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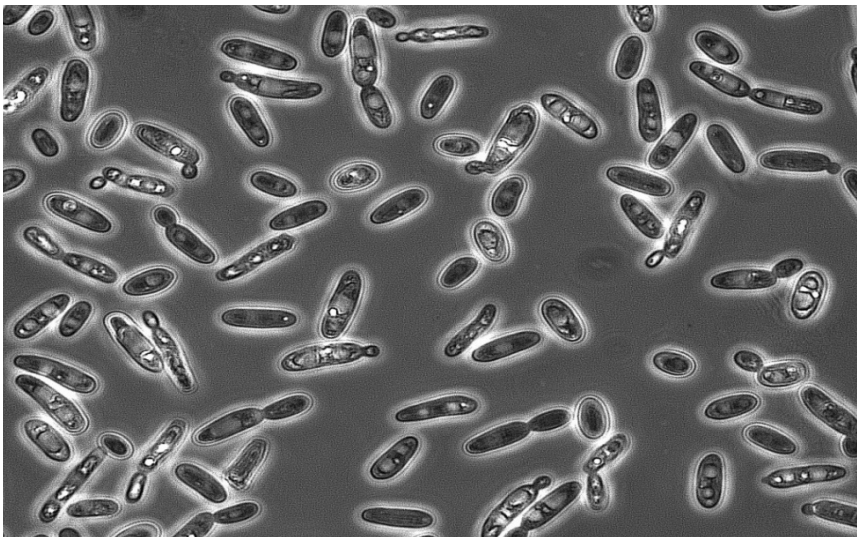

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THESIS

"Brettanomyces in Wine, a Fault or Not?"

INTRODUCTION

Motivation for choosing the subject.

Brettanomyces (or Brett) in wine is a topical problem nowadays because of its obscure nature, the rapid spread throughout the wine world and various opinions on this subject. Generally speaking, nowadays there are three major groups of wine experts who have ever dealt with Brettanomyces. The first group believes that Brettanomyces in wine is a fault, in any concentration. The second group thinks that Brettanomyces in low concentration adds some complexity to the wine. The third group considers Brettanomyces to be an expression of terroir and part of nature. Needless to say, that there are also a lot of wine lovers who have no idea what Brettanomyces is. Who is right and who is wrong?

Reasons why the chosen subject is topical nowadays.

Brettanomyces is reported to be involved in spoilage resulting in off-flavour in wine.[1]

While it is widely considered a spoilage yeast since it can produce off-flavours in wines, there are producers who suggest that at low levels some of the diverse flavours produced by Brettanomyces can improve red wine complexity.[2]

Brettanomyces is a favored topic among wine geeks, who'll often enter into lengthy discussions about whether a certain wine is Bretty or not.[3]

Of all wine faults, Brettanomyces is one of the most multifaced – and one of the most fascinating, partly because it is one of those "faults" that in some contexts can be regarded as a positive. Indeed, there are many sought-after, expensive wines that owe some of their character to Brettanomyces. It is also a controversial topic, with some arguing that Brettanomyces is to be avoided at all costs, while others think a less dogmatic approach is more in order.[4]

Wines from Chateau de Beaucastel famously have high levels of Brettonomyces – but they are rightly regarded as superb wines.[5]

To some, the distinctive earthy, slightly animal-like characteristics of many past vintages of Beaucastel have reflected an expression of terroir, or even the higher-than-average Mourvedre content of this wine. Others think it's because of Brettanomyces infection.[6]

Charles Collins, an American wine collector, says: "You should, however, give up the myth that the odd flavors are due to terroir –they aren't."[7]

Why is Brettanomyces a problem?

The reason Brett is a problem in winemaking is that it is annoyingly resilient, sitting around, biding its time, and then growing in conditions where virtually nothing else can. While it is seen in white wines (albeit very rarely), it is predominantly a red-wine problem. And the

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[1] cf. OIV (2014), Online
[2] cf. Robinson, J. (2015), p. 108
[3] cf. Goode, J. (2014), p. 155
[4] cf. Goode, J. (2014), p. 150
[5] cf. Bird, D. (2010), p. 257
[6] cf. Goode, J. (2014), p. 155
[7] Goode, J. (2014), p. 155

reason it is such a problem is that it produces some distinctive flavors that, at higher levels, can ruin wines.[8]

Brettanomyces can create significant levels of volatile phenols in a short period of time and is difficult to manage in the cellar.[9]

It can also be a ticking time bomb.[10] A small population of Brett might be waiting in a barrel or somewhere else in the winery environment, waiting for the right conditions to grow.[11]

Many wine enthusiasts appreciate the complexity that Brettanomyces brings to a finished wine.[12]

In wines of lesser intensity (with less body and extraction), the "Brettiness" can be overpowering, and indeed ruinous, to the final product's sale value.[13]

It is so difficult to predict what will happen to an unfiltered wine in the bottle, which may be complex at bottling, and horrendous one year later.[14]

In 2014 International Organization of Vine and Wine (OIV) adopted the Code of good vitivinicultural practices in order to avoid or limit contamination by Brettanomyces. This code determines the measures to be set up in vineyards and in wine cellars in order to contribute to reducing the risks linked to the presence of Brettanomyces.[15]

The objective of this work is to explore the nature of Brettanomyces, its affection on wines, discuss some problematic issues and to answer the question of whether it is a fault, terroir or a complexing agent. Furthermore, a practical blind tasting experiment will be carried out with wines affected by Brettanomyces to see how different people react to its by-products (4-ethylphenol and 4-ethylguaiacol) and to define if people are able to tell good quality wines from faulty ones. A mini questionnaire, considering tasters' wine experience (ranging from highly experienced to less experienced people) is devised.

An extensive research into microbiological wine phenomenon, Brettanomyces, as well as an analysis of wine experts' opinions, scientific papers and wine literature will be conducted.

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[8] cf. Goode, J. (2014), p. 150
[9] cf. Robinson, J. (2015), p. 108
[10] cf. Murphy, L. (2009), Online
[11] cf. Arvik, T., Henick-Kling, T. (2005), p. 4
[12] cf. Lucy Joseph, C.M., Albino, E.A., Ebeler, S.E., Bisson, L.F. (2015), p. 379
[13] cf. Arvik, T., Henick-Kling, T. (2005), p. 2
[14] cf. Arvik, T., Henick-Kling, T. (2005), p. 3
[15] cf. OIV (2014), Online

THE NATURE OF BRETTANOMYCES

Historical background of Brettanomyces.

Brettanomyces was first discovered by the brewing industry as an important component in British and Belgian beer styles in the early years of the twentieth century. Interestingly, brewers commonly refer to Brett character in beer as being "vinous".[16]

The name Brettanomyces was officially given to the "Torula-like" yeast that was characterized by N. Hiltje Clausen, in 1904.[17]

The first isolation of Brettanomyces in wine was in the 1930s by Krumbholz and Tauschanoff.[18]

Brettanomyces bruxellensis was described in wines for the first time in the middle of the twentieth century by Agostino (1950), Barret (1950) and Peynaud and Domercq (1956). In the 1990's, several studies focused on Brettanomyces and on volatile phenol production.[19]

What is Brettanomyces?

Brettanomyces is a genus of yeast (that is, a unicellular type of fungus, not a bacterium). While several species names are commonly used, the current classification has the wine-relevant Brett as just two species, Brettanomyces bruxellensis and Brettanomyces anomala, with the former by far the most important.[20]

Brettanomyces has a highly diverse genetic background and a diverse physiology.[21]

Brettanomyces is both an anaerobic and an aerobic organism. It is a resourceful microbe that can utilize a number of substrates at low levels and under restrictive conditions.[22]

Where is Brettanomyces found?

This yeast is found in wines and beers. It can grow in certain wines, and not in others, but we have no clear understanding as to why. Red wines are its favorite.[23]

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[16] cf. Goode, J. (2014), p. 150
[17] cf. Arvik, T., Henick-Kling, T. (2005), p. 2
[18] cf. Robinson, J. (2015), p. 108
[19] cf. Renouf, V., Lonvaud-Funel, A., Coulon, J. (2007), p. 162
[20] cf. Goode, J. (2014), p. 150
[21] cf. Lucy Joseph, C.M., Albino, E.A., Ebeler, S.E., Bisson, L.F. (2015), p. 379
[22] cf. Robinson, J. (2015), p. 108
[23] cf. Margalit, Y. (2012), p. 239

Brett is ubiquitous. That is to say, anywhere in the world that you look for it, if you look hard enough, you will find it. In the vineyards, in water, in soil, in the grape must, in the cold room, fermentation tanks, on concrete floors, and in barrels.[24]

Matt Thomson, an expert on the subject, says: "If someone went and had a decent look for Brettanomyces in the forests in France, they might find it there. I think there is a decent chance that Brett is resident in perhaps some forests."[25]

Brettanomyces strains can be found in young wines or in those of more than thirty years old. Different strains can be found within several cellars. This suggests a great diversity range within Brettanomyces with sometimes one strain representing one vineyard plot. Strains isolated from grapes could also be detected on tank surfaces and barrels, but only if the material has been previously used.[26]

So far, Brettanomyces has never been found on the surface of or inside new material before its first contact with wine. Wine strains are therefore able to colonize oenological material progressively through wine contact.[27]

There is a widespread misconception that Brett contamination is a hallmark of wineries with poor hygiene. Brett has been identified in every wine region where people have looked for it. Matt Thomson thinks that oak is largely to blame for many infections, because Brett can live in the oak and it is almost impossible to get out by cleaning.[28]

Different forms – Brettanomyces/Dekkera.

The double name [Brettanomyces/Dekkera] of these yeast genera refers to different forms of the same microorganism. The asexual form (budding reproduction) is called Brettanomyces, while the sexual form (sporulation) is called Dekkera.[29]

Although the number of Brettanomyces/Dekkera species has expanded and contracted due to changes in methods of identification, of the five species currently recognized, Brettanomyces (Dekkera) bruxellensis is the one most commonly found in wines worldwide.[30]

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[24] cf. Arvik, T., Henick-Kling, T. (2005), p. 3
[25] Goode, J. (2014), p. 154
[26] cf. Renouf, V., Lonvaud-Funel, A., Coulon, J. (2007), p. 164
[27] cf. Renouf, V., Lonvaud-Funel, A., Coulon, J. (2007), p. 165
[28] cf. Goode, J. (2014), p. 153
[29] cf. Margalit, Y. (2012), p. 239
[30] cf. Robinson, J. (2015), p. 108

Suitable environment for Brettanomyces to flourish.

Brett likes oak. It particularly likes toasted new barrels, and has been found as much as ¼ inch deep in staves.[31]

The conditions which are most suitable for Brett growth in the barrels are: wine with high pH (above 3.6), sufficient oxygen supply, higher temperatures (above 20C) and low concentration of SO2 below (15-20) ppm free. And even under good conditions, it grows slowly, without revealing its potential risk.[32]

Brettanomyces is capable of developing under strictly anaerobic conditions in supposedly dry wines and can produce excessive quantities of ethylphenols. The growth of Brettanomyces is supported by the fermentation of small quantities of residual sugars.[33]

Brettanomyces multiplying in the cellar and in the bottle if the infected wine is not filtered, dosed with SO2 or treated with dimethyl dicarbonate (DMDC).[34]

New barrels are more permeable to oxygen and can bring new substrates. They can lead to the maintenance of high levels of acetic acid bacteria and yeast, notably Brettanomyces, during the first period of their use.[35]

Key by-products of Brettanomyces.

There are four key by-products of Brettanomyces growth which can affect the flavor and aroma of a wine: esterases, volatile fatty acids, tetrahydropyridines, and, arguably the most important, volatile phenols. Two critical volatile phenol compounds have been isolated from Brettanomyces activity: 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG). 4-EP is often described as introducing an "animal", "medicinal", and "sweaty saddle" flavor to wine. Its presence is an almost certain indicator of a Brettanomyces infection. 4-EG in wine has a more appealing smoky, spicy, clove-like aroma.[36]

In Brett-infected wines 4-EP and 4-EG typically occur together.[37]

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[31] cf. Goode, J. (2014), p. 154
[32] cf. Margalit, Y. (2012), p. 240
[33] cf. Chatonnet, P., Dubourdieu, D., Boidron, J.N. (1995), p. 467
[34] cf. Murphy, L. (2009), Online
[35] cf. Renouf, V., Lonvaud-Funel, A., Coulon, J. (2007), p. 167
[36] cf. Robinson, J. (2015), p. 108
[37] cf. Goode, J. (2014), p. 151

Ways of defining the presence of Brettanomyces in wine.

Because of the high spoilage potential by Brettanomyces, there is a demand for a fast and reproducible monitoring method. The earlier their detection in wine, the better the chances for winemakers to prevent further growth and spread of this yeast to other batches.[38]

Nowadays, molecular tools based on DNA analysis are used. It is indeed possible to detect and to identify specifically Brettanomyces by a species-specific PCR targeting a DNA region of the Brettanomyces genome.[39]

Another method of identification of Brettanomyces is based on polymorphism in the rRNA internal transcribed spacer region. This method provides a fast and reliable way to identify Brettanomyces yeasts by assembling the four specific primers.[40]

Furthermore, quantitative real time PCR proposed by Phister and Mills and by Delaherche makes it possible to detect specifically and instantaneously the presence and number of Brettanomyces cells in wine.[41]

Brettanomyces and Dekkera yeasts can also be identified in wines by removing the fatty acids from their cell membranes, and, after their derivatisation as methyl esters, quantifying the type and amount of each by gas chromatography.[42]

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[38] cf. Egli, C.M., Henick-Kling, T. (2001), p. 241
[39] cf. Renouf, V., Lonvaud-Funel, A., Coulon, J. (2007), p. 162-163
[40] cf. Egli, C.M., Henick-Kling, T. (2001), p. 246
[41] cf. Renouf, V., Lonvaud-Funel, A., Coulon, J. (2007), p. 163
[42] cf. Suarez, R., Suarez-Lepe, J.A., Morata, A., Calderon, F. (2007), p. 13

AFFECTION OF BRETTANOMYCES ON WINES

Ways of contaminating wine.

Previous investigations have pointed out the bad hygienic conditions of the cellar as the main factor of Brettanomyces development in wine.[43]

Brettanomyces gets established when SO2 is not used appropriately, when barrels or infected containers are neglected and allowed to absorb oxygen from the air.[44]

The reincorporation of lees during ageing can sometimes bring Brettanomyces in wines.[45]

The distribution and infection of Brettanomyces appears to be local in certain wineries, and it is transmitted within the winery through the equipment, barrels and tanks.[46] Matt Thomson goes further: "If you adopt practices that allow cross-contamination of barrels, you will get more brett."[47]

How does Brettanomyces act?

Brett grows slowly, it is tough, and doesn't need much to feed on.[48]

The first sign is reduced varietal character, followed by the degradation of certain fruity aromas by esterases present in this yeast. Esterases are enzymes that cause the breakdown of esters, a chemical group important in conferring fruitiness. Thus grape varieties such as Pinot Noir are particularly badly hit by Brett, because it loses its bright cherry and violet characters, and this loss of fruit can be an early cue for the presence of Brett while it is in barrel.[49]

Among processes that deteriorate wine quality, the production of volatile phenols by Brettanomyces species is widespread and increasingly problematic.[50]

Like any good survivor, Brettanomyces eats what it finds. With Brettanomyces, small amounts of fermentable sugars (0.1 g/L) and high concentrations of ethanol (up to 14%) can act as fuel.[51]

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[43] cf. Renouf, V., Lonvaud-Funel, A., Coulon, J. (2007), p. 168
[44] cf. Arvik, T., Henick-Kling, T. (2005), p. 3
[45] cf. Renouf, V., Lonvaud-Funel, A., Coulon, J. (2007), p. 167
[46] cf. Margalit, Y. (2012), p. 239
[47] Goode, J. (2014), p. 153-154
[48] cf. Goode, J. (2014), p. 150
[49] cf. Goode, J. (2014), p. 151
[50] cf. OIV (2014), Online
[51] cf. Arvik, T., Henick-Kling, T. (2005), p. 3

It can utilize the last bit of sugar that is present in the wine that other yeasts cannot, and can also grow using other wine components as food.[52]

After alcoholic fermentation, the second fermentation, called malolactic fermentation, performed by lactic acid bacteria and mainly the Oenococcus oeni species often occurs. This is the second key stage for Brettanomyces development in wine.[53]

Brettanomyces yeasts grow in wine mainly during barrel aging.[54]

The work of Mark Sefton has shown that oak barrels, which are infected with Brettanomyces cannot be effectively sterilized. Neither careful washing and rinsing with sulfited water, nor shaving and firing, or ozone treatment can sterilize a barrel.[55]

What does Brettanomyces add to wine?

Brettanomyces can make some red wines smell like a horse blanket that has been stored in a metal Band-Aids box.[56]

Volatile phenols, mainly 4-ethylphenol and 4-ethylguaiacol, are produced from p-coumaric acid and ferulic acid respectively after enzymatic decarboxylation (cinnamate decarboxylase, PAD) and reduction (vinylphenol reductase, VPR). These precursors are naturally present in grape must.[57]

Ethylphenols, which may range from a few micrograms up to several milligrams per liter, affect the wine's quality, even in quite small quantities, giving it "animal" phenolic odors, or even "barnyard" and "stable" smells at higher concentrations.[58]

Brettanomyces yeast can also produce a significant amount of acetic acid at levels of a few g/l, adding its share to the accumulation of this fault agent in wine.[59]

Olfactory and organoleptic effects of Brettanomyces on wine (positive and negative).

UC Davis Dr. Linda Bisson and UC Davis Viticulture & Enology Department staff member Lucy Joseph released a Brett aroma wheel around the start of 2013. The wheel is the result of a study the two performed on a collection of 83 Brett strains. The wheel divides Brett aromas

[52] cf. Goode, J. (2016), p. 99
[53] cf. Renouf, V., Lonvaud-Funel, A., Coulon, J. (2007), p. 165
[54] cf. Egli, C.M., Henick-Kling, T. (2001), p. 241
[55] cf. Arvik, T., Henick-Kling, T. (2005), p. 4
[56] cf. Arvik, T., Henick-Kling, T. (2005), p. 1
[57] cf. OIV (2014), Online
[58] cf. Chatonnet, P., Dubourdieu, D., Boidron, J.N. (1995), p. 463
[59] cf. Margalit, Y. (2012), p. 240

into main categories such as "animal," which is further divided by wet dog, sweaty/sour, urine, fecal, barnyard and horse. The other categories are savory, woody, putrid, chemical/solvent, veggie, fruity, floral, spice, fermentation, dairy and earthy. However, Brett is still too unpredictable for Bisson to recommend winemakers to begin flirting with it to try and hit some of those more pleasant sounding parts of the aroma wheel.[60]

I guess the idea of creating the aroma wheel was to show that Brett could bring positive aromas such as fruity, floral and spicy as well as negative ones. It is not only about bad smelling aromas. The only matter we cannot control is when good smelling aromas will become evil-smelling ones.

There is a research into screening for Brettanomyces with positive aroma characteristics. In total, 95 Brettanomyces strains [from UC Davis Department of Viticulture and Enology Wine Yeast and Bacteria Collection] were surveyed to identify those that gave consistently positive aroma characters. A panel of five sensory evaluators was used to determine both the aroma descriptors and the positive and negative perception of aromas from the Brettanomyces strains grown in defined medium supplemented with the three aromatic amino acids, phenylalanine, tryptophan, and tyrosine. Eleven strains produced little or no aroma and were excluded from the analysis. None of the strains were rated as producing only positively perceived aromas by any of the five evaluators. The majority, i.e., approximately two-thirds, of the strains gave a negative or mixed aroma perception. Approximately one-third of the strains tested (i.e., 31) received all three possible aroma scores: positive, mixed, and negative.[61]

The issue of adding complexity to the wine.

While most people won't enjoy a really stinky wine, low levels of Brett might not be a problem. In fact, a little Brett might even add complexity to certain robust styles of wines.[62]

Bob Cartwright, previously senior winemaker of Leeuwin Estate in Western Australia's Margaret River region, acknowledges that, "A lot of winemakers like to have some as complexing character. The question is how much it too much?"[63]

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[60] cf. Adams, A. (2013), Online
[61] cf. Lucy Joseph, C.M., Albino, E.A., Ebeler, S.E., Bisson, L.F. (2015), p. 381
[62] cf. Goode, J. (2014), p. 155
[63] Goode, J. (2014), p. 156

All compounds produced by Brettanomyces in low concentration add to the wines some kind of "complex" nose, which then, as the infection advances, turns out to be a fault.[64]
Peter Gago, chief winemaker for Penfolds in Australia, speaks of the issues winemakers face in dealing with Brett: "We consider that if it is detectable, then it's a flaw. Without wanting to get into detection/recognition thresholds, we think that the argument that Brett adds a touch of complexity has been historically overplayed and misleading".[65]

A perception threshold of defects in wine affected by Brettanomyces.

As with other volatile odorants, people differ widely in their sensitivity to these molecules, and each individual shows a range of different thresholds. For example, the threshold for detecting an odorant differs from the threshold for recognition of the same odorant. A useful sensory threshold to use for 4-ethylphenol is 425 µg/L (a microgram is one millionth of a gram). At this concentration and beyond, a wine will typically be noticeably Bretty. Bellow this concentration, the character of the wine may be changed but people won't, on average, recognize that this is due to 4EP. Pascal Chatonnet, who did pioneering work on the problem of Brett in Bordeaux wines, found that 425 µg/L of 4EP negatively affected the sensory property of the wines from this region that he tested (above the limit threshold of 425 µg/L wine's aroma acquires significant unpleasant characteristics[66]). For 4EG, thresholds are in the range of 100 µg/L. But specifying thresholds is an inexact science. These may be altered by the style of the wine and the presence of other volatile compounds. Others have suggested levels as low as 300 µg/L and even 150 µg/L for 4EP.[67]

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[64] cf. Margalit, Y. (2012), p. 240
[65] Murphy, L. (2009), Online
[66] cf. Chatonnet, P., Dubourdieu, D., Boidron, J.N. (1995), p.467
[67] cf. Goode, J. (2014), p. 151

PROBLEMATIC ISSUES WITH BRETTANOMYCES

How common is Brettanomyces?

Brett is highly prevalent, and represents an increasing problem, even in New World countries such as Australia. "We first started raising this as an issue five years ago", says AWRI's Peter Godden. "Since then, AWRI has started a major project looking at Brettanomyces". As a scientist, he feels that for such an important issue, this is a relatively under-researched area.[68]

With rising standards of winemaking worldwide, it is a surprise to hear that Brett is on the increase. There seem to be two contributing factors to this rise. First, there is the current trend for "natural" wines. "Minimalist winemaking is a perfect recipe for bretty wine," says Godden. Second, there is the move towards "international" styles of red wine, made in an extracted style from super ripe grapes. "These are higher in pH and are richer in polyphenols", explains Randall Grahams.[69]

Methods of prevention, controlling/monitoring Brettanomyces.

Few stabilization techniques are available to limit and prevent Brettanomyces growth in wine, SO2 addition being mostly used. Only molecular SO2 is effective against microorganisms.[70]

Adequate cellar hygiene and carefully controlled SO2 use in the wines and barrels effectively prevent the development of these yeasts and keep the ethylphenol content of the red wines to trace level (only a few µg/L).[71]

A weak point during wine production is the interval between ending the alcoholic fermentation and finishing MLF. At this time the SO2 level must remain low, in order not to inhibit the ML bacteria. During this period, which may take a few weeks or more, the wine is vulnerable, and the Brett yeast can take hold in the wine with long-term results. It is therefore highly recommended that commercial ML bacteria be used to encouraged MLF as soon as possible after alcoholic fermentation. Then, as soon as it is finished, SO2 should be added.[72]

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[68] Goode, J. (2014), p. 152
[69] Goode, J. (2014), p. 152
[70] cf. Coulon, J., Raffestin, V., Bellan, M., Lonvaud-Funel, A. (2013), p. 1
[71] cf. Chatonnet, P., Dubourdieu, D., Boidron, J.N. (1995), p. 464
[72] cf. Margalit, Y. (2012), p. 240

Brettanomyces development can be tempered by SO2 addition if a sufficient molecular concentration of 0.8 mg/l is achieved. However differences do exist among strains in terms of cellular multiplication and cultivability.[73]

Fast fermentations will allow early SO2 addition, protecting the wine. The second crucial step occurs during ageing, where Brettanomyces should be carefully monitored and traditional oenological practices used to reduce its occurrence.[74]

Exposure of the wine to oxygen while in the barrels should be reduced if the winery is facing this problem. It was found that oxygen is a supporting factor in Brettanomyces activity, increasing its grows.[75]

More radical treatments like heat treatments or filtration are effective against Brettanomyces. Wines in which the intrinsic microbial ecosystem is imbalanced are more favorable to contamination. These microbial stabilization methods should be considered only before bottling, the probability of re-contamination being then lower.[76]

Other preventing steps include completing fermentation so there is no residual sugar left in the wine to act as a substrate, and maintaining lower temperatures in the barrel, vat, or tank. Low cellar temperatures seem to make quite a difference.[77]

Chemical alternatives to SO2 can also be considered. DMDC (dimethyl dicarbonate or Velcorin® or E242) is a chemical conservative which shows remarkable antimicrobial activities. It inhibits the enzymes involved in glycolysis.[78]

A new product has recently become available for the control of Brettanomyces in wine. It is called No Brett Inside, and it is a preparation of chitosan, isolated from fungus Aspergillus nigrans. "Sniff' Brett" is another tool for winemakers hoping to control Brett. It comes in the form of small vials, which contain a liquid culture medium containing coumaric acid. Wine is added, and after two or three days' incubation you can use your sense of smell to pick up the

[73] cf. Coulon, J., Raffestin, V., Bellan, M., Lonvaud-Funel, A. (2013), p. 4
[74] cf. Renouf, V., Lonvaud-Funel, A., Coulon, J. (2007), p. 168
[75] cf. Margalit, Y. (2012), p. 240
[76] cf. Renouf, V., Lonvaud-Funel, A., Coulon, J. (2007), p. 167
[77] cf. Goode, J. (2014), p. 156-157
[78] cf. Renouf, V., Lonvaud-Funel, A., Coulon, J. (2007), p. 167

presence of Brett, whose aromatic impact is vastly enhanced by the culturing process. Sniff' Brett is useful for smaller wineries without proper lab facilities.[79]

One more effective method to prevent its growth is sterile filtration. This is not always practical, at least in case where red wines are not filtered (or are filtered just by a coarse filtration) in order to minimize wine stripping.[80]

Hygiene is an important factor in controlling the spread of Brettanomyces in the cellar. Areas that provide suitable niches for Brettanomyces are must lines, dirty crush equipment, barrels, or any tank or transfer line that is not cleaned effectively.[81] However, Brett can be monitored, but not controlled.[82]

So many people, so many opinions on Brettanomyces.

Regarding the "good Brett" idea, some people believe it to be an excuse for producing a faulty wine.[83]

All in all, so many people, so many opinions about Bretattanomyces. Some people like it, others hate it, but many people still don't understand it. Wine tasters have different perception, experience and wine educational background, thus there is a heated discussion nowadays. Needless to say, everyone is entitled to have his or her opinion on the subject. As for me, the more I learn about wine the more I am convinced of my personal opinion – Brettanomyces is a fault in any receptive concentration. If many people like Bretty wines, then there has to be a market for them - demand and supply. Linda Bisson says: "Spoilage is in the eye of the beholder".[84]

Even wine professionals comment at times that it is a matter of personal taste.[85] The wine you drink is the wine you deserve. It is up to consumers to discourage bad winemaking: the quality of wine will improve when they make up their minds to drink better wine and when they are also prepared to pay for that extra quality.[86]

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[79] cf. Goode, J. (2014), p. 157
[80] cf. Margalit, Y. (2012), p. 239
[81] cf. Robinson, J. (2015), p. 108
[82] cf. Arvik, T., Henick-Kling, T. (2005), p. 3
[83] cf. Arvik, T., Henick-Kling, T. (2005), p. 6
[84] Adams, A. (2013), Online
[85] cf. Robinson, J. (2015), p. 591
[86] cf. Peynaud, E. (1987), p. 10

Many French winemakers think Brett is part of their terroir and the reason their wines taste as they do, and they're not alone. Moreover, it is proved that U.S. consumers have already figured this out. UC Davis professor Linda Bisson went out to buy wines described online by consumers as "typical Bordeaux" and discovered that they were just loaded with 4-EP and 4-EG. So typical Bordeaux, for many people, already equals Brett.[87]

"When you give people a bretty wine to smell, they associate it with France, and of course the French don't like that," says Linda Bisson,"They associate it with spoilage."[88]

However, since the early 1990s Brett has become much rarer in Bordeaux mainly due to the groundbreaking work of Dr. Pascal Chatonnet.[89]

Are peoples' palates spoiled?

I also strongly believe, especially listening to those who are talking about horse manure bringing charm to the wine, that a lot of people have spoiled noses and palates or they may not be ready for quality wines. People are talking nonsense.

They have to realize that Brettanomyces is a fault. It has nothing to do with terroir. People should say No to Bretty wines and then we will see how winemakers will take steps to get rid of it. Otherwise why should their bother? People drink their Bretty wines and pay for it lots of money.

The lack of knowledge about Brettanomyces.

A lack of knowledge about Brettanomyces and most importantly about quality wines seems to be the core of the problem. For instance in Russia, the majority of regular wine drinkers have no idea what it is, only a few know just the basics. Even many wine experts have very poor knowledge of it. Unfortunately, in Russian wineries nobody conducts laboratory analysis to identify the presence of Brettanomyces and its by-products in wines, they just don't have such lab equipment and methodology. It took me a lot of efforts to find a lab in Russia which agreed to create a methodology designed specifically for my experiment.

In sommelier schools worldwide there is usually no such subject as practical learning of wine faults, in many restaurants sommeliers don't know how Brettanomyces smells. The subject of

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[87] cf. Blake Gray, W. (2013), Online

[88] McIntyre, D. (2013), Online

[89] cf. Goode, J. (2014), p. 154

Brettanomyces, along with other faults, should at least be taught in sommelier schools, both theory and practice.

A PRACTICAL TASTING EXPERIMENT OF THE BRETTANOMYCES PERCEPTION

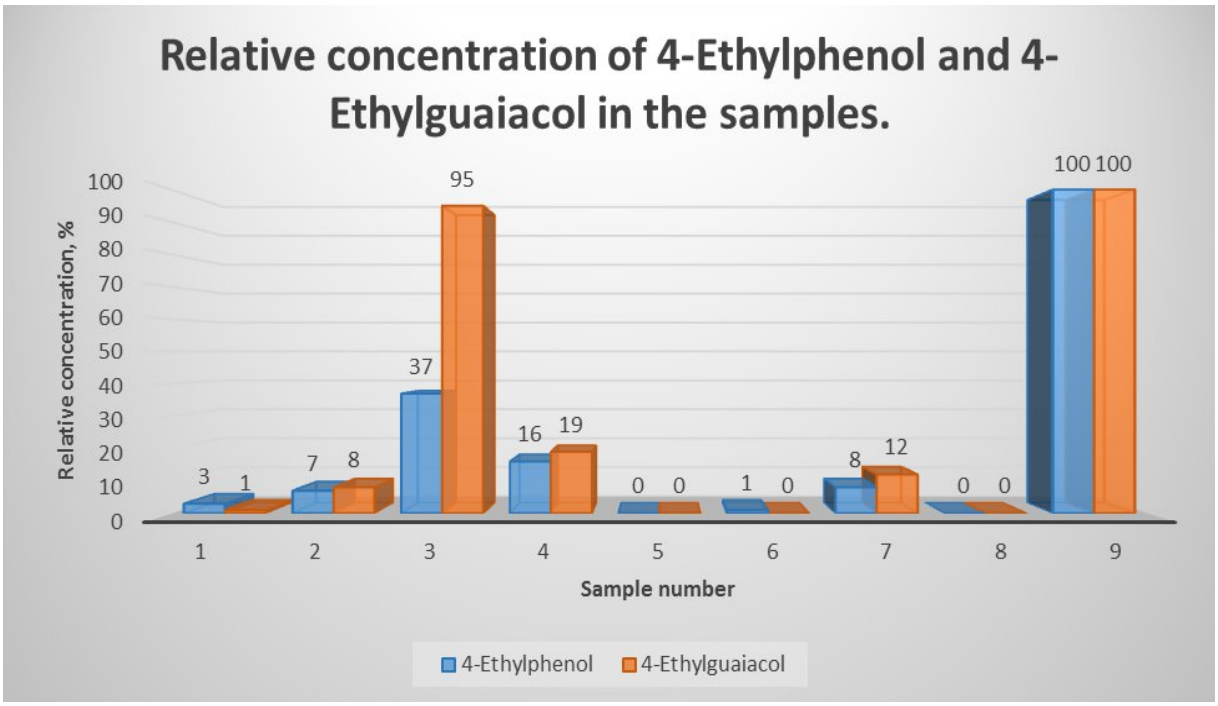
A panel of 13 sensory evaluators[90] (6 winemakers from Russia, France and Italy; 1 sommelier; 2 vinegrowers; 2 wine lovers and 2 laymen) was asked to taste blind 9 red wines[91] to identify personal preferences for specific wines. The evaluators didn't know the real purpose of the tasting experiment. The panel was provided with a mini questionnaire containing 2 questions for each wine: 1. All in all, do you like the nose of this wine?, 2. All in all, do you like the taste of this wine?. At the end of the tasting there were 4 concluding questions: 1.Which wine(s), if any, do you find fruity? 2. Which wine(s), if any, do you find complex? 3.Which wine do you like most? 4.Which wine do you like least? The most important questions were about the nose of each wine and the most liked wine, other questions were irrelevant. Close attention was paid to winemakers' answers that were expected to recognize Brettanomyces.
Before being tasted all the samples had been sent to the lab of the "North Caucasian Regional Research Institute of Horticulture and Viticulture" to have a chemical analysis to confirm the presence of 4-EP and/or 4-EG and then to define their quantity (µg/L) in the tasted wines. This is the only lab in Russia which agreed to conduct this chemical analysis, having worked out specific methodology.
The first part of the analysis was completed quickly. However, it took the lab approximately 15 more weeks to complete the second part which was to identify the amount of 4-EP and 4-EG in µg/L as a control measurement of neat substances of 4-EP and 4-EG was required but there were no neat substances in Russia and the lab had to order it from the EU.[92]

Results and Conclusion.

[90] For the list of participants and the photo report see the Attachment №1 and №3
[91] For the list of wines see the Attachment №2
[92] The results of the second part were revealed during the viva voce exam at Weinakademie Osterreich in Rust

It's understood that 13 evaluators are probably not enough, but still show the general direction.

The results of the analysis are presented in the lab reports.[93]



Sample number	Mass concentration, µg/L	
	4-Ethylphenol	4-Ethylguaiacol
1	46	2
2	109	14
3	**548**	**157**
4	239	31
5	1	1
6	8	1
7	119	20
8	0	1
9	**1489**	**165**

According to the lab reports 4-EP and 4-EG were identified in wines №1,2,3,4,7,9, where wines №3 and №9 had the highest relative concentration (in %) and mass concentration (the

[93] For the lab reports and the official translations of them see the Attachments №4, №5, №6, №7

definition error makes 20%) higher than 425 µg/L for 4-EP and 100 µg/L for 4-EG respectively. Ethylphenols identified in red wines come from a development of yeasts of the genus Brettanomyces.[94]

7 evaluators out of 13 liked the nose of the wine №3 (winemakers-4, sommelier-1, vinegrowers-2, winelovers-0, laymen-0).

8 evaluators out of 13 liked the nose of the wine №9 (winemakers-4, sommelier-1, vinegrowers-1, winelovers-0, laymen-2).

5 evaluators out of 13 liked the wine №9 the most (winemakers-3, sommelier-1, vinegrowers-0, winelovers-0, laymen-1).

Nobody liked the wine №3 the most.

Moreover:

3 evaluators out of 13 found the wine №3 complex (winemakers-3, sommelier-0, vinegrowers-0, winelovers-0, laymen-0).

6 evaluators out of 13 found the wine №9 complex (winemakers-4, sommelier-1, vinegrowers-1, winelovers-0, laymen-1).

It clearly shows that the majority of the most experienced evaluators couldn't recognize Brettanomyces and tell good wines from faulty ones. Moreover, they liked the nose of faulty wines and found them complex. It could be an indicator of their lack of knowledge and experience in this context.

CONCLUSION

The organoleptic deterioration of wine caused by the formation of volatile ethylphenols is a serious economic problem, especially with respect to high quality wines that require long maturation periods in oak casks.[95]

Brettanomyces is very dangerous. As much as it's reviled, Brettanomyces still has its supporters among those who think a little bit of barnyard or wet dog imparts a distinct identity to their wines. A "touch" of Brett can be dangerous because a population could change or

[94] cf. Chatonnet, P., Dubourdieu, D., Boidron, J.N., Pons, M. (1992), p. 174

[95] cf. Suarez, R., Suarez-Lepe, J.A., Morata, A., Calderon, F. (2007), p. 18

grow to overwhelm the wine. The first year could be nice and interesting, but by the second vintage the Brett population could have exploded and by the third "it's going to be, 'Oh my God! What happened?'"[96]

However, in 2013 UC Davis, which had previously always called Brettanomyces in wine a "spoilage organism", for the first time acknowledged that Brett is an important part of some wines' terroir. UC Davis tested 83 strains of Brett and 17 - more than 20% were regarded as giving more positive impact than negative.[97]

Brett can occur naturally in the vineyard and winery, and those familiar with Old World reds from Bordeaux, the Rhône Valley, Rioja, Piedmont and some Burgundy, appreciate the leathery, earthy notes, usually attributing them to terroir – the soils, climate and other growing conditions of the vineyard. Brett shows up in New World wines too, from Australia, New Zealand, Washington State, California and elsewhere, and wines from South Africa have been thumped in the UK press for burnt rubber notes, thought by some to be linked to Brett.[98]

The conclusion seems to be that Brettanomyces is widespread, and virtually every barrel of red wine has the potential to go Bretty. Create the right environment for it, and you will have a Brett infection. Thus the key objective for winemakers isn't to create a sterile winery, which will never happen, but to make sure that their barrels aren't a receptive environment for Brett to grow in.[99]

Beaucastel's Marc Perrin believes: "There are certainly some Brettanomyces in every natural wine, because Brettanomyces is not a spoilage yeast (as many people think) but one of the yeasts that exists in winemaking. Some grapes, like Mourvedre, are richer in 4EP 'precursors' than others and we have a high percentage of these grapes in our vineyard. Of course, you can kill all natural yeasts, then use industrial yeast to start the fermentation, saturate the wine with SO2, and then strongly filtrate your wine. There will then be no remaining yeasts, but also no taste and no typicite. That is the difference between natural wine and industrial wine, between craftsmanship and mass-market product".[100]

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[96] Adams, A. (2013), Online
[97] cf. Blake Gray, W. (2013), Online
[98] cf. Murphy, L. (2009), Online
[99] cf. Goode, J. (2014), p. 154
[100] cf. Goode, J. (2014), p. 155

Richard Gibson echoes more revolutionary sentiments: "Currently, in Australia the move is to eradicate brett".[101]

It seems that there are more questions related to Brettannomyces than answers. In my humble opinion, the problem of Brettanomyces spreading is underestimated; it could be the next epidemic like phylloxera. It is spreading very fast through the wine world; many wine producing countries are already on alert. There is even the Brett Police in California established to eradicate it.

Before conducting my practical experiment, I asked a winemaker from one of the leading wineries in Russia how they do a Brett analysis of their wines. He replied they do it only organoleptically, relying on their senses. Later that winemaker participated in my blind tasting experiment. He liked the nose of a Bretty wine. Moreover, he chose it as the most liked wine out of nine though the lab proved it to be faulty. The experiment shows that there is a lack of knowledge and Brett-related experience even among winemakers. Many experienced tasters are not able to recognize Brettanomyces and to tell good quality wines from faulty ones. It is quite dangerous to rely on your senses especially if you have undeveloped and uncalibrated ones! After the tasting we had an argument and he said that a lot of people drink Bretty wines, so we cannot say it is a bad thing. It is a pity to hear such statement from a winemaker who is supposed to be quality oriented.

To eradicate Brettanomyces it is highly important to educate about that fault winemakers, sommeliers and wine experts. Special educational programs are to be developed. Winemakers are to improve their qualification on a regular basis.

Wines where Brettanomyces is totally absent throughout the whole production process are rare and aiming for a complete absence of this microorganism in wine is unrealistic. Winemakers only have the option of limiting and controlling its multiplication.[102]

Basically, that statement reveals that most of the time consumers drink faulty wines.

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[101] Goode, J. (2014), p. 156

[102] cf. Renouf, V., Lonvaud-Funel, A., Coulon, J. (2007), p. 162

While vintners might personally appreciate a leathery character in their reds, the Brett Police – winemaking peers and certain critics – will condemn them for what they consider to be evidence of winemaking fault.[103]

Emile Peynaud said that some people would have it that a slightly high level of volatile acidity is necessary, that it accentuates the bouquet of some wines or even takes its place. Such people are bad tasters who are talking nonsense. Either they lack sensitivity or else they do not know how to tell good from bad.[104]

This situation is reminiscent of Brettanomyces. A quality wine is all about fruitiness, pleasant aromas and cleanliness, but some winemakers are trying to mask their mistakes talking about the addition of complexity. We are probably just being fooled. Even if the wines are appreciated by some wine drinkers.

It is also a matter of the wine culture in a specific country or a region and current generation. Older generation of Bordeaux is used to consume lots of Bretty wines, so for them it could probably be totally fine.

It would be a wise decision of the governmental bodies to make it the law to conduct a special check-up of bottles of wines for the presence of the main Brett by-products (4-EP and/or 4-EG) before releasing them for sale as in the case of the maximum permitted levels of volatile acidity and SO2.

Only setting the maximum permitted limit of threshold of 4-EP and/or 4-EG wouldn't solve the problem because today it can be ok, but tomorrow it can increase dramatically.

Most wineries have had some experience trying to control Brettanomyces in their cellars. Several wineries we have worked with swore us to secrecy about their "contamination problem." Some said that they had been "working with Brett" to make more complex wines, but now, it was just an overwhelming problem in some of their reds.[105]

A major research in this relation shows that Brettanomyces strains can produce a wide variety of odoractive compounds under different conditions and suggest that many factors influence the formation of a "good" or "bad" odor from this yeast. This study suggests that the absence

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[103] cf. Murphy, L. (2009), Online
[104] cf. Peynaud, E. (1987), p. 178
[105] cf. Arvik, T., Henick-Kling, T. (2005), p. 1

of negative characters rather than the aroma intensity of positive characters is most closely associated with positive sensory perception of Brettanomyces strains.[106]

I completely disagree with the conclusion. A bad smelling aroma even in a very low concentration is still a bad smelling aroma and a negative characteristic, and the absence of "strongly" negative aromas will not make it any better.

A good wine is first of all one which has a pleasant bouquet.[107]

Professional tasters must be aware of wine faults to be able to tell a good quality wine from a faulty wine, and not to be misled by the talks about terroir features. It is not terroir, it is a fault.

The role of tasting expertise is the exercise of quality control. Its function is to judge whether a wine is free of faults.[108]

Bordeaux consultant Denis Dubourdieu said Brett is a fault, but also part of the fabric of Bordeaux. "Without a doubt, Brett is seen as a flaw by Bordeaux winemakers," he says. "It is a matter of great concern to us, as we pick riper berries than ever."[109]

Here is another nebulous issue: Few people are able to smell every aspect of the typical Brett defects in a red wine. Some sense the purely "medicinal" odor of 4-ethylphenol and little of the "green apple" and "wet goat" aromas of valeric and isovaleric acids, each produced to various degrees. Still others sense an odor of "animal" and "barnyard".[110]

There are many – including Emile Peynaud – who argue that the denotation of quality is essentially the subjective enjoyment of pleasure.[111] The most important quality dimension for consumers is the amount of pleasure afforded by the wine.[112] If some people like Brettanomyces in their wines, that is totally fine with me. However, I would enjoy Brett-free and quality wines.

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[106] cf. Lucy Joseph, C.M., Albino, E.A., Ebeler, S.E., Bisson, L.F. (2015), p. 386
[107] cf. Peynaud, E. (1987), p. 48
[108] cf. Peynaud, E. (1987), p. 145
[109] Murphy, L. (2009), Online
[110] cf. Arvik, T., Henick-Kling, T. (2005), p. 2
[111] cf. Robinson, J. (2015), p. 591
[112] cf. Robinson, J. (2015), p. 592

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APPENDIX

Attachment №1

The list of participants at the blind tasting experiment on April 28th, 2017

Taster №	Name, Surname	Relation to the Wine World	Country of Origin
1	Frank Duseigneur	Winemaker	France
2	Oleg Nichvidyuk	Winemaker	Russia
3	Georges Blanck	Winemaker	France
4	Stanislav Osmolovskiy	Layman	Russia
5	Valery Tomilin	Sommelier	Russia
6	Matteo Coletti	Winemaker	Italy
7	Leonid Fadeev	Winelover	Russia
8	Dmitry Muzyukov	Vinegrower	Russia
9	Alexandre Mourousy	Winemaker	France
10	Mary Nichvidyuk	Winelover	Russia
11	Kirill Bardakov	Winemaker	Russia
12	Evgeniy Mikheev	Vinegrower	Russia
13	Alexander Ukhanov	Layman	Russia

Attachment №2

The list of wines tasted at the blind tasting experiment on April 28th, 2017 (in numerical order).

1. Castro Ventosa, “El Castro de Valtuille” Joven, Bierzo DO, 2015, Spain. RP 90. Price paid in Russia 13€.
2. Domaine Chante Cigale, Chateauneuf-du-Pape AOC, 2013, France. RP 87. Price paid in Russia 58€.
3. Domaine Usseglio Raymond & Fils, Chateauneuf-du-Pape AOC Rouge, 2013, France. RP90. Price paid in Russia 74,50€.
4. Perrin et Fils, “Chateau de Beaucastel” Rouge, Chateauneuf-du-Pape AOC, 2013, France. RP 93. Price paid in Russia 171,50€.
5. Domaine Grand Veneur, “Les Origines”, Chateauneuf-du-Pape AOC, 2012, France. RP 93. Price paid in Russia 101,50€.
6. Domaine de Pignan, Coralie at Floriane, Chateauneuf-du-Pape AOC, 2012, France. RP92. Price paid in Russia 74€.
7. Gabriel Meffre, “Laurus”, Chateauneuf-du-Pape AOC, 2011, France. RP 87. Price paid in Russia 76€.
8. Le Celestiere, Chateauneuf-du-Pape AOC Tradition, 2009, France. RP 90. Price paid in Russia 33€.
9. Chateau La Lagune, Haut-Medoc, 2004, France. RP 90. Price paid in Russia 263€.

Attachment №3

A photo-report of the blind tasting experiment on April 28th, 2017

ФГБНУ СКЗНИИСиВ
Научный центр «Виноделие»
350901, г. Краснодар, ул. им. 40-летия Победы, 39,
т. (861) 252-58-77

Заключение №2

от 5 мая 2017 г.

Заказчик: АО «Скалистый берег», г. Анапа.

Задачей исследования являлось определение содержания в представленных заказчиком 9 образцах вина 4-этилфенола и 4-этилгваякола.

Анализ проводился методом газовой хромато-масс-спектрометрии на приборе Clarus 600T (PerkinElmer, США) при следующих условиях: жидкостная экстракция исследуемых соединений, полярная капиллярная колонка Elite-Wax ETR (PerkinElmer, США), газ-носитель гелий, ионизация электронным ударом 70 эВ. Идентификация соединений осуществлялась сканированием в полном ионном токе при помощи библиотеки NIST 2009, для количественного определения применялся более чувствительный анализ по выбранным ионам. В связи с отсутствием чистых веществ было проведено относительное количественное определение с использованием циклогексанола в качестве внутреннего стандарта.

На рис. 1 и 2 представлены результаты идентификации целевых веществ при сканировании образца №9, содержавшего максимальные концентрации 4-этилфенола и 4-этилгваякола. Оба вещества были идентифицированы с вероятностью, близкой к 100%.

Результаты количественного анализа приведены в табл. и на рис. 3. Представлено относительное содержание каждого компонента в процентах между пробами с максимальной и минимальной концентрациями.

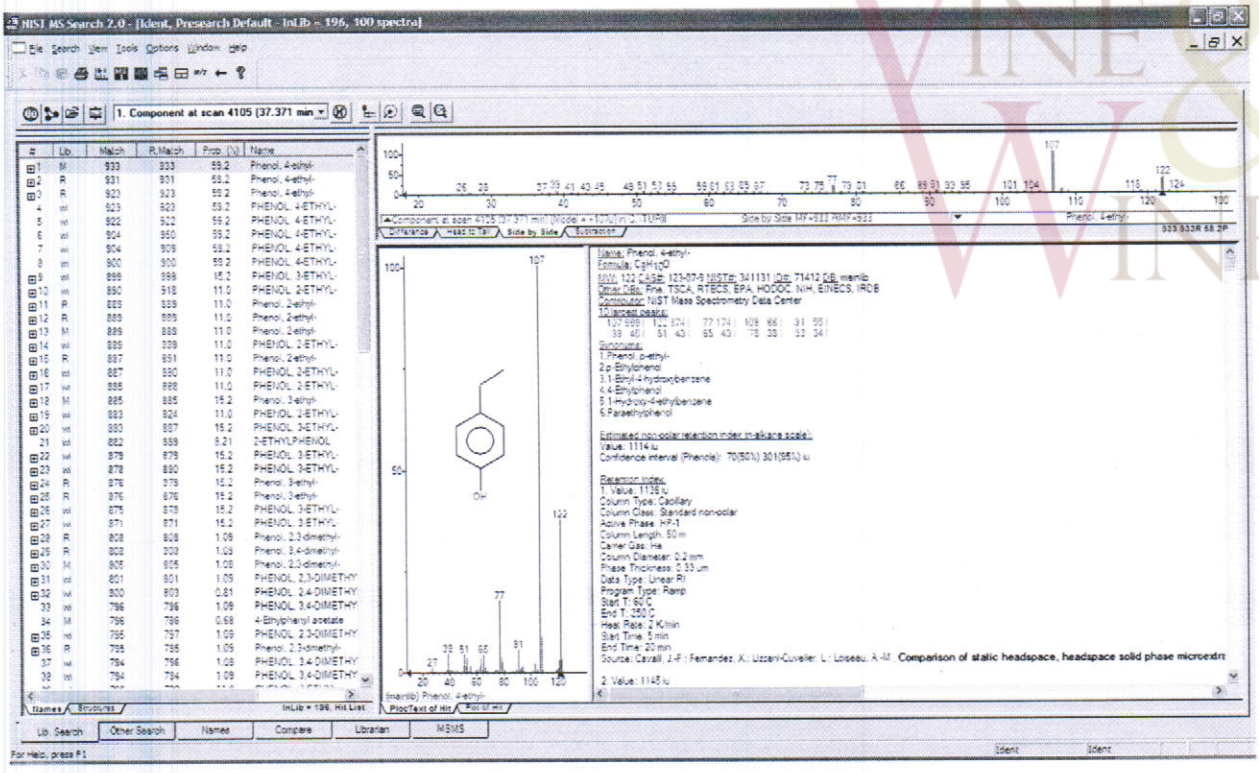

Рисунок 1 – Идентификация 4-этилфенола

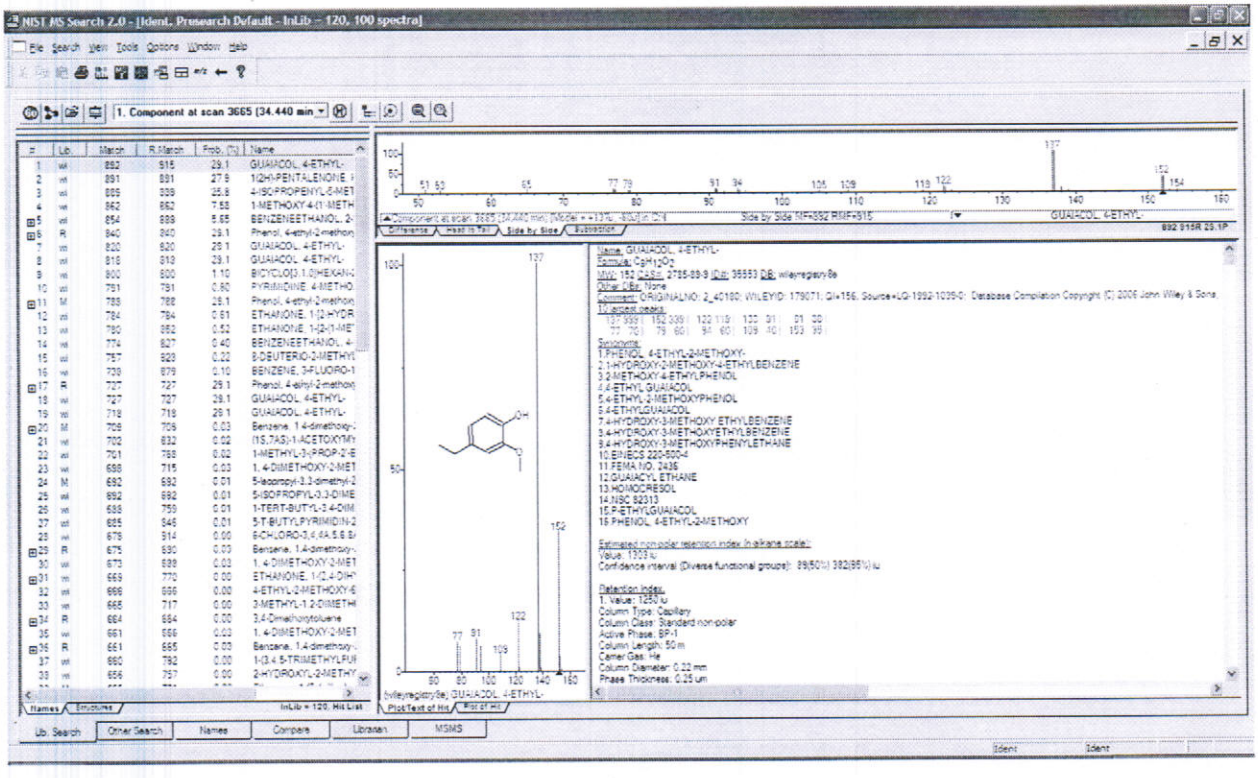

Рисунок 2 – Идентификация 4-этилгваякола

Таблица - Относительное содержание целевых компонентов

№ пробы	Относительное содержание, %	
	4-этилфенол	4-этилгваякол
1	3	1
2	7	8
3	37	95
4	16	19
5	0	0
6	1	0
7	8	12
8	0	0
9	100	100

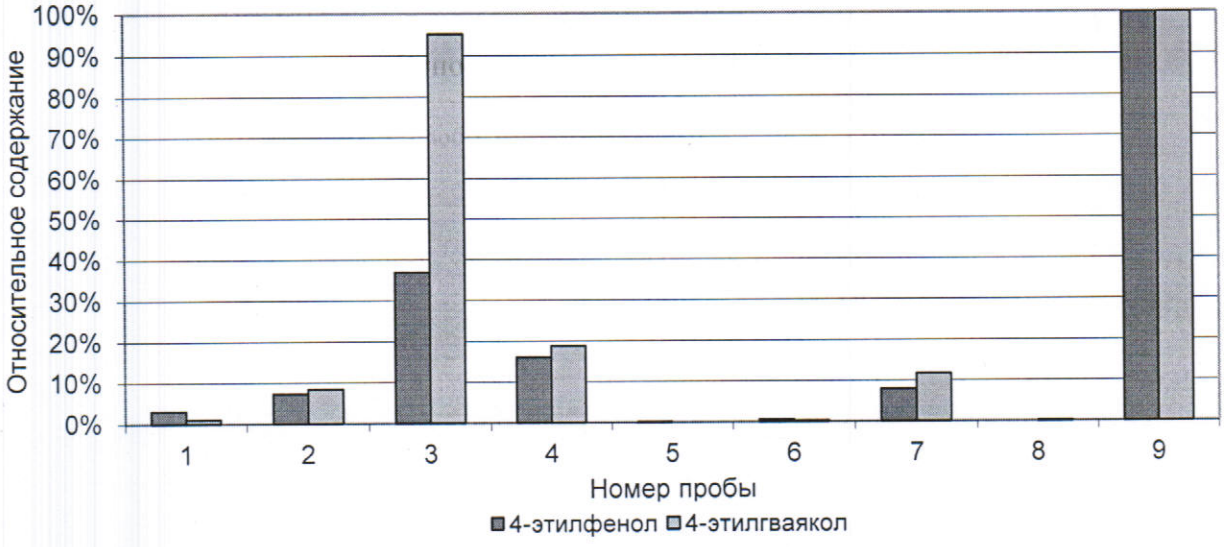

Рисунок 3 – Относительное содержание 4-этилфенола и 4-этилгваякола в представленных образцах.

Старший научный сотрудник, к.т.н. М.Г. Марковский

FGBNU SKZNIICiV
Scientific centre "Winemaking"
39, 40-letiya Pobedy str., 350901 Krasnodar
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The Summary №2
of May 5, 2017

The customer: «Rocky coast» JSC, Anapa.

The research was to determine the presence of the 4-Ethylphenol and 4-Ethylguaiacol in 9 samples of wine presented by the customer.
The analysis was made by a method of gas chromatography–mass spectrometry (GC-MS) through device Clarus 600T (PerkinElmer, the USA) under following conditions: fluid extraction of the investigated compounds, polar capillary column Elite-Wax ETR (PerkinElmer, the USA), gas carrier helium, electron ionization by potential of 70eV. Identification of the compounds was carried out by scanning in total ionic current by means of library NIST 2009, more sensitive analysis was applied to quantitative definition of the selected ions. Due to the lack of pure materials a relative quantitative definition has been made with use of cyclohexanol as the intrinsic standard.

Pic. 1and 2 are presenting results of identification of the targeted substances when scanning sample №9 containing the maximum concentration 4-Ethylphenol and 4-Ethylguaiacol. Both substances have been identified with the probability close to 100 %.
Results of quantitative analysis are reflected in the Table below and on pic. 3. It contains the relative concentration of the each component in percentage between the probations held with maximum and minimum concentrations.

See the picture
Below the picture, Picture 1-Identification of the 4-Ethylphenol

See the picture
Below the picture, Picture 2-Identification of the 4-Ethylguaiacol

TABLE – Relative concentration of the targeted components

№ Probe	Relative concentration, %	
	4-Ethylphenol	4-Ethyulguaiacol
1	3	1
2	7	8
9	37	95
4	16	19
5	0	0
6	1	0
7	8	12
8	0	0
9	100	100

See the picture

Probe No. □ 4-Ethylphenol □ 4-Ethylguaiacol

Below the picture, Picture 3-IRelative concentration of the 4-Ethylphenol and 4-Ethylguaiacol in the given samples.

Senior researcher, PhD of techn. sciences signature /M.G. Markovskiy/

Seal : Federal budgetary scientific establishment, North-Caucasian zonal scientific-researching Institute for gardening and viticulture; city of Krasnodar SC Winemaking.

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Заключение № 3

от 22 августа 2017 г.

Заказчик: АО «Скалистый берег», г. Анапа.

Задачей исследования являлось определение содержания в представленных заказчиком 9 образцах вина 4-этилфенола и 4-этилгваякола.

Анализ проводился методом газовой хромато-масс-спектрометрии на приборе Clarus 600T (PerkinElmer, США) при следующих условиях: жидкостная экстракция исследуемых соединений, полярная капиллярная колонка Elite-Wax ETR (PerkinElmer, США), газ-носитель гелий, ионизация электронным ударом 70 эВ. Идентификация соединений осуществлялась сканированием в полном ионном токе при помощи библиотеки NIST 2009, для количественного определения применялся анализ по выбранным ионам. Определение концентраций соединений проведено на основе аналитических стандартов 4-этилфенола и 4-этилгваякола (Sigma-Aldrich), предоставленных заказчиком.

На рис. 1 и 2 представлены результаты идентификации целевых веществ при сканировании образца № 9, содержавшего максимальные концентрации 4-этилфенола и 4-этилгваякола. Оба вещества были идентифицированы с вероятностью, близкой к 100%.

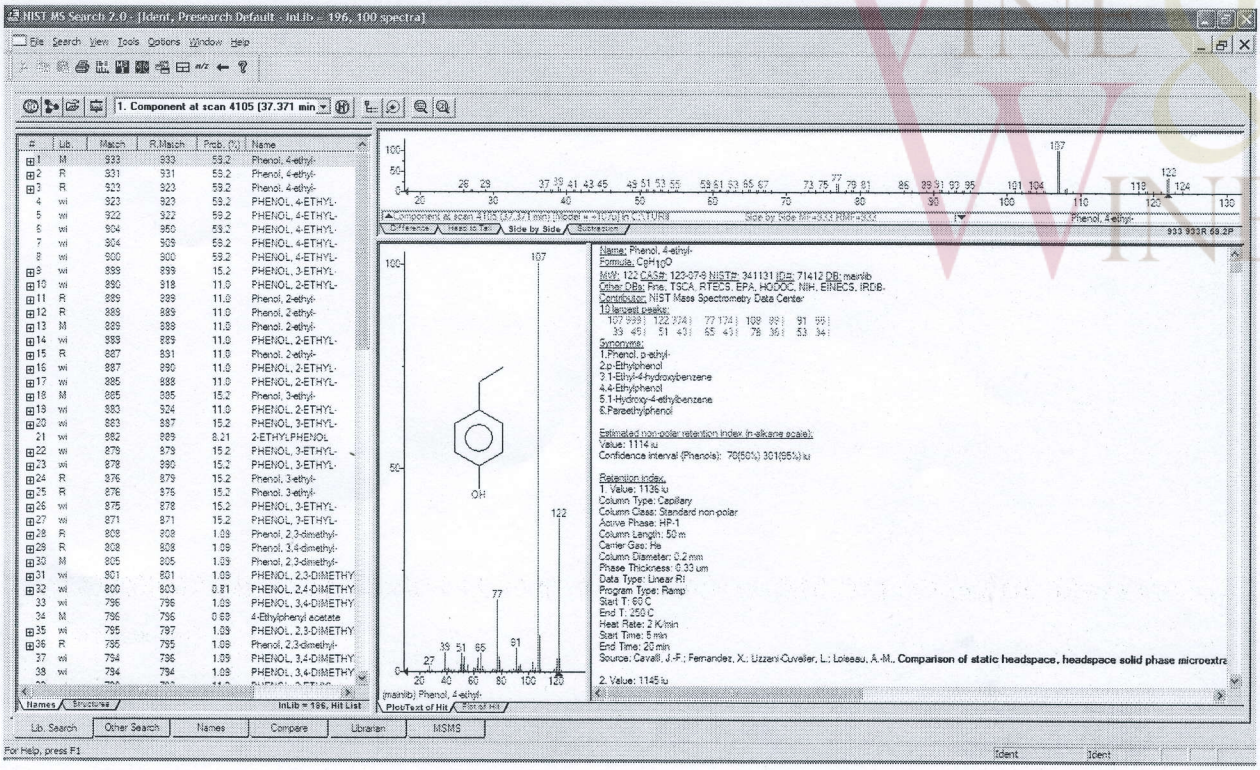

Рисунок 1 – Идентификация 4-этилфенола

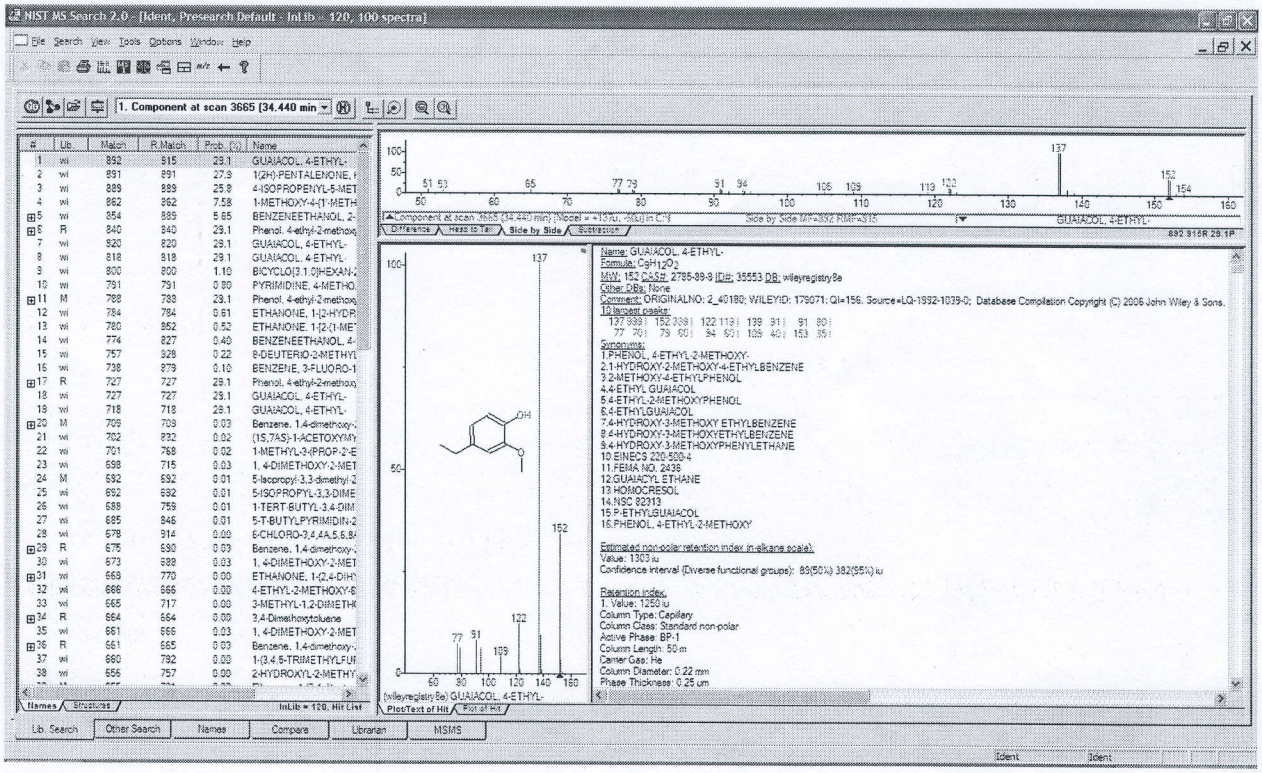

Рисунок 2 – Идентификация 4-этилгваякола

Результаты количественного анализа приведены в таблице. Погрешность определения составляет 20%.

Таблица – Массовая концентрация целевых компонентов

№ пробы	Массовая концентрация, мкг/л	
	4-этилфенол	4-этилгваякол
1	46	2
2	109	14
3	548	157
4	239	31
5	1	1
6	8	1
7	119	20
8	0	1
9	1489	165

Зав. НЦ Виноделие

ФЕДЕРАЛЬНОЕ ГОСУДАРСТВЕННОЕ БЮДЖЕТНОЕ НАУЧНОЕ УЧРЕЖДЕНИЕ «СЕВЕРО-КАВКАЗСКИЙ ФЕДЕРАЛЬНЫЙ НАУЧНЫЙ ЦЕНТР САДОВОДСТВА, ВИНОГРАДАРСТВА, ВИНОДЕЛИЯ» г.КРАСНОДАР НЦ «Виноделие»

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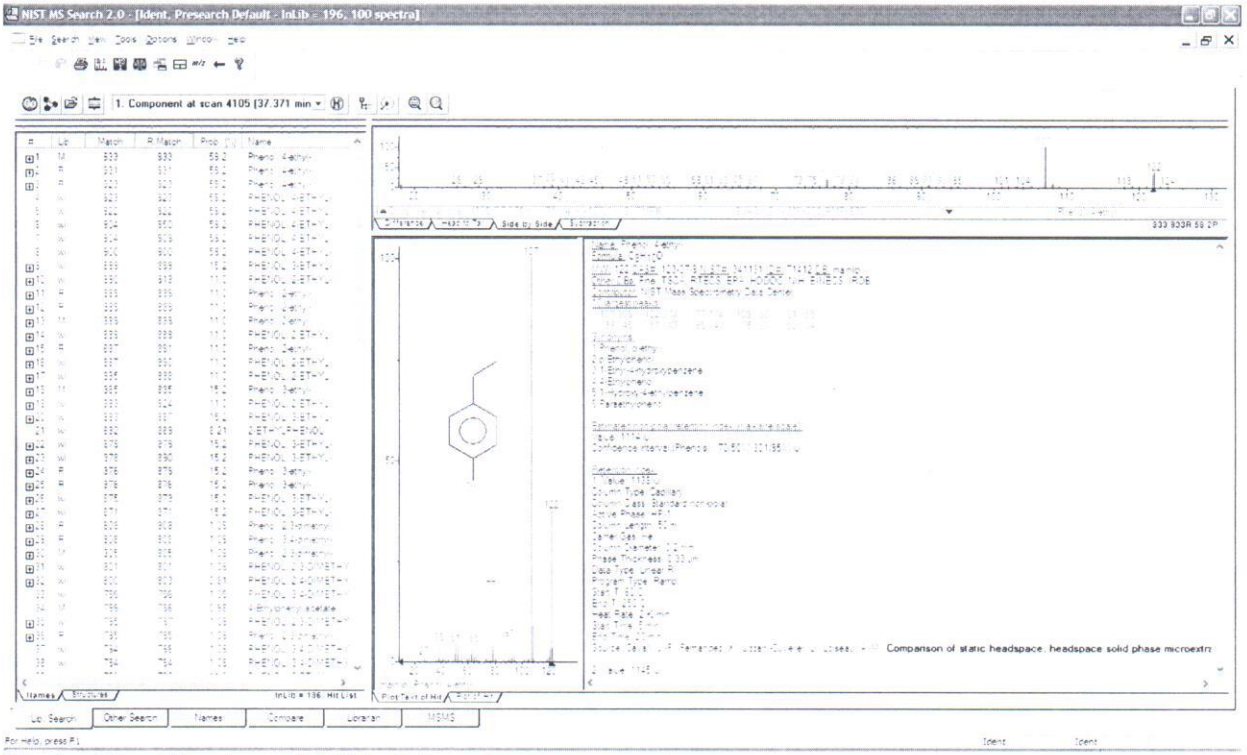
Summary № 3

of August 22, 2017

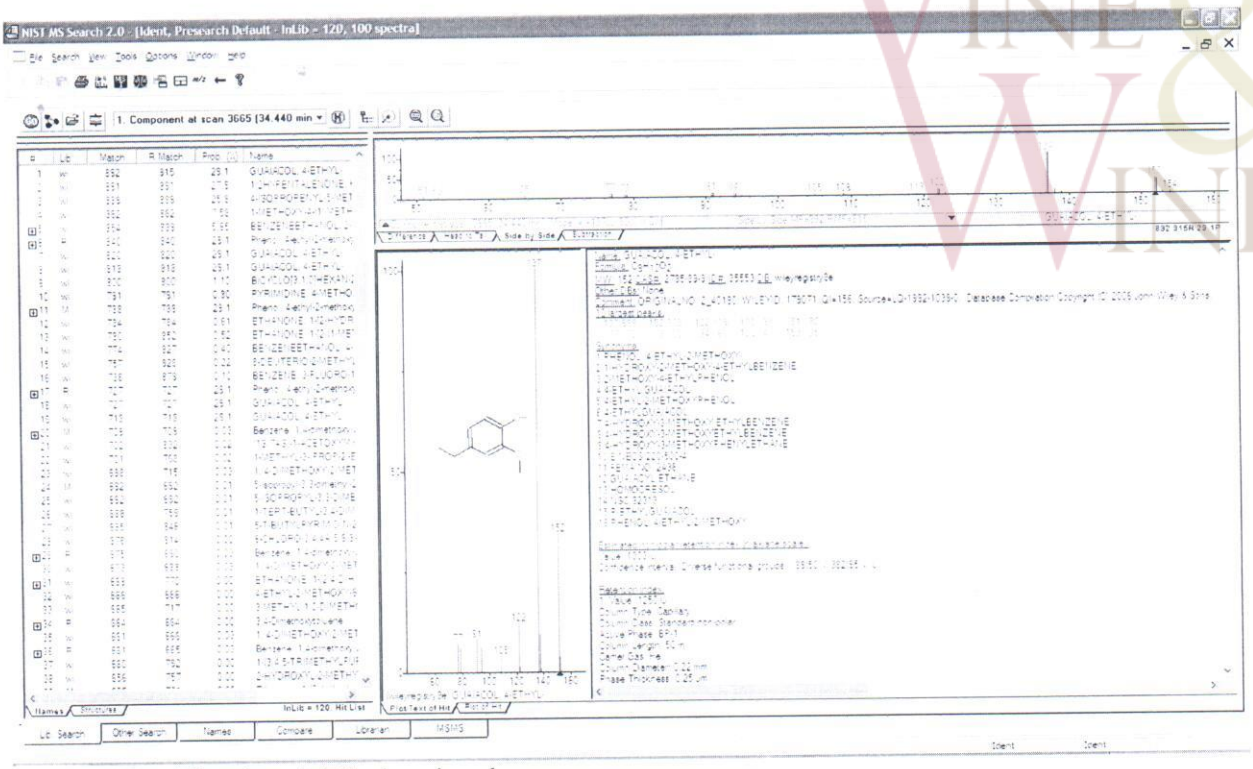
The customer: «Rocky coast» JSC, Anapa.

The research was to determine the presence of the 4-Ethylphenol and 4-Ethylguaiacol in 9 samples of wine presented by the customer.
The analysis was made by a method of gas chromatography–mass spectrometry (GC-MS) through device Clarus 600T (PerkinElmer, the USA) under following conditions: fluid extraction of the investigated compounds, polar capillary column Elite-Wax ETR (PerkinElmer, the USA), gas carrier helium, electron ionization by potential of 70eV. Identification of the compounds was carried out by scanning in total ionic current by means of library NIST 2009, for quantitative definition we analyzed the selected ions. The concentration of the compounds was evaluated on the basis of analytic standards 4-Ethylphenol and 4-Ethylguaiacol (Sigma-Aldrich)that were submitted by the Customer.

Pic. 1and 2 are presenting results of identification of the targeted substances when scanning sample №9 containing the maximum concentration 4-Ethylphenol and 4-Ethylguaiacol. Both substances have been identified with the probability close to 100 %.



Pic.1 - Identification of 4-Ethylphenol



Pic. 2 - Identification 4-Ethylguaiacol

Results of quantitative analysis are shown in the table. The definition error makes 20 %.

The table - Mass concentration of the targeted components

Probe No	Mass concentration, µg/L	
	4-Ethylphenol	4-Ethylguaiacol
1	46	2
2	109	14
3	548	157
4	239	31
5	1	1
6	8	1
7	119	20
8	0	1
9	1489	165

Head of the SC Winemaking signature /T.I.Guguchkina/

Seal : Federal budgetary scientific establishment, North-Caucasian zonal scientific-researching Institute for gardening and viticulture; city of Krasnodar SC Winemaking.

This translation is complete and construed in Bureau of Translations of Anapa town-resort .
Senior translator- Pavelko Alexander Yuriyevich.
Translator's signature Pavelko A.Y. / /
Date translated: August 22, 2017 www.rbsproj.com
The translation bears a stamp and a seal to its effect