Special Issue: Novel Advances in Agricultural Science and Technology for Sustainable Farming in Tropical Region

Autoclaving Cooling Method for Optimizing Resistant Starch Content and Physicochemical Properties of Modified Tannia Flour

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Abstract | While bacteria-modified tannia (*Xanthosoma sagittifolium* (L.) Schott) flour has enhanced properties, it is open to further improvement. Resistant starch type III is beneficial in the attempts to control healthy body weight, blood sugar level, and digestion. Clarifies Autoclaving-Cooling process in 1 to 3 cycles, this research analyzed tannia starch fermented for 36 h with three microbial starters: *Lactobacillus plantarum*, lactic acid bacteria, and Bimo-CF. A randomized design involving nine treatments and three replications was executed to observe resistant starch content, swelling power, water solubility, color characteristics, and texture. All in all, the review reveals insight inti the Autoclaving-Cooling process boosted the resistant starch content in modified tannia starch from 22.79 % to 24.22 %, reduced the swelling power and water-soluble index from 29 % to 32 %, and toned down the brightness of color.

Received | November 05, 2024; **Accepted** | March 24, 2025; **Published** | April 10, 2025
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Citation | Wira, W., N. Harini, D. Damat, B.Y. Ariadi, E.S. Savitri, N.N. Huu, T.A. Pakarti and I.N. Sobach. 2025. Autoclaving cooling method for optimizing resistant starch content and physicochemical properties of modified tannia flour. *Sarhad Journal of Agriculture,* 39 (Special issue 1): 123-130.
DOI | https://dx.doi.org/10.17582/journal.sja/2023/39/s1.123.130
Keywords | Blue taro, Healhty starch, Improve resistant starch, Prevent diabetes mellitus, Taro kimpul, *Xanthosoma sagittifolium*

Introduction

The study titled autoclaving-cooling method for optimizing resistant starch content and physicochemical properties of modified tannia flour aims to explore the potential of tannia (*Xanthosoma sagittifolium* (L.) Schott) as a functional food ingredient. Tannia is an underutilized tuber crop rich in carbohydrates, yet its applications in food products remain limited. In addition to improved resistant starch, the modification also enhances physicochemical properties in flour (Isra *et al*., 2023; Rojas-Molina *et al*., 2020; Zhang *et al*., 2015; Zheng *et al*., 2020). The advantages of resistant starch (later referred to as RS) for health has been reported in a number of researches among them are Ashwar *et al*. (2016), Lockyer and Nugent (2017), and Perera *et al*. (2010).

Fermentation is crucial for transforming starch, as lactic acid bacteria convert complex starches into simpler sugars and organic acids, enhancing digestibility and nutritional value while developing unique flavors and textures. Understanding fermentation mechanisms is vital for optimizing food production and improving starchy ingredients' properties. The autoclaving-cooling method further enhances RS content by heating starch under high pressure, followed by cooling to promote retrogradation, resulting in a more structured starch. Optimizing this process effectively increases RS content, improving the nutritional value and functionality of food products.

Resistant starch type III, formed through retrograded amylose, is widely used to prevent type 2 diabetes mellitus (T2DM) and obesity (Liu *et al*., 2020). This process requires no chemical treatment, utilizing only high-pressure autoclave heating followed by refrigerator cooling, allowing hot water-suspended starch to retrograde as it cools. Recommended by Bojarczuk *et al*. (2022), Ratnaningsih *et al*. (2020), and Setiarto *et al*. (2018), the autoclaving-cooling method offers flexibility in adjusting raw materials, temperature, duration, and cycles for optimal RS content. Heating above the gelatinization temperature disrupts hydrogen bonds in amylopectin, releasing amylose (Wang *et al*., 2022). While varying autoclaving durations (15 min, 30 min, 1 h, and 2 h) affect RS production (Ashwar *et al*., 2016), the number of cycles has been found to have no significant impact (Simons *et al*., 2018).

Part of cocoyam corm, tannia also known as tannia cocoyam in English and taro kimpul in Indonesian has a variation in chemical compositions of 63 % to 85 % moisture, 4 800 $kJkg^{-1}$ to 5 210 $kJkg^{-1}$ energy (James *et al*., 2013), 0.10 % to 0.11 % fat, 0.42 % to 1.01 % sugar, 13 % to 29 % starch, 0.60 % to 1.18 % dietary fiber, 1.4 % to 3.0 % protein and 0.60 % to 1.3 % ash (Kaushal *et al*., 2015). Researches on modified flour have been performed on tuber crops (Budiarti *et al*., 2022; Damat *et al*., 2021a, b; Setiarto *et al*., 2018; Syafutri *et al*., 2018; Wang *et al*., 2022; Wira *et al*., 2024), confirming its feasibility to be raw material in an assortment of food products. Natural fermentation, enzyme fermentation and acetylated-oxidized-acid thinned modification treatments (Obadina *et al*., 2016) are listed to be methods to produce Modification of Tannia Flour (MOTIF). Meanwhile, Igbabul *et al*. (2014) have confirmed that Lactic Acid Bacteria (LAB) is effective in restraining the growth of spoilage bacteria and pathogens while, at the same time, boosting immune competence and antibiotics production.

Combining autoclaving-cooling process and bacterial administration is deduced to be potential in optimizing the physicochemical properties of MOTIF. Selecting three starters– *Lactobacillus plantarum*, LAB, and Bimo-CF – to work in 36 h fermentation, this study aims to evaluate the RS content, color characteristics, swelling power, water solubility, and texture via Scanning Electron Microscope (SEM).

Materials and Methods

Materials

Tannia sample was gained from a traditional market in Malang, Indonesia (coordinates S 7°59'10.4748" and E 112°38'0.474"). Bimo-CF starter fermented seeds generally used in cassava flour modification was obtained from the Center for the Implementation of Agricultural Instrument Standards in Bogor, Indonesia, while LAB and *L. bulgaricus* starters were provided by IPB University, Bogor, Indonesia.

Preparing the treatments, tannia tubers were ground, washed, and dried to reach the moisture content of 25 %. Amount of 5 mL of each bacterium was added to 100 g sample and fermented for 36 h before autoclave heated (Hirayama HVE 50, Japan) at 120 °C for 15 min and room temperature cooled for 1 h. Samples were further cooled in a refrigerator (Sharp SJ-236 MG-GB/GR, Japan) at 5 °C with varied duration referring to each autoclaving-cooling cycle, and then oven dried (Binder ED53 572 °F, USA).

Methods

Operating randomized nested design, two factors were determined accordingly. Factor one (S) was based on three starter types, covering S1 (*L. bulgaricus*), S2 (LAB), and S3 (Bimo-CF starter). Factor two (F) referred to three cycles applied in Autoclaving-Cooling process, comprising F1 (one cycle), F2 (two cycles), and F3 (three cycles). All combinations are as shown in Table 1.

The two research factors generated nine treatment combinations, of each were taken three times, to make a total of 27 samples.

The procedure for testing resistant starch using the Agilent Cary 60 UV-Vis involves dissolving the starch sample in a buffer solution, measuring the absorbance of the solution in a cuvette, and analyzing the data to calculate the concentration of resistant starch based on the measured absorbance.

Table 1: *Detail combination of the experiment.*

Code	Treatment
B1C1	*L. bulgaricus* AC one cycles
B1C2	*L. bulgaricus* AC two cycles
B1C3	*L. bulgaricus* AC three cycles
B2C1	Lactic acid bacteria AC one cycles
B2C2	Lactic acid bacteria AC two cycles
B2C3	Lactic acid bacteria AC three cycles
B3C1	Bimo-CF starter AC one cycles
B3C2	Bimo-CF starter AC two cycles
B3C3	Bimo-CF starter AC three cycles

The formula for calculating resistant starch (RS) typically involves measuring the concentration of glucose produced after treatment with specific enzymes. Here is the basic formula commonly used to calculate the resistant starch content:

Resistant starch calculation Equation 1:

$$RS = \frac{(CxV)}{W} \times 100 \quad ....(1)$$

Where; RS= Resistant starch content (in g/100 g of sample), C= Concentration of glucose measured (in mg/mL), V= Volume of the solution used for measurement (in mL), W= Weight of the sample (in g).

This formula allows for the determination of the resistant starch content based on the glucose released during the enzymatic digestion process.

Swelling power and water-solubility analyses

Following the procedure explained by Jia *et al.* (2023), the liquid supernatant of each sample was transferred to a container, weighed, and heated to find the constant weight (W1). The sediment left in the test tube was also weighed (Ws)15, and the water content was analyzed using a moisture analyser (OHAUS MB25, USA). Swelling power rates were determined by Equation 2 (Budiarti *et al.*, 2022), while water-soluble indexes were assessed with Equation 3 (Antille *et al.*, 2023).

$$SP = \frac{Ws}{(0.1 \times (100\,\% - WSI))}\left(\frac{g}{g}\right) \quad ...(2)$$

$$WSI = \frac{W1}{0.1} \times 100\,\% \quad ...(3)$$

Color characteristics

Employing Lab Color Flex EZ spectrophotometer (Rojas-Molina *et al.*, 2020), the chromameter was calibrated to standard white before utilizing Hunter color system of L* (white), a* (red), b* (yellow) (WR10 Colorimeter, Japan).

SEM analysis

Substances as small as 80 mesh sieve can be revealed by SEM to recognize (Mohammed and Abdullah, 2018), and it has become the reason why texture detail and complexity of each sample were also scrutinized through SEM images (FEI Inspect-S50, USA).

The compiled data on the tested physical and chemical properties of MOTIF were charted in Microsoft Excel before carried out through ANOVA (Setyobudi *et al.*, 2024; Tonda *et al.*, 2022). LSD test at 5 % level followed should be significantly different or at 1 % level if very significantly different (Damat *et al.*, 2024).

Results and Discussion

Resistant starch

The combination of bacterial starter types and autoclaving-cooling cycles applied on MOTIF manifested RS contents as recorded in Figure 1.

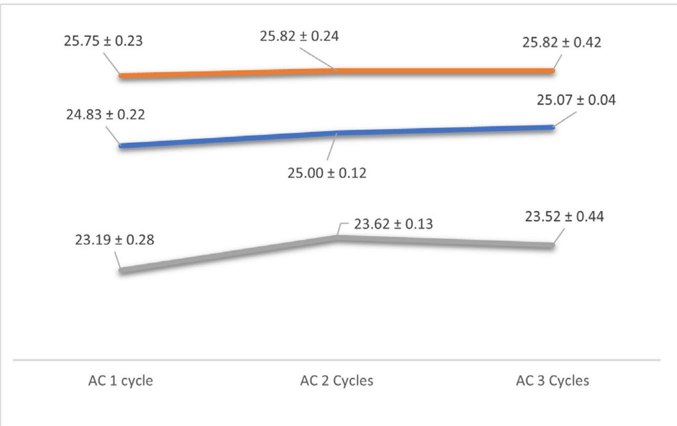

Figure 1: *Resistant starch levels.*

Figure 1 shows that LAB-three cycles treatment has the highest boost (25.82 %), followed by *L. bulgaricus*-three cycles (25.07 %) and Bimo-CF-two cycles (23.52 %). This study confirmed that the autoclaving-cooling with cycles method had significant effect with a 95 % confidence level in increasing the levels of resistant starch in MOTIF. Autoclaving-cooling causes retrogradation of amylose fraction over a certain period, affecting starch digestibility.

In general, while the RS content of MOTIF with autoclaving-cooling process is higher by 22.79 % to 24.22 % compared to one without the process (Wira *et al*., 2024), the samples involving bacteria contain 5.7 % more RS when undergoing it. Mutlu *et al*. (2018) underlined an increase of RS content in corn flour by 2 % after three autoclaving-cooling cycles, while Zheng *et al*. (2020) showed that autoclaving optimized RS content in rice grain through its theoretical value to go as high as 17.57 %. Isra *et al*. (2023) have confirmed that Autoclaving-cooling is a starch physical modification technique widely used to analyze the increase in resistant starch levels in foodstuffs, The result showed that the autoclaving-cooling method had a significant effect in increasing level resistant starch and prebiotic properties (SDM 6.633; 95 % CL:5.286 to 7.980; P<0.001) making a single cycle sufficient for maximum RS production and no additional RS is formed in the next cycles. However, branched amylopectin (DP 25 to 36) of 6.24 % in wheat of two (corn and bran n.d.) can be hydrolyzed up to 15.64 % by the pullulanase enzyme from *Streptococcus thermophilus* (Budiarti *et al*., 2022; Zabar *et al*., 2008).

Swelling power and water solubility

The swelling power rates of MOTIF with bacterial starter and autoclaving-cooling cycle combined treatments are listed in Figure 2.

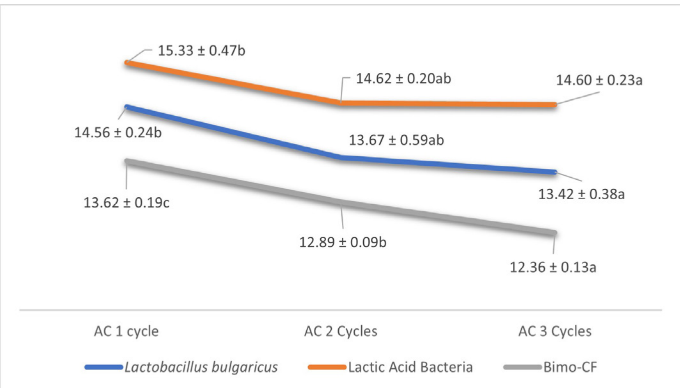

Figure 2: *Swelling power.*

Figure 2 reveals that the swelling power is ranged from 12.36 g to 15.33 g, with LAB-one cycle treatment holding the highest value (15.33 g) and Bimo-CF-three cycles of the lowest value (12.36 g), while ANOVA test came out with significant difference (P < 0.05). Autoclaving-cooling process decreases MOTIF's swelling power from the average of 18.37 g to 13.90 g or down to 32 % when compared to unprocessed one (Wira *et al*., 2024). It is still higher than the swelling power of sweet potato, of which varieties are ranged insignificantly between 3.40 (gg^{-1}) and 3.67 (gg^{-1}). Dewayani *et al*. (2023) have confirmed that modified taro starch has higher swelling power than cassava flour due to less intermolecular association and lower amylose content.

Water-soluble index (WSI) is determined by the amount of dry solids found after evaporating the sample's supernatant in water absorption test and delivered as a percentage of dry solids in 2.5 g of sample (Chisenga *et al*., 2019). The water-soluble index (WSI) rates of MOTIF with bacterial starter and autoclaving-cooling cycle combined treatments are depicted in Figure 3.

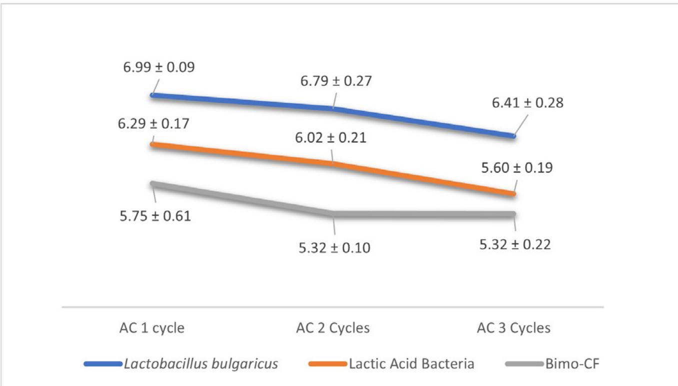

Figure 3: *Water-soluble index.*

Spanning between 5.32 % and 6.99 %, the highest WSI value (6.99 %) of MOTIF was found in *L. bulgaricus* one cycles treatment with 36 h fermentation, while the lowest (5.32 %) was in *L. bulgaricus* one cycles treatment with 36 h fermentation with no significant difference (P< 0.05). Autoclaving-cooling process decreases MOTIF's WSI from the average of 7.78 % to 6.05 % or down to 29 % when compared to unprocessed one (Wira *et al*., 2024). Nevertheless, its water solubility is generally higher compared to those of cassava flour and sweet potato flour. Specifically on sweet potato flour of assorted varieties, the WSI rates are insignificantly ranged from 8.61 % to 9.57 % (Kusumayanti *et al*., 2015). However, WSI of MOTIF is lower when compared to one of modified Bangka Sago starch *Metroxylonsagu* Rottb (Syafutri *et al*., 2018).

Starch solubility is influenced by inter-associative forces in amorphous and crystalline starch domains as well as the presence of other components such as phosphorus (Ofori *et al*., 2020). The same study also emphasized that fermentation in starch leads to gelatinization, forcing starch granules to swell and burst and then hydrated.

Color characteristics (L, a*, b*)*

The combination of bacterial starter types and autoclaving-cooling cycles applied on MOTIF presented color characteristics as illustrated in Table 2.

Table 2: *Color test (L*, a*, b*).*

Code	Color
B1C2	L* 84.65; a* 7.50; b* 15.33
B1C3	L* 84.88; a* 7.49; b* 15.25
B2C1	L* 84.83; a* 7.51; b* 15.24
B2C2	L* 84.80; a* 7.57; b* 15.29
B2C3	L* 84.58; a* 7.67; b* 15.69
B3C1	L* 84.89; a* 7.54; b* 15.24
B3C2	L* 84.41; a* 7.55; b* 15.61
B1C1	L* 84.56; a* 7.65; b* 15.84

From Table 2, it can be deduced that the highest average value (84.89) was found in Bimo-CF- one cycle treatment, while the lowest average value (84.41) was in Bimo-CF two cycles. Autoclaving-cooling process has made the treatments generally reduced brightness when compared to MOTIF without the process. A* color analysis indicated that LAB-three cycles treatment had highest average value (7.67) and *L. bulgaricus*-three cycles with the lowest average value (7.49). B* color analysis came out with Bimo-CF-three cycles at the highest average value (15.84) and LAB-one cycle as well as Bimo-CF-one cycle at the lowest average value (15.24). For all L*, a*, and b* components, this color indicated no significant difference. It is therefore concluded that starter and cycle factors involved have no effect to the color test (Hayati *et al*., 2020).

Scanning electron microscope (SEM)

Scanning Electron Microscopy (SEM) is one of the most widely used methods to analyze the imaging characteristics of micro and nano particles in solid objects. Its ability to expose a minuscule article at the resolution of 10 nm equals 100 Å is principal.

The combination of bacterial starter type and Autoclaving Cooling cycles on the Scanning Electron Microscope (SEM) of Modified Tannia Flour is presented in Figure 4.

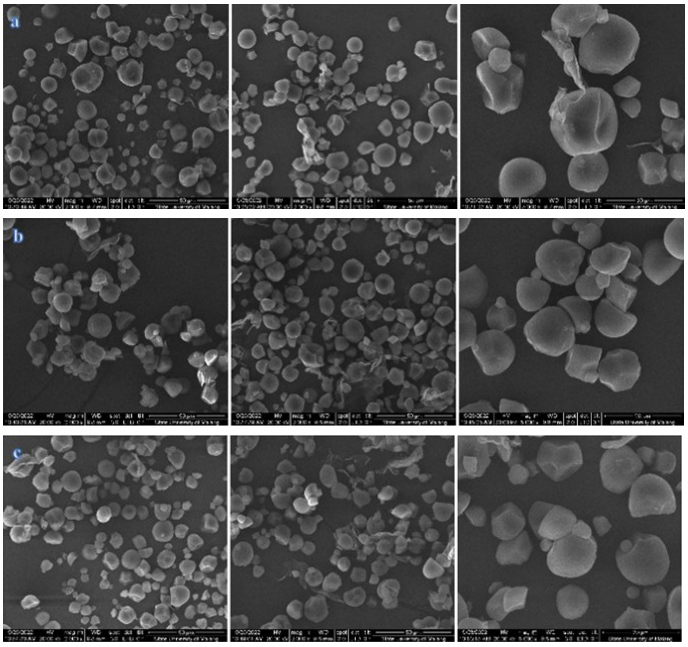

Figure 4: *Scanning Electron Microscopy (SEM) images of modified tannia flour: (a) L. bulgaricus-two cycles; (b) Lactic acid bacteria-two cycles; (c) Bimo-CF-two cycles.*

Figure 4 represents growth rings (Figure 4), where the radial organization of the amylopectin within such structures is thought to cause optical polarization due to the visible optical polarization to be in the order of the wavelength of the visible light (100 nm to 1 000 nm) (Pérez and Bertoft, 2010). At this level, the structure of LAB-two cycles treatment looks tighter compared to the other two. Most of the starch granules consist of alternating amorphous and semi-crystalline shells with a thickness of between 100 nm and 400 nm.

Conclusions and Recommendations

This study confirmed that the autoclaving-cooling with cycles method had significant effect with a 95 % confidence level in increasing the levels of resistant starch in MOTIF. The highest resistant starch total value is of 25.82 %, found in tannia flour modified with lactic acid bacteria and two cycles of autoclaving-cooling process. This finding brings a new prospect to light where modified tannia flour containing high resistant starch should be able to serve as a substitute in probiotic products, such as analog rice, aiming to lower the consumer's glycemic index.

Acknowledgements

The authors would like to express their appreciation and gratitude to Dr. Roy Hendroko Setyobudi for

his assistance in editing, arranging, and guiding the submission and publication of this manuscript.

Novelty Statement

Previous studies (Wahyuni *et al.*, 2020; Ratnaningsih *et al.*, 2020; Setiarto *et al.*, 2018) have documented that modification of tannia flour and tubers through fermentation by using a number of bacteria has been widely researched; however, attempts in improving physicochemical quality and resistant starch type III in tannia cocoyam using the autoclaving-cooling method has not been carried out.

Authors Contribution

Wirawan Wira: Designed the study, conceptualized and elaborated the intellectual content, performed literature search, manuscript preparation, and manuscript revision.
Noor Harini, Damat Damat, and Bambang Yudi Ariadi: Research supervision and manuscript review.
Evika Sandi Savitri and Nguyen Ngoc Huu: Elaborated on the intellectual content, performed the literature search and manuscript review.
Budi Santosa: Data analysis and visualization.
Indah Nur Sobach: Administration, Turnitin, and Grammarly check.
Trias Agung Pakarti: Formatted manuscript and English edited.
All authors have read and approved the final manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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