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SUMMARY of Ph.D. THESYS
Obtaining different types of wine products from honey

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CLUJ-NAPOCA
2015
INTRODUCTION

Honey wine or mead is one of the first alcoholic beverages ever known by men. It is obtained from honey fermentation, being possible to obtain various types and different names depending of geographical cultural regions, existing proves that this beverage was very appreciated even by our ancestors, the dacians.

As it was said before, mead is the result of alcoholic fermentation, by mixing honey in different proportions with water and pollen as a fermentation agent. *Pollen* can replace different yeasts, and the fermentation process can be stimulated by nutritive agents.

The main objective of this thesis was to obtain honey wine by fermenting honey, as the main raw material, implementing a proper technological process as required quality legislation (ISO 22000; ISO 9001), and to establish the parameters of the fermentation process.

**Thesis structure**: The thesis is compose from two main parts: “**Actual state of research**” and “**Own research**”, including materials and methods used, results obtained and the conclusions of the study including in this part future research due to be taken in postdoc program.

First part (**Actual state of research**) includes 6 chapters:

**Chapter 1 – Honey wine or mead. History**

Includes a brief history of honey wine, with historical and archeological references regarding the presence of this beverage in worldwide cultures.

**Chapter 2 – Honey, main raw material**

It refers at honey organoleptic and physical properties (color, aroma, taste, consistency, wettability, thermic conductivity and turbidity), and chemical properties (sugars, water, enzymes, proteins, organic acids, minerals, vitamins) of honey used in mead production. Also, in this chapter it is approached therapeutic aspects by the point if view of antibacterial components as well as European and international normative regarding honey quality.
Chapter 3 – Main ferments used in mead fermentation

In this chapter are debated main ferments elected to be used in the process of mead making: *Saccharomyces cerevisiae* yeast and *pollen*, which was used in ancient times as ferment.

Chapter 4 – Studies regarding the impact of different additives used in honey must fermentation process

It emphasizes the use of some additives (substitute substances) to help achieve complete cycle of fermentation process, and to fill fermentation environment gaps – which means honey must is poor in thin nutrients needed by yeasts.

Chapter 5 – Fermentation evolution

Describe the steps of forming and fermenting honey must, referring at wine making technology, where the studies regarding this phenomenon are very extensive. It starts by describing the fermentation process and the changes that occur during alcoholic fermentation.

Chapter 6 – Final product. Actual state of research

In this chapter it is described the actual state of research regarding honey wine or mead, and a short review of all scientific publications on this topic, making an inventory of research findings and future directions.

Part two (Own research) comprises seven chapters (7-13) and the conclusions of the thesis:

Chapter 7 – Materials and methods

This chapter refers to the material used and the methods of analysis addressed the raw materials, auxiliary materials, honey musts and final products of honey wine. The following determinations were made:
- HMF determination using high performance liquid chromatography coupled with UV-VIS detector with photodiode array (HPLC - PDA);
- Individual sugar content determination using high performance liquid chromatography coupled to refractive index (HPLC-IR);
- Total polyphenols determination;
- Flavones determination;
- DPPH determination;
- FRAP determination;
- Alcoholic concentration determination;
- Determination of NO$_2^-$ from water;
- Determination NO$_3^-$ of from water;
- Determination of NH$_4^-$N from water;
- Determination of from water;
- Determination of pH, free acidity, lactones acidity;
- Mold and yeast determination;
- Determination of water microorganisms. Membrane filtration technique.

Chapter 8 – Technologic process of honey wine making

It has been followed the reception protocol of raw materials and auxiliary materials (Ch. 8.1.) According to the technological scheme there were identified the critical control points (Ch. 8.2.) and they has been initiated research in order to eliminate PCC1 by implementing innovative methods of honey sterilization to obtain an aseptic fermentation broth (Ch. 8.2.1.):

- Honey sterilization using ultra fast freezing (Ch. 8.2.1.1.);
- UV honey sterilization (Ch. 8.2.1.2.);
- Honey sterilization using alcohol (Ch. 8.2.1.3.).

After removing the last obstacle we moved to the analysis of honey mash (Ch. 8.3.). The fermentation process is described in detail (Ch. 8.4.) from the beginning of the fermentation process, to the environment clarification process, making a parallel between the two types of yeast used (Saccharomyces cerevisiae and pollen) (Ch. 8.5.).
Chapter 9 – Honey wine. Physicochemical and organoleptic data interpretation

Unfortunately, the rules of alcoholic fermentation of grape must do not apply equally to honey must due to lack of raw materials and whimsical elements necessary for the alcoholic fermentation. For this reason two different mixes of fermentation have been tested (Ch. 9.1.), the results can be observed in Table 1:

<table>
<thead>
<tr>
<th>Details</th>
<th>Fructose (%)</th>
<th>Glucose (%)</th>
<th>Galactose (%)</th>
<th>Turanose (%)</th>
<th>Maltose (%)</th>
<th>Trehalose (%)</th>
<th>Isomaltose (%)</th>
<th>Erlose (%)</th>
<th>Total sugars (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Must miere</td>
<td>11,50</td>
<td>7,79</td>
<td>0,04</td>
<td>0,68</td>
<td>0,47</td>
<td>0,19</td>
<td>0,20</td>
<td>0,45</td>
<td>21,32</td>
</tr>
<tr>
<td>M1 mix</td>
<td>0,10</td>
<td>0,04</td>
<td>-</td>
<td>0,26</td>
<td>0,44</td>
<td>0,16</td>
<td>0,11</td>
<td>-</td>
<td>1,02</td>
</tr>
<tr>
<td>M2 mix</td>
<td>11,33</td>
<td>8,31</td>
<td>-</td>
<td>0,25</td>
<td>0,35</td>
<td>0,16</td>
<td>0,08</td>
<td>-</td>
<td>20,48</td>
</tr>
</tbody>
</table>

Glucidic spectrum for the products fermented by two different types of additives

The curve of the formation of alcohol was analyzed (Fig. 1) in the case of fermentation with *Saccharomyces cerevisiae* (Chap. 9.3.), and how it decreases the pH (Fig. 2) in tandem with the increase of alcohol concentration.

![Fig. 1. Alcohol formation curve during 45 days of fermentation](image-url)
Sensory profile of the products obtained were analyzed according to standard industry applied oenological organoleptic analysis (Fig. 3). We opted for the five points scoring to better compare pairs of products which is the focus of this chapter.

**Fig. 2.** Descend curve of pH during 45 days of fermentation

**Fig. 3.** Comparative senzorial diagram of the products fermented by Saccharomyces cerevisiae (left) and pollen (right)
Further, previously mentioned properties were analyzed for the products made from linden honey wine (Ch. 9.5.) and honeydew honey (Ch. 9.6.).

**Chapter 10 – Mead types**

Harmonious composition, the ability to develop many product variations, taking advantage of attractiveness and originality of organoleptic characteristics of this product represent alluring features in expanding the study to develop a whole new category of pallets to be studied. In this case, we developed 7 recipes of honey wine based on the promising results obtained in the initial stage of the research.

**Chapter 11 – Honey wine faults**

Organoleptic defects can occur due to changes caused by various unwanted chemical interactions between chemical compounds must and environmental factors. Also there are cases where defects are caused by organoleptic properties characteristics of raw materials or materials.

Another cause of defects is the failure of environmental sanitation technology or various external contamination. Inoculation with harmful micro-organisms through the auxiliary material (spices, grains, fruits) is also possible.

**Chapter 12 – Honey wine. Functional food**

The functional properties of honey is transmitted without being adversely affected by factors inherent in the finished product. To this is added the products resulting from alcoholic fermentation and physicochemical and biochemical processes occurring maturation and aging of wine.

**12.1. Polyphenols and flavones**

After fermentation the amount of polyphenols and flavanols decreases both without any added product, and in those with added. This applies largely to fermentation performed by
Saccharomyces cerevisiae, as pollen by its contribution of polyphenols, flavones and they send much of the finished product, which is present in large quantities even after fermentation.

12.2. Antioxidant activity

12.2.1. DPPH

AOA capacity of products obtained by fermentation of Saccharomyces cerevisiae comparative with pollen is different: the lowest they have are those fermented with Saccharomyces cerevisiae particularly simple recipe without additives (EC50 (%) -52.73 % and a value of inhibition 972% ) as counterpart fermented pollen product has a value of 2.94 times higher oxidizing activity (EC50 - 17.93 %) inhibition with a percentage of 27.9%.

12.2.2. FRAP

The results of antioxidant activity by FRAP method are similar to those of DPPH method, where honey has the highest value (123.29 mMTr / kg ) in the process of diluting the concentration decreases. The greatest value of the finished products AOA has the wine with added plum concentrate fermented with Saccharomyces cerevisiae (6.40 mMTr/ l), concentration greater even than homologous product fermented with pollen (5.19 mMTr / l).

Chapter 13 – Brand new marketing

The brand is the identity of the product. Generally a brand promotes a certain concept, reputation and quality. To promote wine from honey have chosen the concept of "Dacian’s legacy" in order to promote national identity, is recorded in historical sources that wine from honey represent in Dacian’s culture a drink base being known throughout Europe as a magic liquor prepared from honey.

Partial conclusions

The physico-chemical analyzes performed on the raw materials, intermediate products and finished products showed the following:

- The amount of sugars present in the worth is directly proportional to the value of
the alcoholic strength of the final product;

- pollen fermented wines have an alcohol concentration higher than the fermented with *Saccharomyces cerevisiae*, which is explained by the additional intake of carbohydrates that brings the addition of pollen;

- fermentation with *Saccharomyces cerevisiae* is safer and easier to control, especially if the sterilization of raw materials is performed to achieve an fermentation environment conducive without competing harmful microorganisms;

- The pH and acidity directly influences the fermentative process and its duration being essential factors in the fermentation of honey;

- correction option of honey mash by adding various auxiliary materials aids showed a considerable improvement flavones and total polyphenol content and antioxidant power also increased compared to the simple recipe. In simple recipe case wine antioxidant power kept although it is slightly lower than the initial worth before fermentation. For fermented with pollen loads were recorded much higher amounts of polyphenols, flavonols and antioxidant power than the simple product fermented with *Saccharomyces cerevisiae*.

Organoleptic analysis of finished products was done in accordance with the current legislation and have revealed the following:

- *Pollen* can be used as a developer of flavor. It decisively influences the odorant Sensations, floral notes, emphasizing olfacto - gustatory qualities of the raw material. Notes odoriferous of polyforal, lime and sugar reported in sensory evaluation of wines fermented with pollen exceed the intensity of the sensations of the wines fermented with *Saccharomyces cerevisiae*;

- *Saccharomyces cerevisiae* gives pronounced astringency. After five months of aging was reported in increased feelings tasting sweetener in fermented products for both yeast and especially a reduction of acidity and astringency sensations for lots fermented by *Saccharomyces cerevisiae*. High acidity and pronounced astringency the result of fermentation with *Saccharomyces cerevisiae* makes these products to be appreciated for lack of "sweetness" and alcoholic strength which gives corpulence wines;

- As an adjunct to ferment, fermented products with added grape must showed
Improved features. This can be explained by the fact that the addition is suitable for this type of yeast being used in wine making industry fund in the fermentation of grape must.

SELECTIVE BIOGRAPHY


