CHAPTER 4

Mead Production: Tradition Versus Modernity

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Contents

Abstract

Honey is a natural product with recognized physical and chemical properties, which contribute to its biological activity. However, honey is currently being sold at low prices, making it imperative to find alternatives to make apiculture a viable national enterprise. One of these alternatives could be mead production.

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Despite the excellent properties of honey, mead production faces several problems, namely, delays and "pouts" fermentations, lack of product uniformity, and production of yeast off-flavors. Many factors might be related with these problems, such as honey variety, temperature, medium composition (vitamin and nitrogen content), fermentative yeast, and pH. Due to all these factors, mead production has decreased over the years. To overcome this situation, more research is needed to optimize the production of this beverage.

This chapter presents a comprehensive review of previous research on mead production. It will focus on honey characterization and mead production. The first section covers honey composition and the way this affects honey properties, as well as important parameters that are indicators of honey quality. The second section discusses mead production, including fermentative microorganisms, fermentation conditions, and required postfermentation adjustments and maturation conditions. The final section focuses on the problems that must be surpassed and what the future holds for mead production.

I. INTRODUCTION

Mead is a traditional alcoholic beverage obtained by fermenting mead wort that contains 8–18% (v/v) ethanol. Its production has been known since ancient times. However, mead fermentation and maturation requires an extended period, often lasting several months to years. Mead still remains a relatively empirical and manual exercise, requiring large capacity vessels and the investment of considerable capital in terms of the raw material. In addition, the fermentation rate depends on several factors, such as honey variety, yeast strain, yeast nutrition, and pH. Due to the lack of scientific investigation, mead production has suffered in comparison with other alcoholic beverages and so more research is needed to optimize the production process.

The present study presents a comprehensive review of the scientific and technical research on the mead production. It is divided in two sections:

I. *Honey characterization*—as the raw material of mead its attributes greatly affect the production and characteristics of mead; important parameters that are indicators of honey quality are also discussed and
II. *Mead production*—under this heading are discussed fermentative microorganisms, fermentation conditions, postfermentation adjustments, and maturation conditions.

In conclusion, problems that must be surmounted relative to the future of mead production are discussed.

II. HONEY CHARACTERIZATION

Honey is a natural product used since the beginning of human society. It has had great popularity in Egyptian, Arab, Greek, and other civilizations. Even today, honey plays an important role in human nutrition, commonly used as a sweetener and an ingredient in desserts. It is one of the products most referred in old traditional medicines, due its therapeutic potential, notably in treating respiratory and gastrointestinal illnesses. Recently, it has proven effective in healing wounds and burns, in acute and chronic gastric lesions, and as an antimicrobial agent (Al-Mamary *et al*., 2002; Mulu *et al*., 2004). The healing capacity of honey appears to be related with its physical and chemical properties (Basualdo *et al*., 2007).

According to Portuguese law (Law-Decree nº 214/2003, September 18), honey is defined as a ''natural sweet substance, produced by *Apis mellifera* bees, from the nectar of plants, secretions of its living parts or from the excretion of sucking insects of plants.''

Honey bees form nectar into honey by a process of regurgitation and store it as a primary food source in wax honeycombs inside the beehive. According to the source, honey can be divided into different types:

Honeydew honey, that is prepared from secretions of living parts of plants or excretions of plant-sucking insects (Hemiptera) and
floral honey made by honeybees from the nectar of blossoms.

According to the method of production or presentation, honey may be classified as comb honey, honey with pieces of honeycomb, honey drained, centrifuged honey, pressed honey, or filtered honey.

A. Honey composition and its relation with physicochemical properties

Honey composition varies, depending on floral origin, the climate, environmental and seasonal conditions, as well as agricultural practices (Al-Mamary *et al*., 2002; Anklam, 1998; Arráez-Román *et al*., 2006; Azeredo *et al*., 2003; Baltrušaityté *et al*., 2007; Küçük *et al*., 2007). Honey contains about 200 different substances (Al-Mamary *et al*., 2002; Arráez-Román *et al*., 2006; Küçük *et al*., 2007), carbohydrates being the main constituents, followed by the minerals, proteins, vitamins, lipids, organic acids, amino acids (Finola *et al*., 2007), phenolic compounds (flavonoids and phenolic acids), and other phytochemicals (Bertoncelj *et al*., 2007).

1. Carbohydrates

Carbohydrates are the major constituents of honey, corresponding to ~95–99% of the dry matter (Olaitan *et al*., 2007). In average terms, they are composed mainly of fructose (38.2 g/100 g), glucose (31.3 g/100 g),

TABLE 4.1 Composition of nectar honey (g/100 g), mean values. The minimum and maximum values are also presented (*source*: Bogdanov, 2009).

	Nectar honey	Minimum–maximum
Moisture content	17.2	15–20
Fructose	38.2	30–45
Glucose	31.3	24–40
Sucrose	0.7	0.1–4.8
Other disaccharides	5.0	28
Melezitose	<0.1	–
Erlose	0.8	0.56
Others oligosaccharides	3.6	–
Total sugars	79.7	–
Minerals	0.2	0.1–0.5
Amino acids, proteins	0.3	0.2–0.4
Acids	0.5	0.2–0.8
pH	3.9	3.5–4.5

and sucrose (0.7 g/100 g; Table 4.1). The other carbohydrates in honey constitute about 12% by mass (Iurlina and Fritz, 2005). They include disaccharides, such as maltose, isomaltose, trisaccharides, and tetrasaccharides (Anklam, 1998).

Reducing sugars, mainly fructose and glucose, are the major constituents of honey (Küçük *et al.*, 2007), often being in a ratio of 1.2/1 (de Rodriguez *et al.*, 2004). Portuguese law established a minimum of 60 g reducing sugars per 100 g honey. The proportion of fructose, in relation to glucose, depends on the nectar source (Anklam, 1998). Multifloral honeys tend to have similar contents of fructose and glucose, whereas in monofloral forms, the content of fructose is significantly higher. Fructose/glucose ratio can influence the flavor of honey since fructose is sweeter than glucose (Finola *et al.*, 2007). Moreover, honeys with higher fructose/glucose ratios remain liquid for longer periods because glucose is less soluble in water than fructose. Thus, the glucose content in honey significantly influences crystallization (de Rodriguez *et al.*, 2004; Finola *et al.*, 2007).

2. Moisture content

Water is the second major component of honey (17.2 g/100 g; Table 4.1). It depends not only on environmental factors, such as the weather and humidity inside the hive, but also on the treatments applied during nectar and honey collection and storage (Olaitan *et al.*, 2007). It is an important quality parameter because it predicts the shelf life of the product and the capacity of the honey to remain stable and free from fermentation. Higher

water content increases the probability that the honey will commence fermentation during storage (Vargas, 2006). Because part of the water is bound to sugars, it is unavailable to microorganisms. The available water (free water content) that determines water activity (a_w) is one factor that influences the microbial stability of honey (*Note*: other factors that also influence the microbial stability of honey are pH, phenolic compounds, and the content on hydrogen peroxide). The a_w value of honey can vary between 0.55 and 0.75. Honeys with an $a_w \leq 0.60$ are microbiologically stable. Regardless, the simple and fast measurement of the water content has proven sufficient for assaying the fermentation risk of honey.

Viscosity is one of the physical properties of honey that depends on its water content (Olaitan *et al*., 2007). Moreover, the content of insoluble matter in water present in the honey is an important method for detecting impurities. The impurities content must not exceed the maximum allow limit of 0.1 g/100 g.

3. Organic acids

Organic acids are responsible for the acidity of honey and contribute considerably to its unique flavor (Anklam, 1998). Organic acid content of about 0.57% consists primarily of gluconic acid. It is a by-product of the enzymatic action of glucose oxidase on glucose (Olaitan *et al*., 2007). Other organic acids identified up to the present are the pyruvic acid, malic acid, citric acid, succinic acid, and fumaric acid.

The observed variation on the acidity content may be associated with harvest conditions (de Rodriguez *et al*., 2004) and the type of flower. Organic acids have been used to discriminate honeys according to their botanical and/or geographical origin. Acidity and humidity are lower in multifloral honeys (Küçük *et al*., 2007). Organic acids also influence the color and flavor of honeys. Finola *et al*. (2007) observed an inverse relationship between free acidity and the ash content of honey, indicating that the acidity decreases slightly with an increase in the ash content. Dark-colored honeys contain more minerals.

According to de Rodriguez *et al*. (2004), the presence of xerotolerant yeasts in honey may be responsible for increasing its acidity. Thus, values below the limit of 50 mEq of acid per 1000 g indicate the absence of undesired fermentation (Finola *et al*., 2007).

The pH of honey ranges between 3.4 and 6.1 with an average of 3.9 (Iurlina and Fritz, 2005). However, the pH is not directly related to acidity, due to the buffering action of acids and minerals found in honey (de Rodriguez *et al*., 2004). Its acidity is due to the presence of organic acids, particularly gluconic acid, pyruvic acid, malic acid, and citric acid. These are in equilibrium with lactones or esters, as well as to inorganic ions, such as, phosphate or chloride (Anklam, 1998).

4. Minerals

Minerals are present in honey on small amounts and vary between 0.04% in light honey to 0.2% in some dark honeys (Anklam, 1998), generally corresponding to their respective ash contents (Finola *et al.*, 2007). Potassium is the most abundant mineral, but others include calcium, copper, iron, manganese, and phosphorus (Olaitan *et al.*, 2007). The mineral content of honey can provide indications about environmental pollution as well as geographic origin (Anklam, 1998; Pohl, 2009).

5. Protein and nitrogen compounds

The protein content of honey is usually around 0.2% (Anklam, 1998; Iurlina and Fritz, 2005). A small portion of this fraction consists of enzymes, notably invertase, diastase, amylase, glucose oxidase, catalase (Anklam, 1998), α-glucosidase, and β-glucosidase (Won *et al.*, 2008). Some are derived from bees, whereas others come from the nectar. Enzyme activity varies among honey samples since the amount of bee saliva, the source of bee enzymes, found in honey varies with the conditions of honey production (Anklam, 1998).

The nitrogen content of honey is low and varies widely, with an average of about 0.04%. This consists mainly of alkaloids, chlorophyll derivatives, amino acids, and amines (Al-Mamary *et al.*, 2002). Of amino acids, proline is dominant, but arginine, tryptophan, and cysteine, whose presence is characteristic of some types of honey, have also been detected (Anklam, 1998). Analysis of the amino acid profile has greater potential in detecting the geographic and botanic origin of honey than the protein composition (Anklam, 1998).

6. Volatile compounds

The volatile constituents of honey are responsible for its characteristic flavor (Finola *et al.*, 2007). Many of these compounds come from the nectar of flowers. More than 300 compounds have been identified, including acids, alcohols, ketones, aldehydes, esters, and terpenes (Castro-Vásquez *et al.*, 2009). The presence of these compounds can provide information about the botanical source of the honey (Escriche *et al.*, 2009).

7. Phenolic compounds

Honey contains an extensive diversity of phenolic compounds as secondary constituents, notably flavonoids and phenolic acids (Arráez-Román *et al.*, 2006; Baltrušaityté *et al.*, 2007; Estevinho *et al.*, 2008). The flavonoid content reaches about 6000 mg/kg, consisting mainly of flavanones and flavones (Anklam, 1998). The main flavonoids are myricetin, tricetin, quercetin, hesperidin, luteolin, kaempferol, pinocembrin, chrysin, pinobanksin,

genkwanin, naringenin, and galangin (Anklam, 1998; Baltrušaityté *et al.*, 2007; Bertoncelj *et al.*, 2007; Estevinho *et al.*, 2008; Yao *et al.*, 2004).

These phenolic constituents have been used as identifying botanical and geographic markers. For honeys of particular botanical origin, such as heather honey, orange tree honey, sunflower honey, etc., it is possible to determine patterns of characteristic flavonoids. These data can also be used in determining geographic origin (Anklam, 1998). For example, the flavone hesperidin can be used as a marker for orange tree honey; luteolin for lavandula honey; quercetin for sunflower honey, and 8-methoxy-kaempferol is the major compound in rosemary honey (Baltrušaityté *et al.*, 2007).

The concentration of phenolic acids can vary from 0.01 to 10 ppm (Anklam, 1998). The dominant acids are gallic acid and *p*-coumaric acid, followed by the caffeic, ferulic, ellagic, chlorogenic, syringic, vanillic, cinnamic, and *p*-hydroxybenzoic acids (Baltrušaityté *et al.*, 2007; Bertoncelj *et al.*, 2007; Estevinho *et al.*, 2008). The high concentrations of benzoic, phenylacetic, mandelic, and β-fenil lactic acids (Anklam, 1998) can be used in the identification of heather honey.

Apart from flavor and aroma, color is a characteristic that permits floral origin identification. Color can range from white-water, extra-white, white, extra clear amber, light amber, amber, and dark amber (Bertoncelj *et al.*, 2007). This property is related to the mineral content, pollen, and phenolic compounds present in the honey (Baltrušaityté *et al.*, 2007; Bertoncelj *et al.*, 2007). Beyond varying with the botanical origin, color also depends on its age and storage conditions. During storage, browning/darkening of honey is due to Maillard reactions, caramelization of fructose, and polyphenolic reactions, depending on storage temperature and/or duration (Bertoncelj *et al.*, 2007). However, transparency or clarity of honey depends on the amount of suspended particles, such as pollen (Olaitan *et al.*, 2007).

8. Other compounds

Vitamin content is generally low in honey and varies with the floral origin (Valbuena, 1992). Vitamins C, B (thiamine), and B_2 complex, such as riboflavin, nicotinic acid, and pantothenic acid have been detected (Olaitan *et al.*, 2007). Ascorbic acid (a powerful antioxidant that confers physiological protection against degenerative diseases and processes caused by oxidative stress) is the only vitamin present in appreciable amounts in nectar and honey, with content reaching 0.5% or more, depending on floral origin (Kesić *et al.*, 2009).

B. Indicators of honey quality

The quality of honey (nectar and honeydew) is determined by its sensorial, physical, and chemical properties. The last two are evaluated using standard parameters established by the Codex Alimentarius (2001) and

TABLE 4.2 Legal criteria for the composition of honey. *Source*: Portuguese legislation—Law-Decree nº 214/2003, September 18.

Parameters	Specification	
	Nectar honey	Honeydew honey
Sugars (glucose + fructose)	Min 60 g/100 g	Min 45 g/100 g
Sucrose	Maximum 5 g/100 g	
Water	Maximum 20%	
Insoluble materials in water	0.1 g/100 g	
Electric conductivity	Maximum 0.8 mS/cm	Min 0.8 mS/cm
Free acids	Maximum 50 mEq/1000 g	
Diastase activity (Schade scale)	Minimum 8 or 3 if HMF $\leq$ 15 mg/kg	
Hydroxymethylfurfural (HMF)	Maximum 40 mg/kg	

by Portuguese Law (Law-Decree nº 214/2003, September 18; Table 4.2). This includes pH, water content, reducing sugars, sucrose, ash, materials insoluble in water, mineral content, electrical conductivity, acidity, and hydroxymethylfurfural (HMF), as well as the diastase activity. Nectar and honeydew honeys must be in accordance with these legal frameworks.

Regarding sucrose, the maximum content permitted is 5 g/100 g (Table 4.2), with exceptions for some types of honey. High sucrose content can mean that honey collection occurred prematurely and the sucrose was not completely dissociated into glucose and fructose by the action of invertase secreted by the hypopharyngeal glands of bees (Sodré *et al.*, 2007).

Diastase activity and HMF content are indicators of the freshness and are useful tools to detect heat-induced defects and improper storage (de Rodriguez *et al.*, 2004; Küçük *et al.*, 2007). The HMF is an organic compound derived from dehydration of sugars, and diastase activity measures the action of a group of amylases. Diastase activity should register a minimum of 8 on the Schade scale and HMF a maximum of 40 mg/kg (Table 4.2). HMF can be formed by the dehydration of a hexose under acidic conditions or by Maillard reactions. Temperature and storage period may increase the level of HMF. High-quality honey has a high diastase activity and a low concentration of HMF (Küçük *et al.*, 2007). Diastase activity is related to thermal exposure but is not adequate to detect honey origin (Anklam, 1998). Nevertheless, Küçük *et al.* (2007) observed that the floral type is an important factor for diastase activity, even if in an indirect way.

Electrical conductivity is directly related to the concentration of mineral salts, organic acids, and proteins and may be useful in identifying floral origin (Acquarone *et al.*, 2007). Honeydew honeys should register

more than 0.8 mS/cm; nectar honeys less. Exceptions are *Arbutus*, *Erica*, *Eucalyptus*, *Tilia*, *Calluna vulgaris*, *Leptospermum*, and *Melaleuca* honeys (Bogdanov *et al*., 1997, 2004).

In order to classify honeys in terms of monofloral or multifloral, pollen analysis is widely used. The identification and quantification of pollen grains in honey sediment (melisopallynological) is still the most important method in establishing botanical origin (Anklam, 1998). The development of new, alternative methods, such as isotope ratio, other than carbon (White, 2000), or use of an electronic tongue (Dias *et al*., 2008) are still in their initial stages. ''Monofloral'' honey is used to describe honey produced primarily from a single plant species. Generally, monofloral honey has at least 45% of particular pollen; however, this percentage varies with the floral source. For example, chestnut tree honey needs to have at least 90% of pollen of *Castanea sativa* to be considered monofloral (Anklam, 1998), whereas lavandula honey needs only to possess 15% *Lavandula* sp. pollen grains to be considered monofloral (Maia *et al*., 2003; Russo-Almeida and Paiva, 1996). Monofloral honeys are in higher consumer demand, which means that they also have a higher commercial value for the producer than honeys from mixed botanical sources. They can thus be considered as premium products.

C. Microbiota of honey

The microbial content in honey may be classified as primary or secondary. The primary sources include pollen, the bee digestive tract, dust, air, soil, and flowers (Olaitan *et al*., 2007; Snowdon and Cliver, 1996). Common examples are bacteria (*Bacillus*, *Micrococcus*), yeast (*Saccharomyces* spp.), and fungal spores (*Aspergillus*).

Osmotolerant yeasts can grow at low pH values and are not inhibited by high osmotic concentrations, as those present in honeys mainly due to sucrose and other osmotically active sugars. Thus, their presence is a potential problem because their growth is limited only by the amount of water available. Some conditions, such as high humidity, moderate temperature, occurrence of crystallization, a high yeast count, and high contents of some minerals and nitrogen promote the fermentation of honey (Snowdon and Cliver, 1996). The main yeast found in honey is *Saccharomyces* spp. However, species of *Debaromyces*, *Hansenula*, *Lipomyces*, *Pichia*, *Schizosaccharomyces*, *Torula*, and *Zygosaccharomyces* have been isolated (Snowdon and Cliver, 1996).

Yeasts metabolize sugars in honey, producing acids, gas, and other products that make honey unfit for consumption. Unlike yeasts and molds, bacteria can survive in honey but are unlikely to grow (Snowdon and Cliver, 1996). Growth of pathogenic bacteria has not been detected in honey. Thus, a high bacterial count is indicative of recent contamination

from a secondary source (Iurlina and Fritz, 2005). This could come from humans, packaging, and other equipment, be wind borne, come from soil, insects, animals, or water. Some bacterial isolated from honey have included *Escherichia coli* and species of *Bacillus*, *Clostridium*, *Enterobacter*, *Klebsiella,* and *Proteus* (Iurlina and Fritz, 2005). Bacterial endospores, particularly those of the genera *Bacillus* and *Clostridium*, are usually the most common (Finola *et al*., 2007). The most common species of *Bacillus* are *B. cereus*, *B. megaterium*, *B. pumilus*, and *B. coagulans* (Lopez and Alippi, 2009).

III. MEAD PRODUCTION

A. Introduction

Mead is a yeast-derived alcoholic beverage varying in alcohol content between 8% and 18% (Navrátil *et al*., 2001). It is produced from a dilute solution of honey, obtained by adding an adequate amount of water or fruit juice. Honey can be diluted in different proportions, for example, 1:0.5, 1:1, 1:2, and 1:3 (honey:water). Each produces different types of mead.

In traditional mead, small amounts of fruits, spices, and herbs are added, but their incorporation should not mask the honey flavor and aroma (McConnell and Schramm, 1995). According to method of production, mead can be classified in different ways. Pyments, cysers, melomels, and metheglin are meads that include the addition of grapes, apples, other fruits, and spices, respectively. Spiced pyment can be classed as a hippocras (McConnell and Schramm, 1995).

B. Fermentative microorganisms

The yeasts used in the production of mead are usually strains of *Saccharomyces cerevisiae*, similar to that used in wine, beer, and champagne productions. These yeasts metabolize sugars, such as glucose and fructose, resulting in the formation of ethanol and carbon dioxide. Nevertheless, the yeast *Hansenula anomala* had also given good results (Qureshi and Tamhane, 1987).

Strains of *S. cerevisiae* used include C11-3 (Navrátil *et al*., 2001), BRL-7 (Qureshi and Tamhane, 1986), and UCD522 (Mendes-Ferreira *et al*., 2010) from culture collections, as well as commercial strains, such as Premier cru (Pereira *et al*., 2009) and ENSIS-LE5 (Roldán *et al*., 2011).

Nevertheless, honey and wine musts have different compositions in regard with sugar content (nearly 3 times higher in the former) and nitrogen concentrations (about 100 times higher in the last). Thus, wine

yeast strains are not necessary optimally suitable for mead production. In order to circumvent this problem, yeasts isolated from honey had been studied in relation to their fermentative abilities (Pereira *et al*., 2009). In order to get more knowledge on this subject, Pereira (2008) and Pereira *et al*. (2009) made isolates of yeasts present in honey to study their stress resistance. This type of analysis can be used as a criterion for selecting yeasts of enologic value, since yeast fermentation performance and resistance to stress are related (Zuzuarregui and del Olmo, 2004). Five strains isolated from Portuguese honey, as well as reference and commercial strains were used by Pereira (2008) and Pereira *et al*. (2009) to study and compare their behaviors.

Seven *S. cerevisiae* strains were characterized relative to their resistance to sulfur dioxide (since it is a desirable feature in the fermentative yeast strains), ethanol (where tolerance is an indispensable property due to the high concentrations reached by the end of fermentation (Carrasco *et al*., 2001)), and osmotic stress (due to the high osmotic potential of mead at the commencement of fermentation). Pereira (2008) and Pereira *et al*. (2009) verified that significant differences did not exist between the strains. *S. cerevisiae* strains isolated from honey were similar to commercial and reference strains—all appearing to be suitable for mead production.

Other studies have investigated microorganisms inducing alcoholic fermentation of beverages in tropical and subtropical areas. For example, *S. cerevisiae* ET99, isolated from ogol, an indigenous Ethiopian honey wine (Teramoto *et al*., 2005), have yielded promising results. However, more studies are required to isolate strains ideal for mead production.

C. Fermentation conditions

Due to the high sugar contents involved in the mead production, fermentation tends to be slow and requires a yeast strain as well as pH, temperature, and growth conditions that are optimal.

During mead fermentation, several problems are generally encountered. For example, the anticipated alcohol content may not be achieved within the time desired. There may also be a lack of uniformity in the final product, due to differences in water content of the honey used. In some situations, such as worts with high sugar contents, successive addition of honey is needed to avoid premature termination of fermentation. This likelihood of stuck fermentation is increased as most mead is made empirically, without adjustments. This can lead to subsequent yeast refermentation and secondary fermentations by lactic and acetic acid bacteria. These can undesirably increase acidity and the production of volatile esters (Casellas, 2005). The presence of these compounds alters

the organoleptic quality of mead, in particular its aroma and flavor, making its consumption unpleasant.

The production of mead involves several steps. The first step involves must preparation. Depending on the style desired, the honey is diluted with water or juice, and a nutrient mixture added. This may include $(NH_4)_2SO_4$, $CaSO_4$, $(NH_4)_3PO_4$, $NH_4H_2PO_4$, $(NH_4)_2HPO_4$ (DAP), K_3PO_4, $MgCl_2$, $MgSO_4 \cdot 7H_2O$, $NaHSO_4$, citric acid, sodium citrate, tartaric acid, potassium tartrate, potassium sodium tartrate 4-hydrate, malic acid, vitamins (biotin, pyridoxine, thiamin), myo-inositol, and peptone or commercially available yeast energizers (McConnell and Schramm, 1995; Mendes-Ferreira *et al.*, 2010; Navrátil *et al.*, 2001; Pereira *et al.*, 2009). Recently, Roldán *et al.* (2011) analyzed the influence of pollen addition to mead elaboration, showing that it improved fermentation rates, alcohol yield, and final sensory attributes. Pollen addition also reduced mead's total acidity, possibly by supplementing its potassium and calcium content. These could have lead to salinization, reducing acidity (Roldán *et al.*, 2011).

The must is subsequently sterilized, boiling being the most commonly used method (McConnell and Schramm, 1995; Navrátil *et al.*, 2001; Ukpabi, 2006). Heat treatments also have the potential to alter the antioxidant capacity by changing their phenolic profiles (Wintersteen *et al.*, 2005). However, other techniques are described in the literature. These include the use of metabisulfite (sodium or potassium salts or in commercial form as Campden tablets)—releases sulfur dioxide that either kills or inactivates most microbes (McConnell and Schramm, 1995; Roldán *et al.*, 2011), sulfur dioxide gas (Pereira *et al.*, 2009; Ukpabi, 2006), pasteurization (McConnell and Schramm, 1995; Mendes-Ferreira *et al.*, 2010), and ultrafiltration, with a 50-kDa molecular weight cutoff (McConnell and Schramm, 1995). Some of these methods also promote the removal of proteins by denaturation and coagulation, resulting in more rapid clarification during maturation.

After sterilization, yeast is added to initiate fermentation. McConnell and Schramm (1995) recommend inoculation with no less than 10% by volume. Moreover, as the pH of honey is naturally low and because it is poorly buffered, the pH of must may drop during fermentation to a point limiting yeast efficiency. pH reduction can result from the synthesis of acetic and succinic acids by the yeast cells (Sroka and Tuszynski, 2007). While a rapid decline in pH inhibits undesirable microbial activity (Sroka and Tuszynski, 2007), it also reduces the dissociation of fatty acids in the wort, potentially slowing yeast metabolic action. For this, addition of a buffer is important to maintain the pH within a range of 3.7–4.0 throughout fermentation (McConnell and Schramm, 1995). Calcium carbonate, potassium carbonate, potassium bicarbonate, and tartaric acid are potential candidates. However, as some of these salts can add a bitter–salty

flavor, if overused, no more than what is just needed is recommended (McConnell and Schramm, 1995).

Fermentation has been variously recommended to occur at: ambient temperature (10–21 °C; McConnell and Schramm, 1995); 25, 30, 35, and 40 °C (Navrátil *et al*., 2001); 25–26 °C (Ukpabi, 2006); 27 °C (Pereira *et al*., 2009); and 22 °C (Mendes-Ferreira *et al*., 2010).

Because honey is the only source of organic flavorants and sugar, its composition clearly sets limits on the final quality of mead. For example, Pereira (2008) demonstrated that between a dark (heather) and light (rosemary) honey, the heather honey produced the more appreciated mead. This occurred, regardless of yeast strain or supplement. To date, yeast strains isolated from honey have not shown any advantage over easily obtained commercial strains.

Gomes (2010) has studied the optimization of fermentation conditions, in terms of temperature and salt concentration, involving heather honey and a commercial supplement, Enovit® salt. Two commercial *S. cerevisiae* strains, Fermol® Reims Champagne (Pascal Biotech®) and ICV® D47, were used. Three fermentation temperatures and three salt additions (60, 90, and 120 g/hL) were used (20, 25, and 30 °C). Although both yeast strains seem to be appropriate for mead production, models developed for Fermol® Reims Champagne (Pascal Biotech®) predicted better its fermentation behavior than those developed for ICV D47®. Moreover, it was shown that the best results in terms of ethanol, acetic acid, and glycerol productions, as well as sugar consumption were obtained for nutrient concentrations between 85 and 100 g/hL and temperatures between 24 and 29 °C (Gomes, 2010).

Another important aspect in mead fermentation relates to changes in organic acid content. Sroka and Tuszynski (2007) observed high levels of medium-chain fatty acids, such as octanoic, decanoic, and dodecanoic acids. These are believed to be able to arrest fermentation. Increasing concentrations of medium-chain fatty acids in cell membrane reduces their hydrophobic characteristics, impacting enzyme function and reducing the cell's ethanol tolerance (Sroka and Tuszynski, 2007). Mendes-Ferreira *et al*. (2010) further reported that the conversion of medium-chain fatty acids into their corresponding ethyl esters was strongly affected by the presence of organic acids (potassium tartrate and malic acid).

Some studies on continuous mead production have been performed, involving *S. cerevisiae* immobilized in calcium alginate gels (Qureshi and Tamhane, 1985) or calcium pectate (Navrátil *et al*., 2001). In the first situation, alcohol production was stable within a pH range of 2.5–6.0, with an optimum at pH 5.5, and a temperature range of 18–30 °C, with a sharp increase at 35 °C (Qureshi and Tamhane, 1985). Mead production proceeded continuously for more than 3 months and experienced less

contamination and secondary fermentation than associated with traditional procedures. In an experiment performed by Navrátil *et al*. (2001), using cells immobilized in calcium pectate in a two-column system, fermentation began to slow after only 60 h (30 °C). Ethanol production fell to about 50% of its maximal rate within 120 h. Much work still needs to be done before it could become commercially viable.

D. Postfermentation adjustments and maturation conditions

At the completion of fermentation, mead undergoes a period of maturation that includes clarification and filtration. These are obligatory despite their increasing production costs.

For clarification, bentonite is often used (McConnell and Schramm, 1995; Pereira *et al*., 2009; Roldán *et al*., 2011), as well as gelatin (Roldán *et al*., 2011).

Aging is important in mead production, particularly in relation to the development of aroma compounds, particularly ethyl acetate. Aging usually lasts between 1 and 10 years. Nevertheless, caution is required as ethyl acetate is sometimes considered an off-flavor, with a solvent-like odor (Mendes-Ferreira *et al*., 2010). Roldán *et al*. (2011) has observed that ethyl acetate content is related to the acetic acid content—meads with higher volatile acidity had higher ethyl acetate values.

In order to reduce the duration of aging, Qureshi and Tamhane (1986) used a two-packed bed column reactors in series, separately containing immobilized *H. anomala* (yeast strain able to produce ethyl acetate) and immobilized *S. cerevisiae*. They also found that the rate of ethanol production was higher in a double column reactors in series than in a single column reactor with both yeast species coimmobilized.

In an experiment, Gomes (2010) produced distinctive meads by varying when fermentation was stopped and whether brandy was added. Of these, the most appreciated by a Portuguese consumer panel was the sweetest, independent of the alcohol content. The sweetest mead was obtained after prematurely stopping fermentation early by the addition of brandy (77% alcohol). The product had an alcohol content of 18% (v/v) and a sweetness equivalent to 8° Baume. The alcohol content was then adjusted to 18%, 20%, and 22% (v/v).

These results indicate that high sugar content is an important requisite to the Portuguese mead consumer. These results are similar to those reported by Vidrih and Hribar (2007), where the test panel preferred mead with higher reducing sugar contents. In another preference study, Roldán *et al*. (2011) found that mead produced with the addition of pollen was rated higher than those without pollen addition. This is likely due to the sourness and lack of desirable flavors in the latter.

To extend shelf life, it may be necessary to perform a secondary racking or filtration, and essential to use airtight (anaerobic) bottling (Ukpabi, 2006). A second racking within 4–6 months, followed by bottling has been recommended by Morse and Hooper (1985), cited by Ukpabi (2006), in USA and Europe.

In the production of tropical meads, Ukpabi (2006) recommends the use of sturdy bottles to avoid bottle breakage, due to possible high internal pressure (from fermentation gases) if refermentation occurs, as well as the use of cork or equivalent for stoppering the bottles.

In evaluating mead quality, Kahoun *et al*. (2008) suggest determining HMF and phenolic contents. High concentrations of HMF and absence of most common phenolics are an indicator of excessive heating during mead production. Moreover, detection of abnormally high concentrations of some compounds, or even their presence, may be indicative of adulteration. Possible examples are the detection of high concentrations of vanillin (Kahoun *et al*., 2008). Although it may be found in honey or propolis (resinous mixture collected by honey bees from tree buds, sap flows, etc.), it only occurs naturally in trace amounts. The presence of ethylvanillin, not naturally found in honey, would also be an indicator of adulteration.

IV. FINAL CONSIDERATIONS

Mead seems to be a good option for increasing the income of honey producers, allowing the development of a beverage little known in some countries but possessing great commercial potential. Introducing new mead products to the markets (e.g., sweet, dry, with fruit juice) may help producers increase the current low economical value of mead. This is also in line with the present situation of consumers demanding more options and a willingness to try new products.

However, for mead production to become profitable, it is necessary to decrease production time. A major concern in mead fermentation is the notoriously long period required to reach completion. Although fermentation rate depends on the honey variety and its characteristics, through proper selection of yeast strain and fermentation conditions, such as, mixing during fermentation, yeast nutrition, and pH's control, it may be possible to dramatically increase fermentation rate.

In relation to yeast strain selection, few studies have been conducted on this subject. It will be necessary to find/isolate yeast strains more resistant to, and with better fermentation performance under the harsh conditions of mead production, such as high osmotic values and low nitrogen content. Breeding may be an option.

As honey composition varies, mead producers must take this into account when adding supplements to create optimal fermentation

conditions. Many of the distinctive flavorants in mead owe their origin to the botanical and geographic origin of the honey. During fermentation, some of these compounds may act as carbon and nitrogen sources for yeast metabolism, or be chemically transformed. Thus, considerable research is still needed to characterize both honey and mead constituents that are responsible for its organoleptic properties. This is a precondition for regulating mead's sensory attributes. Because few organoleptic studies involving mead have been performed, knowledge of consumer tastes is still ill-defined, as well as what how postfermentation adjustments and maturation conditions might affect consumer preference.

In conclusion, mead research remains an area with great potential with many questions still needing answers.

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