RINGKASAN

Nira kelapa adalah getah yang berasal dari bunga pohon kelapa yang masih kuncup. Pemanfaatan nira kelapa yang paling menonjol adalah sebagai bahan baku dalam pembuatan gula merah. Selain itu nira kelapa juga digunakan dalam pembuatan tuak, *cimplung*, sirup nira kelapa, permen maupun gula kelapa cair. Pemanfaatan nira kelapa yang masih belum banyak diteliti adalah pemanfaatannya sebagai sumber yang potensial untuk isolasi *Saccharomyces cerevisiae*. Hal ini karena nira kelapa memiliki kandungan gula yang cukup tinggi yang sangat dibutuhkan untuk pertumbuhan berbagai jenis mikrobia termasuk *S. cerevisiae*.

S. cerevisiae adalah yeast yang sangat bermanfaat bagi kehidupan manusia dan terdapat melimpah dalam habitat nabati seperti nira kelapa. Penerapan *S. cerevisiae* tidak hanya pada bidang pangan tetapi juga pada bidang non pangan seperti untuk produksi bioetanol. Penerapan *S. cerevisiae* di bidang pangan seperti pada pembuatan produk pangan fermentasi baik yang tradisional maupun non tradisional. Keuntungan makanan fermentasi antara lain akan memperbaiki sistem kekebalan tubuh, menaikkan nilai gizi dan memperbaiki cita rasa makanan. Akan tetapi produksi makanan fermentasi biasanya menggunakan starter yang viabilitasnya dan tingkat kemurniannya rendah. Hal ini memunculkan masalah dan ancaman yang serius dari produk pangan fermentasi.

Mengingat ketersediaan nira kelapa di Desa Susukan Kecamatan Sumbang cukup terjamin maka perlu dilakukan penelitian untuk menganalisis potensi nira kelapa sebagai sumber *S. cerevisiae*, mengisolasi serta mengidentifikasi *S. cerevisiae* yang sesuai untuk diterapkan di industri makanan, memproduksi starter *S. cerevisiae* dan melakukan optimasi pembuatan starter menggunakan perangkat lunak Design Expert v.13.

Penelitian dimulai dengan pengambilan sampel menggunakan teknik *simple random sampling*. Sampel nira kelapa yang digunakan dalam penelitian ini adalah nira asli, nira sulfit dan nira organik sadapan pagi dan sore. Sampel nira kelapa dimasukkan ke dalam botol plastik steril selanjutnya dibawa ke Laboratorium Teknologi Pertanian menggunakan kotak es (*ice box*). Analisis sampel nira kelapa meliputi warna, aroma dan kenampakan, % brix, pH, total gula, gula reduksi, air,total mikrobia, total yeast, total bakteri, persentase yeast dan persentase bakteri. Analisis sensori, % brix dan pH dilakukan *in situ*. Data dianalisis menggunakan uji anova, uji t data bebas dan korelasi Pearson. Isolasi dilakukan dengan menggunakan teknik *streak plate*. Identifikasi morfologi didasarkan pada pengamatan makroskopis dan mikroskopis. Isolat terpilih kemudian diidentifikasi secara molekuler dengan teknik PCR konvensional. Identifikasi fisiologis strain GNS3, GNS9 dan GNS14 meliputi resistensi suhu, pH, NaCl dan etanol, glukosa dan metabisulfit. Jumlah yeast dihitung menggunakan metode *pour plate* pada PDA. Optimasi pembuatan starter *S. cerevisiae* GNS9 dilakukan dengan metode *Central Composite Design-Response Surface Methodology.* Faktor yang dioptimasi pada penelitian ini adalah lama inkubasi, lama pengeringan dan suhu pengeringan. Ada pun respon yang diamati adalah viabilitas dan kemurnian. Verifikasi perlakuan terbaik dilakukan sebanyak 3 kali.

Karakterisasi starter komersial meliputi warna, bentuk, bau, keberadaan benda asing, kadar air, jumlah N dan kandungan *E. coli*.

Hasil penelitian menunjukkan bahwa nira asli sadapan sore berwarna krim sampai cokelat muda, beraroma netral sampai khas, buih sedikit sampai cukup, persentase brix 16,26±1,14% dan kadar gula total 8,15±0,05%. Total yeast dan persentase yeast nira asli sadapan sore hari adalah 5,82±0,12 log cfu/ml dan 73,53±1,51%. Nira kelapa potensial sebagai sumber *S. cerevisiae* terutama nira asli sadapan sore hari adalah sumber *S. cerevisiae* yang paling potensial. Hal ini karena nira asli sadapan sore hari mempunyai total yeast dan persentase yeast yang lebih tinggi dibandingkan nira sulfit dan nira organik. Hasil analisis korelasi Pearson menunjukkan bahwa komposisi kimiawi, kondisi agroklimat dan waktu sadap berkorelasi dengan profil mikrobia dengan tingkat keeratan sedang sampai kuat. Hal ini menunjukkan bahwa profil mikrobia nira kelapa dipengaruhi oleh komposisi kimiawi, kondisi agroklimat dan waktu sadap.

Sebagian besar sel berbentuk oval. Ukuran sel bervariasi, panjang antara 12,5 – 17,8 µm dan lebar antara 0,8 – 4,6 µm. Selnya berwarna putih krem dan permukaannya kusam. Hasil identifikasi molekuler menunjukkan bahwa GNS1, GNS3, GNS4, GNS9 dan GNS14 adalah strain baru *S. cerevisiae* dengan 400 – 600 bpDNA. Hasil konstruksi pohon filogenetik menunjukkan bahwa strain GNS9 adalah strain yang secara genetik paling berbeda dengan kontrol. Sedangkan strain GNS1, GNS3, GNS4 diduga adalah strain yang sama. Hasil uji resistensi menunjukkan bahwa strain GNS9 adalah strain *S. cerevisiae* yang mempunyai resistensi tinggi terhadap suhu 40^{o}C, NaCl 9%, etanol 10%, glukosa 40%, dan metabisulfit 500 ppm. Total yeast strain *S. cerevisiae* GNS9 pada suhu 40^{o}C 8,35±0,96 log cfu/ml, NaCl 9% 8,51±1,14 log cfu/ml, etanol 10% 8,45±1,10 log cfu/ml, glukosa 40% 8,65±1,52 log cfu/ml dan metabisulfit 500 ppm 8,35±1,28 log cfu/ml.

Kondisi proses terbaik pada pembuatan starter *S. cerevisiae* GNS9 adalah pada lama inkubasi 45,089 jam, lama pengeringan 35,270 jam dan suhu pengeringan 44,880^{o}C. Lama pengeringan dan suhu pengeringan menurunkan viabilitas starter *S. cerevisiae* GNS9 masing-masing sebesar 6,5% dan 14%. Pengaruh lama inkubasi, lama pengeringan dan suhu pengeringan terhadap respon viabilitas dapat digambarkan melalui sebuah model matematik kuadratik Y (Viabilitas) = 85,35 – 0,2197A – 0,7589B – 0,0266C – 0,1250AB + 0,6250AC + 0,6250BC – 0,2327A^2 – 0,5862B^2 – 0,9398C^2. Lama inkubasi akan menurunkan tingkat kemurnian starter *S. cerevisiae* GNS9 antara 8 - 10%. Pengaruh lama inkubasi, lama pengeringan dan suhu pengeringan terhadap respon kemurnian dapat digambarkan melalui sebuah model matematik kuadratik Y (Kemurnian) = 90,65 – 1,13A – 0,3894B – 0,0732C – 0,6250AB + 0,1250AC + 0,1250BC – 1,02A^2 – 0,3120B^2 – 0,1352C^2. Starter *S. cerevisiae* GNS9 mempunyai viabilitas 85,333±1,95%, kemurnian 90,333±1,84%, warna putih, bentuk bundar, bau normal, bebas benda asing, kadar air 7,56±0,83%, jumlah N 6,35±0,47%, dan kandungan *E. coli* 2 APM/g.

Kata kunci : nira kelapa, isolasi, identifikasi, *S. cerevisiae*, RSM

SUMMARY

Coconut sap is a sap that comes from coconut tree flowers that are still in bud. The most prominent use of coconut sap is as a raw material for making brown sugar. Apart from that, coconut sap is also used in making palm wine, cimplung, coconut sap syrup, candy and liquid coconut sugar. The use of coconut sap that has not been widely studied is its use as a potential source for isolating *Saccharomyces cerevisiae*. This is because coconut sap has a fairly high sugar content which is needed for the growth of various types of microbes including *S. cerevisiae*.

S. cerevisiae is a yeast that is very beneficial for human life and is found abundantly in vegetable habitats such as coconut sap. The application of S. cerevisiae is not only in the food sector but also in non-food sectors such as bioethanol production. The application of S. cerevisiae in the food sector is in the manufacture of fermented food products, both traditional and non-traditional. The benefits of fermented food include improving the immune system, increasing nutritional value and improving the taste of food. However, fermented food production usually uses starters whose viability and purity levels are low. This raises problems and threats from fermented food products.

Considering that the availability of coconut sap in Susukan Village, Sumbang District is quite guaranteed, it is necessary to carry out research to analyze the potential of coconut sap as a source of *S. cerevisiae*, isolate and identify *S. cerevisiae* for application in the food industry, produce *S. cerevisiae* starters and optimize the manufacture of starters using tools Design Expert v.13 software.

The research began with sampling using simple random sampling techniques. The coconut sap samples used in this research were original sap, sulfite sap and organic sap harvested in the morning and evening. Samples of coconut sap were put into sterile plastic bottles and then taken to the Agricultural Technology Laboratory using an ice box. Analysis of coconut sap samples includes colour, aroma and appearance, % Brix, pH, total sugar, reducing sugar, water, total microbes, total yeast, total bacteria, percentage of yeast and percentage of bacteria. Sensory analysis, % Brix and pH were carried out in situ. Data were analyzed using anova test, independent data t test and Pearson correlation. Isolation was carried out using the streak plate technique. Morphological identification is based on macroscopic and microscopic observations. The selected isolates were then identified molecularly using conventional PCR techniques. Physiological identification of strains GNS3, GNS9 and GNS14 includes resistance to temperature, pH, NaCl and ethanol, glucose and metabisulfite. The amount of yeast was calculated using the pour plate method on the PDA. Optimization of making commercial starters was carried out using the Central Composite Design- Response Surface Methodology method. The factors optimized in this research were incubation time, drying time and drying temperature. The responses observed were viability and purity. Verification of the best treatment was carried out 3 times. Characterization of starter *S. cerevisiae* GNS9 includes colour, shape, odor, presence of foreign matter, water content, amount of N and *E. coli* content.

The results of the research showed that the original afternoon sap was cream to light brown in color, had a neutral to distinctive aroma, had little to enough foam, a Brix percentage of 16.26 ± 1.14% and a total sugar content of 8.15 ± 0.05%. The total yeast and yeast percentage of native sap in the afternoon tap were 5.82 ± 0.12 log cfu/ml and 73.53 ± 1.51%. Coconut sap has the potential to be a source of *S. cerevisiae*, especially native sap from the afternoon tap is the most potential source of *S. cerevisiae*. This is because the original sap tapped in the afternoon has a higher total yeast and yeast percentage than sulphite sap and organic sap. The results of Pearson correlation analysis showed that chemical composition, agro-climatic conditions and tapping time were correlated with the microbial profile with a moderate to strong level of closeness. This shows that the microbial profile of coconut sap is influenced by chemical composition, agro-climatic conditions and tapping time.

Most cells are oval in shape. Cell size varies, length between 12.5 – 17.8 µm and width between 0.8 – 4.6 µm. The cells are creamy white and have a dull surface. Molecular identification results show that GNS1, GNS3, GNS4, GNS9 and GNS14 are new strains of *S. cerevisiae* with 400 – 600 bpDNA. The results of the phylogenetic tree construction showed that the GNS9 strain was the strain that was most genetically different from the control. Meanwhile, strains GNS1, GNS3, GNS4 are thought to be the same strain. The resistance test results showed that the GNS9 strain is an *S. cerevisiae* strain that has high resistance to temperatures of 40^{o}C, 9% NaCl, 10% ethanol, 40% glucose, and 500 ppm metabisulfite. Total yeast strain *S. cerevisiae* GNS9 at 40^{o}C 8.35±0.96 log cfu/ml, 9% NaCl 8.51±1.14 log cfu/ml, 10% ethanol 8.45±1.10
log cfu/ml, 40% glucose 8.65±1.52 log cfu/ml and metabisulfite 500 ppm 8.35±1.28 log cfu/ml.

The best process conditions for making *S. cerevisiae* GNS9 starter are incubation time of 45.089 hours, drying time of 35.270 hours and drying temperature of 44.880^{o}C. Drying time and drying temperature reduced the viability of *S. cerevisiae* GNS9 starter by 6.5% and 14%, respectively. The influence of incubation time, drying time and drying temperature on the viability response can be described through a quadratic mathematical model Y (Viability) = 85.35 – 0.2197A – 0.7589B – 0.0266C – 0.1250AB + 0.6250AC + 0, 6250BC – 0.2327A^{2} – 0.5862B^{2} – 0.9398C^{2}. Long incubation will reduce the purity level of *S. cerevisiae* GNS9 starter between 8 - 10%. The influence of incubation time, drying time and drying temperature on the purity response can be described through a quadratic mathematical model Y (Purity) = 90.65 – 1.13A – 0.3894B – 0.0732C – 0.6250AB + 0.1250AC + 0, 1250BC – 1.02A^{2} – 0.3120B^{2} – 0.1352C2. *S. cerevisiae* GNS9 starter has a viability of 85.333±1,95%, purity of 90.333±1,84%, white colour, round shape, normal odour, free of foreign matter, water content of 7.56±0.83%, total N of 6.35±0.47%, and *E. coli* content of 2 APM/g.

Key words: coconut sap, isolation, identification, *S. cerevisiae*, RSM