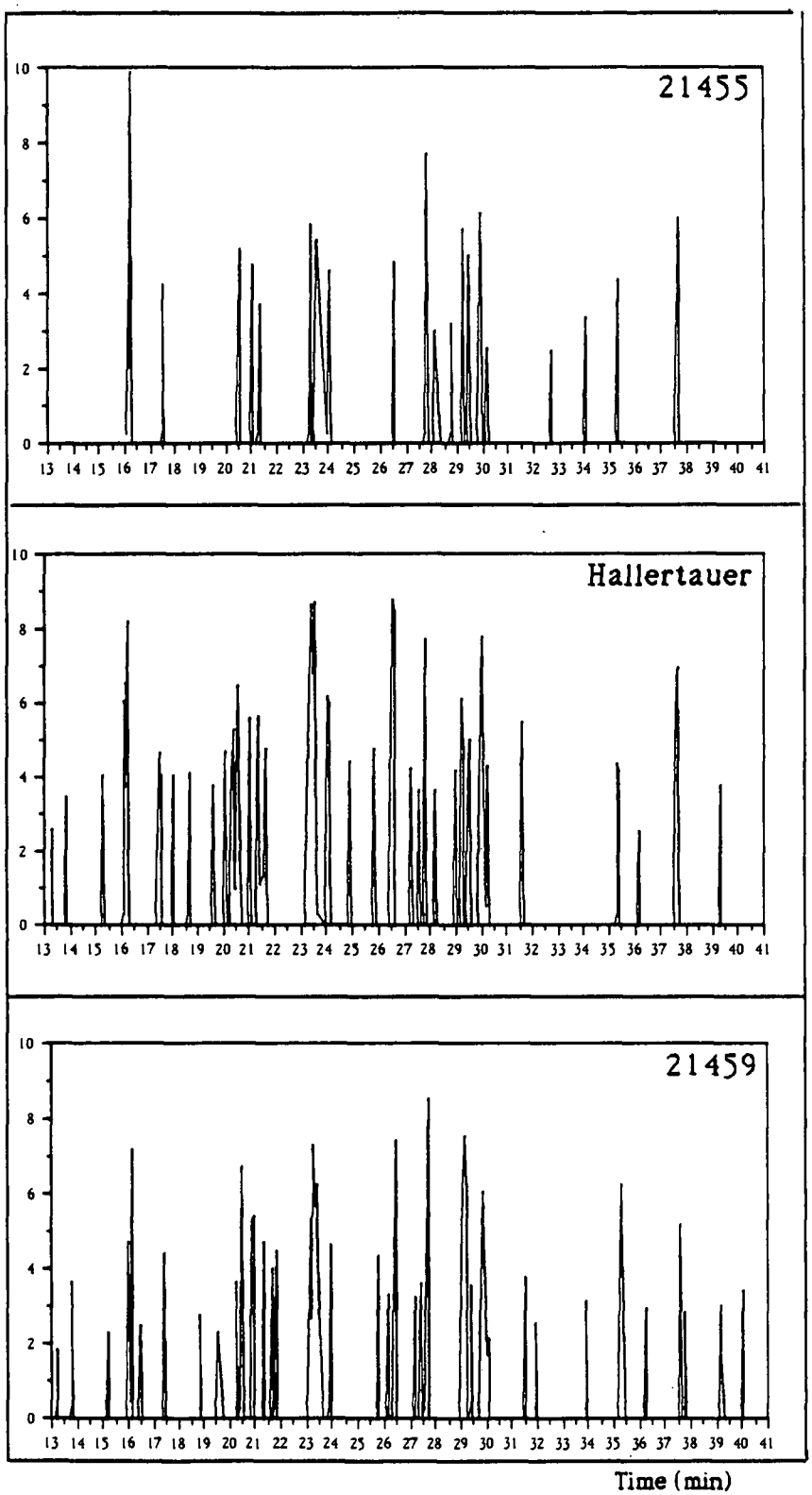

Fig.4 - Aromagrams for oxygenated fractions. Subject 1.

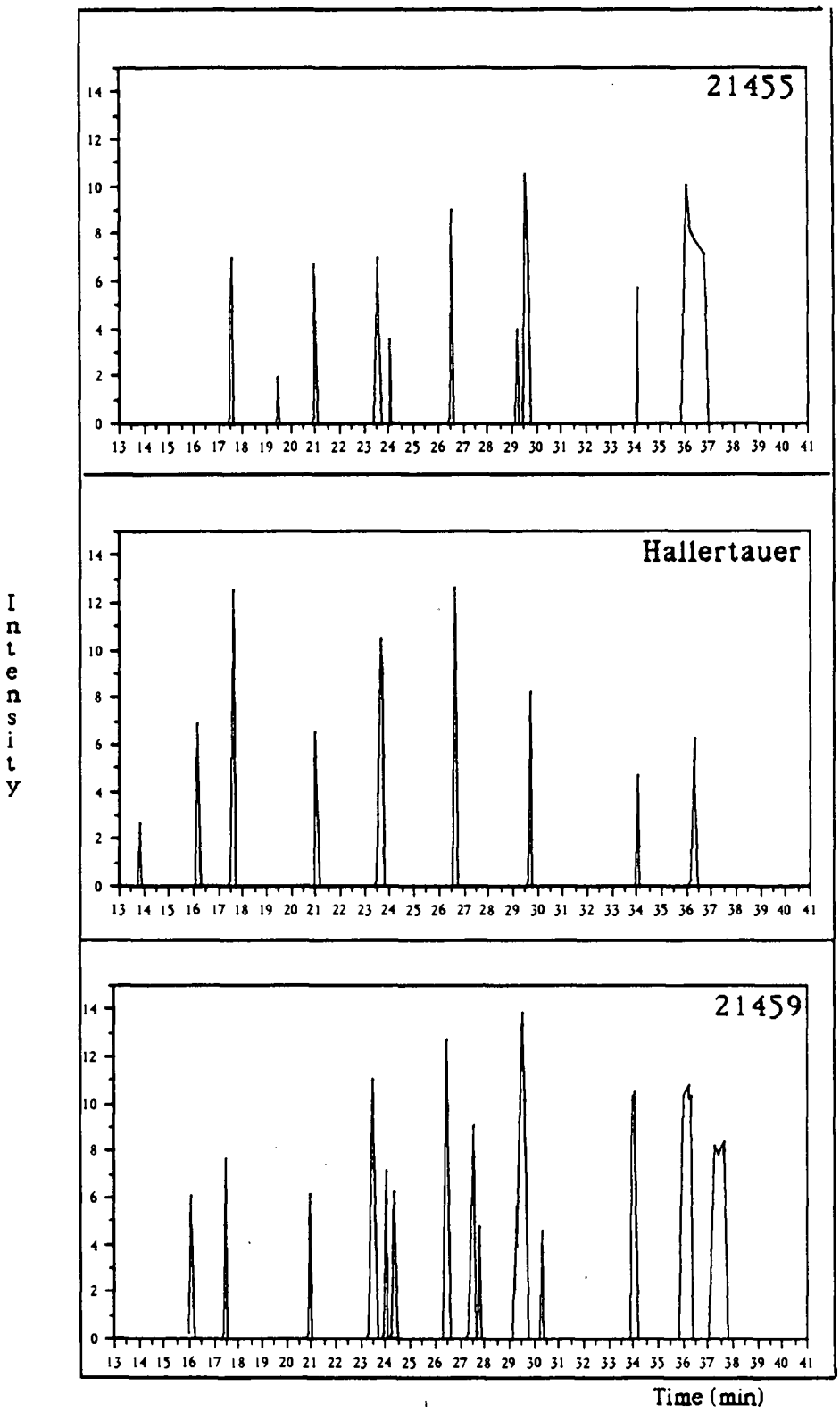

Fig.5- Aromagrams for oxygenated fractions. Subject 2.

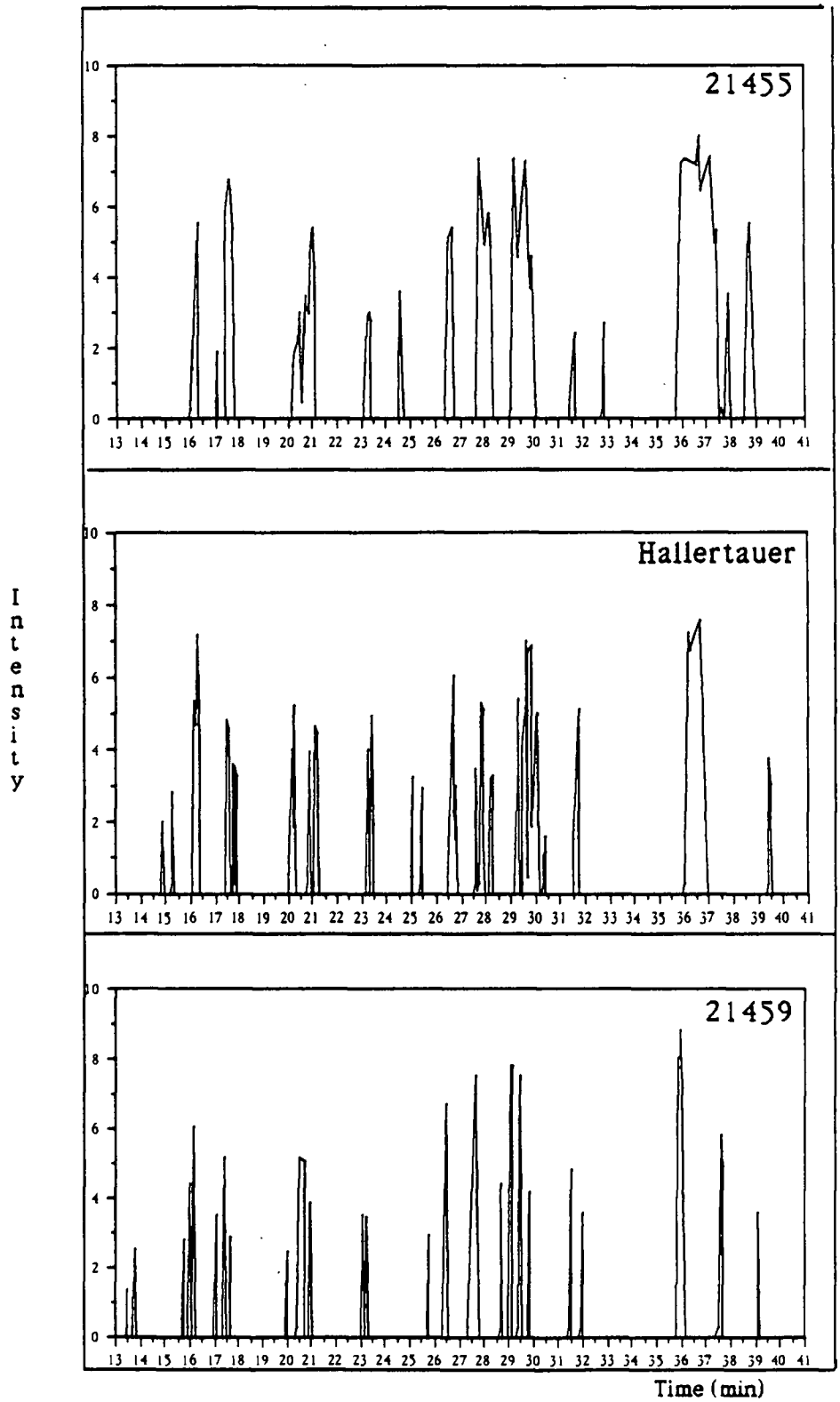

Fig.6 - Aromagrams for oxygenated fractions. Subject 3.

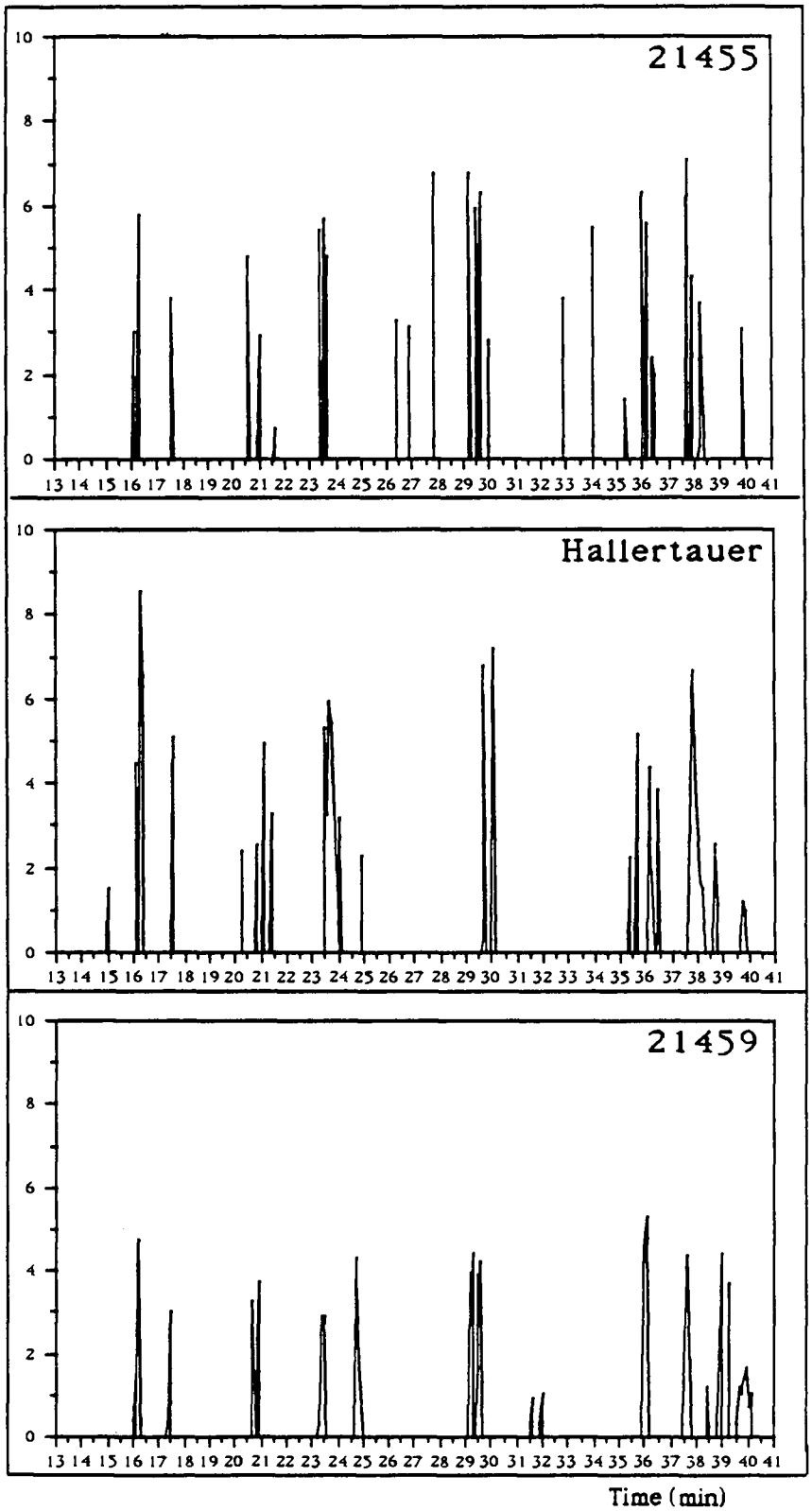

Fig.7 - Aromagrams for oxygenated fractions. Subject 4.

Fig.8 - Consensus aromagrams for oxygenated fractions. A, B, C, D, E, and underlined numbers are unique peaks in each variety.

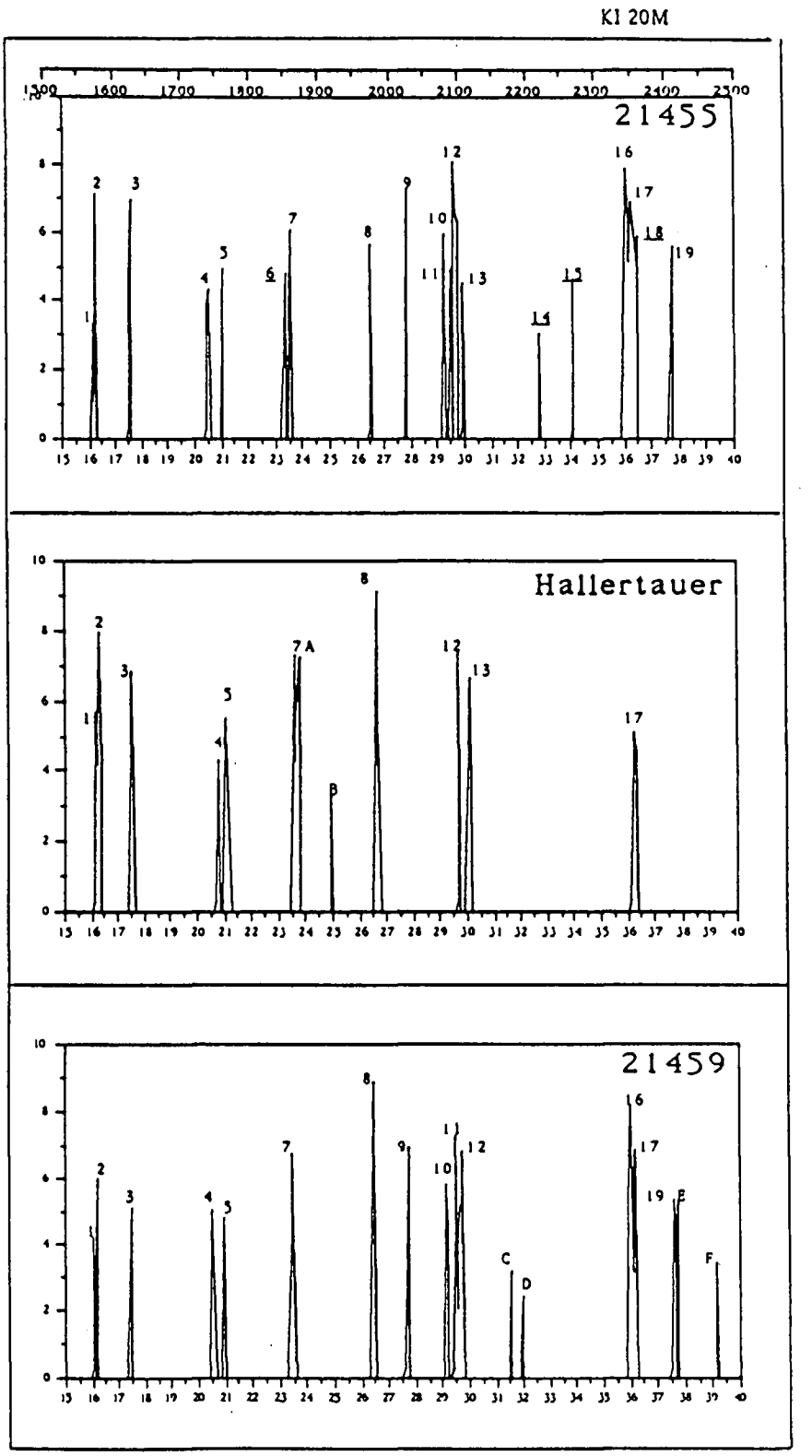

Fig.8

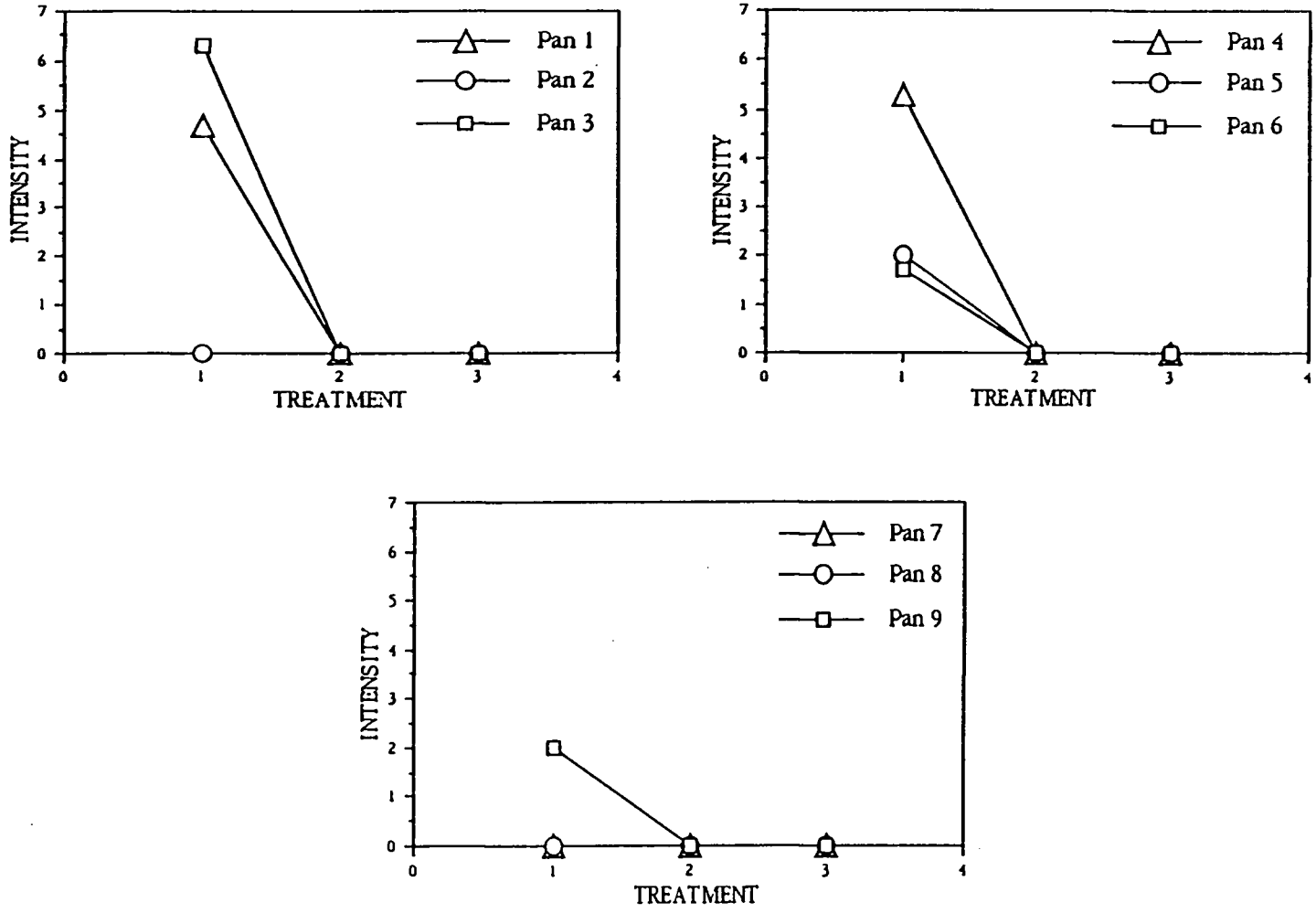

Fig.9 - Interaction panelist x treatment for the vitamin B attribute for oxygenated fractions based on the whole descriptive sensory panel data. Treatments: 1= Hallertauer, 2= U.S.D.A. 21459, and 3= U.S.D.A. 21455.

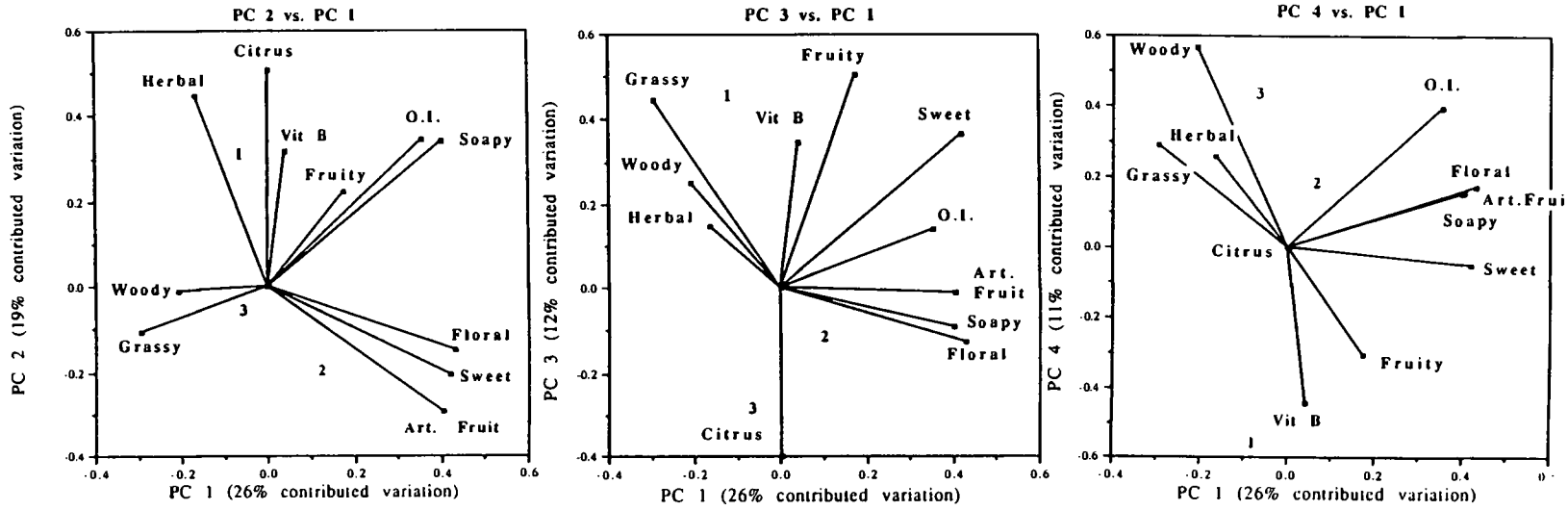

Fig.10 - Principal component analysis graphs for oxygenated fractions based on whole descriptive sensory panel data. Treatments: **1**= Hallertauer, **2**= U.S.D.A. 21459, and **3**= U.S.D.A. 21455.

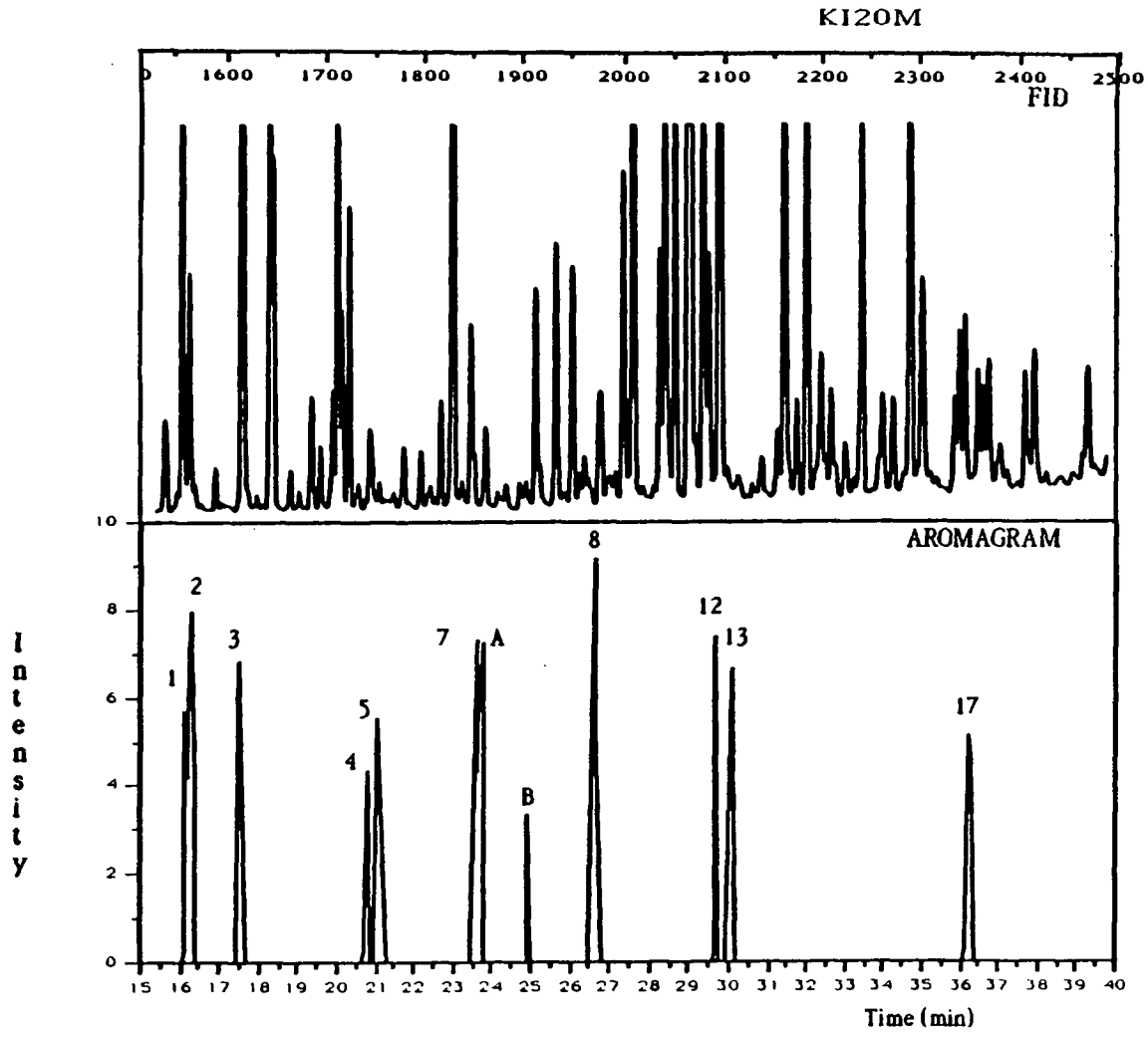

Fig.11 - FID chromatograms and consensus aromagrams for Hallertauer oxygenated fraction. Peaks 4, A and B are unique to this variety.

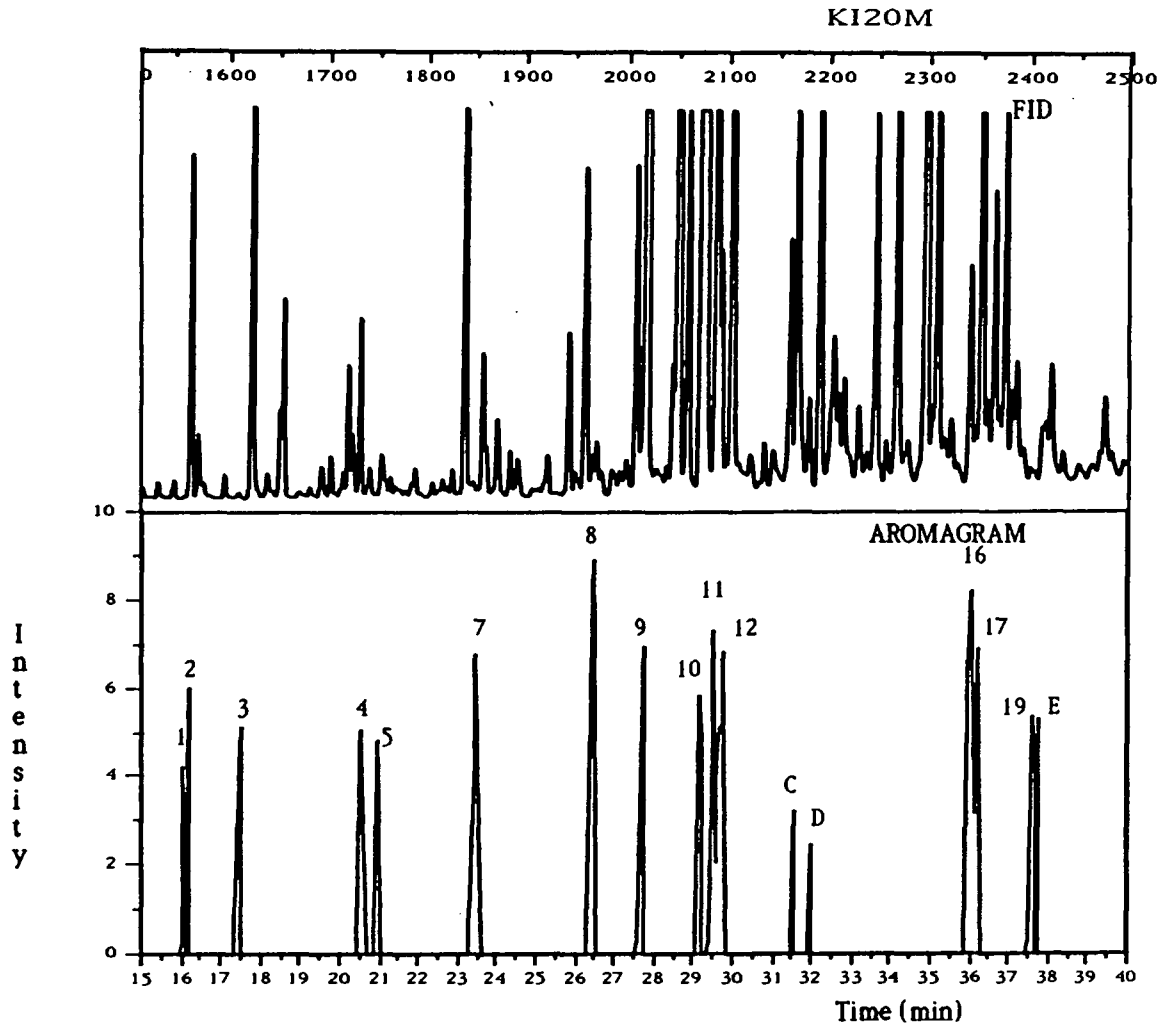

Fig.12 - FID chromatograms and consensus aromagrams for U.S.D.A. 21459 oxygenated fraction. Peaks C, D, and E are unique to this variety.

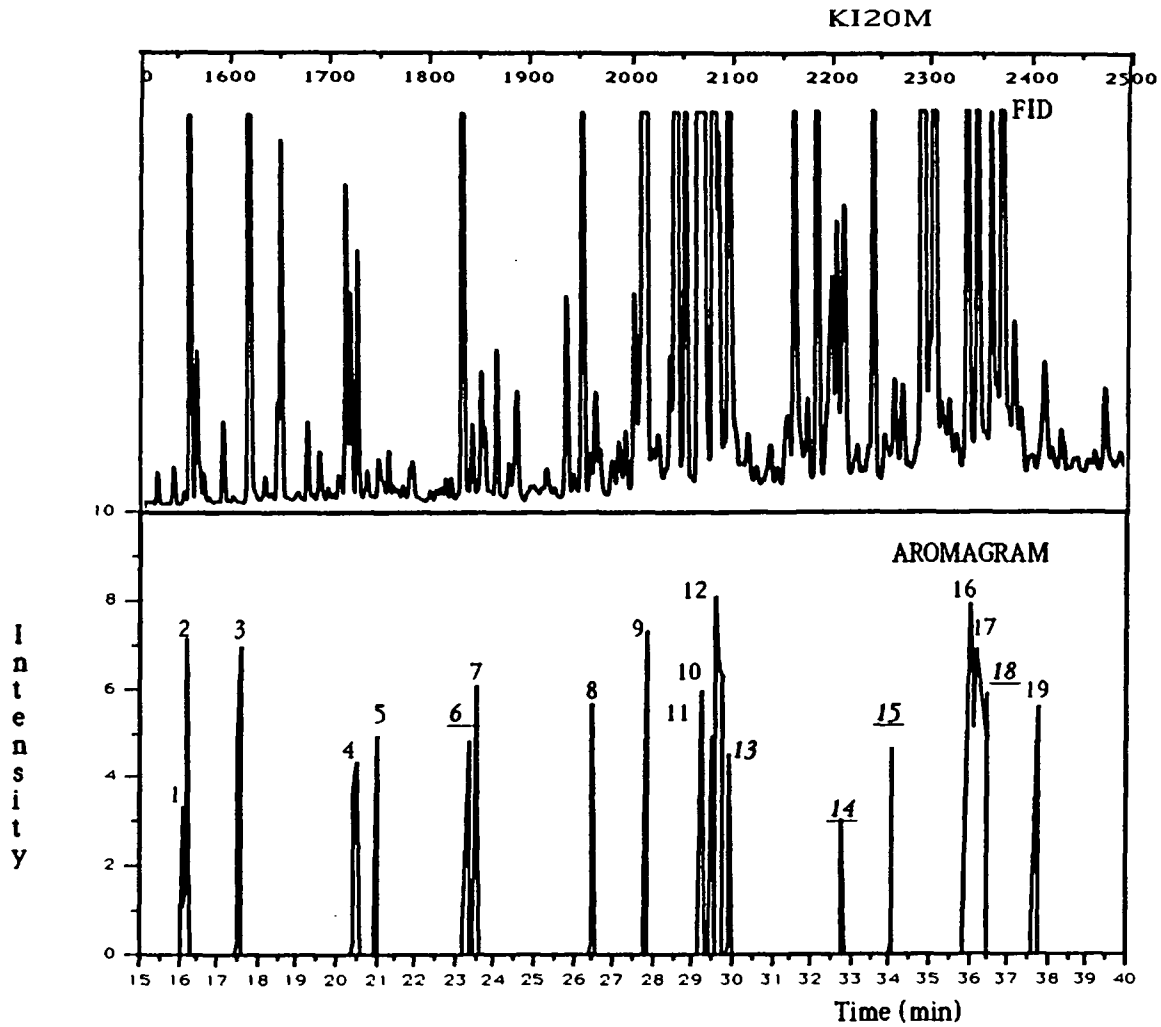

Fig.13 - FID chromatograms and consensus aromagrams for U.S.D.A. 21455 oxygenated fraction. Underlined peaks are unique to this variety.

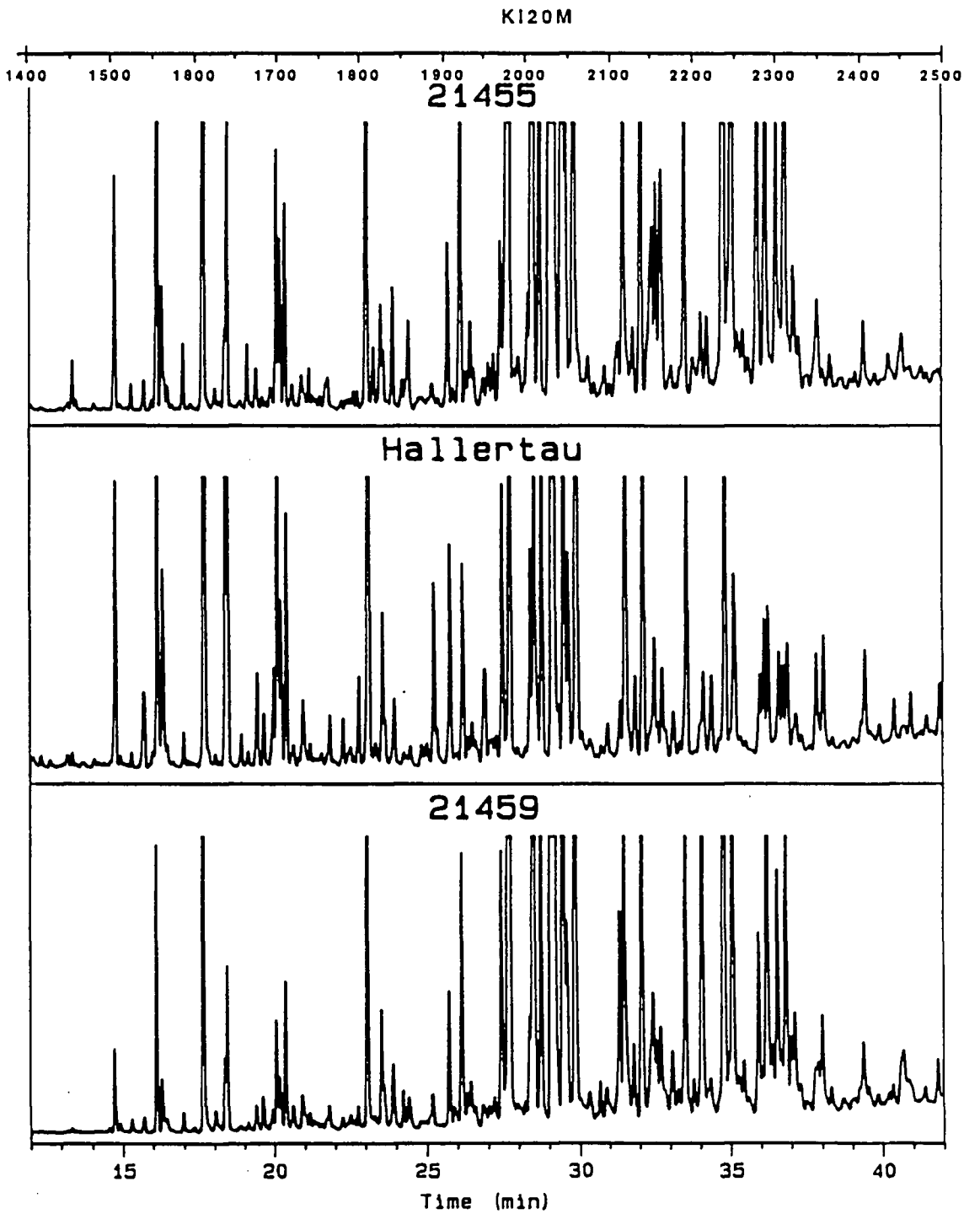

Fig.14 - FID chromatograms for hop oil oxygenated fractions. Odor active region.

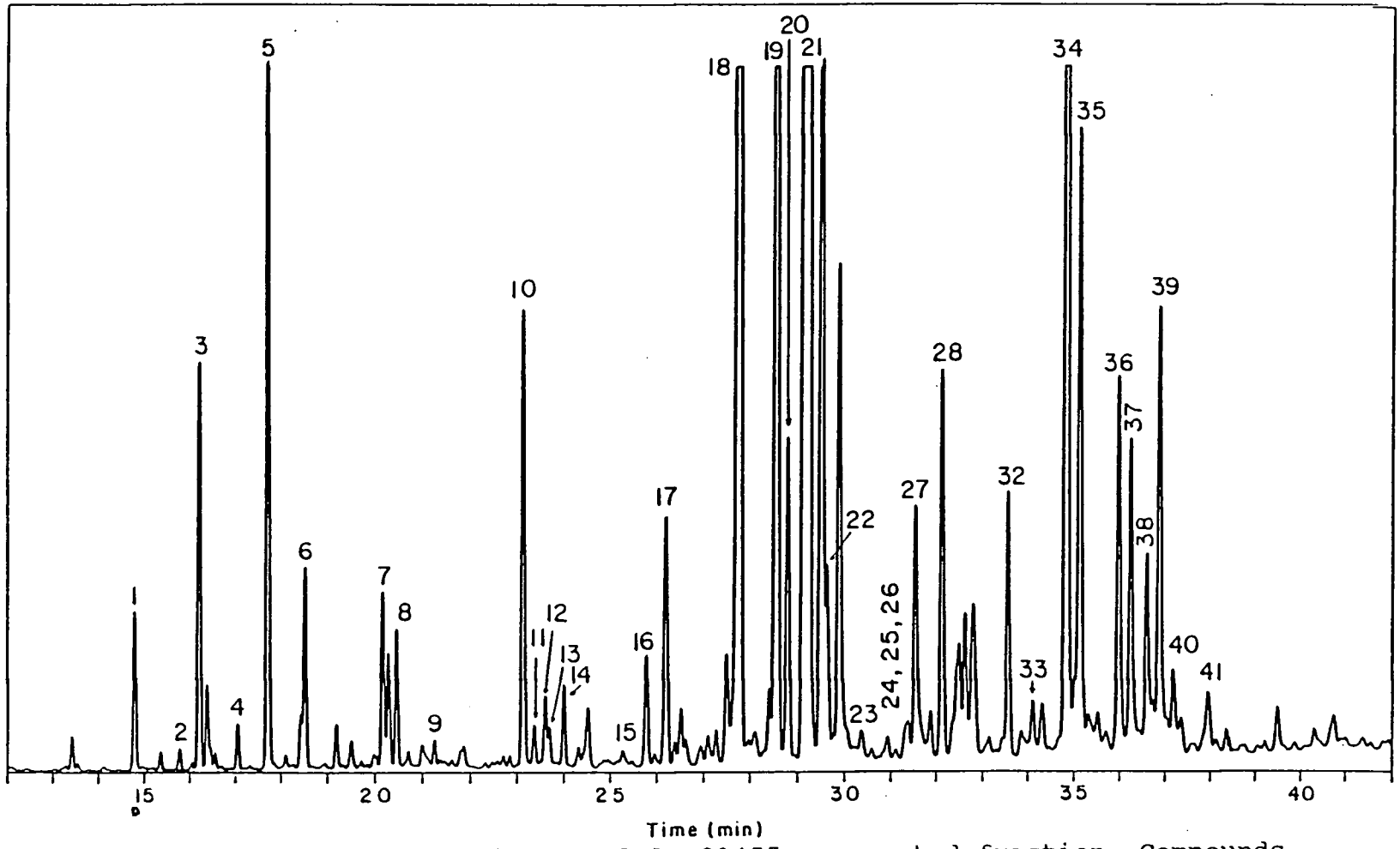

Fig. 15 - FID chromatogram for U.S.D.A. 21455 oxygenated fraction. Compounds identified are listed in Table 23.

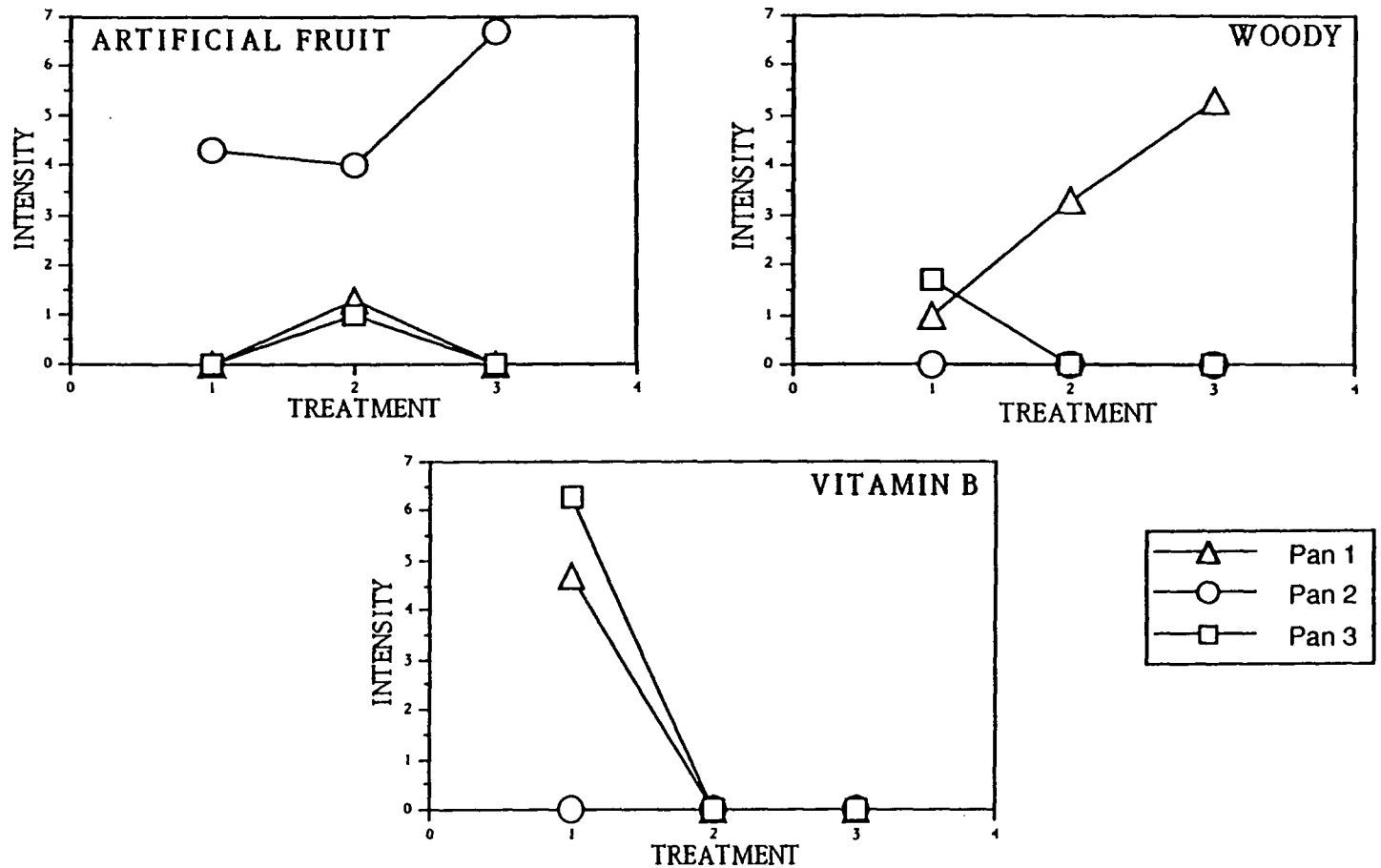

Fig.16 - Interaction panelist x treatment for the fruity, woody, and vitamin B attributes for oxygenated fractions based on the descriptive sensory panel ratings of the three panelists that participated in the sniffing of the GC effluents. Treatments:1= Hallertauer, 2= U.S.D.A. 21459, and 3= U.S.D.A 21455.

Table 1. Definitions of the Intensity Standards[1] Used by the Descriptive Sensory Panel During the Evaluation of Hop Oil Oxygenated Fractions.

Intensity (Scale Value)	Reference Aroma	Reference Preparation
Slight (3)	Oil	Total impact of the oil aroma from 30 mL of Saffola, 100% Safflower oil (Westley Foods, Inc., City of Industry, CA).
Moderate (7)	Orange	Total impact of the orange aroma from 30 mL of Hi-C Orange Drink (Coca Cola Foods, Inc., Plymouth, Fl).
Large (11)	Grape	Total impact of the grape aroma from 30 mL of Welches 100 % Natural Grape Juice (Welch Foods, Inc., Westfield, NY).
Extreme (15)	Cinnamon	Total impact of the cinnamon in Big Red chewing gum (W.M. Wrigley's Jr. Co., Chicago, IL).

1- All Intensity Standards were prepared fresh each day and served in standard wine glasses covered with a watch glass at room temperature. The oil, orange, and grape juice samples were stored until their use in 60 mL glass containers with a minimal headspace at - 10°C.

Table 2. Definitions of the Attribute Reference Standards[1] Used by the Descriptive Sensory Panel During the Evaluation of Hop Oil Oxygenated Fractions.

Attribute	Reference Preparation
Overall Aroma	Total impact of all aromas.
Soapy	Aroma intensity which yields an irritating piercing or reflex response. Reference prepared by imbibing an aroma paper[2] stick on 90% pure Methyl Laureate (Eastman).
Herbal	Primary aroma of a reference prepared by mixing 1 g each of Sassafras, Chamomile, Rosehips, Hibiscus and 2 g of Lemon Grass.
Woody	Primary aroma of a reference prepared by imbibing an aroma paper stick in Cedar Wood Essential Oil (Frontier Herbs, Norway, IA 52318).
Grassy	Primary aroma of a reference prepared by: 1- Fifty ml of a solution 0.01 % of cis-3-hexenal (95%, Bedoukian Research, Inc., Danbury, CT), in spring water. 2- Sixty ml of macerated straw or hay in spring water.
Citrus	Primary aroma of a reference prepared by combining 2 half wedges of orange, grapefruit and lemon (Sunkist Growers, Inc., Los Angeles, CA).
Sweet	Primary aroma of 10 ml of honey (Sue Bee, Grade A White, Pure Clover Honey, Sioux Honey Assn., Sioux City, IA).
Fruity	Primary aroma of a reference prepared by: 1- Three 1cm. dices of cooked, dried, apricots (Del Monte Co., San Francisco, CA). 2- One half canned Bartlett pear (Del Monte Co., San Francisco, CA), diced in 1 cm cubes. 3- One quart fresh Jonathan apple diced in 1 cm cubes.

Table 2. (cont.)

Attribute	Reference Preparation
Artificial Fruit	Primary aroma of a reference prepared by: 1- 100 g of Fruit Loops (Kellog Co., Battle Creek, MI). 2- 15 g of Mixed Berry Jello (General Foods Co., White Plains, NY).
Floral	Primary aroma of a reference prepared by imbibing an aroma paper stick in carnation or rose essence (Uncommon Scents, Eugene, OR).
Vitamin "B"	Primary aroma in a reference prepared by 2 halved tablets of Stresstabs 600 (Lederle Laboratories Division, Pearl River, NY), soaked with few drops of spring water.

1- All the Reference Standards were evaluated in 350 mL amber glasses covered with an aluminum foil lid and served at room temperature.
2- Fragance Test Filters (Orlandi, Inc., Farmingdale, NY).

Table 3. Chemical Composition of Hops*

Varieties	% α-acids[1]		% ß-acids[2]		Oil cont.[3]		H.S.I.[4]	Cohumulone Ratio[5]	Oxyg. Fraction[6]	% Oxyg. Fraction
	IS	DW	IS	DW	IS	DW				
U.S.D.A. 21455	2.9	3.1	5.1	5.6	0.70	0.77	0.55	23	0.34	35.4
HALLERTAUER	4.6	5.0	3.3	3.6	0.70	0.77	0.37	33	0.12	11.7
U.S.D.A. 21459	2.0	2.2	2.9	3.2	0.30	0.33	0.55	80	0.15	31.7

1- % α-acids calculated on internal standard (IS) and dry weight (DW) basis.
2- % ß-acids calculated on internal standard (IS) and dry weight (DW) basis.
3- Oil content (expressed in ml/100 g hops), determined on internal standard (IS) and dry weight (DW) basis.
4- Hop storage index.
5- Expressed as % of α-acids.
6- Expressed in ml/100 g of hops.
*- Analyses conducted by Gail Nickerson, Agricultural Chemistry, Oregon State University.

Table 4. Descriptors Assigned to the Following Compounds Spiked in Water During the Training Sessions for the Sniffing of the Chromatographic Effluents

Compound	Descriptor
Geraniol (98%) (Aldrich Chemical CO., Milwaukee, WI)	Floral, citrus
Linalool (97%) (Aldrich Chemical Co., Milwaukee, WI)	Floral/fruity/citrus
Humulene monoepoxides[1] I, II, III	Musty/floral/spicy Woody, cedar oil
Humulene diepoxides[1] A and B	Medicinal, plastic
Caryophyllene Oxide[1]	Floral/spicy Cheap perfume
Citral (97%) (Fluka, Ronkonkoma,NY)	Floral, citrus, spicy
Citronellal (90%) (Fluka, Ronkonkoma, NY)	Floral, sweet, citrus, pine
ß-Ionone (90%) (Fluka, Ronkonkoma, NY)	Violets, floral, musty, sweet
Geranyl iso-butyrate[1]	Citrus, roses, floral,
Geranyl acetate (95%) (Carl Roth, K.G.)	Geranium, floral, roses, citrus

1- Synthesized at Agricultural Chemistry Dept., OSU.

Table 5 . Definitions of the Attribute Reference Standards[1] Used During the Sniffing of the Chromatographic Effluents for Hop Oil Oxygenated Fractions

Attribute	Reference Preparation
Rancid	Primary aroma of a reference prepared by 30 mL of rancid oil (Saffola 100% Safflower oil, Westley Foods, Inc., Plymouth, FL).
Cheesy	Primary aroma of a reference prepared by 50 mL of a solution 0.01% of Butyric acid (Aldrich, 99%, Gold Label, Milwakee, WI).
Fresh Vegetative	Primary aroma of a reference prepared by 3 sliced fresh sweet peas.
Cooked Vegetative	Primary aroma of a reference prepared by 50 g of canned green beans (S&W, French Style, Fine Foods, Inc., San Ramon, CA).
Spicy	Primary aroma of a reference prepared by: 1- One cinnamon stick (Spice Islands, Specialty Brands, San Francisco, CA). 2- Three grams of nutmeg (Schilling, McCormik & Co., Inc., Baltimore, MD). 3- Three grams of anise seeds (The R.T. French Co., Rochester, NY). 4- Imbibing an aroma[2] paper stick in Eugenol (Aldrich, 99%, Milwaukee, WI).
Minty	Primary aroma of a reference prepared by: 1- Two grams of mint leaves (Nichols Garden Nursery, Albany, OR).
Fruity	Primary aroma from a reference prepared by: 1- One half fresh Jonathan apple sliced in 1 cm cubes. 2- One half fresh Granny Smith apple sliced in 1 cm cubes. 2- One quart fresh banana (Dole, Inc., Honolulu, Hawaii).
Tobacco	Primary aroma of one cigarette Camel (R.J. Reynolds Tobacco Co., Winston, Salem, NC).

Table 5. (cont.)

Attribute	Reference Preparation
Floral	Primary aroma of a reference prepared by imbibing an aroma paper stick on: 1- carnation 2- magnolia 3- rose 4- violet essences (Uncommon Scents, Eugene, OR).
Piney	Primary aroma of a reference prepared by 50 ml of pine leaves macerated with spring water for 1 min. in a blender.
Citrus	Primary aroma of a reference prepared by combining two half wedges of grapefruit, orange and lemon (Sunkist Growers, Inc., Los Angeles, CA).
Butter	Primary aroma of a reference prepared by 30 g of 100% butter (Darigold AA grade, Seattle, WA).
Burned Matches	Primary aroma of one burned match (Diamond Brands, Inc., Minneapolis, MN).
Prunes	Primary aroma of a reference prepared by three 1 cm cubes of prunes (Del Monte Co., San Francisco, CA).
Cooked Fruit	Primary aroma of a reference prepared by cooking during 5 min.: 1- Three prunes (Del Monte Co., San Francisco, CA). 2- Three dried apricots (Del Monte Co., San Francisco, CA). 3- One half Jonathan apple diced in 1 cm cubes.
Vinyl	Primary aroma of a plastic toy.
Musty	Primary aroma of a reference prepared by soaking with spring water five halves of filberts and let stand until mold developed on them.
Woody	Primary aroma of a reference prepared by imbibing an aroma paper stick in Cedar Wood Essential Oil (Frontier Herbs, Norway, IA 52318).

1- All the References Standards were evaluated in 350 ml amber glasses covered with an aluminum foil lid and served at room temperature.
2- Fragrance Test Filters (Orlandi, Inc., Farmingdale, NY).

Table 6. F-values and Significance Levels for Oxygenated Hop Oil Fraction Attributes from ANOVA for Panelists (PAN), Treatment (TRT), Replications (REP), and Interaction Sources

	SOURCE OF VARIATION					
ATTRIBUTE	**PAN[1]**	**TRT[2]**	**REP[3]**	**PANxTRT**	**PANxREP**	**TRTxREP**
Overall Intensity	3.11**	0.91^{ns}	1.55^{ns}	0.78^{ns}	0.77^{ns}	0.41^{ns}
Soapy	3.73**	1.20^{ns}	1.47^{ns}	0.88^{ns}	0.39^{ns}	0.66^{ns}
Herbal	4.01***	0.60^{ns}	0.62^{ns}	0.63^{ns}	0.70^{ns}	1.10^{ns}
Woody	2.72*	1.82^{ns}	0.58^{ns}	1.75^{ns}	1.04^{ns}	0.75^{ns}
Grassy	2.13*	0.61^{ns}	0.50^{ns}	0.58^{ns}	0.88^{ns}	1.27^{ns}
Citrus	11.37***	0.64^{ns}	0.68^{ns}	0.75^{ns}	0.41^{ns}	1.14^{ns}
Sweet	8.04***	0.83^{ns}	1.13^{ns}	1.03^{ns}	1.01^{ns}	0.47^{ns}
Fruity	1.82^{ns}	2.30^{ns}	0.88^{ns}	0.73^{ns}	1.33^{ns}	0.29^{ns}
Artificial Fruit	5.50***	0.40^{ns}	0.50^{ns}	1.29^{ns}	1.71^{ns}	3.49*
Floral	3.30**	1.16^{ns}	0.60^{ns}	0.89^{ns}	0.44^{ns}	1.41^{ns}
Vitamin B	1.00^{ns}	8.90**	1.00^{ns}	4.97***	1.00^{ns}	0.22^{ns}
Degrees of Freedom	8	2	2	16	16	4

*,**,*** Significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, respectively.

ns Not significant

1 Means based on scores from nine panelists.

2 Hallertauer; U.S.D.A. 21459; U.S.D.A. 21455

3 Three replications per treatment.

Table 7. Attribute Means,[1,2] (Standard Deviations), and LSD[3] Values From Whole Descriptive Panel for Hop Oil Oxygenated Fractions

	VARIETIES			
Attribute	**Hallertauer**	**USDA 21459**	**USDA 21455**	**LSD Values**
Overall Intensity	9.0 (1.63)	8.9 (1.95)	8.8 (2.66)	---
Soapy	4.2 (3.03)	4.3 (2.64)	5.1 (2.87)	---
Herbal	2.3 (2.08)	2.2 (2.08)	2.3 (2.03)	---
Woody	1.1 (1.64)	1.5 (2.14)	2.2 (2.20)	---
Grassy	1.7 (2.22)	1.4 (2.19)	1.4 (2.06)	---
Citrus	2.9 (2.57)	3.0 (2.31)	3.2 (2.17)	---
Sweet	2.4 (2.47)	2.5 (2.56)	2.2 (2.31)	---
Fruity	2.5 (2.33)	2.1 (2.46)	1.6 (1.89)	---
Artificial Fruit	2.1 (2.64)	2.6 (2.76)	2.1 (2.83)	---
Floral	1.7 (2.28)	2.8 (2.78)	2.8 (2.53)	---
Vitamin B	2.4[a] (2.78)	0.0[b] (0.00)	0.0[b] (0.00)	1.338

a,b Means with different superscripts within the same row are significantly different from one another.

1 Sixteen point intensity scale (0 = none, 15 = extreme)

2 Means calculated with nine panelists

3 LSD at $p \leq 0.05$

Table 8. Factor Loadings from Principal Component Analysis on Whole Descriptive Sensory Panel Data for Hop Oil Oxygenated Fractions

	PRINCIPAL COMPONENT			
Attribute	**PC1**	**PC2**	**PC3**	**PC4**
Overall Intensity	0.354	0.343	0.141	0.397
Soapy	0.401	0.340	-0.092	0.150
Herbal	-0.166	0.447	0.149	0.256
Woody	-0.208	-0.009	0.252	0.569
Grassy	-0.295	-0.104	0.445	0.289
Citrus	0.004	0.509	-0.399	0.001
Sweet	0.419	-0.206	0.367	-0.054
Fruity	0.174	0.223	0.506	-0.303
Artificial Fruit	0.404	-0.239	-0.125	0.158
Floral	0.430	-0.150	-0.126	0.174
Vitamin B	0.041	0.311	0.348	-0.445
Descriptor for Principal Component	Floral Artificial Fruit Sweet Soapy OI	Citrus Herbal OI Soapy	Fruity Grassy Sweet Vitamin B vs. Citrus	Woody OI vs. Vitamin B Fruity
Eigen value	2.893	1.811	1.650	1.230
Cumulative Proportion (%)	26.2	45.4	60.5	71.6

Table 9. Discriminant Variables from Stepwise Discriminant Analysis on Descriptive Sensory Panel Data for Hop Oil Oxygenated Fractions. a = Whole Panel, b = Three Common Panelists

a. Whole Panel

Step #	Variable Entered	F value, Significance[2]	Partial R-square
1	Vitamin B	20.90***	0.542
2	Soapy	2.96[1]	0.200
3	Woody	2.42[1]	0.202

b. Three Common Panelists

Step #	Variable Entered	F value, Significance[2]	Partial R-square
1	Vitamin B	14.24***	0.349
2	Soapy	2.88[1]	0.072
3	Floral	2.79[1]	0.059

*** significant at ($p \leq 0.001$)
1 significant at ($p \leq 0.15$)
2 significance level to enter and to stay a variable set up at ($p \leq 0.15$)

Table 10. Retention Times[1] (RT), Kovats'[2] Indexes (KI_{20M}), Descriptors[3] and Identified Compounds[4] for Odor Active Peaks Detected at Least Fifty Percent of the Time for Each Subject During the Sniffing of the Chromatographic Effluent for the Oxygenated Fraction of Hallertauer Hop Oil.

RT (min) (KI20M)	SUBJECTS 1	2	3	4	POSSIBLE COMPOUND RESPONSIBLE FOR ODOR
13.33 (1458)	**Fruity** Citrus	*	*	*	
13.85 (1485)	**Fresh green Vegetative**	**Solvent** Floral	*	*	
14.95 (1521)	*	*	**Coconut Soap** Floral	**Floral** Rancid	2 - DECANONE (1518)
15.32 (1570)	*	*	**Floral-Musty** Lemon-Vinyl	*	
16.13 (1560)	**Musty-Rancid** **I** **I** **I**	**Woody** Floral	**Vinyl-Butter** Floral **I** **I**	**Musty**	9 - METHYL -DECAN -2 - ONE (1559)
16.27 (1564)	**Floral/Fruity/ Citrus**	*	**Floral/Fruity/ Citrus**	**Floral/Fruity/ Citrus**	LINALOOL (1563)
17.51 (1600)	**Floral** Minty-Fresh **I**	**Sweet-Fruity** Floral-Citrus	**Vinyl** Coconut Soap	**Floral/Citrus** Carnation	N.I. (1589)
17.60 (1607)	**Rancid-Spoiled** Fruity	*	*	*	
17.74 (1613)	*	*	**Coconut** Rancid	*	
17.95 (1622)	**Floral-Fruity** Vinyl	*	**Rancid** Coconut Soap Vinyl	*	2 - UNDECANONE (1613)
18.64 (1651)	**Prunes** Vegetative	*	*	*	METHYL - 4 - DECENOATE (1643)
19.64 (1692)	**Floral** Cardboard	*	*	*	
20.16 (1712)	**Vinyl-Herbal** **Musty-Sweet**	*	**Floral-Rancid** Vinyl **I** **I**	*	METHYL -4,8 - DECADIENOATE (1712)
20.27 (1716)	*	*	**Floral** Rancid	**Fruity**	

Table 10 (cont.)

RT (min) (KI20M)	SUBJECTS 1	2	3	4	POSSIBLE COMPOUND RESPONSIBLE FOR ODOR
20.44 (1722)	**Rancid** I I I	*	*	*	2 - DODECANONE (1723)
20.75 (1734)	**Putrid**	*	**Cheesy-Putrid**	**Rancid/Putrid** Floral - ?	
21.06 (1755)	**Minty**	**Anise** Floral	**Lemon** Floral-Minty	**Citrus-Orange** Anise - Floral	CITRAL (1743-1752)
21.40 (1758)	**Vinyl** I I I	*	*	**Floral-Anise**	
21.70 (1768)	**Vinyl** Prunes-Fruity	*	*	*	
23.32 (1829)	**Rancid** I I	*	**Floral-Vinyl** Citrus	*	2 -TRIDECANONE (1822)
23.59 (1840)	**Floral** I I	**Apple** I I	**Floral** Citrus-Vinyl	**Floral** Herbal-Musty I	GERANYL ISOBUTYRATE (1832)
23.77 (1847)	**Prunes-Fruity**	**Floral**	*	**Tobacco**	N.I. (related to Geraniol) (1841)
24.05 (1850)	**Floral** Carnation-Roses	*	*	**Prunes**	GERANIOL (1856)
24.95 (1893)	**Rancid**	*	**Floral**	**Herbal?-?**	N.I. (Geraniol ester) (1890)
25.44 (1912)	*	*	**Musty/Floral** Soapy	*	
25.89 (1929)	**Fresh Veget.** Floral-Vinyl	*	*	*	*SESQUITERPENE EPOXIDE* (1927)
26.70 (1964)	**Floral**	**Roses**	**Clean fresh Floral**	*	
27.27 (1988)	**Vinyl-Fresh** Piney	*	*	*	

Table 10 (cont.)

RT (min) (KI20M)	SUBJECTS 1	2	3	4	POSSIBLE COMPOUND RESPONSIBLE FOR ODOR
27.62 (1999)	**Vinyl** Fruity	*	**Floral** Violets	*	
			I		
27.84 (2008)	**Musty/Floral/ Spicy**	*	**Musty/Floral/ Spicy** Piney	*	CARYOPHYLLENE OXIDE (2005)
28.24 (2025)	**Fresh green vegetative**	*	**Vinyl** Floral	*	2-PENTADECANONE (2025)
29.00 (2056)	**Fresh green vegetative**	*	*	*	HUM. MONOEPOXIDE I (2048)
29.27 (2067)	**Musty/Floral/ Spicy**	*	**Musty/Floral/ Spicy**	*	
			I		
29.54 (2076)	**Prunes** Fruity	*	**Musty/Floral/ Spicy**	*	HUM. MONOEPOXIDE II (2076)
29.67 (2083)	*	**Soapy/Floral**	**Musty/Floral/ Spicy**	**Musty/Floral/ Spicy**	
			I		
29.84 (2090)	*	*	**Musty/Floral/ Spicy**	*	HUM. MONOEPOXIDE III (2093)
			I		
30.07 (2099)	**Floral** Perfume	*	**Floral-Vinyl** Butter	**Clean floral**	*DELTA CADINOL* (2100)
	I				
	I				
30.31 (2109)	**Musty/Floral/ Spicy**	*	**Floral**	*	*EPICUBENOL* (2109)
31:71 (2168)	**Fresh Veget.** Herbal	*	**Floral** Vinyl	*	HUMULOL (2162)
34.05 (2266)	*	**Soapy/Floral**	*	*	N.I.? (2266)
35.35 (2322)	**Floral** Fruity-Sweet	*	*	**Spicy**	*FUSED POLYCYCLIC ALCOHOL* (2322)
35.69 (2337)	*	*	*	**Herbal** Rancid-Prunes	

Table 10 (cont.)

RT (min) (KI20M)	SUBJECTS 1	2	3	4	POSSIBLE COMPOUND RESPONSIBLE FOR ODOR
36.19 (2360)	**Floral/Fruity**	**Soapy/Floral**	**Musty/Floral/ Spicy**	**Fruity/Musty**	*HUMULENOL ISOMER* (2351)
			I		
36.40 (2364)	*	*	**Floral/Musty/ Spicy**	**Musty/Floral Spicy**	HUMULENE DIEPOXIDE A (2362)
			I	**I**	
36.49 (2368)	*	*	**Musty/Floral/ Spicy**	**Musty/Floral/ Spicy**	
			I		
36.67 (2380)	*	*	**Musty/Floral/ Spicy** Piney		HUMULENE DIEPOXIDE B (2377)
37.75 (2452)	**Fruity-Citrus**	*	*	**Floral/Citrus** Floral-Prunes Citrus	*SESQUITERPENE ALCOHOL* (2402)
38.64 (2511)	*	*	*	**Soapy-Musty** Vegetative	
39.37 (2560)	**Spoiled Apple**	*	**Cooked Fruit**	*	

1- Retention times and KI_{20M} are averaged over the subjects.
2- KI_{20M} means Kovats' Indexes on Carbowax 20M phase.
3- Descriptors in **Bold** are those more frequently assigned to the peak.
(?) in the descriptors column means that the odor was detected but it could not be described.
(I) means that the peak has two maximum, that is the odor intensity never reached a value of zero in that region.
(*) means that the odor was not detected.
4- Compounds that were identified in the region where the odor occurred. Those in *Italics* are tentatively identified.
5- Intensity values registered in a sixteen points intensity scale (0 = none, 15 = extreme).

Table 11. Retention Times[1] (RT), Kovats'[2] Indexes (KI_{20W}), Descriptors[3] and Identified Compounds[4] for Odor Active Peaks Detected at Least Fifty Percent of the Time for Each Subject During the Sniffing of the Chromatographic Effluent for the Oxygenated Fraction of the U.S.D.A. 21459 Hop Oil.

RT (min) (KI20M)	SUBJECTS 1	2	3	4	POSSIBLE COMPOUND RESPONSIBLE FOR ODOR
13.35 (1470)	**Fresh Veget.** Fruity	*	**Floral**	*	
13.81 (1485)	**Fresh Veget.**	*	**Floral** Vinyl-Spicy	*	
15.19 (1530)	**Roses-Musty Rancid-Herbal**	*	*	*	
15.77 (1549)	*	*	**Floral**	*	2 - NONANOL (1537)
16.05 (1558)	**Musty** Rancid	**Solvent** Green	**Vinyl-Floral**	**Floral-Musty**	9 - METHYL - DECAN -2-ONE (1559)
	I				
16.19 (1562)	**Floral/Fruity/ Citrus**	*	**Floral/Fruity/ Citrus**	**Floral/Fruity/ Citrus**	LINALOOL (1563)
16.49 (1572)	**Floral** Fresh Veget.	*	*	*	
17.07 (1589)	*	*	**Floral** Fruity	*	
17.46 (1601)	**Fruity** Floral Vegetative	**Fruity-Floral** Sweet	**Floral** Perfume Cooked Fruit	**Fruity-Floral**	
17.67 (1610)	*	*	**Rancid** Vinyl-Coconut	*	2 - UNDECANONE (1613)
18.86 (1660)	**Spoiled Fruit**	*	*	*	
19.53 (1680)	**Rancid** Musty-Cooked	*	*	*	
20.03 (1707)	**Vegetative**	*	**Cooked Fruit** Vinyl	*	METHYL - 4,8 - DECADIENOATE (1712)
20.28 (1716)	**Rancid**	*	*	*	
20.56 (1726)	**Rancid**	*	**Cheesy-Leather**	**Cheesy-Putrid** Rancid	2 - DODECANONE (1723)
20.98 (1750)	**Minty**	Minty-Anise Spicy	**Rancid-Soapy**	**Citrus**	CITRAL (1744-1752)
21.35 (1756)	**Minty** Fruity Fresh Veget.	*	*	*	

Table 11 (cont.)

RT (min) (KI20M)	SUBJECTS 1	2	3	4	POSSIBLE COMPOUND RESPONSIBLE FOR ODOR
21.66 (1767)	**Vinyl-Minty Sweet**	*	*	*	
21.82 (1783)	**Fruity-Sweet**	*	*	*	
23.12 (1821)	**Vinyl** Fresh Veget. I	*	**Floral**	*	2 - TRIDECANONE (1822)
23.24 (1826)	**Rancid** I I	*	**Floral** Fruity	*	
23.46 (1835)	**Prunes**	**Apple** I **Tobacco**	*	**Tobacco**	GERANYL ISOBUTYRATE (1831) *TRIDECEN - 2 - ONE* (1844)
23.96 (1855)	**Floral**	**Floral**	*	*	GERANIOL (1856)
24.38 (1871)	*	**Floral**	*	*	
24.73 (1884)	*	*	*	**Fruity-Prunes**	
25.77 (1926)	**Fresh Veget.**	*	**Floral** Geranium	*	*SEQUISTERPENE EPOXIDE* (1928)
26.15 (1942)	**Vinyl** Floral	*	*	*	
26.50 (1956)	**Fresh Clean Floral**	**Fresh Floral** Roses	**Clean Fresh Floral**	*	
27.18 (1982)	**Vinyl** Floral	*	*	*	
27.51 (1995)	**Floral/Musty/ Spicy** I	**Floral** Roses-Geranium	*	*	
27.74 (2004)	**Floral/Musty/ Spicy**	**Floral/Musty/ Spicy**	**Floral/Musty/ Spicy**	**Floral/Musty/ Spicy**	CARYOPHYLLENE OXIDE (2006)
28.71 (2042)	*	*	**Floral/Fruity**	*	2-PENTADECANONE (2025)

Table 11 (cont.)

RT (min) (KI20M)	SUBJECTS 1	2	3	4	POSSIBLE COMPOUND RESPONSIBLE FOR ODOR
29.17 (2063)	**Floral/Musty/ Spicy**	**Syrupy** I	**Musty/Floral/ Spicy**	**Floral**	HUM. MONOEPOXIDE I (2049)
		I			
29.44 (2074)	**Prunes**	**Soapy/Floral** Fruity	**Musty/Floral/ Spicy**	**Musty/Floral/ Spicy**	HUM. MONOEPOXIDE II (2078)
		I		I	
29.75 (2086)	**Vinyl** I	**Soapy/Floral**	**Musty/Floral/ Spicy**	**Musty/Floral/ Spicy**	HUM. MONOEPOXIDE III (2093)
	I				
30.10 (2100)	**Musty/Floral/ Spicy**	**Soapy/Floral**	*	*	*DELTA - CADINOL* (2113)
31.55 (2161)	**Fresh Veget.**	*	**Soapy** Vinyl	**Floral**	*SPATHULENOL* (2156) HUMULOL (2161)
31.98 (2179)	**Egg Yolk**	*	**Egg Yolk**	?	*T - CADINOL* (2185)
33.97 (2319)	**Fruity** Fresh Veget.	**Floral**	*	*	N.I.
35.29 (2319)	**Fresh Veget.** Green-Minty		*	*	
36.01 (2351)	*	**Musty/Floral** I	**Musty/Floral/ Spicy**	**Musty/Floral/ Spicy** Woody	*HUMULENOL ISOMER* (2350)
		I		I	
		I		I	
36.20 (2360)	**Floral/Fruity/ Musty**	**Musty/Floral**	*	**Musty/Floral/ Spicy** Woody	HUMULENE DIEPOXIDE A (2362) HUMULENE DIEPOXIDE B (2377)
37.62 (2427)	**Floral-Musty**	**Fruity-Floral** Geranium	**Floral** Vinyl	**Vinyl** Rancid Musty	*SESQUITERPENE ALCOHOL* (2403)
		I	I		
		I	I		
37.74 (2433)	**Musty/Floral/ Spicy**	**Floral** Musty	**Musty/Floral/ Spicy**	*	N.I. (similar to Humulenol II) (2441)
38.37 (2449)	*	*	*	**Rancid ?**	

Table 11 (cont.)

RT (min) (KI20M)	SUBJECTS 1	2	3	4	POSSIBLE COMPOUND RESPONSIBLE FOR ODOR
38.94 (2480)	*	*	*	**Fruity** Prunes-Floral	
39.18 (2501)	**Spoiled Apple**	*	**Caramel** Licorice	*	
39.81 (2580)	*	*	*	**Floral** I	
40.06 (2590)	**Musty/Floral/ Spicy**	*	*	**Vinyl?**	

1- Retention times and KI_{20M} are averaged over the subjects.
2- KI_{20M} means Kovats Indexes on Carbowax 20M phase.
3- Descriptors in **Bold** are those more frequently assigned to the peak.
(?) in the descriptors column means that the odor was detected but it could not be described.
(I) means that the peak has two maximum, that is the odor intensity never reached a value of zero in that region.
(*) means that the odor was not detected.
4- Compounds that were identified in the region where the odor occurred. Those in *Italics* are tentatively identified.
5- Intensity values registered in a sixteen points intensity scale (0 = none, 15 = extreme).

Table 12. Retention Times[1] (RT), Kovats'[2] Indexes (KI_{20M}), Descriptors[3] and Identified Compounds[4] for Odor Active Peaks Detected at Least Fifty Percent of the Time for Each Subject During Sniffing of the Chromatographic Effluent for the Oxygenated Fraction of the U.S.D.A. 21455 Hop Oil.

RT (min) (KI20M)	SUBJECTS 1	2	3	4	POSSIBLE COMPOUND RESPONSIBLE FOR ODOR
16.09 (1559)	**Rancid** **Musty**	*	**Vinyl** Floral	Musty Sweet	9 - METHYL - DECAN -2-ONE (1560)
16.23 (1563)	**Floral/Fruity/ Citrus**	*	**Floral/Fruity/ Citrus**	**Floral/Fruity/ Citrus**	LINALOOL (1563)
17.10 (1590)	*	*	**? - Plums Floral?**	*	N.I. (1586)
17.55 (1605)	**Vegetative** Fruity	**Floral** Roses	**Floral** Violets-Citrus	**Floral** Anise Fruity	2 - UNDECANONE (1609)
19.48 (1685)	*	**Sweet** Maltol-Candy	* *	* *	METHYL - 4,8 - DECADIENOATE (1708)
20.55 (1726)	**Rancid**	*	**Rancid** Prunes-Leather **I** **I**	**Spicy** ? Rancid	2 - DODECANONE (1719)
20.75 (1734)	*	*	?	*	
21.01 (1749)	**Fresh Veget.** Rancid	**Anise-Solvent** Fruity	**Floral** **Leather**	**Floral/Spicy**	CITRAL? (1739 - 1748)
21.47 (1760)	**Minty** **Fresh Veget.**	*	*	**Fresh Veget.** Spicy	
23.33 (1832)	Rancid	*	**Floral/Violets** Rancid	**Violets** Spicy	GERANYL ISOBUTYRATE (1827)
23.54 (1838)	**Prunes** Floral	**Apple/Floral** Fresh Floral	*	**Floral** Spicy/Coffee	N.I. (similar to Geraniol) (1836)
23.68 (1843)	*	*	*	**Prunes-Oranges** Floral/Spicy	*TRIDECEN - 2 ONE* (1840)
24.05 (1858)	**Floral** Roses-Geranium	**Fruity-Roses?**	*	*	GERANIOL (1851)

Table 12. (cont.)

RT (min) (KI20M)	SUBJECTS 1	2	3	4	POSSIBLE COMPOUND RESPONSIBLE FOR ODOR
26.50 (1957)	**Floral**	**Floral** Fruity-Citrus Roses	**Fresh Floral** Violets Perfume Magnolia I	**Floral** Citrus	N.I. (1939)
26.79 (1967)	*	*	**Musty/Floral**	**Musty**	
27.83 (2008)	Cheap perfume **Musty/Floral/ Spicy**	*	**Musty/Floral** Old Perfume I I	**Musty/Floral Cloves**	CARYOPHYLLENE OXIDE (2001)
28.19 (2023)	**Fresh Veget.** Green-Floral	*	**Musty/Floral**	*	2-PENTADECANONE (2023)
29.25 (2066)	**Musty/Floral** Fruity	**Soapy/Floral**	**Musty/Floral Spicy** Violets Old Perfume I	**Floral/Musty/ Spicy**	HUM. MONOEPOXIDE I (2045)
29.49 (2076)	**Prunes**	*	**Floral/Musty/ Spicy** I	**Musty/Floral/ Spicy**	HUM. MONOEPOXIDE II (2071)
29.60 (2080)	*	**Soapy/Floral**	**Musty/Floral/ Spicy** I	**Musty/Floral/ Spicy**	HUM. MONOEPOXIDE III (2085)
29.94 (2094)	**Vinyl** Spicy	*	**Musty/Floral/ Spicy**	**Musty/Floral/ Spicy Fresh Floral**	
30.19 (2104)	**Floral** Green Vegetative	*	*	*	*DELTA - CADINOL* (2106)
31.67 (2166)	*	*	**Rancid-Butter** Coconut Soap	*	HUMULOL (2156)
32.80 (2213)	**Sulfur Burned matches**	*	**Putrid** Rancid	**Rancid** Putrid	*ALPHA - CADINOL* (2200) *T - MUUROLOL?* (2200) *TORREYOL* (2207)

Table 12. (cont.)

RT (min) (KI20M)	SUBJECTS 1	2	3	4	POSSIBLE COMPOUND RESPONSILE FOR ODOR
34.06 (2267)	**Floral**	**Floral** Roses	*	**Citrus-Floral** Geranium-Orange	N.I. (2263)
35.33 (2321)	**Fresh Veget.** Floral-Vinyl Artif. Fruit	*	*	?	
36.04 (2353)	*	**Floral-Musty**	**Musty/Floral/ Spicy**	**Musty/Floral/ Spicy** Cloves	*HUMULENOL ISOMER* (2344)
		I	I		
		I	I		
		I			
36.20 (2360)	*	**Soapy/Floral** Musty	**Musty/Floral/ Spicy**	**Musty/Floral/ Spicy**	HUMULENE DIEPOXIDE A (2356)
		I	I		
		I	I		
36.48 (2372)	*	**Soapy** I	**Musty/Floral/ Spicy**	**Musty/Floral/ Spicy**	HUMULENE DIEPOXIDE B (2377)
		I	I		
		I	I		
36.92 (2391)	*	**Soapy**	**Musty/Floral/ Spicy**	*	*FARNESOL H* (2383)
			I		
37.44 (2410)	*	*	**Musty/Floral/ Spicy**	*	SESQUITERPENE ALCOHOL? (2395)
			I		
37.76 (2427)	**Floral** Citrus	*	**Floral**	**Floral-Perfume** Citrus	N.I. (2420)
37.90 (2427)	*	*	**Floral**	**Floral** Citrus	
38.20 (2446)	*	*	*	?	
38.79 (2472)	*	*	?	*	
39.88 (2580)	*	*	*	**Putrid-Rancid**	
41.84 (2593)	*	**Sweet-Prunes** Fruity	*	*	

1- Retention times and KI_{20M} are averaged over the subjects.

Table 12. (cont.)

2- KI_{20M} means Kovats' Indexes on Carbowax 20M phase.
3- Descriptors in **Bold** are those more frequently assigned to the peak.
(?) in the descriptors column means that the odor was detected but it could not be described.
(**I**) means that the peak has two maximum, that is the odor intensity never reached a value of zero in that region.
(*) means that the odor was not detected.
4- Compounds that were identified in the region where the odor occurred. Those in *Italics* are tentatively identified.
5- Intensity values registered in a sixteen points intensity scale (0 = none, 15 = extreme).

Table 13. Total Number of Odor Active Peaks Detected by Each Subject During the Sniffing of the Chromatographic Effluents for Hop Oil Oxygenated Fractions.

	SUBJECTS			
VARIETIES	**1**	**2**	**3**	**4**
HALLERTAUER	36	10	31	23
U.S.D.A. 21459	38	17	26	20
U.S.D.A. 21455	20	14	26	26
AVERAGE	31.3	13.7	27.7	23.0

Table 14. Number of Peaks and Total Area[1] in Aromagrams for Hop Oil Oxygenated Fractions

	HALLERTAUER	U.S.D.A 21459	U.S.D.A. 21455
Peaks	12	17	19
Total Area	3616	4500	4245

1- Areas calculated using the special data collection software.

Table 15. Odor Intensities, Descriptors and Possible Chemical Compounds Responsible for the Odor Active Peaks Present in the Three Hop Varieties Studied. 1 = HALLERTAUER, 2 = U.S.D.A. 21459, 3 = U.S.D.A. 21455

Peak #	Time[1] (KI_{SOL})	Intensities[2] (sd) Var. 1	Var. 2	Var. 3	Avg. Int.	Descriptor[3]	Identified Compound[4]
1	16:09 (1559)	5.7 (1.00)	4.2 (1.91)	3.3 (1.33)	4.4 (1.22)	Musty-Rancid	9-Methyl-Decan-2-One
2	16:23 (1563)	7.9 (0.72)	6.0 (1.33)	7.1 (2.44)	7.0 (1.00)	Floral/Fruity/ Citrus	Linalool
3	17:51 (1602)	6.8 (3.90)	5.1 (2.01)	6.9 (1.72)	6.3 (1.01)	Floral	2-Undecanone
4	20:60 (1729)	4.3 (2.04)	5.0 (1.71)	4.3 (0.93)	4.5 (0.42)	Rancid-Cheesy	2-Dodecanone
5	21:00 (1751)	5.5 (0.83)	4.8 (1.31)	4.9 (1.62)	5.1 (0.40)	Minty-Anise Citrus-Spicy	Citral B
7	23:53 (1838)	7.3 (2.51)	6.7 (4.12)	6.1 (0.84)	6.7 (0.60)	Prunes-Apples Tobacco	N.I.(similar to Geraniol)
8	26:57 (1958)	9.1 (3.33)	8.9 (3.30)	5.6 (2.41)	7.9 (2.00)	Floral	N.I.
12	29:67 (2083)	7.3 (0.78)	6.8 (3.01)	8.1 (2.22)	7.4 (0.72)	Musty/Floral/ Spicy	Humulene Monepox.III
17	36:20 (2360)	5.1 (2.62)	6.9 (1.54)	6.9 (3.92)	6.3 (1.03)	Musty/Floral/ Spicy	Humulene Diepox. A

1- Time (min) and KI_{SOL} averaged over the times and KI_{SOL} of the three varieties.
2- Determined on a sixteen point intensity scale, (0=none, 15=extreme). Values for each variety are averaged over panelists that detected the odor.
3- Most frequent descriptor assigned to the peak.
4- Most probable compound responsible for the odor detected.
(N.I.) means that the compound was investigated but it could not be identified.

Table 16. Odor Intensities, Descriptors and Possible Chemical Compounds Responsible for the Odor Active Peaks Present in Two Out of the Three Varieties. 1 = HALLERTAUER, 2 = U.S.D.A. 21459, 3 = U.S.D.A. 21455

A. Variety 1 va. Variety 3

Peak #	Time[1] (KI_{zon})	Intensities[2] (SD) Var. 1	Var. 3	Avg. Int.	Descriptor[3]	Identified Compound[4]
13	30:00 (2095)	6.7 (1.53)	4.5 (1.71)	5.6 (1.50)	Vinyl-Floral	δ-Cadinol

B. Variety 2 va. Variety 3

Peak #	Time (KI_{zon})	Intensities (SD) Var. 2	Var. 3	Avg. Int.	Descriptor	Identified Compound
9	27:79 (2006)	6.9 (1.92)	7.3 (0.54)	7.1 (5.92)	Musty/Floral/ Spicy	Caryophyllene Oxide
10	29:21 (2065)	5.8 (2.32)	5.9 (1.54)	5.9 (0.14)	Musty/Floral/ Spicy	Humulene Monoepox.I
11	29:46 (2075)	7.2 (4.70)	5.0 (0.73)	6.1 (1.50)	Musty/Floral/ Spicy	Humulene Monoepox.II
16	36:03 (2352)	8.2 (2.63)	7.9 (2.32)	8.1 (0.20)	Musty/Floral/ Cloves	Humulenol Isomer
19	37:69 (2427)	5.3 (1.61)	5.6 (1.83)	5.5 (0.20)	Floral	N.I.

1- Time (min) and KI_{zon} averaged over the times and KI_{zon} of the three varieties.
2- Determined on a sixteen point intensity scale (0 = none, 15 = extreme). Values for each variety are averaged over panelists that detected the odor.
3- Most frequent descriptor assigned to the peak.
4- Most probable compound responsible for the odor detected. (N.I.) means that the compound was investigated but it couldn't be identified.

Table 17. Unique Peaks Detected by Three Out of Four of the Subjects During the Sniffing of the Chromatographic Effluent for HALLERTAUER Hop Oil (Oxygenated Fraction).

Peak #	Time (min) (KI_{20M})	Descriptor	Intensity (SD)	Identified Compounds (KI_{20M})
4?	20:75 (1734)	Rancid-Putrid Cheesy	4.3 (2.04)	2-Dodecanone (1723)
A	23:77 (1847)	Tobacco-Floral Prunes	7.2 (1.91)	*Tridec-?-en-2-One* (1844) Geraniol (1856)
B	24:95 (1893)	Rancid-Floral	3.3 (1.81)	N.I. (Geraniol ester) (1890)

1- ? means uncertainty in the assignment of the peak.
2- Time (min) and KI_{20M} averaged over the subjects that detected the odor. KI_{20M} means Kovats' index on Carbowax 20 M column.
3- Most frequent descriptor assigned to the peak.
4- Determined on a sixteen point intensitiy scale (0 = none, 15 = extreme). Values averaged over subjects that detected the odor.
5- Most probable compound responsible for the odor detected. Compounds in Italic were tentatively identified. (N.I.) means that the compound was investigated but it could not be identified.

Table 18. Unique Peaks Detected by Three Out of Four of the Subjects During the Sniffing of the Chromatographic Effluent for U.S.D.A 21459 Hop Oil (Oxygenated Fraction).

Peak #	Time[1] (min) (KI_{20M})	Intensity[2] (SD)	Descriptor[3]	Identified Compound[4] (KI_{20M})
C	31:55 (2161)	3.2 (2.01)	Fresh vegetative Soapy	N.I. (2137) *Spathulenol* (2156) Humulol (2161)
D	31:98 (2179)	2.4 (1.30)	Egg yolk	____
E	37:74 (2433)	5.3 (2.44)	Musty/Floral/ Spicy	N.I.(similar to Humulenol II) (2441)

1- Time (min) and KI_{20M} averaged over the subjects that detected the odor. KI_{20M} means Kovàts' Index on Carbowax 20 column.
2- Determined on a sixteen point intensity scale (0 = none, 15 = extreme). Values averaged over the subjects that detected the odor.
3- Most frequent descriptor assigned to the peak.
4- Most probable compound responsible for the odor. (N.I.) means that the compound was investigated but it could not be identified.
____ means that no compound was identified in that region. Compounds in Italic are tentatively identified.

Table 19. Unique Peaks Detected by Three Out of Four of the Subjects During the Sniffing of the Chromatographic Effluent for U.S.D.A. 21455 Hop Oil (Oxygenated Fraction)

Peak #	Time[1] (min) (KI_{20M})	Intensity[2] (SD)	Descriptor[3]	Identified Compound[4] (KI_{20M})
6	23:33 (1832)	4.8 (1.51)	Rancid-Violets	2-Tridecanone (1817) Geranyl Isobutyrate (1827)
14	32:80 (2213)	3.0 (0.72)	Rancid-Sulfur Putrid	*α-Cadinol* (2200)? *Torreyol* (2207)?
15	34:06 (2267)	4.6 (1.33)	Floral-Roses Citrus	N.I. (2263)
18	36:48 (2372)	5.6 (2.44)	Musty/Floral/ Spicy	Humulene Diepox. B (2377)

1- Time (min) and KI_{20M} averaged over the subjects that detected the odor. KI_{20M} means Kovàts' Index on Carbowax 20 column.
2- Determined in a sixteen point intensity scale (0 = none, 15 = extreme). Values are averaged over the subjects that detected the odor.
3- Most frequent descriptors assigned to the peaks.
4- Most probable compound responsible for the odor detected. Compounds in Italic are tentatively identified. (N.I.) means that the compound was investigated but it could not be identified.

Table 20. Descriptors[1], Intensities[2], Area Percent[3] and Concentration of the Identified Compounds for the Odor Active Peaks Detected by Three Out of Four of the Subjects During the Sniffing of the Chromatographic Effluents for HALLERTAUER Hop Oil (Oxygenated Fractions)

Peak #	RT(min) (KI_{zou})	Descriptors	Intensity (SD)	% Total Area	Identified Compounds (KI_{zou})	Conc. (ppm oil)
1	16:13 (1560)	**Musty** Rancid Soapy-Woody I	5.7 (1.00)	3.8	9-Methyl-Decan-2-One (1559)	62
2	16:27 (1564)	**Floral/ Fruity/ Citrus**	7.9 (0.72)	13.1	Linalool (1563)	1696
3	17:51 (1600)	**Floral** Vegetative Citrus Fruity Carnation Vinyl	6.8 (3.90)	7.6	N.I.[4] (1589)	116
					2-Undecanone (1613)	5144
4	20:75 (1734)	**Rancid** Putrid Cheesy	4.33 (2.04)	4.0	2-Dodecanone (1723)	798
5	21:06 (1755)	**Minty** Anise Lemon Citrus Floral	5.5 (0.83)	8.9	Citral B (1752)	333.5
7	23:59 (1840)	**Prunes-Apples** Vinyl-Herbal	7.3 (2.51)	8.0	Geranyl Isobutyrate (1832)	60
					N.I. (1841	498
A	23:77 (1847)	**Tobacco Floral** I	7.2 (1.20)	13.2	*Tridec-?-en-2-One* (1844)	115
					Geraniol (1856)	275
B	24:95 (1893)	**Rancid-Floral** Vinyl-Herbal	3.3 (0.94)	3.5	N.I.(Geraniol Ester) (1890)	92
8	26.70 (1964)	**Clean Fresh Floral** Roses	9.1 (3.33)	14.4	_____	
12	29:67 (2083)	**Musty/ Floral/ Spicy**	7.3 (0.78)	5.1	Humulene Monoep. II (2076)	1633
					Humulene Monoep. III (2093)	789
13	30:07 (2099)	**Floral** Fresh-Vinyl Musty/Fl/Sp	6.7 (1.53)	9.5	*Delta Cadinol* (2100)	140
17	36:19 (2360)	**Musty/ Floral/ Spicy**	5.1 (2.62)	8.8	*Humulenol Isomer* (2351)	369
					Humulene Diepoxide A (2362)	627

1- Descriptors in **bold** are the most frequent assigned to the correspondent peak.
2- Intensities registered in a sixteen point intensity scale (0 = none, 15 = extreme). Intensities are averaged over subjects.
3- Area percent calculated with the Data Collection Software.
4- N.I. means that the compound was investigated but it could not be identified. Compounds in *Italic* are tentatively identified.
5- I means that the intensity of the odor never reached intensity zero in this region.

Table 21. Descriptors[1], Intensities[2], Area Percent[3] and Concentration[6] of the Identified[5] Compounds for the Odor Active Peaks Detected by Three Out of Four of the Subjects During the Sniffing of the Chromatographic Effluents for U.S.D.A. 21459 Hop Oil (Oxygenated Fraction)

Peak #	RT(min)[4] (KI_{FSOT})	Descriptor	Intensity (SD)	% Total Area	Identified Compound (KI_{FSOT})	Conc. (ppm in Oil)
1	16:05 (1559)	**Musty-Rancid** Vinyl-Floral	4.2 (1.91)	1.6	9-Methyl-Decan-2-One (1559)	8.1
2	16:19 (1562)	**Floral/Fruity/ Citrus**	6.0 (1.33)	3.1	Linalool (1563)	883
3	17:46 (1601)	**Floral** Fresh Veg. Vinyl-Fruity Fruity	5.1 (2.00)	3.3	2-Undecanone (1613)	1967
4	20:56 (1726)	**Rancid** Putrid-Cheesy Leather	5.0 (1.71)	5.5	2-Dodecanone (1723)	494
5	20:98 (1750)	**Minty** Spicy-Anise Solvent-Citrus	4.8 (1.31)	3.3	Citral B (1750)	183
7	23:46 (1835)	**Prunes-Apples** Floral Butter	6.7 (4.12)	10.2	Geranyl Isobutyrate (1830) N.I. (related to Geraniol) (1841)	63 453
8	26:50 (1956)	**Fresh Clean Floral**	8.9 (3.30)	10.1		1123
9	27:74 (2004)	**Musty/Floral/ Spicy** Cheap Perfume	7.0 (1.92)	7.1	Caryophyllene Oxide (2006)	9349

Table 21 (cont.)

Peak #	RT(min) (KI_{20M})	Descriptor	Intensity (SD)	% Total Area	Identified Compound (KI_{20M})	Conc. (ppm in Oil)
10	29:17 (2063)	**Musty/Floral/ Spicy**	5.8 (2.32)	7.0	Humulene Monoepoxide I (2049)	1335
11	29:44 (2074)	**Musty/Floral/ Spicy**	7.3 (4.70)	8.1	Humulene Monoepoxide II (2078)	2818
		I				
12	29:75 (2086)	**Musty/Floral/ Spicy**	6.8 (3.00)	10.3	Humulene Monoepoxide III (2082)	649
C	31:55 (2161)	**Fresh Vegetative Soapy**	3.2 (2.01)	1.8	*Spathulenol* (2156)	941
					Humulol (2161)	1805
D	31:98 (2179)	**Egg Yolk**	2.4 (1.30)	0.8		
16	36:01 (2351)	**Musty/Floral/ Spicy**	8.2 (2.63)	7.3	*Humulenol Isomer* (2350)	912
17	36:20 (2360)	**Musty/Floral/ Spicy** Woody-Floral	6.9 (1.54)	11.8	Humulene Diepoxide A (2362)	2698
19	37:62 (2427)	**Fruity-Floral**	5.3 (1.61)	5.9	*Sequisterpene Alcohol* (2403)	552
E	37:74 (2433)	**Musty/Floral/ Spicy** Floral	5.3 (2.44)	1.7	N.I. (similar to Humulenol II) (2441)	615 1186

1- Descriptors in **bold** are the most frequent descriptors assigned to the peak.
2- Intensities registered on a sixteen point intensity scale (0 = none, 15 = extreme). Intensities are averaged over subjects. (I) means that the intensity of the odor never reached zero in this region.
3- Area Percent calculated with the Data Collection Software.
4- Time (min) averaged over panelists- KI_{20M} are Kovats Index on carbowax 20M column.
5- N.I. means compound was investigated but it could not be identified.
* Compounds in *Italic* are tentatively identified.
6- Concentration calculated using Internal Standard.

Table 22. Descriptors[1], Intensities[2], Area Percent[3] and Concentration[6] of the Identified[5] Compounds for the Odor Active Peaks Detected by Three Out of Four of the Subjects During the Sniffing of the Chromatographic Effluents for U.S.D.A. 21455 Hop Oil (Oxygenated Fraction)

Peak #	RT(min)[4] (KI_{FSOT})	Descriptor	Intensity (SD)	% Total Area	Identified Compound (KI_{FSOT})	Conc. (ppm in Oil)
1	16:09 (1559)	**Musty-Rancid** Vinyl-Floral	3.3 (1.33)	2.5	9-Methyl-Decan-2-One (1560)	45
		I				
2	16:23 (1605)	**Floral/Fruity/ Citrus**	7.1 (2.44)	4.7	Linalool (1563)	1870
3	17:55 (1605)	**Floral** Fresh Veg. Vinyl-Fruity Fruity	6.9 (1.72)	5.0	2-Undecanone (1609)	3738
4	20:55 (1726)	**Rancid** Putrid Leather	4.3 (0.93)	5.2	2-Dodecanone (1719)	692
5	21:01 (1749)	**Anise** Spicy Solvent	4.9 (1.62)	2.9	Citral B (1748)	232
6	23.33 (1832)	**Rancid-Violets** Prunes-Musty Butter	4.8 (1.51)	3.8	2-Tridecanone (1817) Geranyl Isobutyrate (1827)	2362 194
7	23:54 (1838)	**Prunes-Apples** Fruity	6.1 (0.84)	5.1	N.I. (related Geraniol) (1836) *Tridec-?-en-2-one* (1840)	351
8	26:5 (1957)	**Floral** Roses	5.6 (2.41)	4.4		1583
9	27:83 (2008)	**Musty/Floral/ Spicy** Cheap Perfume	7.3 (0.54)	4.9	Caryophyllene Oxide (2001)	20355
10	29:25 (2066)	**Musty/Floral/ Spicy** Violets	5.9 (1.54)	5.2	Humulene Monoepoxide I (2048)	1906

Table 22 (cont.)

Peak #	RT(min) (KI_{20M})	Descriptor	Intensity (SD)	% Total Area	Identified Compound (KI_{20M})	Conc. (ppm in Oil)
11	29:49 (2076)	**Musty/Floral/ Spicy**	4.9 (0.73)	3.1	Monoepoxide II (2071)	5466
12	29:60 (2080)	**Musty/Floral/ Spicy**	8.1 (2.22)	8.3	Humulene Monoepoxide III (2085)	309
13	29:94 (2094)	**Vinyl** Floral-Musty	4.5 (1.71)	3.3	Delta-Cadinol (2106)?	165
14	32:80 (2213)	**Putrid** Rancid-Sulfur Burned Matches	3.0 (0.72)	1.2	*Alpha-Cadinol* (2200)? *Torreyol* (2207)?	1234
15	34:06 (2267)	**Floral-Roses** Citrus	4.6 (1.33)	1.6	N.I. (2263)	432
16	36:04 (2353)	**Musty/Floral/ Spicy** Soapy I	7.9 (2.32)	10.2	*Humulenol Isomer* (2344)	2439
17	36:20 (2360)	**Musty/Floral/ Spicy** I	6.9 (3.9)	5.6	Humulene Diepoxide A (2360)	1992
18	36:48 (2372)	**Musty/Floral/ Spicy**	5.8 (2.44)	16.0	Humulene Diepoxide B (2377)	1186
19	37:76 (2427)	**Floral** Fresh Veget. Vinyl	5.67 (1.83)	6.0	N.I. (2431)	1061

1- Descriptors in **bold** are the most frequent descriptors assigned to the peak.
2- Intensities registered on a sixteen point intensity scale (0 = none, 15 = extreme). Intensities are averaged over subjects. (I) means that the intensity of the odor never reached zero in this region.
3- Area Percent calculated with the Data Collection Software.
4- Time (min) averaged over panelists- KI_{20M} are Kovats Index on carbowax 20M column.
5- N.I. means compound was investigated but it could not be identified.
* Compounds in *Italic* are tentatively identified.
6- Concentration calculated using Internal Standard.

Table 23. Chemical Composition of the Hop Oil Oxygenated Fractions Analyzed. 1 = U.S.D.A. 21455, 2 = HALLERTAUER, 3 = U.S.D.A. 21459

Compound (KI_{20M})	Concentration[1] (ppm in oil)		
	Var 1	Var2	Var3
1- 2-Decanone* (1518)	125.7	923.4	264.14
2- 9-Methyl Decan-2-One (1558)	44.5	61.9	8.1
3- Linalool* (1562)	1869.9	1696.0	883.2
4- N.I. (1588)	234.20	116.4	68.3
5- 2-Undecanone* (1611)	3738.5	5143.9	1967.3
6- Methyl-4-Decenoate (1643)	991.2	1443.6	206.3
7- Methyl-4,8-Decadienoate (1711)	874.2	1464.3	366.5
8- 2-Dodecanone* (1722)	692.1	797.5	494.0
9- Citral* (1742) (1751)	156.0 232.2	80.9 333.5	60.1 182.9
10- 2-Tridecanone* (1820)	2362.5	3675.1	1868.4
11- Geranyl iso-butyrate* (1830)	194.3	59.61	62.80
12- N.I. (similar to Geraniol) (1839)	351.0	498.3	453.0
13- **Tridec-?-en-2-one** (1842)	178.0	114.5	136.0
14- Geraniol* (1854)	410.6	274.8	325.8
15- N.I. (Geraniol ester) (1888)	410.6	274.8	325.8
16- **Sesquiterpene epoxide** (1926)	669.3	827.1	515.6
17- Ketone (N.I.) (1942)	1582.7	730.1	1123.5
18- Caryophyllene Oxide* (2004)	20355	2548.4	9348.9
19 2-Pentadecanone* (2037)	8927.6	1549.7	4823.2
20- Hum.monoepox. I* (2048)	1906.5	1325.4	1335.5

Table 23 (cont.)

Compound (KI_{20M})	Concentration[1] (ppm in oil)		
	Var 1	Var2	Var3
21- Hum.monoepoxide II* (2075)	5466.3	1633.0	2818.2
22- Hum.monoepoxide III* (2083)	939.7	789.35	649.0
23- **δ-Cadinol** (2103)	164.6	139.5	11.5
24- **Epicubenol** (2112)	190.05	135.5	18.57
25- **Hop Furanone Z** (2128)	109.8	57.1	145.8
26- **Spathulenol** (2156)	2192.8	271.0	941.3
27- Humulol (2161)	2192.8	2215.3	1805.4
28- **ζ-Cadinol** (2183)	2730.7	1809.3	1820.6
29- **Sesquiterpene alcohol** (2198)	1765.5	526.5	858.3
30- **α-Cadinol** or **ζ-Muurolol** (2204)	1233.9	131.9	352.9
31- **Torreyol** (2210)	1934.9	392.3	464.3
32- **ζ-Muurolol** (2244)	1595.5	1409.4	1480.8
33- N.I. (2267)	432.0	437.7	2398.5
34- Humulenol II (2298)	11072.3	4814.4	2157.5
35- **Fused Polycyclic alcohol** (2311)	4141.3	1941.8	1059.3
36- **Humulenol Isomer** (2348)	2439.2	368.7	911.9
37- Hum.diepoxide A* (2361)	1991.7	627.3	2697.6
38- Hum.diepoxide B* (2377)	1185.7	563.7	1199.3
39- Farnesol H (2387)	2790.4	530.7	1500.2
40- **Sesquiterpene alcohol** (2401)	409.7	303.1	551.77
41- N.I. (simmilar to to Humulenol II) (2441)	1061	542.8	615.3

1- Concentration calculated using internal standard method.
(*) means that the identity was confirmed using standards.
Compounds in ***bold*** are those that were tentatively identified.
(N.I.) means that the compound was investigated but not identified.yy

Table 24. **Correlation Matrix Among the Sensory Attributes Evaluated by the Descriptive Sensory Panel (Three Common Panelists) and the Odor Intensities of the Peaks Detected During the Sniffing of the GC Effluents for Hop Oil Oxygenated Fractions**

	OI	Soapy	Herbal	Woody	Grassy	Citrus	Sweet	Fruity	Ar.Fr.	Floral	Vit.B
P1	*	NS	**	*	NS	NS	NS	NS	NS	NS	NS
P2	+	NS	*	*	**	NS	NS	NS	NS	NS	NS
P3	NS	+	NS	NS	NS	+	(**)	NS	*	NS	NS
P4	*	NS	*	NS	+	NS	NS	**	NS	NS	NS
P5	NS	NS	*	NS	+	*	NS	NS	NS	NS	NS
P6	NS	+	NS	NS	NS	NS	NS	NS	NS	NS	NS
P7	+	NS	NS	NS	*	NS	NS	**	(*)	NS	NS
P8	NS	NS	*	NS	NS	*	NS	NS	NS	+	NS
P9	NS	+	NS	NS	NS	NS	NS	NS	NS	NS	(*)
P10	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	(*)
P11	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	(*)
P12	NS	NS	NS	NS	(**)	NS	NS	(*)	NS	NS	NS
P13	NS	NS	NS	NS	+	NS	NS	NS	NS	(+)	*
P14	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
P15	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
P16	NS	NS	(+)	(+)	(**)	NS	NS	NS	NS	+	NS
P17	(+)	NS	NS	(+)	NS	NS	NS	NS	NS	NS	NS
P18	(+)	+	NS	NS	NS	NS	NS	NS	+	NS	NS
P19	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	(*)
PA	NS	NS	NS	NS	+	NS	NS	+	NS	NS	***
PB	NS	NS	NS	NS	+	NS	NS	NS	NS	NS	*
PC	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS
PD	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS
PE	+	NS	NS	NS	NS	NS	NS	NS	**	NS	NS

*,**,*** significant at ($p \leq 0.05$, 0.01, 0.001) respectively
+ significant at ($p \leq 0.15$)
NS not significant
() negatively correlated
P1 - PE Odor active peaks.

Table 25. Correlation Matrix Among the Odor Intensities of the Peaks Detected During the Sniffing of the Chromatographic Effluent for Hop Oil Oxygenated Fractions.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	PA	PB	PC	PD	PE
P1	1																							
P2	+	1																						
P3	NS	NS	1																					
P4	+	NS	NS	1																				
P5	NS	NS	+	NS	1																			
P6	NS	NS	NS	NS	NS	1																		
P7	*	+	NS	*	NS	NS	1																	
P8	NS	NS	NS	*	NS	NS	NS	1																
P9	+	NS	NS	NS	NS	NS	NS	NS	1															
P10	NS	NS	NS	NS	NS	NS	NS	NS	***	1														
P11	(+)	NS	NS	NS	NS	NS	+	NS	**	***	1													
P12	NS	(+)	NS	NS	NS	NS	NS	NS	NS	NS	NS	1												
P13	NS	*	NS	NS	NS	NS	NS	NS	+	*	+	NS	1											
P14	NS	NS	NS	NS	NS	NS	NS	NS	+	NS	+	NS	NS	1										
P15	NS	NS	NS	NS	(+)	NS	NS	NS	NS	NS	NS	NS	NS	NS	1									
P16	*	**	NS	NS	+	NS	*	NS	NS	+	**	NS	(+)	NS	NS	1								
P17	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	+	NS	NS	NS	NS	1							
P18	(+)	NS	*	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	+	NS	NS	+	1						
P19	NS	NS	NS	NS	(+)	+	NS	NS	**	***	**	NS	(+)	+	+	+	NS	NS	1					
PA	NS	NS	NS	NS	NS	NS	+	NS	(*)	(*)	(*)	NS	*	NS	NS	NS	NS	NS	(*)	1				
PB	*	NS	NS	NS	NS	NS	+	NS	(**)	(***)	(**)	NS	NS	NS	NS	(+)	NS	NS	(***)	**	1			
PC	NS	NS	NS	NS	NS	NS	NS	NS	NS	+	NS	NS	NS	NS	NS	NS	(+)	NS	NS	NS	NS	1		
PD	NS	NS	NS	NS	NS	NS	NS	NS	NS	+	NS	NS	NS	NS	NS	NS	(+)	NS	NS	NS	NS	NS	1	
PE	NS	NS	NS	NS	NS	NS	NS	NS	NS	+	NS	NS	(+)	NS	NS	NS	(+)	NS	NS	NS	NS	***	***	1

*,**,*** significant at ($p < 0.05$, 0.01, 0.001) respectively
+ significant at ($p \leq 0.15$)
() negatively correlated
-PA-PE-P6-P14-P15-P18 unique peaks to just one hop oil variety
-others peaks that are common to two or three varieties studied

Table 26. **Correlation Equations Between the Sensory Attributes Evaluated by the Three Common Panelists in the Descriptive Sensory Panel and the Odor Active Peaks Detected by the Same Panelists During the GC-Effluent Sniffing Procedure of the Hop Oil Oxygenated Fractions.**

Attribute	Equation (SE)[a]	$C_{(p)}$	F value Model	Cumulative R-square Model
Overall Intensity	6.08 + 0.34 P2 + (1.56) (0.20) 0.28 P4 - 0.11 P18 (0.23) (0.13) + 0.33 PE (0.18)	2.5	4.88[1]	P4 0.508 P18 0.633 PE 0.703 P2 0.829
Soapy	2.45 + 0.60 P3 + (1.06) (0.22) 0.49 P14 (0.15)	102	10.04*	P14 0.487 P3 0.769
Herbal	-4.94 + 0.36 P1 + (1.23) (0.17) 0.42 P4 + 0.99 P5 (0.17) (0.25)	16	21.59**	P1 0.645 P5 0.835 P4 0.928
Woody	-5.01 +0.89 P2 (1.95) (0.27)	1.5	10.84*	P2 0.608
Grassy	2.72 - 0.26 P12 - (0.24) (0.05) 0.18 P16 (0.03)	4.8	39.85***	P2 0.729 P12 0.879 P16 0.949 - P2 0.930
Citrus	2.92 + 0.43 P5 + (0.56) (0.13) 0.12 P8 (0.04)	2.0	17.67**	P5 0.632 P8 0.855

Attribute	Equation	$C_{(p)}$	F value Model	Cumulative R-square Model	
Fruity	-2.29 + 0.56 P4 + 0.35 P7 (1.08) (0.30) (0.19)	6.0	10.50*	P7 P4	0.651 0.778
Artif. Fruity	-0.83 + 1.14 P3 - 0.55 P7 (3.20) (0.54) (0.23)	1.4	8.72*	P7 P3	0.554 0.744
Sweet	6.20 - 9.99 P3 (1.35) (1.25)	0.9	22.5*	P3	0.641
Floral	1.55 + 0.98 PE (0.39) (0.20)	1.0	23.33**	PE	0.769
Vitamin B	0.12 + 0.69 PA (0.39) (0.12)	1.4	36.33***	PA	0.834

*,**,*** significant at ($p \leq 0.05$, 0.01, 0.001), respectively
1 significant at ($p \leq 0.15$)
a Standard Error (SE) for the estimated regression coefficients

Table 27. Correlation Matrix Among the Sensory Attributes Determined by the Whole Descriptive Sensory Panel During the Evaluation of Hop Oil Oxygenated Fractions.

	OI	Soapy	Herbal	Woody	Grassy	Citrus	Sweet	Fruity	A.Fr.	Floral	Vit.B
OI	1										
Soapy	***	1									
Herbal	NS	NS	1								
Woody	NS	NS	NS	1							
Grassy	NS	(**)	NS	NS	1						
Citrus	NS	+	**	NS	(*)	1					
Sweet	*	NS	(*)	NS	NS	(+)	1				
Fruity	NS	NS	NS	NS	NS	NS	**	1			
A.Fr.	NS	NS	(+)	NS	NS	NS	***	NS	1		
Floral	*	*	NS	(+)	(*)	NS	**	NS	***	1	
Vit.B	NS	NS	NS	NS	NS	NS	NS	+	NS	NS	1

*,**,*** significant at ($p \leq 0.05, 0.01, 0.001$)
+ significant at ($p \leq 0.15$)
NS not significant
OI Overall Intensity
() negatively correlated
1- correlation coefficient

Table 28. Correlation Matrix Among the Sensory Attributes Determined by the Three Common Panelists During the Evaluation of Hop Oil Oxygenated Fractions.

	OI	Soapy	Herbal	Woody	Grassy	Citrus	Sweet	Fruity	A.Fr.	Floral	Vit.B
OI	1										
Soapy	NS	1									
Herbal	+	NS	1								
Woody	NS	NS	+	1							
Grassy	NS	NS	**	*	1						
Citrus	NS	NS	*	NS	+	1					
Sweet	NS	NS	NS	NS	NS	NS	1				
Fruity	NS	NS	+	NS	*	NS	+	1			
A.Fr.	NS	NS	NS	NS	NS	NS	(**)	(*)	1		
Floral	NS	NS	NS	NS	NS	NS	NS	NS	NS	1	
Vit.B	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1

*,**,*** significant at ($p \leq 0.05, 0.01, 0.001$)
· significant at ($p \leq 0.15$)
NS not significant
OI Overall Intensity
() negatively correlated
1- correlation coefficient

Table 29. **Means[1], (Standard Deviations), and t-Values for the Comparison of the Scores Assigned by the Three Common Panelists and by the Whole Descriptive Sensory Panel to Each Attribute on the Evaluation of Hop Oil Oxygenated Fractions**

Attribute	Mean Group1 (n = 3)	Mean Group2 (n = 9)	t value
Overall Intensity	9.9 (0.77)	9.0 (1.31)	1.958^{ns}
Soapy	5.8 (0.32)	4.5 (1.73)	1.689^{ns}
Herbal	3.1 (1.75)	2.3 (1.33)	1.277^{ns}
Woody	1.3 (1.70)	1.6 (1.32)	0.494^{ns}
Grassy	1.0 (1.15)	1.5 (1.04)	1.050^{ns}
Citrus	5.5 (0.69)	3.1 (1.98)	5.853***
Sweet	1.6 (1.33)	2.4 (2.32)	0.847^{ns}
Fruity	1.9 (1.72)	2.4 (1.00)	0.226^{ns}
Artif. Fruit	1.9 (1.72)	2.3 (2.16)	0.339^{ns}
Floral	2.4 (1.64)	2.4 (1.47)	0.014^{ns}
Vitamin B	1.2 (1.09)	0.8 (0.81)	1.049^{ns}

*,**,*** significant at $p \leq 0.5, 0.01, 0.001$, respectively.
ns not significant.
1 means computed over replications and treatment for each panelist and attribute.
Group 1 three common panelists.
Group 2 whole panel (nine panelists).

Table 30. F values and Significance Levels for Oxygenated Hop Oil Fraction Attributes from ANOVA for Panelists (PAN), Treatment (TRT), Replications (REP) and Interaction Sources

	SOURCE OF VARIATION					
ATTRIBUTE	**PAN[1]**	**TRT[2]**	**REP[3]**	**PANxTRT**	**PANxREP**	**TRTxREP**
Overall Intensity	1.05^{ns}	0.65^{ns}	1.95^{ns}	1.52^{ns}	0.97^{ns}	1.67^{ns}
Soapy	1.27^{ns}	1.93^{ns}	2.18^{ns}	0.73^{ns}	0.13^{ns}	0.77^{ns}
Herbal	41.20**	1.31^{ns}	2.80^{ns}	1.32^{ns}	0.54^{ns}	1.14^{ns}
Woody	14.28*	0.44^{ns}	0.61^{ns}	4.54*	1.13^{ns}	1.37^{ns}
Grassy	10.80*	1.73^{ns}	1.90^{ns}	0.15^{ns}	0.38^{ns}	1.35^{ns}
Citrus	5.57ns	1.04^{ns}	1.00^{ns}	0.44^{ns}	0.51^{ns}	1.60^{ns}
Sweet	8.00*	2.64^{ns}	1.35^{ns}	0.45^{ns}	0.67^{ns}	0.40^{ns}
Fruity	12.73*	1.50^{ns}	0.95^{ns}	1.03^{ns}	0.43^{ns}	0.32^{ns}
Artificial Fruit	107.69***	0.38^{ns}	3.25^{ns}	5.34*	0.84^{ns}	2.89^{ns}
Floral	6.40^{ns}	2.45^{ns}	1.47^{ns}	1.14^{ns}	0.44^{ns}	0.36^{ns}
Vitamin B	97.00***	1.84^{ns}	3.00^{ns}	97.00***	1.00^{ns}	3.00^{ns}
Degrees of Freedom	2	2	2	4	4	4

*,**,*** Significant at $p \leq 0.05$, 0.01, 0.001 respectively.

ns Not significant

1 Means based on scores from three panelists.

2 HALLERTAUER; U.S.D.A. 21459; U.S.D.A. 21455

3 Three replications per treatment.

Table 31. Attribute Means[1,2] and (Standard Deviations) Based on Three Panelists for Hop Oil Oxygenated Fractions

Attribute	VARIETIES		
	Hallertauer	USDA 21459	USDA 21455
Overall Intensity	9.8 (1.27)	10.2 (1.92)	9.7 (3.74)
Soapy	5.4 (1.81)	5.0 (1.65)	6.9 (1.83)
Herbal	3.7 (1.50)	2.6 (2.13)	2.9 (1.83)
Woody	0.9 (1.36)	1.1 (2.26)	1.8 (2.73)
Grassy	1.7 (2.06)	0.4 (1.33)	0.9 (1.76)
Citrus	5.6 (1.23)	5.2 (1.48)	5.6 (1.01)
Sweet	1.1 (1.54)	1.9 (1.96)	1.7 (2.0)
Fruity	2.1 (2.67)	1.8 (2.08)	2.2 (3.34)
Artificial Fruit	1.4 (2.18)	2.1 (2.08)	1.6 (1.94)
Floral	1.6 (2.00)	4.1 (2.98)	1.6 (1.94)
Vitamin B	3.7 (2.91)	0.0 (0.00)	0.0 (0.00)

1 Sixteen point intensity scale (0 = none, 15 = extreme)

2 Means calculated with three panelists

3 There were no significant differences among the attributes means for each variety.

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