Effects of Mung Bean, Soy Bean and Red Kidney Bean on Mead Production

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ABSTRACT

Nitrogen sources are important in wine production as they involve in yeast metabolism especially the biosynthesis of building blocks. Mead or honey wine was produced using *Saccharomyces cerevisiae* as a starter culture. Various concentrations of three types of beans including mung bean, soy bean and red kidney bean were investigated their effects on mead fermentation. After 30 days of fermentation at 25^{o}C, mead samples were harvested and analyzed. It was found that all meads had ethanol content of 10.8 – 11% (v/v). Interestingly, the antioxidant activity of mung bean mead was significantly higher than that of soy bean and red kidney bean ($p < 0.05$).

Keywords: Antioxidant, Bean, Mead

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Introduction

Wine is normally referred to an alcoholic beverage made from fermented grapes (Johnson, 1989). Yeasts metabolize sugars in grapes and convert them to ethanol and carbon dioxide in anaerobic condition. Unique characteristic of wine is dependent on varieties of yeast strains and grapes. Furthermore, honey, fruits or grains can be used as raw materials of wine making instead of grapes (Mills *et al*., 2008). In wine fermentation process, nitrogen sources are added for yeast growth promotion, hence, unique flavor and aroma of wine will be obtained. Yeast extract, peptone or ammonium phosphate have been normally used in wine fermentation process (Sewsuwan, 2013; Sukmuang, 2015).

Mead (honey wine) is a traditional alcoholic beverage. Generally, mead production involves the addition of nutrients to diluted honey, pasteurization, yeast inoculation, fermentation and removal of impurities (Iglesias *et al*., 2014). Due to the high antioxidant activity in mead, drinking one or two glasses of mead per day has been believed to reduce cholesterol levels in blood, and may reduce risks of cardiovascular disease, atherosclerosis, hypertension, certain type of cancer, type 2 diabetes, neurological disorders and some metabolic syndromes (Guilford and Pezzuto, 2011; Percival *et al*., 2014).

Beans are among the most versatile and commonly edible foods throughout the world. These economical foods have a potential to improve the diet quality (Robinson, 2013). Numerous studies have indicated that incorporation of beans into the diets could aid in the prevention and management of diseases and/or symptoms such as diabetes mellitus, obesity and cancer (Bennink and Rondini, 2008). Mung bean (*Vigna radiata* (L.) Wilczek.), soy bean (*Glycine max* (L.) Merr.) and red kidney bean (*Phasecolus valgaris* L.) contain high levels of vitamin A, flavonoids and phenolic compounds such as lutein, zeaxanthin and carotene. These compounds act as protective scavengers against oxygen-derived free radicals and reactive oxygen species (ROS) that play a role in aging and various disease processes (Rudrappa, 2016).

Objective of the study

To evaluate the antioxidant activity of bean containing mead.

Methodology

1. The ability to produce ethanol from honey by *Saccharomyces cerevisiae* using bean as a nitrogen source

1.1 Preparation of a starter culture, *Saccharomyces cerevisiae*

Honey was diluted with sterile water at a ratio of 1:4. Subsequently, peptone and yeast extract, 0.2% (w/v), were added before autoclaving. When cooled down to room temperature, the prepared honey was then inoculated with *Saccharomyces cerevisiae* and incubated at 25^{o}C for 9 hours to obtain the log phase of yeast growth. Turbidity of yeast culture was measured using a spectrophotometer at a wavelength of 660 nm. The optical density of yeast suspension was adjusted to 1.0 when used.

1.2 Ethanol fermentation process

Honey was diluted with sterile water at ratio of 1:4. The diluted honey, 850 ml, was then filled in each Erlenmeyer flask. Various concentrations; 0, 0.2, 0.4, 0.8, 1.6 and 3.2% (w/v); of mung bean, soy bean or red kidney bean were added and the media were then adjusted the pH to 6.5 before autoclaving at 121°C, 15 psi for 15 minutes. When cooled down, each flask was inoculated with 1% (v/v) inoculum size of *Saccharomyces cerevisiae* (provided by the Microbiology section, Department of Biology, Faculty of Science, Chiang Mai University). All fermentation flasks were incubated at 25°C for 30 days. At the end of fermentation, samples were collected to determine quantities of brix (°) by a refractometer (Trans Instrument®, Singapore), pH by a pH meter, ethanol content by an ebulliometer (Dujardin Salleron®, Paris) and reducing sugar content by a DNS method.

2. Determination of reducing sugar by a DNS method

A mead sample, 1 ml, was added into a test tube prior to addition of DNS solution, 3 ml, and well mix with a vortex mixer. The mixtures were then boiled for 5 minutes before immediately cooling down using a tub of cold water. Then, 6 ml of distilled water were added into each tube, mixed well and measured the absorbance at a wavelength of 540 nm against the reagent blank (Miller, 1959).

3. Determination of the antioxidant activity in mead

2, 2-Diphenyl-1-piccrylhydrazyl radical (DPPH) solution, 0.1 mM in methanol, was prepared. Gallic acid, 0.01 – 0.1 mg/ml, was used as a standard. Each test tube was added with a sample or gallic acid and DPPH solution volume of 0.5 and 1.5 ml, respectively. Well mixed tubes were incubated in the dark for 20 minutes. Afterwards, the absorbance of the sample (A_{sample}) was measured using a spectrophotometer at 517 nm against ethanol blank. Methanol was used as a negative control ($A_{control}$). The half maximal inhibitory concentration (IC_{50}) and the antioxidant activity were calculated (Shekhar and Anju, 2014) according to the equations below:

$$\text{DPPH inhibition (\%)} = \frac{A_{517control} - A_{517sample}}{A_{517control}} \times 100$$

$$\text{Antioxidant activity (\%)} = \frac{IC_{50}\ \text{control}}{IC_{50}\ \text{sample}}$$

Results

1. Mead production by *Saccharomyces cerevisiae* using beans as nitrogen source

After 30 days of fermentation, all meads obtained from fermentation of mung bean, soy bean or red kidney bean containing honey had been determined the amounts of ethanol, brix and reducing sugar, and the changing of pH as well as the antioxidant activity. It was found that the ethanol content of all meads were ranging between 10.8 and 11.0% (v/v) with types and concentrations of beans were insignificantly affected ($p > 0.05$) (Figure 1). Nevertheless, pHs of fermentation broths were significantly decreased ($p < 0.05$) after 30 days of fermentation. The fermentation broth of soy bean containing honey exhibited the highest pH reduction by 2 pH units (Figure 2).

Sugars in honey fermentation broths were utilized by *S. cerevisiae* resulting in the lower amount of sugar content expressed as brix and reducing sugar values. *S. cerevisiae* converted sugars into ethanol and other metabolites with significantly influenced by types and concentrations of beans ($p < 0.05$) (Figure 3-4). After 30 days of fermentation, the highest conversion rate, 45.4%, was found in mung bean containing mead.

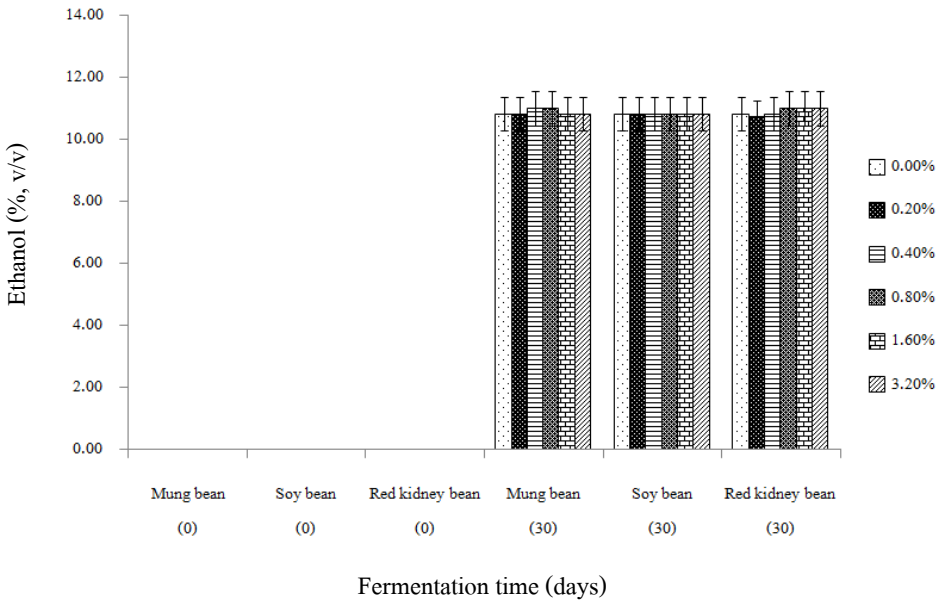

Figure 1. Effect of types and concentrations of beans on ethanol production. The fermentations were conducted at 25^{o}C for one month. The ethanol content was measured by an ebulliometer.

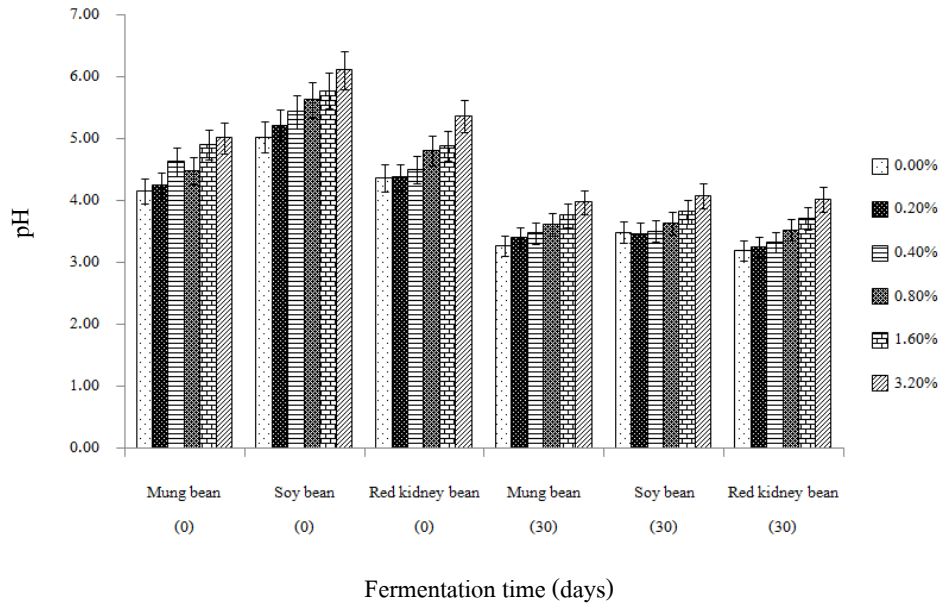

Figure 2. Changes of pH in mead production using various concentrations of mung bean, soy bean and red kidney bean as nitrogen sources. The fermentations were conducted at 25°C for one month.

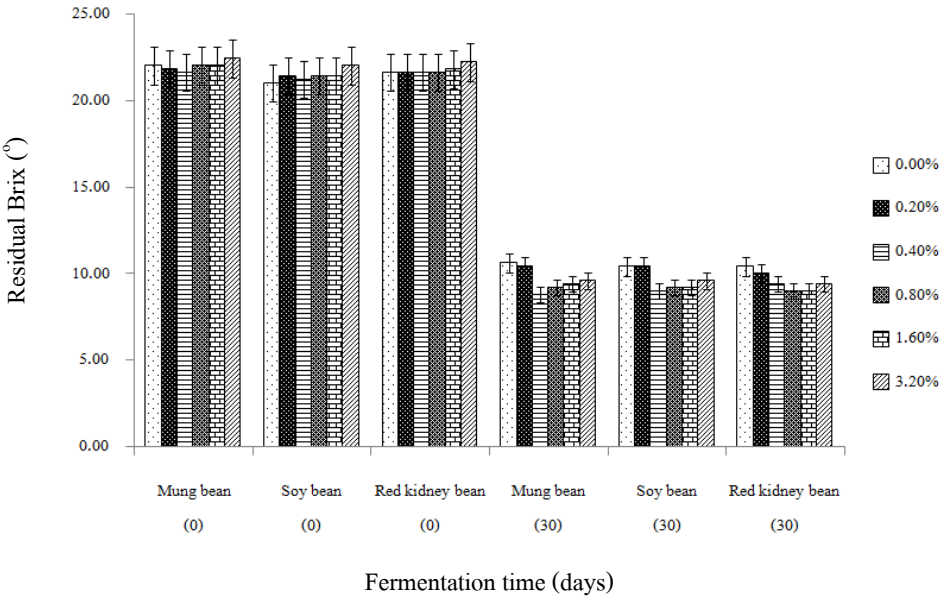

Figure 3. Changes of brix (°) in mead production using various concentrations of mung bean, soy bean and red kidney bean as nitrogen sources. The fermentations were conducted at 25°C for one month.

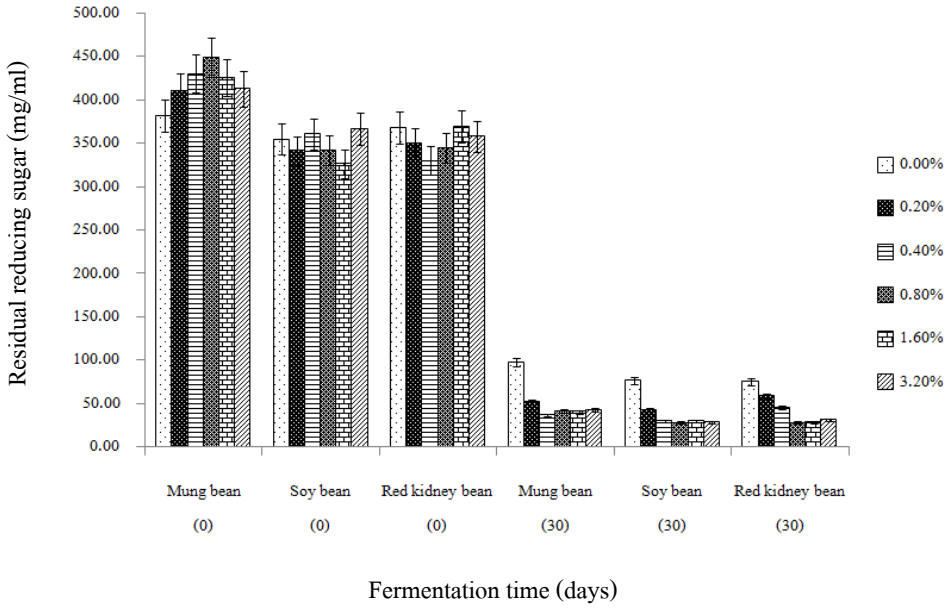

Figure 4. Changes of reducing sugars in mead production using various concentrations of mung bean, soy bean and red kidney bean as nitrogen sources. The fermentations were conducted at 25°C for one month.

2. Antioxidant activity investigation in mead

All meads obtained exhibited the antioxidant activity which was significantly ($p < 0.05$) highest in mung bean, 3.2% (w/v), containing mead, 652.3 ± 19.3 mg gallic acid/ml. Concentrations of beans were also significantly influenced the activity of antioxidant in mung bean and red kidney bean containing meads but not in soy bean containing mead (Figure 5).

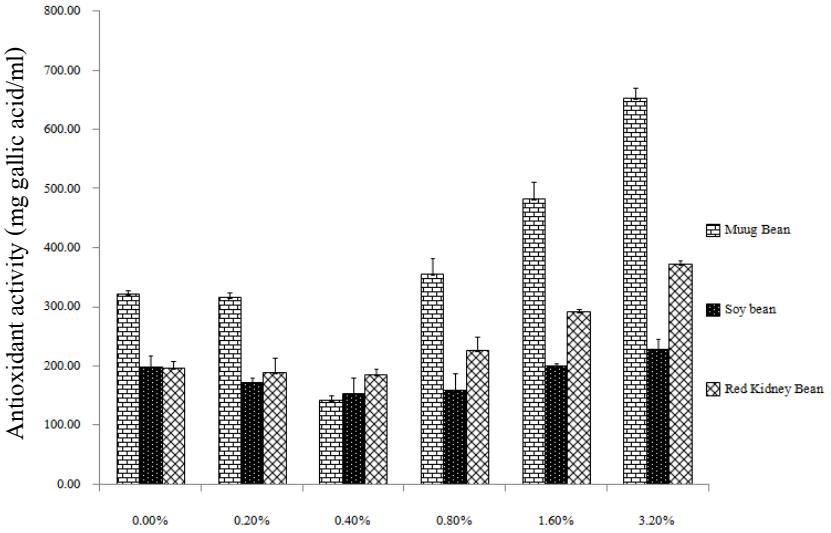

Figure 5. The antioxidant activity of mung bean, soy bean and red kidney bean containing meads. The fermentations were conducted at 25°C for one month.

Discussion

Concentrations of mung bean, soy bean or red kidney bean were not affected the ethanol production due to the fact that beans are rich in nitrogen content that can be used as a nitrogen source but not the carbon source which is responsible for ethanol production. Mubarak (2005) reported that nutrients in beans was damaged by heat. However, the ethanol contents in all mead obtained were insignificantly different demonstrating that nitrogen sources in beans were not affected ethanol fermentation in *S. cerevisiae*. On the other hand, Sewsuwan (2013) and Sukmuang (2015) found that yeast extract affected the ethanol content during fermentation process. Similarly, Pramanik and Rao (2005) suggested that the addition of nitrogen source into mead fermentation should be optimized due to the inhibitory effect of high quantity of nitrogen source on *S. cerevisiae* growth.

When increased concentration of beans, pHs of fermentation broths were increased as well. Bridges and Mattice (1939) demonstrated that pH in beans were between 6.0 and 6.6. Moreover, it was found that the concentrations of beans had no effect on sugar content in meads. Dahiya *et al.* (2015) reported that beans consisted of carbohydrate and protein which were not dissolved. Nevertheless, the concentration of beans affected the reducing sugar content because beans included polysaccharides which could be hydrolyzed by heat (Lorenz and Johnson, 1972).

The mung bean mead provided the highest antioxidant activity possibly because of phenolic compounds found in mung bean. Sonklin *et al.* (2012) reported that pulp of mung bean had 94.93% (w/w) of antioxidant content. Rudrappa (2016) found that mung bean was rich of antioxidants including vitamin B6 (pyridoxine), vitamin B1 (thiamin) and vitamin C. Moreover, Akond *et al.* (2011) reported that red kidney bean consisted of anthocyanin, a well-known antioxidant; hence, study of mead fermentation using mung bean as a nutrient source would be further focused in the future.

Conclusions

Types and concentrations of beans are not affected ethanol content but the antioxidant activity in mead. Mung bean has the highest antioxidant activity.

Acknowledgements

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References

Akond A.S.M.G.M., Khandaker L., Berthold J., Gates L., Peters K. and Delong H. Anthocyanin, total polyphenols and antioxidant activity of common bean. American Journal of Food Technology 2011; 6(5): 385-394.

Bennink M. and Rondini E. Dry beans and human health. The bean institute. Frazee, Minnesota; 2008. Pp. 82-97.

Bridges M.A. and Mattice M.R. Over two thousand estimations of the pH of representative food. American Journal of Digestive Diseases 1939; 9: 440-449.

Dahiya P.K., Linnemann A.R., Vanboekel M.A.J.S., Khetarpaul N., Grewal R.B. and Nout M.J.R. Mung bean: technological and nutritional potentail. Critical Reviews in Food Science and Nutrition 2015; 55: 670-688.

Guilford J.M. and Pezzuto J.M. Wine and health: a review. American Journal of Enology and Viticulture 2011; 62(4): 471-486.

Iglesias A., Pascoal A., Choupina A.B., Carvalho C.A., Feás X. and Estevinho L.M. Developments in the fermentation process and quality improvement strategies for mead production. Molecules 2014; 19: 12577-12590.

Johnson H. Vintage: the story of wine. Simon and Schuster 1989; 6-11.

Lorenz K. and Johnson J.A. Strach hydrolysis under high temperatures and pressures. American Association of Cereal Chemists 1972; 49: 616-628.

Miller G.L. Use of dinitrosalicyclic acid reagent for determination of reducing sugar. Analytical chemistry 1959; 31(3): 426-428.

Mills D.A., Phister T., Neeley E. and Johannsen E. Wine fermentation. Molecular Techniques in the Microbial Ecology of Fermented Foods 2008; 162-192.

Mubarak A.E. Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. Food Chemistry 2005; 89: 489-495.

Percival S.S., Sims C.A. and Talcott S.T. Immune benefits of consuming red muscadine wine. Food Science and Human Nutrition Department 2014; 1-4.

Pramanik K. and Rao D.E. Kinetic study on ethanol fermentation of grape waste using *Saccharomyces cerevisiae* yeast isolated from toddy. India.:The Institution of Engineers Publications 2005; 85: 53-58.

Robinson J.G. All about beans nutrition, health benefits, preparation and use in menus. Fargo, North Dakota: North Dakota State University; 2013. pp. 3-7.

Rudrappa U. Health benefits of green beans [online] 2016 [cited 2016 Dec 20]. Available from: http://www.nutrition-and-you.com/green_beans.html.

Sewsuwan M. Isolation and screening of microbes for vinegar production from honey. Master of Science Thesis in Applied Microbiology Faculty of Science Chiang Mai University 2013. pp. 77-79.

Shekhar T.C. and Anju G. Antioxidant activity by DPPH radical scavenging method of *Ageratum conyzoides* Linn. leaves. American Journal of Ethnomedicine 2014; 1(4): 244-249.

Sonklin C., Laohakunjit N. and Kerachoechuen O. Antioxidant activity of enzymatic mung bean meal protein hydrolysate. Agricultural Science Journal 2012; 43(2): 521-524.

Sukmuang W. Isolation and screening of yeasts for production of amla mead. Master of Science Thesis in Applied Microbiology Faculty of Science Chiang Mai University 2015. pp. 49-51.