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Full Length Research Paper

Mead features fermented by *Saccharomyces cerevisiae* (lalvin k1-1116)

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Alcoholic beverages are produced practically in every country in the world representing a significant percentage of the economy. Mead is one of the oldest beverages and it is easily obtained by the fermentation of a mixture of honey and water. However, it is still less studied compared to other beverages and does not have industrialized production. It is prepared as a handmade product. The origin of the honey used to formulate the mead creates differences on the final product characteristics. In this study, fermentation occurred at temperatures of about 22.1 ± 0.4°C after a previous pasteurization and inoculation. *Saccharomyces cerevisiae* (K1-LALVIN 1116) was used to produce the mead that was prepared in order to obtain dry mead by mixing 200 g L^{-1} of honey in water. For better results inorganic salts were used [$(NH_4)_2SO_4$: 0.2 g L^{-1}, $(NH_4)_2HPO_4$: 0.02 g L^{-1}]. The results at the end of the process were a mead with: 12.5 ± 0.4°GL; pH 3.33; low amounts of high alcohols and methanol and great quantity of esters, that provide a nailing flavor to the beverage. Low production cost and simplicity of the fermentation process may represent good alternative for producers using honey also as raw material in the production of mead.

Key words: Mead, fermentation, beverage, honey, sugarcane, *Saccharomyces cerevisiae*.

INTRODUCTION

Alcoholic beverages are produced in almost all countries worldwide and represent an important percentage on world´s economy. Due to their diversity, a lot of them as beer, wine and distilled beverages have known technologies and usual processes, while others, with less significance, either by the costs of the raw material, acceptance or popularity, are less known and studied.

The "honey wine", known as mead, is considered as one of the oldest beverages, invented thousands of years ago, maybe older than wine and probably the precursor of beer (Steinkraus, 1983; Pereira, 2008), and it is obtained by the fermentation of a mixture of honey and potable water. Mead do not have an industrialized production, shows little representation and is characterized to be an alcoholic beverage with great quality, however, with little economic expression. It´s production is generally done in handmade character, using pure yeast cultures or commercial biological barm as *Saccharomyces cerevisiae* and *Saccharomyces uvarum*.

Honey, the main material for mead elaboration, is exclusively made by *Apis mellifera* and results, specially, from two reactions that nectar suffers, inside bee´s honey sacks, after been collected from flowers. One of these reactions, from enzymatic nature (chemical), occurs by the transformation of saccharose to glucose and fructose by the invertase enzyme action that hinders the crystallization of sugars even at high concentrations. Another two enzymatic reactions occur with less intensity and consist in the transformation of the starch from nectar to maltose by the amylase and glucose in gluconic acid and hydrogen peroxide by glucose oxidase action (Norman,

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1994; Lengler, 2005).

The main components of honey are carbon hydrates (carbohydrates) that match 95 to 99% of dried matter (about 70%) essentially, fructose (38.4%), glucose (30.3%), saccharose (1.3%) and disaccharides as maltose and isomaltose, trisaccharides, polysaccharides (12%) (Olaitan et al., 2007; Iurlina and Fritz, 2005; Anklan, 1998; Crane, 1983). Water is the second most important compound of honey, ranging from 0.5 to 0.6 water activity (Aw).

This parameter interferes on the viscosity of honey. The organic acids constitute about 0.57% of honey, influencing the pH (3.5 to 4.8) and acidity. Honey is generally acidic due to the presence of acids as citric, malic, succinic, formic, acetic, lactic, and piroglutamic acids. Besides these, the amino acids and gluconic acid are also responsible for the honey acidity (Olaitan et al., 2007; Steinkraus et al., 1971; Amaral and Alves, 1979).

Small amounts of minerals are detected in honey, varying between 0.04% (clear honey) and 0.2% (dark honey), and potassium is the mineral found in larger quantity (Anklan, 1998).

Proteins occur about 0.2% and arise mainly from bees and plants. However, a little portion corresponds to enzymes which include invertase, diastase, glucose oxidase, catalase, α-glucosidase, β-glucosidase e-amylase (Anklan, 1998; Iurlina and Fritz, 2005; Won et al., 2008).

Honey, a natural source of antioxidants, is an effective compound that helps to reduce heart diseases risk, cataracts, inflammation and other pathologies, that can avoid food damage by oxidative reactions as enzymatic browning of fruits and vegetables, the lipid oxidation in meat (Arraéz-Román et al., 2006) and inhibit the pathogenic microorganisms growing (Bertoncelj et al., 2007).

The flavor of honey comes from its acids. The color is also directly linked to the occurrence of these acids, therefore, the darker the honey, the bigger is mineral salts presence also the more intense the flavor.

Physicochemical characteristics of honey can vary according to their botanical origin, weather, humidity, altitude and the manipulation, as the explored area by bees (Wiese, 2000). However, there are more than 100 volatile compounds that determine the honey's flavor (Steinkraus et al., 1971; Lengler, 2005). These characteristics are determined "*terroir*". Wines that have *terroir* are products with all requirements to be recognized with geographic indications as qualifier to determine origin. Such products combine the interaction of "weather *x* soil *x* variety *x* know-how *x* etc.", giving origin, differentiation and identity to the products (Tonietto, 2007).

Most of the flowers' features which the nectar is extracted by bees are maintained on honey and, consequently, on the mead. The difference in the characteristics of the mead derives from the origin of the honey (from eucalyptus, orange flowers, wild plants, sugarcane agricultural areas, etc), by adding fruits or not and the initial sugar concentration of the must.

Diluted in water, honey becomes unsatisfactory for fermentation needing the addition of mineral salts to improve the performances of fermentation (Maugenet, 1964).

Substrates of disabilities of nitrogen and phosphorus, normally, extend the fermentation although most of the yeasts have the capacity to synthesize the vitamins needed to their metabolisms (Steinkraus and Morse, 1966). Extended fermentations can promote the yeasts' autolysis and propitiate bacterial contaminations that cause unpalatable flavors (Adams and Niesen, 1963; Morse and Steinkraus, 1975).

Growth of yeasts during the fermentation process is affected from several conditions as oxidative and osmotic stress, ethanol concentration, proteins, etc. (Zuzuarregui and DelOlmo, 2004).

The initial sugar concentration in the must determines the final alcoholic graduation and the type of mead (dry, medium dry or sweet). After a previous must's pasteurization and yeast's inoculation, the fermentation has to proceed at temperatures between 19.6 to 22.8°C for an adequate fermentation process. High values of temperature as 32.0 to 38.0°C provide unwanted fermentations speed and the growth of a large number of bacteria that affect the alcoholic fermentation.

The choice of the yeast to the fermentation honey process is an important stage, because different types show different resistance to acidity and alcohol concentration. The present research aimed to follow the technology of mead production by the process of physicochemical parameters measurement. Table 1 describes the mead physicochemical medium composition and Table 2 shows the mead's chemical features established by Brazilian Decree 410/74.

The literature about honey fermentation studies is narrow and, in the few countries that it occurs (the production of mead), there is a lack of scientific researches to base the production (Pereira, 2008). Due to the acceptation and qualities of honey, the development of derivate products can improve commercial gains of honey producers by its diversification and incorporation into feed habits.

MATERIALS AND METHODS

Wine yeast, *Saccharomyces cerevisiae* LALVIN K1-V1116 was used for the development of the process. To prepare the "must", sugarcane honey obtained from the local commerce was used and it was sized to obtain dry mead (low residual sugar content). For this, the honey was mixed with water to obtain a soluble solids concentration about 20% (20º Brix) and added $(NH_4)_2SO_4$ (0.2 gL^{-1}) and $(NH_4)_2HPO_4$ (0.02 g L^{-1}) to stimulate the growth and fermentation by yeasts.

During fermentation the must was conditioned into a sterilized glass container which joined 8% of the total volume of yeast culture. A device was adapted to the glass with the must for the siphoning to avoid external contaminations and to facilitate sampling for periodical analysis to follow the process. The fermentation process was maintained at 22.1 ± 0.04°C for a period of 17 days. The anaerobiosis condition was established by the siphon shutoff linked to the container to relieve the CO_2 pressure.

During the fermentation process, analyses of density (by the weighing of 100.0 mL of the must) were made, pH by digital

Table 1. Mead physicochemical medium composition.

Compound	Minimum	Maximum
Alcohol (%)	12.2	20.8
pH	2.9	3.75
Total acidity (g L^{-1})	2.20 (36.636 mEq L^{-1})	7.08 (117.902 mEq L^{-1})
Volatileacidity (g L^{-1})	0.14 (2.331 mEq L^{-1})	0.779 (12.973 mEq L^{-1})
Residual sugar (%)	2.5	27.8
Acetaldehyde (mg L^{-1})	18.2	125.5
Ashes (%)	0.046	0.520
Calcium (%)	0.41	5.11
Magnesium (%)	0.43	2.03
Potassium (%)	8.62	74.19
Sodium (%)	1.24	14.02

Steinkraus and Morse (1973).

Table 2. Legislation's standards to mead production (Decree 410/74).

Parameter	Maximum (g 100 ml^{-1})	Minimum (g 100 ml^{-1})
Ethylalcohol	14.00	4.00
Total acidity	9.75	3.75
Fixedacidity	—	2.25
Volatileacidity	1.20	—
Reduceddryextract	—	7.00
Total sulfate	1.00	—
Sulfurdioxide	0.35	—
Total Chlorides	0.50	—
Ashes	—	1.50

Barone (1994).

determination, yeast's feasibility stained with erithrosine (Neubauer chamber), alcoholic strength (ebuliometer 3300), and total sugars (ºBrix) by refractometer visualization, and turbidity (NTU) by turbidmeter MA TB1000 measurement. After fermentation, higher alcohols were quantified by Gas chromatography (GC).

RESULTS AND DISCUSSION

Sugarcane is an important renewable resource usually used in Brazil to produce sugar, ethanol and alcoholic beverage among others. A lot of by-products of it have been studied and availed also some unusual as its bagasse. However, after the burning process of the sugarcane, a common Brazilian stage before harvest, bees use exudate broth to make honey. Due to the heat of burning process, the concentration of furfural becomes high, generating a low price and low quality honey (Ribeiro et al., 2012).

Literature evidences present that different types of honey generally are rich in sugar, acids, minerals, aromatic substances, enzymes, vitamins and others (White, 1979), characterized as a potential stuff to alcoholic fermentation. The sugarcane honey used in this work is in agreement with the legislation range established and can therefore be used to elaborate an alcoholic beverage mixing it with water (Table 3).

In the intent to monitor the ongoing process, periodic analyses were performed on fermentation must involving some characteristics parameters of alcoholic batch fermentation. As a result the decay of density from 1.072 g mL^{-1} to 0.985 g $mL^{-1}$was measured, explained by the increase of alcohol concentration that is, in the final of the process, was 12.7 ºGL, a level within the legislation standards establishments (Decree 410/74).

To ensure a high quality production, raw materials with defined parameters are required to avoid losses during the process or unpleasant features in the products. The physicochemical analyses indicated by the Brazilian legislation for honey quality control are established as to maturity (reducing sugars, humidity, apparent sucrose), purity (water insoluble solids, minerals or ashes, pollen) and deterioration (free acidity, diastatic activity and hydroxymethylfurfural (HMF)) (MAPA, 2010).The quantity of HMF in sugarcane honey is usually high even with values lightly above the Brazilian legislation prohibiting its sale however the dilution with water to obtain the must

Table 3. Average of the main compounds of honey and the characterization of the studied sugarcane honey.

Composition	Average*	Sugarcane honey
Fructose (%)	38.19	37.8
Glucose (%)	31.28	31.0
Sucrose (%)	1.31	2.9
pH	3.91	4.4
Total acidity (mEq Kg^{-1})	29.33	28.0
Waste (ashes, %)	0.169	0.9
Amylase value	20.8	37.1

*Camargo (1972).

Table 4. Final physicochemical features from sugarcane honey mead.

Feature	Unit	Result		
		Laboratory	Gas Chromatography - 1	Gas Chromatography - 2
Ethylic alcohol	%	12.70	11.62	11.84
Final pH		3.33		
Total acidity	mEq L^{-1}	38.768		
Fixed acidity	mEq L^{-1}	19.983		
Volatile acidity	mEq L^{-1}	18.784		
Turbidity	NTU	16.2		
Dried reduced extract	g 100 ml^{-1}	2.364		
Final density	g L^{-1}	985.0		
Ethyl acetate	mg 100 ml^{-1} AA	221.733	15.562	39.572
Lactic acid	mML^{-1}	12.80		
Ashes	%	0.027		
Aldehyde	mg 100 ml^{-1} AA		47.433	6.296
Acetone	mg 100 ml^{-1} AA		0.000	0.000
Acrolein	mg 100 ml^{-1} AA		0.000	0.000
Methanol	mg 100 ml^{-1} AA		0.000	0.000
Higher alcohols	mg 100 ml^{-1}AA		129.320	117.895

and to produce mead decreases the HMF concentration to low amounts becoming it in agreement to the legislation.

Honey intrinsic properties can affect the microorganisms' survival by bacteriostatic or bactericide action and low pH and high sugar content can prevent the growth of many microorganisms (Iurlina and Fritz, 2005). Moreover, honey presents a large concentration of enzymatic antioxidants (glucose oxidase and catalase) and non-enzymatic (ascorbic acid, flavonoids, phenolic acids derived from carotenoids, organic acids and Maillard reaction's products) (Meda et al., 2005; Baltrušaitytė et al., 2007).

The mead obtained by the sugarcane honey fermentation showed results that permit and infer that an alcoholic beverage made with the parameters showed on this work could obtain great results of ethylic alcohol, including the absence of methanol and great quality of flavors derivatives from the presented esters, showed by the ethyl acetate quantity that is responsible for the flavors of this beverage (Andrade et al., 2008; Barcelos et al., 2007). The most is characterized by the low pH derived from honey's organics acids combination that can influence the fermentation. However, the fermentation tax of mead depends mainly on the variety of honey, strain of yeast, the culture medium composition and the extracellular pH (Navrátil et al., 2001). The presence of these factors is fundamental to improve the cellular activity and can become the process more effective, reducing costs (Sroka and Tuszyński, 2007). The initial must of the mead here studied presented pH 4.00, showing a drop to 3.20 ± 0.03 after the beginning of the microbial activities. The final pH was 3.33. Another relevant variant to a fermentative process is the temperature of exposure of the experiment, because high temperatures influence negatively the biochemistry of fermentation. The average of honey characteristics described by Camargo (1972) and the honey used in this work are presented in Table 3 showing that the composition of the sugarcane honey used was within the range and the final results of mead fermentation are described in Table 4. The acidity and dried exact of the final product need more studies and corrections with tartaric acid, a common used acid on traditional wines. These two features are important to promote the longevity of the mead, more studies using tartaric acid to correct the acidity, and other dilutions to increase the dried reduced extract can help to improve the quality of the beverage, making it marketable (Daudt and Fogaça, 2008).

Honey derivate products development can raise the value of these products and improve the profits on the honey products trade, expanding source of income for beekeepers. The use of honey from noblest sources as orange flowers honey, wild flowers and others can produce better quality mead (Nogueira-Couto, 1996). The fermented products, even with good quality, must be acepted as members of food habits to become competitive in trade. The consumer acceptation has primordial importance on the process development, improvement and maintenance of food products quality. To make it happen is necessary a statistic evaluation to represent the consumer population of the product. These results are essential to measure the commerce potential and approval of the product (Meilgaard et al., 1991; Macfie and Thomson, 1994).

Conclusion

The use of raw materials with low costs as sugarcane honey, that eventually show quantities of HMF higher than legislation requirements, can be used to obtain products with better quality and added value, improving the income of beekeepers and generating a great opportunity to enlarge the range of items for sale. The results obtained in this work show that the mead acquired from sugarcane honey has the potential to become a high quality beverage, with physicochemical characters included into the legal parameters of the Brazilian food legislation. Some new studies are needed to get it better in the fixed acidity, to ensure a beverage capable of aging that can improve more on the quality, flavor, price and earnings to the producer.

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