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Technical parameters affecting agave Wine Fermentation

Nguyen Phuoc Minh

Binh Duong University, Vietnam

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Agave(*Agave tequilana*) is one of underutilized plants available in tropical and sub-tropical regions. This plant has different phytochemical constituents, specially inulin and fructan. It's also a rich source of carbohydrate appropriate for wine fermentation. This research evaluated different technical parameters affecting to physicochemical and organoleptic attributes of wine. Agave leaves were frozen, defrosted, pressed into mash, treated with Pectinex 0.2 % at 37 ^{o}Cfor 6 hours. The agave mash treated by Pectinex would be filtered with water in material: solvent 1.0:2.5. The agave filtrate was adjusted to pH 4.3 by Na_2CO_3 10%. The total soluble solide (TSS) of agave filtrate was adjusted to 19 ^{o}Brix bysaccharose (99 % purity). The enriched agave filtrate was pasteurized at 90 ^{o}C for 4 minutes and then cooled to ambient temperature (30±0.5 ^{o}C). The pasteurized agave filtrate was inoculated with the activated yeast 2.0% (12 log cfu/ml). The main fermentation was conducted in a 5L bioreactor at temperature 29 ^{o}C for 12 days. The secondary fermentation was lasted for 5 weeks at 9.5 ^{o}C with the support of 2 % chitosan as clarifying agent. The final wine was filtered, filled into glass bottle and pasteurized at 85^{o}C for 5 minutes before storage.

Keywords: Agave, chitosan, fermentation, Pectinex, wine, yeast

INTRODUCTION

Fermentation required simple equipment to preserve fruits in the form of drink and beverage at a later date and to ensure food security. Wine with alcohol content around 7-8 % could be considered as a functional beverage with numerous healthy advantages (Adelakun et al. 2013)**.**Wine production could be prepared from different kinds of fruits and vegetables to receive different flavor, aroma and taste based on raw material (Agostino et al. 2011; Akubor et al. 2013; Aldrete-Tapia et al. 2018; Anjali et al. 2018). The wine was basically conducted by feeding sugar and nutrient into filtrate and yeast, converting sugar into ethanol, carbon dioxide, flavor and aroma (Archibong et al. 2015). Fermentation involved the extracting energy from the oxidation of organic substances such as carbohydrates using an endogenous electron acceptor, usually pyruvate, an organic component. One glucose molecule was broken down into two pyruvate molecules during glycolysis. Fermentation was essential in anaerobic conditions when there was no oxidative phosphorylation to keep the production of Adenosine tri-phosphate by glycolysis. Fermentation was commonly conducted with *Saccharomyces cerevisiae* as starter culture. Yeast could survive in an oxygen deficient environment for a period of time (Aziz et al. 2009). Actually, commercial strains did not completely inhibit wild strains until several days after the process was started. The starter culture should compete with not only non-*Saccharomyces* yeasts, but also with indigenous *S. cerevisiae* strains, which theoretically adapted better to must conditions (Bahareh et al. 2017; Barrajon et al.

2011). In wine fermentation, pH had a down trend, the specific gravity increased and the ethanol increased gradually (Beltrán et al. 2002).

Agave (*Agave tequilana*) belongs to the Agavaceae family. It's identified by fleshy, rigid and hard surfaced leaves growing directly out from the center stock to produce a dense rosette. The pulp of agave is a rich source of saccharine so it has sweet taste and fibrous texture. Its seed can be finely ground into flour and utilized as thickening agent in soup and bread. The flower stalk can be roasted and consumed similar to asparagus. Sap derived from cutting the flower stem can be utilized as syrup (Bingqian et al. 2017). Pina of agave is also a rich source of fructan and inulin. Fructan can be utilized as prebiotic due to its inability of digestion in human gut. Inulin is commonly applied as vaccine adjuvant in the pharmaceutical science. This plant has strong antibacterial, antifungal, molluscicidal, insecticidal, properties. It had been utilized for the production of various alcoholic beverages (Bokulich et al. 2013). Agave juice has a low content of nitrogen. It's compensated by the supplementation of inorganic N-sources, such as ammonium sulfate or ammonium phosphate. The agave juice fermented fructose into ethanol and volatile substances (Capece et al. 2010). The fermentation of agave juice could be conducted by spontaneous yeast or inoculation of selected yeasts (Carrau et al. 2008). Low yield and quality of wine were noticed by spontaneous fermentation (Chen et al. 2012; Díaz-Montaño et al. 2008). Several reports mentioned to the fermentation of agave juice into wine and beer by different *S. cerevisiae* strains (Dittmar et al. 1993; Erten et al. 2006; Escalante-Minakata et al. 2008). Objective of our study was to examine different technical parameters such as enzyme ratio, solid/solvent ratio, pH, initial total soluble solid, temperature/time in filtrate pasteurization, yeast ratio, main fermentation temperature and time, the secondary fermentation time, chitosan supplementation as clarifying agent affecting to ethanol formation, residual sugar and organoleptic properties of wine..

MATERIALS AND METHODS

Material

Agave leaves were collected from gardens in SocTrang province, Vietnam. *Saccharomyces cerevisiae* strain from Vinmec Co. Ltd was utilized as starter culture for juice fermentation.Saccharose was purchased from ThanhThanh Cong Bien Hoa Joint Stock Company. Pectinex® Ultra Clear, a blend of pectinases, hemicellulases and arabinanases, was purchased from Novozymes. Chemical reagents were all analytical grade.

Researching method

Saccharomyces cerevisiae strain from Vinmec Co. Ltd was cultured at temperature 30 °C and shaking 250 rpm for 24 hours in 250 mL Erlenmeyer flask containing propagation medium prepared from ammonium sulfate and ammonium phosphate monobasic. Agave leaves (*Agave tequilana*) were cleaned in fresh water to remove foreign matters before freezing to -18^{o}C. They were defrosted and pressed by single screw extruder to get mash. Mash agave was treated with Pectinex in different ratio (0.05÷0.25 %) at 37 ^{o}Cfor 6 hours. The agave mash treated by Pectinex would be filtered with water in different ratio of material: solvent (1.0:1.0, 1.0:1.5, 1.0:2.0, 1.0:2.5, and 1.0:3.0). The agave filtrate was adjusted to different pH values (4.1÷4.5) by Na_2CO_3 10%. Then the total soluble solide (TSS) of agave filtrate was adjusted to different levels (16÷20 ^{o}Brix) bysaccharose (99 % purity). The enriched agave filtrate was pasteurized in different time and temperature (100/2.0, 95/3, 90/4, 85/5, 80/6, ^{o}C/minutes) and then cooled to ambient temperature (30±0.5 ^{o}C). The pasteurized agave filtrate was inoculated with the activated yeast (12 log cfu/ml) in different ratio (0.5÷2.5 %). The main fermentation was conducted in a 5L bioreactor at temperature (28÷30 ^{o}C) for 15 days. In 3 day-interval, the must was taken to analyze ethanol content, residual sugar, and overall acceptance. The secondary fermentation was lasted for 5 weeks at 9.5 ^{o}C with the support of chitosan (1.0÷3.0 %) as clarifying agent. The final wine was filtered, filled into glass bottle and pasteurized at 85^{o}C for 5 minutes before storage.

Physicochemical, microbial and antioxidant determination

pH was measured by pH meter. Total soluble solid (TSS, ^{o}Brix) was estimated by hand-held refractometer. Yeast population (log cfu/ml) was counted in Neubauer counting chamber. Yeast viability was determined by staining with methylene blue (Evans et al. 2002). Ethanol (g/L) was determined by reversed-phase liquid chromatography using the UV detector (Ferrarini et al. 2007). Residual sugar (g/L) was analyzed by Fiber-optic FT-NIR spectrometry (Fleet 2013). Overall acceptance (sensory score)

was evaluated by a group of panelists using 9 point-Hedonic scale.

Statistical analysis

All experiments were set in 3 replications. The presented data were shown as mean±standarddeviation. Statistical analysis was accounted by the Statgraphics (version 16.2.04, 64-bit).

RESULTS AND DISCUSSION

Pectinase composed of pectin methyl esterase and depolymerase widely utilized in fruit processing industries for extraction and clarification of fruit juice and wine; preparation of pectin free starch; curing of coffee, cocoa and tobacco; refinement of vegetable fibres(Francisco et al. 2010; Fundira et al. 2012; Giri et al. 2013). The enzymatic liquefaction not only improved the overall juice yield and total soluble solid but also upgraded the quality properties of the extracted juice leading to sparkling clarity (Guillermo et al. 2016; Hatice and Ezgi 2017). Table 1 showed the effect of enzyme ratio (%) to total soluble solid (^{o}Brix) and sensory score of agave filtrate. Mash agave was treated with Pectinex in different ratio (0.05÷0.25 %) at 37 ^{o}Cfor 6 hours**.** There was significant difference of total soluble solid and overall acceptance by the ratio of Pectinex supplemented in juice extraction. 0.2 % Pectinex was appropriate to obtain high total soluble solid (11.23±0.04 ^{o}Brix) and sensory score (6.81±0.03). Suspended cloud-causing pectin particles in filtrate could be eliminated by pectinase catalyzed electrostatic destabilization (Heinicke 2001). Degree of enzymatic hydrolysis depended on technical parameters like incubation time, temperature and enzyme ratio (Joseph and John 1987; Joshi and Bhutani 1991). The pectinase in juice production was demonstrated to be beneficial and profitable as it accelerated the juice extraction yield (Khairul et al. 2015).

Solvent-to-solid ratio was demonstrated to have a critical role in extraction efficiency (Kunkee and Vilas 2014). Table 2 proved the impact of solid: solvent ratio to total soluble solid and sensory score of agave filtrate. It was found that high solid to solvent ratio (1.0: 2.5) provided reasonable solute-solvent contact to obtain medium total soluble solid (8.95±0.01 ^{o}Brix) and high sensory score (7.41±0.04). As the solvent volume was increased, more total soluble solid could be extracted (Lowor et al. 2016).

Table 3 demonstrated the role of pH of juice to ethanol formation, residual sugar and sensory score of agave must.*Saccharomyces cerevisiae*required pH 4.3 optimal to produce highest ethanol content (4.83±0.02 g/L), lowest residual sugar (0.81±0.00 g/L) and the best sensory score (8.01±0.01). During alcohol fermentation, an acidic fermentation took place at the same time as the alcoholic fermentation (Manuel et al. 2005).The initial pH of must was a key factor affecting yeast proliferation and ethanol formation. *Saccharomyces cerevisiae* faced low pH stress at the beginning of fermentation. Low initial pH revealed the characteristics of extending yeast lag phase, influencing accumulated mass loss, altering the utilization rate of total soluble solid, enhancing final amount of acetic acid and glycerol, and lowering final content of ethanol and l- succinic acid (Martina et al. 2017).

Saccharomyces cerevisiae was fairly tolerant to high total soluble solid content and adapted well in must with 40 ^{o}Brix. Over this concentration, a certain group of yeasts - the osmophilic - could survive. Few yeast strains could tolerate soluble dry matter 65–70 ^{o}Brix but proliferated very slowly. Table 4 revealed the influence of initial dry matter of juice to ethanol formation, residual sugar and sensory score of agave must. There was significant difference of ethanol content, residual sugar content and overall acceptance by the initial dry matter. 19 ^{o}Brix was optimal for initial fermentation to obtain the highest ethanol content (6.97±0.00g/L), high residual sugar (1.14±0.03g/L) and highest sensory score (8.40±0.01). Total soluble solid was one of the most important factors influenced to alcohol fermentation (Beltrán et al. 2002). Dry matter was a key precursor nutrient affecting to the physicochemical and organoleptic qualities of fruit wine. It revealed a strong correlation to the wine attributes (Mateo et al. 2001). Higher total soluble solid retarded the growth and proliferation of yeast (Matteo et al. 2019). Saccharose should be supplemented into the fermentation batch to support for the fruit having low natural dry matter (Manuel et al. 2005).

It's necessary to pasteurize filtrate before fermentation to ensure probability for starter strain dominate to establish appropriate quality control points (Maurizio et al. 2016). Table 5 presented the effectiveness of temperature/time during juice pasteurization to ethanol, residual sugar and sensory score of agave must. There was significant difference of ethanol content; residual sugar and sensory score in agave must by temperature/time in filtrate pasteurization. Pasteurization of 90 ^{o}C for 4 minutes was adequate to obtain high ethanol content

(8.06±0.03 g/L), low residual sugar (0.44±0.02g/L) and high sensory score (8.23±0.00). On average, the density of yeast cells inoculated as starter culture was approximately 5×10^6 cfu/mL, hence the wild yeast load should not surpass 5×10^5cfu/ mL at the initial fermentation (Mena et al. 2012).

Saccharomyces cerevisiae played important role in wine fermentation as its ability to reproduce much faster (Manue et al. 2005). Yeast needed soluble dry matter, moisture, and optimal temperature for its growth and proliferation.

Table 1: Effect of enzyme ratio (%) to total soluble solid (°Brix) and sensory score of agave filtrate

Pectinex (%)	0.05	0.10	0.15	0.20	0.25
TSS (°Brix)	8.46±0.03^{c}	9.12±0.01^{bc}	10.06±0.02^{b}	11.23±0.04^{ab}	12.01±0.03^{a}
Sensory score	6.15±0.01^{b}	6.34±0.04^{ab}	6.52±0.01^{ab}	6.81±0.03^{a}	6.90±0.02^{a}

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 2: Effect of solid:solvent to total soluble solid (°Brix) and sensory score of agave filtrate

Solid: solvent	1.0:1.0	1.0:1.5	1.0:2.0	1.0:2.5	1.0:3.0
TSS (°Brix)	11.23±0.04^{a}	10.63±0.02^{ab}	9.46±0.03^{b}	8.95±0.01^{bc}	8.12±0.02^{c}
Sensory score	6.81±0.03^{c}	6.98±0.01^{bc}	7.12±0.02^{b}	7.41±0.04^{a}	7.25±0.00^{ab}

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 3: Effect of pH to ethanol (g/L), residual sugar (g/L) and sensory score of agave must

pH	4.1	4.2	4.3	4.4	4.5
Ethanol (g/L)	3.52±0.01^{c}	4.16±0.03^{bc}	4.83±0.02^{a}	4.64±0.03^{ab}	4.38±0.00^{b}
Residual sugar (g/L)	1.67±0.00^{a}	1.30±0.02^{ab}	0.81±0.00^{c}	0.97±0.01^{bc}	1.12±0.02^{b}
Sensory score	7.73±0.02^{c}	7.89±0.00^{b}	8.01±0.01^{a}	7.94±0.03^{ab}	7.80±0.01^{bc}

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 4: Effect of initial dry matter (°Brix) of filtrate to ethanol (g/L), residual sugar (g/L) and sensory score of agave must

Initial dry matter (°Brix)	16	17	18	19	20
Ethanol (g/L)	4.83±0.02^{c}	5.49±0.01^{bc}	6.15±0.03^{b}	6.97±0.00^{ab}	7.24±0.02^{a}
Residual sugar (g/L)	0.81±0.00^{c}	0.97±0.02^{bc}	1.06±0.01^{b}	1.14±0.03^{ab}	1.20±0.01^{a}
Sensory score	8.01±0.01^{b}	8.15±0.00^{ab}	8.27±0.02^{ab}	8.40±0.01^{a}	8.42±0.03^{a}

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 5: Effect of temperature/time in filtrate pasteurization to ethanol (g/L), residual sugar (g/L) and sensory score of agave must

Pasteurization (°C/min)	100/2	95/3	90/4	85/5	80/6
Ethanol (g/L)	6.97±0.00^{c}	7.15±0.02^{bc}	8.06±0.03^{a}	7.83±0.02^{ab}	7.52±0.01^{b}
Residual sugar (g/L)	1.14±0.03^{a}	1.02±0.01^{ab}	0.44±0.02^{c}	0.65±0.01^{bc}	0.81±0.02^{b}
Sensory score	8.40±0.01^{a}	8.31±0.00^{ab}	8.23±0.00^{ab}	8.10±0.03^{b}	8.07±0.00^{b}

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 6: Effect of the inoculated yeast ratio (%) at density 12 log cfu/ml to ethanol (g/L), residual sugar (g/L) and sensory score of agave must

Yeast ratio (%)	0.5	1.0	1.5	2.0	2.5
Ethanol (g/L)	8.06±0.03^{b}	8.24±0.01^{ab}	8.65±0.00^{ab}	8.91±0.02^{a}	8.93±0.01^{a}
Residual sugar (g/L)	0.44±0.02^{a}	0.36±0.00^{b}	0.25±0.01^{bc}	0.19±0.00^{c}	0.18±0.03^{c}
Sensory score	8.23±0.00^{ab}	8.37±0.02^{a}	8.11±0.03^{b}	7.98±0.01^{bc}	7.87±0.02^{c}

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 7: Effect of the main fermentation temperature (°C) to ethanol (g/L), residual sugar (g/L) and sensory score of agave must

Temperature (%)	28.0	28.5	29.0	29.5	30.0
Ethanol (g/L)	8.99±0.01^{ab}	9.07±0.00^{a}	9.14±0.01^{a}	9.03±0.00^{ab}	8.91±0.02^{b}
Residual sugar (g/L)	0.17±0.03^{a}	0.13±0.02^{a}	0.11±0.03^{a}	0.15±0.03^{a}	0.19±0.00^{a}
Sensory score	8.04±0.00ab	8.09±0.02b	8.14±0.02a	8.05±0.02ab	7.98±0.01b

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 8: Effect of the main fermentation time (days) to ethanol (g/L), residual sugar (g/L) and sensory score of agave must

Main fermention time (days)	3	6	9	12	15
Ethanol (g/L)	7.06±0.03^{c}	7.89±0.02^{bc}	8.42±0.00^{b}	8.86±0.02^{ab}	9.14±0.01^{a}
Residual sugar (g/L)	0.62±0.02^{a}	0.53±0.01^{ab}	0.41±0.01^{b}	0.24±0.01^{bc}	0.11±0.03^{c}
Sensory score	7.54±0.01^{c}	7.83±0.00^{bc}	8.01±0.03^{b}	8.37±0.03^{a}	8.14±0.02^{ab}

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 9: Effect of the secondary fermentation time (weeks) to ethanol (g/L), residual sugar (g/L) and sensory score of agave wine

Secondary fermention time (weeks)	1	2	3	4	5
Ethanol (g/L)	8.86±0.02^{b}	8.91±0.01^{ab}	8.93±0.03^{ab}	8.96±0.00^{a}	8.97±0.01^{a}
Residual sugar (g/L)	0.24±0.01^{a}	0.21±0.00^{ab}	0.19±0.02^{ab}	0.17±0.03^{b}	0.15±0.00^{b}
Sensory score	8.37±0.03^{c}	8.43±0.02^{bc}	8.51±0.01^{b}	8.63±0.02^{ab}	8.72±0.01^{a}

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 10: Effect of chitosan (%) as clarifying agent on sensory score of wine under secondary fermentation time

Chitosan (%)	1.0	1.5	2.0	2.5	3.0
Sensory score	8.77±0.02bc	8.82±0.02b	8.91±0.02a	8.87±0.02ab	8.65±0.02c

Note: the values were expressed as the mean of twenty two samples; the same characters (denoted above), the difference between them was not significant (α = 5%).

Yeast flourished in habitat where soluble dry matter was available. The ethanol fermentation of fruit must is initiated by a complex yeast flora including a high proportion of oxidative and weakly fermentative yeasts (Morán-Marroquín et al. 2011). In pure fermentation, the power of inoculated *Saccharomyces cerevisiae* to retard the wild microflora was one of the most important feature determining the starter ability to dominate the process (Nguyen and Viet 2009). The inoculation of must using selected *Saccharomyces cerevisiae* does not ensure its dominance at the end of fermentation (Barrajon et al. 2011). Table 6 mentioned to the efficacy of yeast ratio (12 log cfu/ml) to ethanol formation, residual sugar and sensory score of wine. There was significant difference of ethanol content, residual sugar and sensory score in agave must by the inoculated yeast ratio. 2 % yeast ratio (12 log cfu/ml) inoculated into must would obtain the highest ethanol content (8.91±0.02g/L), low residual sugar (0.19±0.00g/L) and medium sensory score (7.98±0.01). The inoculum ratio was a key variable in fermentation (Nur et al. 2013; NurAliaa et al. 2010). Yeast inoculation strongly effected quality characteristics of wine during fermentation (Obaedo and Ikenebomeh 2009; Ogodo et al. 2015; Papagianni and Moo-Young 2002). The optimal yeast inoculum ratio was very important as dry matter utilization was a balance between biomass accumulation and ethanol release. An adequate inoculum ratio would be a compromise on ethanol formed (Pazhani et al. 2017). A higher level of inoculum resulted in higher fermentation rate (Pérez-Coella et al. 2003). Higher inoculum size resulted in higher yields of glycerol and ethyl alcohol (Phonesavard et al. 2010). Unfortunately, at the higher inoculum size, yeast cells grew not well because of the limited nutrient and not suitable to convert more sugar into ethanol

(Adelakun et al. 2013). Higher alcohols, iso-acids and fatty acids were commonly undesirable constituents in wine even in low amount (Pinkie et al. 2011). There were not significant differences between inoculum size and aroma concentration, except for higher alcohols and ethyl acetate (Pino and Queris 2015; Pongkan et al. 2018). Total diacetyl content strongly accelerated by inoculum size (Radler and Schütz 1982; Ricardo et al. 2012). A consistent increase of desired aroma compounds (esters, lactones and free monoterpenes), and a decrease of less desired compounds (higher alcohols and medium chain fatty acids), was shown at inoculum sizes of 10^5 cells/mL in winemaking (Pérez-Coella and González-Viñas 2003). Satav and Pethe (Robinson et al. 2010) examined wine making from banana fruits. 10% and 15% inoculum concentration resulted similar ethanol amount but 10% induced the better taste than 15%.

Table 7 showed the fluctuation of the main fermentation temperature to ethanol formation, residual sugar and sensory score of wine. There was a slightly significant difference of ethanol content, residual sugar and sensory score in agave must by main fermentation temperature 28±30 ^oC. The optimal temperature for the main fermentation was noticed at 29.0 ^oC to achive ethanol content (9.14±0.01 g/L), residual sugar (0.11±0.03g/L) and sensory score (8.14±0.02). The biochemical process of fermentation released residual heat causing the most out of the ideal temperature range for the wine (Robinson 2006). A lower temperature is highly appreciated as it accelerated the production of esters, aromatic substances, and ethanol. This resulted the wine easier to clear and less susceptible to bacterial cross contamination (Sakhale et al. 2016). Temperature control in wine fermentation was important to enhance yeast proliferation, extract aroma and pigment from the fruit peel, permit emission of favorable by-products, and prevent hot spot that might negatively affected to the yeast cells (Samson et al. 2017). The low temperature and slow fermentation favored the retention of volatile components. High temperature was essential to extract the pigment from the fruit peel (Satav and Pethe 2017).

Table 8 presented the ethanol formation, residual sugar and sensory score of must by the main fermentation time. There was a significant difference of ethanol content, residual sugar and sensory score in agave must by main fermentation time (3÷15 days). The optimal duration for the main fermentation was noticed at 12 days to obtain ethanol content (8.86±0.02g/L), residual sugar (0.24±0.01g/L) and sensory score (8.37±0.03). Micronutrients, non-volatile and volatile components, ketones, lactones, beta-carotenoids, terpenoids, pro-xeronine produced in fermented fruit extract (Scrimgeour et al. 2015).The organic acids existing are cupric and caprylicacids (Smart et al. 1999). Fermentation time significantly affected to titratable acidity and total polyphenol content (Soto et al. 1989).

Table 9 revealed the change of ethanol formation, residual sugar and sensory score of wine under secondary fermentation time. After 5 weeks of ageing, ethanol content and overall acceptance increased (8.97±0.01g/L) and (8.72±0.01) respectively. Meanwhile there was down trend of residual sugar (0.15±0.00g/L) during this period.The alcohol productivity increased slightly in winedue to slow fermentation that happened during ageing (Sreenivas et al. 2004). During secondary fermentation, many reactions caused significant modifications in wine quality attributes such as color, phenol, flavor and aroma (Tzanov et al. 2001). Storage duration contributed a significant role in wine ageing. The volatile composition of wine changed due to various reactions taking place, particularly ester hydrolysis/esterification reactions. During this period, the elevated temperature resulted a significant risk to the physicochemical and organoleptic properties, especially ester composition of wine (Velázquez-Ríos et al. 2019). Wine kept at low temperature had extended shelf life as well as youthful wine aromas (Verbelen et al. 2009; Versavaud et al. 1995). The nutrient in wine was also improved by the release of amino acids and other compounds from yeast (Manuel et al. 2005).

Table 10 proved the effectiveness of chitosan supplementation ratio as clarifying agent on sensory score of wine under secondary fermentation time. 2.0% chitosan was adequate for clarification of protein haze to improve overall acceptance of wine. The clarifying agents bound the target particles to create insoluble aggregates that were subsequently removed from the wine (Wasila et al. 2013). Chitosan prevented protein haze via the semi-precipitation of abundant proteinaceous matter (Xiao et al. 2017). Chitosan as a clarifying agent made a complex with proteins, polyphenols, and others particle matters resulting flocculation and sedimentation therefore inducing in separation of these potential haze precursors (Xingyan et al. 2015). Agave juice was fermented by *Saccharomyces cerevisiae* in

continuous fermentation at two different aeration flow rates, feeding sterilized and non-sterilized media. Aeration produced much more ethanol (37÷40 g/L), reducing sugar consumption (73÷88 g /L). There was no significant difference by the effect of feeding sterilized or non-sterilized medium to the continuous cultures. Supplementing yeast extract and aeration to the continuous fermentation induced in accumulation of ethanol production (88 %) (Zhuang et al. 2011; Guillermo et al. 2016).

CONCLUSION

Agave (*Agave tequilana*) contained various phytochemical constituents. It's a rich source of carbohydrate ideal for wine fermentation. This research successfully examined the possibility of wine fermentation from agave juice. Pectin ratio, solid/solvent ratio, pH, initial total soluble solid, temperature/time in filtrate pasteurization, yeast ratio, main fermentation temperature and time, the secondary fermentation time, chitosan supplementation as clarifying agent were thoroughly verified to during wine fermentation from agave juice. Findings of this research diversified and improved the added value of this underutilized plant.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Nguyen Phuoc Minh arranged the experiments and also wrote the manuscript.

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