Application of autoclaving-cooling cycling treatment to improve resistant starch content of corn-based rice analogues

B Hidayat¹, M. Muslihudin², and S Akmal³
¹,²,³Agricultural Technology Department, State Polytechnic of Lampung
10 Soekarno-Hatta Street, Bandar Lampung 35144, Indonesia

e-mail : beni_lpg@polinela.ac.id

Abstract. Resistant starch is one important component determining the characteristics of a functional food. The aim of the research was to determine the cooling time optimum in the autoclaving-cooling treatment to increase the resistant starch content corn-based rice analogues, with 6 level of cooling time (0 hours/control, 12 hours, 24 hours, 36 hours, 48 hours and 60 hours). The results showed that cooling at 4°C for 60 hours would increase the resistant starch content (6.27% to 15.38%), dietary fiber content (14.53% to 20.17%); and decrease the digestible starch content (61.81% to 52.70%). Cooling time level at 4°C for 24 hours, would increase the sensory score of corn-based rice analogues then back down until cooling time level of 60 hours. Microscopic analysis of granular structure using SEM indicated that cooling time had a linear correlation with cracks intensity on the granule surface of the corn-based rice analogues. The high content of resistant starch showed that the application of cooling time level at 4°C for 24 hours would improve the functional properties of corn-based rice analogues with sensory characteristics remain favorable to panelists.

Keyword : resistant starch, corn-based rice analogues, autoclaving-cooling cycling

1. Introduction
Resistant starch is a starch fraction that can not be digested by enzymes in the small intestine so it has physiological effects that are beneficial to health such as prevention of colon cancer and have hypoglycemic and hypocholesterolemic effects [1]. The content of RS can be used to identify of functional food [2].

Hidayat, et al. [3] reported that corn-based rice analogues processed by granulation method categorized as functional food and has low IG value, one of which is a contribution of a high content of resistant starch. The resistant starch type 3 (RS3) is formed during the processing because of the cooling process stages. Resistant starch type 3 (RS3) is a resistant starch that is formed mainly due to food processing [4].

The high content of resistant starch is determined by the intensity of the autoclaving -cooling process. The autoclaving process is conducted at temperature >100°C, while the cooling process is carried out at 4°C. Increased content of resistant starch as a result of autoclaving-cooling cycles treatment has been reported by Dundar and Gocmen [5]; Lehmann et al. [6]; Vasanthan and Bhattay [7]; Saguillan et al. [8]; Zhao and Lin [9].

The longer the cooling process, the RS 3 will be higher and the product will increasingly have properties as the functional food. But on the other hand, the content of resistant starch will affect the sensory characteristics of the product. The aim of the research was to determine the cooling time optimum in the autoclaving-cooling treatment to increase the resistant starch content of corn-based rice analogues.
2. Materials and methods

2.1 Materials
The raw materials were yellow corn var. Bisi II from Practice Gardens of Lampung State Polytechnic, harvested in January 2017 and cassava starch from a local market. The chemicals used were Starch (GR, Merck), maltose (Sigma M5885), Dinitrosalicylic acid (DNS, Sigma D-0550), glucose (Sigma G8270), termamyl enzyme (α-amylase, Sigma A-4862), pepsin enzyme (Sigma P-7000), amylloglucosidase enzyme (Sigma A-9913), pancreatin enzyme (Sigma P-1750) obtained from PT Elo Karsa, Jakarta.

2.2 Methods
Preparation of modified corn flour. Modified corn flour was prepared by the wet milling and pre-gelatinization method based on Hidayat et al. [10], through the stages of sorting of whole maize, coarse grinding (20 mesh), separation of the epidermis and core by soaking, wet milling, partial pre-gelatinization, drying followed by fine grinding (60 mesh).

Processing of corn-based rice analogues. Corn-based rice analogues were processed by granulation method through the stages of mixing of 70% modified corn flour (MCF) with 30% cassava starch (CS) and addition of water as 45% of the mixture, followed by granulating process with granulator.

Application of autoclaving-cooling cycling treatment to increase the resistant starch content of corn-based rice analogues. Treatment was carried out through the cooking stages by steaming for 30 minutes and cooling stage at 4°C for 0 hours/control, 12 hours, 24 hours, 36 hours, 48 Hours and 60 hours). The cooling stage was done by storing in refrigerator at 4°C with cooling time according to treatment. The treated-corn-based rice analogues then dried at 50°C for 5 hours.

Analysis of corn-base rice analogues. Analysis of corn-base rice analogues was conducted in the form of analysis of functional component, analysis of sensory characteristic, and microscopic analysis of granular structure. Analysis of functional component, included: dietary fiber by the enzymatic method based on Asp [11], resistant starch (RS) and digestible starch (DS) based on Englyst et al. [12]. Sample preparation for sensory analysis was done by cooking with the addition of ½ part water of corn-base rice analogues. Sensory analysis was performed by twenty trained panelists in the form of hedonic test (1-9 scale) for the parameter of eating quality, texture, and taste. Microscopic analysis of granular structure was observed using the SEM technique (SEM ZEISS EVO MA 10).

Data analysis. The analysis was carried out in four replicates for all determinations, except organoleptic and SEM data. The data were analyzed by one-way analysis of variance (ANOVA) and the results are reported as mean ± SD on dry basis (db). The significance of the differences was defined as P<0.05. Data of organoleptic score was the average value of the 20 panelist continued by quantitative description analysis spider web by MS Excel program.

3. Results and Discussion
3.1. Resistant starch content
Increasing of cooling time level was significantly (p<0.05) increase the resistant starch content of corn-based rice analogues from 6.27% to 15.38% (Table 1). The increase of resistant starch content to the longer cooling time was associated with higher percentage of retrograded starch. Because it was formed by processing, the resistant starch type was resistant starch type 3 (RS3). Resistant starch type 3 (RS3) was a resistant starch that was formed mainly due to food processing [2]. Fabbri et al. [13] and Perdon, et al. [14] reported that cooling process will stimulate the formation of RS 3. The formation of RS 3 due to retrogradation process were reported also by Vasanthan and Bhatt [7]; Fredriksson et al. [15]; Farhat et al. [16]; Thompson [17]; Fuentes-Zaragoza et al. [18].
Table 1. Resistant Starch (RS), Digestible Starch (DS), Dietary Fiber (DF) of Corn-Based Rice Analogues on various level of cooling time (mean ± SD), % dry basis

| Level of cooling time (hours) | RS (%)       | DS (%)       | DF (%)       |
|------------------------------|--------------|--------------|--------------|
| 0 (control)                  | 6.27 ± 0.37e | 61.81 ± 0.66e | 14.53 ± 0.17e |
| 12                           | 10.44 ± 0.31d | 57.64 ± 0.63d | 15.48 ± 0.36d |
| 24                           | 12.28 ± 0.44c | 55.80 ± 0.34c | 17.15 ± 0.52c |
| 36                           | 13.87 ± 0.25b | 54.21 ± 0.63b | 18.85 ± 0.22b |
| 48                           | 15.27 ± 0.34a | 52.81 ± 0.49a | 20.15 ± 0.27a |
| 60                           | 15.38 ± 0.15a | 52.70 ± 0.79a | 20.17 ± 0.29a |

Values with the same letters are not significantly different (P > 0.05)

3.2. Digestible starch content
Table 1 shows, the increasing of cooling time level was significantly (p<0.05) decrease the digestible starch content of corn-based rice analogues from 61.81% to 52.70%. The content of digestible starch (DS) had been positively correlated to resistant starch (RS) of corn-based rice analogues. The lower of DS content was due to the rise of RS content (Table 1). Resistant starch on corn-based rice analogues had been dominated by RS 3 as a result of retrogradation. Retrogradation was increase crystallinity degree of starch [19, 20]. As a result, starch becomes less susceptible to hydrolysis with the amylolytic enzymes and decrease of starch digestibility [21, 22]. The decreasing of starch digestibility along with the increasing of RS content, also reported by Chung et al. [23]; Mir et al. [24]; Zhu et al. [25]; and Hsu et al. [26].

3.3. Dietary fiber content
Increasing of cooling time level was significantly (p < 0.05) increase the dietary fiber content of corn-based rice analogues from 14.53% to 20.17% (Table 1). Increasing of the dietary fiber content was closely related to increasing of resistant starch content. The higher of resistant starch content caused the higher of dietary fiber content of corn-based rice analogues. Resistant starch is defined as the amount of degradation product of starch that can not be absorbed by the human intestine and is grouped into dietary fiber [13, 17, 18].

3.4. Sensory characteristic
Eating quality, texture, and taste score of corn-based rice analogues would increase until cooling time level of 24 hours but back down until cooling time level of 60 hours (Fig 1). This showed that cooling time level of 24 hours was the optimum cooling time to get high RS of corn-based rice analogues. Increased sensory score until cooling time level of 24 hours was due to increase of crispy texture on the product [27] which caused the product to be preferred by the panelist. Decreased sensory score after cooling time level of 24 hours was associated with increased RS content. Increased RS content will improve crystallinity degree of starch [19, 20] and decreased swelling index. Increased degree of crystallinity and decreased swelling index will increase the perceived hardness of corn-based rice analogues [28]. Increasing the hardness of corn-based rice analogue would lower the panelist's preference level.
Description of score: 9 (like extremely), 8 (like very much), 7 (like moderately), 6 (like slightly), 5 (neither like nor dislike), 4 (dislike slightly)

**Fig. 1.** Spider web of sensory characteristic of corn-based rice analogues on various level of cooling time (average value of the 20 panelists)

3.5. Granular structure

Microscopic analysis of granular structure using SEM indicates that cooling time had a linear correlation with cracks intensity on the granule surface of the corn-based rice analogues (Fig. 2 and 3). The longer the cooling time, the more granules will broke into small granules. Increasing of cooling time also caused cracks in the granular surface. Cracking and breakage of starch granules due to this heating and cooling treatment also reported by Ashwar et al. [28] and Shin et al. [29].

4. Conclusions

Cooling time level at 4°C for 60 hours would increase the resistant starch content (6.27% to 15.38%), dietary fiber content (14.53% to 20.17%); and decrease the digestible starch content (61.81% to 52.70%). Cooling time level at 4°C for 24 hours, would increase the sensory score of eating quality, texture, and taste of corn-based rice analogues then back down until cooling time level of 60 hours. Microscopic analysis of granular structure using SEM indicated that cooling time had a linear correlation with cracks intensity on the granule surface of the corn-based rice analogues. The cooling time optimum in the autoclaving-cooling treatment to increase the resistance starch content (6.27% to 12.28%) of corn-based rice analogues was 4°C for 24 hours. The high content of resistant starch showed that the application of cooling time level at 4°C for 24 hours will improve the functional properties of corn-based rice analogues with sensory characteristics remain favorable to panelists.

**Fig. 2.** Scanning electron micrographs of corn-based rice analogues on various level of cooling time of: (a) 0 hours/control, (b) 12 hours, (c) 24 hours
5. Acknowledgments

The authors would like to thanks to the Directorate of Research and Community Services, the Ministry of Research, Technology and Higher Education, for funding this research through the Higher Education Competitive Research scheme in 2016 and 2017.

6. References

[1] Lockyer S and Nugent AP 2017 Health effects of resistant starch, review Nutrition bulletin 42 10
[2] Raigond P, Ezekiel R and Raigond B 2015 Resistant starch in food: a review Resistant starch in food: a review 95, 1968-1978
[3] Hidayat B, Akmal S, Muslihudin M and Suhada B 2017 Assessment of Corn-Based Rice Analogues Made from Modified Corn Flour and Cassava Starch Which Processed by Granulation Method as Functional Food Food Science and Quality Management 61 19
[4] Kim SK, Kwak JE and Kim WK 2003 A Simple Method for Estimation of Enzyme-Resistant Starch Content Starch/Stärke 55 366
[5] Dundar AN and Gocmen D 2013 Effects of autoclaving temperature and storing time on resistant starch formation and its functional and physicochemical properties Carbohydrate Polymers 97 764
[6] Lehmann U, Jacobasch G, Schmiedl D and Rossler C 2003 Characterization of Resistant Starch Type III from Banana (Musa acuminate) J Food. 47(1) 60
[7] Vasanthan T and Bhatti RS 1998 Enhancement of Resistant Starch (RS3) in Amylomaize, Barley, Field Pea and Lentil Starches Starch/Stärke 50(7) 286
[8] Saguilan AA, Flores-Huicochea E, Tovar J, Garcia-Suarez F, Gutierrez-Meraz F and Bello-Perez L.A. 2005 Resistant Starch-rich Powders Prepared by Autoclaving of Native and Lintnerized Banana Starch: Partial Characterization Starch/Stärke 57 405
[9] Zhao XH and Lin Y 2009 The impact of coupled acid or pullulanase debranching on the formation of resistant starch from maize starch with autoclaving–cooling cycles Eur. Food Res. Technol. 230 179
[10] Hidayat B, Nurbani K and Surfiana 2013 Characterization of Modified Corn Flour Processed with Partial Pregelatinisation Method Proc. Science and Technology Conference (Lampung University Press) p 884
[11] Asp NG, Johansson CG, Hallmer H and Siljestrom M 1983 Rapid enzymic assay of insoluble and soluble dietary fiber J. Agric. Food Chem. 31 476
[12] Englyst HN, Kingman SM and Cummings JH 1992 Classification and measurement of nutritionally important starch fractions Eur. J Clin Nutr. 46 S33
[13] Fabbri ADT, Schacht RW and Crosby GA 2016 Evaluation of resistant starch content of cooked black beans, pinto beans, and chickpeas NFS Journal 3 8
[14] Perdon AA, Siebenmorgen TJ, Buescher RW and Gbur EE 1999 Starch retrogradation and texture of cooked milled rice during storage Journal of Food Science 64(5) 828

Fig. 3. Scanning electron micrographs of corn-based rice analogues on various level of cooling time of: (d) 36 hours (e) 48 hours, and (f) 60 hours
[15] Fredriksson H, Silverio J, Andersson R., Eliasson AC and Åman, P 1998 The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches Carbohydrate Polymers 35 119

[16] Farhat IA, Protzmann J, Becker A, Valles-Pamies B, Neale R and Hill SE 2001 Effect of the extent of conversion and retrogradation on the digestibility of potato starch Starch/Staerke 53(9) 431

[17] Thompson, DB 2001 Strategies for the manufacture of resistant starch Trends in Food Science and Technology 11(7) 245

[18] Fuentes-Zaragoza E, Sánchez-Zapata E, Sendra E, Sayas E., Navarro C, Fernández-López J and Pérez-Alvarez JA 2011 Resistant starch as prebiotic: A review Starch/Staerke 63(7) 406

[19] Alsaffar AA 2011 Effect of food processing on the resistant starch content of cereals and cereal products – a review International Journal of Food Science and Technology 46 455

[20] Haralampu S 2000 Resistant starch—a review of the physical properties and biological impact of RS3 Carbohydrate Polymers 41(3) 285

[21] Gallant DJ, Bouchet B, Buléon A and Pérez S 1992 Physical characteristics of starch granules and susceptibility to enzymatic degradation European Journal of Clinical Nutrition 46 S3

[22] Buleon A, Colona P, Plancho V and Ball S 1998 Starch granules: structure and biosynthesis International Journal of Biological Macromolecules 23 85

[23] Chung H, Lim HS and Lim S 2006 Effect of partial gelatinization and retrogradation on the enzymatic digestion of waxy rice starch Journal of Cereal Science 43 353

[24] Mir JA, Srikaeo K and García J 2013 Effects of amylose and resistant starch on starch digestibility of rice flours and starches International Food Research Journal 20(3) 1329

[25] Zhu L, Liu Q, Wilson JD, Gu M and Shi Y 2011 Digestibility and physicochemical properties of rice (Oryza sativa L.) flours and starches differing in amylose content Carbohydrate Polymers 86(4) 1751

[26] Hsu RJ, Chen H, Lu S and, Chiang W 2015 Effects of cooking, retrogradation and drying on starch digestibility in instant rice making Journal of Cereal Science 65 154

[27] Homayouni A, Amini A, Keshtiban AK, Mortazavian AM, Esazadeh K and Pourmoradian S 2014 Resistant starch in food industry: A changing outlook for consumer and producer Starch/Stärke 66 102

[28] Ashwar BA, Gani A, Wani IA, Shah A, Masoodi FA and Saxena DC 2016 Production of resistant starch from rice by dual autoclaving-retrogradation treatment: Invitro digestibility, thermal and structural characterization Food Hydrocolloids 56 108

[29] Shin SI, Kim HJ, Ha HH, Lee SH, Moon TW 2005 Effect of Hydrothermal Treatment on Formation and Structural Characteristics of Slowly Digestible Non-pasted Granular Sweet Potato Starch Starch/Stärke 57 421