



# The influence of hydrocolloids on mead wort fermentation



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## ABSTRACT

The purpose of this study was to determine the effect of hydrocolloids on the kinetics of ethanol fermentation in high-sugar mead worts. High osmotic pressure, inhibitors and other factors frequently cause sluggish or stuck mead fermentation. In the experiments, natural hydrocolloids – gum arabic, ghatti, karaya, xanthan gum or carob bean gum – were added in an amount of 0.5 g/L to mead worts. During fermentation, the mass of evolved CO<sub>2</sub> was controlled. Anionic hydrocolloids significantly affected the kinetics of mead wort fermentation, significantly accelerating fermentation and causing higher production of ethanol and reduced volatile acidity and acetic acid synthesis by *Saccharomyces cerevisiae*. Supplementation with gum karaya allowed an increase of ethanol concentration by about 30% compared to control samples. Application of anionic hydrocolloids resulted in significant reduction of amounts of the cations Ca<sup>2+</sup> and Mg<sup>2+</sup> in the solutions, and increased the clarity of young mead.

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## 1. Introduction

Mead is an alcoholic beverage obtained by fermentation of honey (mead) wort, containing between 8% and 18% alcohol by volume. Mead wort is produced by diluting bee honey with the appropriate amount of water. Depending on the proportion of honey and water, different types of mead are obtained, the finest at 1:0.5 ( $V_{\text{honey}}:V_{\text{water}}$ ), or at 1:1, 1:2 and 1:3. Worts that contain a higher concentration of sugar (the 1:0.5 and 1:1 types) are prepared by successive addition of the appropriate portions of honey to the fermenting medium in order to prevent inhibition of fermentation, due to an excessive osmotic pressure (Gogol & Tuszyński, 1996; Ramalhosa, Gomes, Pereira, Dias, & Estevinho,

2011). The relatively long time needed for wort fermentation and mead maturation, ranging from several months (meads made from wort 1:3) to several years (meads made from worts 1:0.5 and 1:1), requires the use of large capacity vessels and also increases the costs of production (Aleksandrowicz, 1988; Iglesias et al., 2014).

Mead worts contain relatively high amounts of amphiphilic medium-chain fatty acids (Sroka & Tuszyński, 2007), and other inhibitors such as hydroxymethylfurfural (Kahoun, Rezková, Veškrnová, Královský, & Holčápek, 2008) and phenolic compounds (Wintersteen, Andrae, & Engeseth, 2005; Švecová, Bordovská, Kalvachová, & Hájek, 2015), which might reduce the yeast growth, the fermentation kinetics and ethanol production. Frequently, during fermentation of high gravity worts, the appropriate alcohol content is not reached within the appropriate time (Iglesias et al., 2014). These problems can be solved by yeast strains isolated from honey (Pereira, Dias, Andrade, Ramalhosa, & Estevinho, 2009), cells adapted to high sugar concentrations (Sawicka, 1970), high cell

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density fermentation (Pereira, Mendes-Ferreira, Oliveira, Estevinho, & Mendes-Faia, 2013) or yeast immobilization in hydrogel beads (Mendes-Ferreira et al., 2010; Pereira, Mendes-Ferreira, Estevinho, & Mendes-Faia, 2014a; Pereira, Mendes-Ferreira, Oliveira, Estevinho, & Mendes-Faia, 2014b). Increasing the attenuation can also be achieved through supplementation of honey wort with assimilable nitrogen compounds, salts and vitamins (Pereira, Mendes-Ferreira, Estevinho, & Mendes-Faia, 2015; Pereira, Mendes-Ferreira, Oliveira, Estevinho, & Mendes-Faia, 2014c).

Natural gums can be classified as uncharged or ionic polymers. Locust bean gum is a neutral polymer. Gum arabic, ghatti, karaya and xanthan are polyelectrolytes containing, among other things, glucuronic acid units. All of the ionic polymers containing functional groups, mainly carboxylic, are capable of dissociation (Pegg, 2012). In the above-mentioned compounds, only gum arabic is used in the wine industry to protect wine against light iron and copper haze, and to prevent the precipitation of substances such as colloidal pigment (O.I.V., 2016). Gum arabic is a branched-chain, complex polysaccharide, either neutral or slightly acidic, a mixed calcium, magnesium and potassium salt of a polysaccharide acid. It is composed of six carbohydrate moieties (galactopyranose, arabinopyranose, arabinofuranose, rhamnopyranose, glucopyranosyl uronic acid and 4-O-methyl glucopyranosyl uronic acid) and also contains a small proportion of protein (0.22–0.39% nitrogen) as an integral part of the structure (Idris, Williams, & Phillips, 1998; Islam, Phillips, Sljivo, Snowden, & Williams, 1997).

Polysaccharides are one of the main groups of macromolecules in wine (Vidal et al., 2004). Groat and Ough (1978) found that various insoluble solids added to clarified grape juice intensified fermentation and more sugars were used than in control samples. High turbidity can increase yeast growth and viability (Boivin, Feuillat, Alexandre, & Charpentier, 1998). So far, no studies have been conducted during fermentation of mead worts with addition of hydrocolloids.

The aim of this study was to determine the effect of addition of selected natural hydrocolloids containing an ionic group (gum arabic, gum ghatti, karaya gum and xanthan gum) and a non-ionic hydrocolloid (locust bean gum) on the kinetics of mead wort fermentation, chemical composition and profile of young meads.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Chemicals

Acetic acid, acetone, citric acid, diammonium hydrogen phosphate(V), ethanol, nitric acid(V) and sodium chloride were purchased from POCh (Gliwice, Poland). Benzyl bromide, benzyl alcohol, glutaraldehyde, osmium tetroxide and all hydrocolloids – carob bean gum (locust bean gum from *Ceratonia siliqua* seeds), gum arabic (from acacia tree), ghatti (from *Anogeissus latifolia*), karaya (from *Sterculia* tree) and xanthan (from *Xanthomonas campestris*) – were from Sigma-Aldrich Chemie (Steinheim, Germany).

#### 2.1.2. Biological material

Free cells of *Saccharomyces cerevisiae* yeast, Johannisberg–Riesling (JR) breed, were obtained from the Center of Industrial Microorganism Collection of the Institute of Fermentation Technology and Microbiology, Technical University of Łódź (collection no. ŁOCK 105).

#### 2.1.3. Mead wort preparation and fermentation

Buckwheat honey (Bartnik Sądecki, Poland) was mixed with potable water (66 mg/L  $\text{Ca}^{2+}$ , 9 mg/L  $\text{Mg}^{2+}$ ) in the proportions 1:2

(v/v), heated and gently boiled for 10 min, then diammonium hydrogen phosphate(V) (0.4 g/L) and citric acid (0.25 g/L) were added; the extract was checked after mixing. Then 0.4 L of hot wort was poured into bottles (0.75 L), and 0.2 g of carob bean gum, gum arabic, ghatti, karaya or xanthan was added. After the wort was cooled down to approximately 30 °C, a precisely defined amount of starter yeast was added (0.5 g of DW per liter), then the bottles were stopped with sterile fermentation trap tubes. The yeast suspension was prepared in a three-stage culture (agar medium, 9% brewer's wort, still culture (10 mL) and shaken culture (100 mL)). The control samples were wort without the addition of hydrocolloids. Fermentation was carried out at 20 °C (refrigerated incubator Q-Cell 240, Equimed, Poland) for about 4 weeks. The weight losses associated with the liberation of carbon dioxide were measured daily. All fermentation experiments were conducted in three repetitions.

After fermentation, the young meads were separated from the sediments by carefully pouring into another vessel and kept for further clarification (sedimentation under gravity) at 4 °C. Clarified meads were subjected to analysis.

### 2.2. Experimental methods

#### 2.2.1. Analytical methods

The ethanol content, pH, total and volatile acidity, and total and reducing sugars were determined using official methods (O.I.V., 2015).

#### 2.2.2. Determination of clarity of young meads

Clarity of the test samples was determined nephelometrically using a Beckman DU650 spectrophotometer. The sample was placed in 1 cm path length cuvettes, and the absorbance was measured at 620 nm against water as a blank.

#### 2.2.3. Acetic acid determination

The analysis was performed by gas chromatography after esterification of the analyzed acid with benzyl bromide and extraction using headspace solid-phase microextraction (HS-SPME-GC) (Sroka & Tuszyński, 2007). The chromatographic analysis was performed using an HP 5890, series II gas chromatograph, with a flame ionization detector (FID) and an HP5 capillary column (30 m × 0.53 mm × 2.65 μm). The temperature of the injector and detector was set to 250 °C. The oven temperature was 40 °C for the first 5 min, with a ramp of 5 °C/min until the temperature reached 160 °C. The carrier gas was helium at 20 mL/min. The recovery of acetic acids was 95.0%.

#### 2.2.4. Determination of metal ions

The content of calcium, magnesium and zinc in the fermented worts was determined by flame atomic absorption spectrometry (Varian AA240FS), with a sample introduction pump system (SIPS), gas flow: 3.5 L/min (acetylene), 14 L/min (air) (Poreda, Tuszyński, Zdaniewicz, Sroka, & Jakubowski, 2013). Before determination, the sample (2 mL volume) was subjected to a process of mineralization, with 5 mL of 65% nitric acid(V) in closed PTFE vessels in a Mars Xpress microwave oven (1200 W, 170 °C, 15 min) The elements were determined under a fast on-line dilution sequence in a single sample aspiration. The standard solution was prepared using Merck standards, after appropriate dilution. The stock solutions contain calcium, magnesium and zinc in quantities of 40, 100 and 8 mg/L, respectively. Mineralized samples were transferred to Eppendorf tubes, diluted with deionized water, and then their absorbance was determined at wavelengths of 422.7 nm (calcium), 202.6 nm (magnesium) and 213.9 nm (zinc).

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