Sifat Fisik dan Kimia *Resistant Starch* Tipe 3 Pati Kacang Tunggak (*Vigna unguiculata*) dengan Perlakuan *Autoclaving-Cooling* Serta Potensinya Untuk Pencegahan *Colitis-Associated Adenocarcinoma* pada Tikus *Sprague-Dawley*

INTISARI

Kacang tunggak (*Vigna unguiculata*) merupakan salah satu jenis kacang-kacangan minor yang dibudidayakan di Indonesia namun pemanfaatannya sebagai bahan pangan masih terbatas. Tingginya kandungan pati pada kacang tunggak dengan kadar amilosa tinggi dan struktur kristalin tipe C mengindikasikan potensinya sebagai alternatif sumber pati dan *resistant starch* (RS). RS merupakan bagian pati yang tidak dapat dicerna di usus halus namun akan difermentasi oleh mikroflora kolon sehingga menghasilkan *short chain fatty acid* (SCFA) yang berperan untuk kesehatan kolon dan organ lain, termasuk untuk mencegah inflamasi di kolon. Salah satu metode untuk meningkatkan kadar RS tipe 3 (RS3) adalah perlakuan fisik seperti *autoclaving-cooling* multisiklus.

Tujuan umum penelitian ini adalah mengevaluasi karakteristik RS3 pati kacang tunggak dengan perlakuan *autoclaving-cooling* serta potensinya untuk pencegahan *colitis-associated adenocarcinoma* pada tikus *Sprague-Dawley*. Tujuan khusus penelitian adalah mengevaluasi: 1) sifat fisik, kimia, *in vitro digestibility,* dan *estimated glycemic index* (eGI) pati alami dari 5 varietas kacang tunggak, yaitu KT4, KT5, KT7, KT8, dan KTL; 2) pengaruh perlakuan jumlah siklus *autoclaving-cooling* pati kacang tunggak varietas terpilih terhadap sifat fisik, kimia, *in vitro digestibility,* dan eGI RS3 yang dihasilkan; dan 3) profil SCFA dan potensi RS3 pati kacang tunggak dengan perlakuan *autoclaving-cooling* terpilih untuk pencegahan *colitis-associated adenocarcinoma* pada tikus *Sprague-Dawley*.

Penelitian ini dilakukan dalam tiga tahap, yaitu 1) Tahap identifikasi sifat fisik, kimia, *in vitro starch digestibility,* dan eGI pati alami dari 5 varietas kacang tunggak (KT4, KT5, KT7, KT8, dan KTL), 2) Tahap modifikasi pati kacang tunggak varietas terpilih dengan variasi jumlah siklus *autoclaving-cooling* terhadap sifat fisik, kimia, *in vitro starch digestibility,* dan eGI RS3 yang dihasilkan, dan 3) Tahap evaluasi potensi RS3 pati kacang tunggak dengan perlakuan *autoclaving-cooling* terpilih untuk pencegahan *colitis-associated adenocarcinoma* pada tikus *Sprague-Dawley*.

Hasil penelitian membuktikan perbedaan varietas kacang tunggak dapat mempengaruhi sifat-sifat fisik, kimia, *in vitro digestibility,* dan eGI pati alaminya. Pati dari lima varietas kacang tunggak mempunyai rendemen 17,78-22,93%, kadar pati dan kadar amilosa tinggi, granula berbentuk oval sampai bulat dengan permukaan halus, dan *Z average* 7,91-15,51µm. WHC dan OHC pati alami kacang tunggak berkisar 0,64-0,99 g/g dan 0,47-0,63g/g. Pola difraksi sinar X menunjukkan struktur kristalin tipe C_A dengan puncak kuat pada 15º, 17º, 18º, dan 23º (2θ). Sifat termal pati kacang tunggak menunjukkan stabilitas termal yang baik dan jumlah rantai pendek amilopektin yang melimpah. Sifat pasting pati kacang

tunggak menunjukkan resistensi tinggi terhadap pembengkakan, viskositas tinggi, namun mudah mengalami retrogradasi. Spektra FT-IR menunjukkan karakteristik *band* yang hampir sama dan mempunyai daerah kristalin dan jumlah *double helix* yang tinggi. Semua pati kacang tunggak dikategorikan sebagai bahan pangan dengan kadar RS sangat tinggi (65,75-76,15%) dan eGI rendah (45,46-48,14). Varietas yang dipilih untuk penelitian tahap kedua adalah KT5 karena kadar RS paling tinggi, SAG paling tinggi, eGI paling rendah, sifat termal lebih baik, dan suhu pasting paling tinggi.

Perlakuan jumlah siklus *autoclaving-cooling* menyebabkan perubahan sifat fisik, kimia, *in vitro digestibility*, dan *estimated glycemic index* RS3 pati kacang tunggak dibandingkan dengan pati alaminya. Jumlah siklus *autoclaving-cooling* menyebabkan peningkatan WHC, OHC, kadar amilosa, suhu pasting, *peak time*, T_p, T_c, jumlah *double helix*, daerah kristalin, dan kadar RS. Sebaliknya, nilai L, derajat putih, kadar pati, ΔH, *final viscosity*, *set-back viscosity*, dan eGI mengalami penurunan dengan semakin banyak jumlah siklus *autoclaving-cooling*. Tipe struktur kristalin berubah dari tipe C_A menjadi campuran tipe B dan V. Siklus *autoclaving-cooling* menyebabkan pergeseran pita dan kedalaman serapan pada spektra FT-IR pati modifikasi, serta meningkatkan jumlah kristalit dan *double helix* dibandingkan dengan pati alaminya. Jumlah siklus *autoclaving-cooling* yang dipilih untuk penelitian tahap III adalah perlakuan *autoclaving-cooling* 1 siklus (AC-1) karena mempunyai kadar amilosa dan RS paling tinggi, eGi rendah, derajat putih, *final viscosity*, dan *set-back viscosity* lebih tinggi daripada perlakuan lain. Kadar RS dan eGI pada AC-1 tidak berbeda dengan RS3 komersial (Novelose 330).

RS3 pati kacang tunggak dengan perlakuan *autoclaving* multisiklus terpilih dapat meningkatkan konsentrasi dan rasio molar SCFA, termasuk butirat, dan ketebalan mukosa kolon, serta mampu memperbaiki kerusakan sel kolon yang mengalami kolitis dan kerusakan sel liver akibat induksi *azoxymethane* pada tikus *Sprague-Dawley*. Hasil penelitian ini mengindikasikan RS pati kacang tunggak, baik pati alami maupun yang dimodifikasi dengan *autoclaving-cooling* 1 siklus, berpotensi untuk pencegahan *colitis-associated adenocarcinoma* secara *in vivo*.

Kata-kata kunci: pati kacang tunggak, RS3, *autoclaving-cooling*, sifat fisik dan kimia, *colitis-associated adenocarcinoma*

Physicochemical properties of type 3 resistant starch from cowpea (*Vigna unguiculata*) starch by autoclaving-cooling treatment and its potency for preventing colitis-associated adenocarcinoma in *Sprague-Dawley* rats

ABSTRACT

Cowpea (*Vigna unguiculata*) is cultivated as a minor pulse in Indonesia but its utilization in food products is limited. High starch and amylose content of cowpea starch indicate its potency as a source of starch and resistant starch. Resistant starch (RS) is a starch that can escape digestion in the small intestine and reach the large intestine to ferment by the gut microflora. This fermentation could produce short chain fatty acid (SCFA), particularly butyrate, which have an important role in maintaining the health of colon and other organs including to prevent colitis. RS formed by processing is defined as RS type 3 (RS3). Among the various methods for increasing RS3, physical modification such as autoclaving-cooling is more preferred because it is easier, lower cost, and more safety than the other methods.

The objectives of research were as follow: 1) to evaluate the physical, chemical, *in vitro* starch digestibility, and estimated glycemic index of native starch from five varieties of cowpea, i.e. KT4, KT5, KT7, KT8, and KTL; 2) to evaluate the effect of the autoclaving-cooling cycles from selected cowpea starch on the physical, chemical, *in vitro* starch digestibility, and estimated glycemic index of RS3; and 3) to evaluate the SCFA profile of RS3 from cowpea starch by selected autoclaving-cooling and its potency for preventing colitis-associated adenocarcinoma in *Sprague-Dawley* rats model.

The research was divided into three stages as follows: 1) characterization of the physicochemical properties, *in vitro* starch digestibility, and estimated glycemic index (eGI) of native starch from five varieties of cowpea, 2) characterization of the physicochemical properties, *in vitro* starch digestibility, and eGI of RS3 from cowpea starch (selected varieties) with variations of autoclaving-cooling cycles, and 3) evaluation of the potency of RS3 from cowpea starch by selected autoclaving-cooling for preventing colitis-associated adenocarcinoma in *Sprague-Dawley* rats model.

The result of the research showed the differences of cowpea varieties could affect the physicochemical properties, *in vitro* starch digestibility, and eGI of native starch. The yield of starch from five varieties of cowpea ranged 17.78-22.93%, high starch and amylose content, shape of granule was oval to round with smooth surface, and Z average 7.91-15.51µm. WHC and OHC of native cowpeas starch ranged from 0.64 to 0.99 g/g and 0.47-0.63 g/g, respectively. The X-ray diffraction pattern showed a C_A-type crystalline structure with a strong peak at 15º, 17º, 18º, and 23º (2θ). The thermal properties of cowpea starch indicated a good thermal stability and abundance of short chain amylopectin. The pasting properties of cowpea starch showed a high resistance to swelling, high viscosity, but easily retrograde. The FT-IR spectra of cowpea starch exhibit a similarity band

characteristics and have a high crystalline regions and double helix. All cowpea starch was categorized as very high RS (65.75-76.15%) and low eGI (45,46-48,14). The variety selected for the second stage of research was KT5 based on highest RS and SAG content, lowest eGI, better thermal properties, and higher pasting temperature.

The number of autoclaving-cooling cycles led to changes in physical, chemical, *in vitro* digestibility, and estimated glycemic index of RS3 cowpea starch compared ith native starch. The number of autoclaving-cooling cycles could increase in WHC, OHC, amylose content, pasting temperature, peak time, Tp, Tc, double helix, crystalline region, and RS content. In contrast, the values of L, white degree, starch, ΔH, final viscosity, set-back viscosity, and eGI decreased with increasing the number of autoclaving-cooling cycles. The crystalline type structure changed from the C_A type to mixture of the B and V type. The autoclaving-cooling cycle could shift the band and the depth of absorption of FT-IR spectra in the modified starch, indicating the increasing of the amount of crystallite and double helix compared with native starch. The number of autoclaving-cooling cycles selected for the third stage of the study was the one cycle autoclaving-cooling treatment (AC-1) based on the highest of, amylose and RS content, white degree, final viscosity, and set-back viscosity than other treatments. RS content and eGI of AC-1 did not differ with commercial RS3 (Novelose 330).

RS of cowpea starch, both native and AC-1, could increase the concentration and molar ratio of SCFA, particularly butyric acid, and the thickness of colonic mucosa, and be able to repair the damage of colon and liver cell in azoxymethane-induced *Sprague-Dawley* rats. The results of this study indicate RS from cowpea starch, both native and retrograded starch, has a potency for the prevention of colitis-associated adenocarcinoma.

Keywords: cowpea starch, RS3, autoclaving-cooling, physicochemical properties, colitis-associated adenocarcinoma