

Article

A Pressure Cook–Cool Process with Coconut Oil and Thai Herbs Enhances Resistant Starch, Antioxidant Activity, and Prebiotic Potential in Khao Dawk Mali 105 (KDML 105)

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Abstract

Khao Dawk Mali 105 (KDML 105) is a widely consumed Thai staple, but conventional cooking yields rapidly digestible starch with limited functional health benefits. This study aimed to formulate pressure-cooked KDML 105 rice as a functional food using extra-virgin coconut oil, citric acid, and Thai herbs (butterfly pea flower or pandan leaf juice). Rice was pressure-cooked, cooled at room temperature, stored at 4 °C for 24 h, and frozen at −20 °C to promote resistant starch (RS) formation. RS content increased from 0.65 g 100 g^{−1} DW in control rice to 1.39 g 100 g^{−1} DW with coconut oil, and to 2.08 and 1.80 g 100 g^{−1} DW when citric acid plus pandan or butterfly pea juice were added, respectively. Coconut oil-treated samples showed higher antioxidant activity in DPPH and FRAP assays, while formulations with butterfly pea or pandan juices additionally reduced ABTS^{•+} radicals. Prebiotic potential was evaluated in vitro using *Levilactobacillus brevis*, *Lactobacillus bulgaricus*, and *Streptococcus thermophilus* grown in MRS medium with rice extracts. All formulations enhanced probiotic growth versus control, indicating that this pressure cook–cool process can produce a ready-to-eat functional rice with improved RS, antioxidant capacity, and probiotic support.

Keywords: resistant starch; Khao Dawk Mali 105; butterfly pea; coconut oil; probiotic

1. Introduction

Rice (*Oryza sativa* L.) plays an important role in the world's food security—supplying about one-fifth of human dietary energy—and remains the principal staple across Southeast Asia and globally. It is an economic crop of great importance in Thailand. Especially Thai fragrant rice, which is widely recognized internationally. Both in terms of consumption quality and unique aroma, one of the famous and highly valued rice varieties is Khao Dawk Mali 105 rice [1].

Khao Dawk Mali 105 rice (KDML105) is one of the varieties of *Oryza sativa* L. suitable for planting in arid areas. It does not require a lot of water, is resistant to acid and saline soils, and responds well to organic fertilizers. Carbohydrates, as the main components in rice, are digested to form sugars, which are used as a source of energy [2]. Most conventional cooking methods still deliver highly digestible starch with limited functional attributes. As consumer demand shifts toward foods that couple nutrition with clear health functionality, rice is an ideal canvas for clean-label reformulation. Process–structure



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relationships in rice can be modified to lower digestibility, increase resistant starch (RS), and enhance antioxidant capacity. RS—the fraction escaping small-intestinal digestion—is increasingly engineered in staple foods. In rice systems, two routes dominate: RS3 from amylose retrogradation during cooling/storage and RS5 from V-type amylose–lipid inclusion complexes [3]. Rice studies show that lipid complexation can reduce digestibility and alter colonic fermentation profiles [4], while citric acid modification shifts digestibility fractions [5] and thermal behavior via formation of ester linkages [6,7].

Resistant starch (RS) is normally present in most of the starchy foods. There are many factors affecting RS content, for example, quality of native starch, such as its amylose: amylopectin ratio, compactness, crystallinity, etc. It serves as a dietary fiber, being a starch portion that remains undigested and unabsorbed in the small intestine of healthy people, but is fermented in the large intestine, yielding short-chain fatty acids with health advantages. In addition to these factors, the digestibility of starch is influenced by the behavior and composition of food, cooking methods, temperatures, post-cooking storage, and the interaction of starch with proteins, lipids, and other carbohydrates [8].

Antioxidant outcomes in cereal matrices are shaped by extraction chemistry and are routinely quantified by DPPH, ABTS, FRAP, and total phenolics/flavonoids (TPC/TFC) using validated colorimetric methods. Antioxidants in the body have several mechanisms to inhibit free radicals, including free radical trapping, inhibition of electron-deficient oxygen, binding to metals with oxidative properties, inhibiting reactions that generate free radicals' synergism, and inhibition of enzyme activity [9,10].

Thai herbs provide polyphenols such as Pandan leaves (*Pandan leaves amaryllifolius* Roxb), Asian pigeon wings (*Clitoria ternatea* L.). There are good sources of bioactive compound providing antioxidant that may modulate redox behavior and microbial growth [11]. This study integrates these levers to generate a practical rice cooking prototype method and evaluates their prebiotic potential in lactic cultures. We selected three benchmark lactic cultures with complementary metabolisms and industrial relevance—*Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Levilactobacillus brevis*.

S. thermophilus and *L. bulgaricus* are thermophilic, homolactic starters with rapid glycolysis (ideal for testing how a prebiotic paces sugar release and moderates acidification), whereas *L. brevis* is obligately heterofermentative, utilizing a broader sugar spectrum (including pentoses) and producing lactate, acetate/ethanol and CO₂—useful for detecting prebiotic support across diverse saccharolytic routes. The well-characterized proto-cooperation between *S. thermophilus* and *L. bulgaricus* (exchange of amino acids, peptides, and formats) makes their growth kinetics highly sensitive to carbon flow, nitrogen availability, and redox buffering—key dimensions that prebiotics are meant to influence. In 2024, Poomsa-ad et al. [12] studied pressure cooking, extra-virgin coconut oil, and 2% citric acid elevate resistant starch concentrate on %RS optimization across cultivars and processing parameters; our work extends to antioxidant capacity (ABTS/FRAP/DPPH; TPC/TFC) and probiotic growth (*S. thermophilus*, *L. bulgaricus*, *L. brevis*), enabling functional product design beyond digestibility control.

This study aims to develop a ready-to-eat KDML 105 platform that enhances resistant starch (RS) via pressure cook-cool processing and wax with extra-virgin coconut oil enabled amylose–lipid complexation, and integrates traditional Thai herbs (pandan, butterfly pea) to tune antioxidant capacity. We then link structure to function by quantifying strain-specific probiotic responses—*S. thermophilus*, *L. bulgaricus*, and *L. brevis*. This study demonstrates that the optimized processing and formulation not only enhanced resistant starch and antioxidant activity but also promoted probiotic growth, providing a practical and economical route for developing ready-to-eat functional rice products to commercial RS-rice in the future.

2. Materials and Methods

2.1. Preparation of Control Samples

KDML 105, serving as the control, was immersed in 250 mL of reverse osmosis water in pressure rice cookers (HOMEMATE, Model: HOM-12LC62, Ningbo City, Zhejiang Province, China) and subsequently cooked under pressure (13 psi or 90 kPa) at 121 °C for 15 min. Subsequently, the cooked rice was placed in a refrigerator and maintained at 4 °C overnight; the rice samples were stored at −20 °C until resistant starch (RS) analysis.

2.2. Preparation of Resistant Starch Cooked Rice Samples

According to Petty patent number 2203002265, Thailand, two hundred grams of KDML 105 rice were thoroughly combined with either 5 mL of extra-virgin coconut oil (Treatment 1), 5 mL of extra-virgin coconut oil, 5 mL of 2% citric acid, and 250 mL of pandan leaf juice (Treatment 2), or 5 mL of extra-virgin coconut oil, 5 mL of 2% citric acid, and 250 mL of butterfly pea flower juice (Treatment 3). The mixture was then soaked for 5 min and subsequently cooked in a pressure rice cooker at 13 psi (90 kPa) and 121 °C for 15 min. Subsequently, the cooked rice was placed in a refrigerator at 4 °C overnight, and the rice samples were stored at −20 °C until RS analysis.

2.3. Preparation of Starch Cooked Rice Samples

A sample of roughly 50 g from each rice variety, after being stored at −20 °C, was pulverized in a grinding mill or electric meat mincer to achieve a particle size of approximately 4.5 mm, then screened through a 1.0 mm sieve, transferred to plastic bags, and stored in a humidity-controlled jar until analysis. The moisture content of dry samples was assessed using AOAC Method 925.10 [13], whereas that of fresh samples was evaluated using lyophilization followed by oven drying, in accordance with AOAC Method 925.10 [13].

2.4. RS Analysis

The RS analysis was performed according to Poomsa-ad et al. The RS content of all samples was estimated using a Megazyme kit (Megazyme, K-RSTAR 08/11, Wicklow, Ireland) with modifications. Briefly, a 100 mg rice flour sample was digested with pancreatic α -amylase containing amyloglucosidase in a 50 mL conical flask at 37 °C and then shaken for 16 h, followed by the addition of 4 mL absolute ethanol (Merck, Darmstadt, Germany) with vigorous stirring. After that, centrifugation at 3000 rpm for 10 min, and suspended in 2 mL 50% ethanol with vigorous stirring, followed by adding 6 mL 50% ethanol and stirring. And then centrifugation at 3000 rpm for 10 min. The supernatant was decanted, and the method was repeated with the residue. The supernatant was decanted and excess liquid was drained out by inverting the tubes on absorbent paper. The pellet was suspended in 2 mL KOH (2 M) (MP Biomedicals) in a test tube and stirred in an ice bath for 20 min. Then, 8 mL of 1.2 M sodium acetate buffer was added while stirring, immediately followed by the addition of 0.1 mL of amyloglucosidase (3300 U/mL). After shaking, the tubes were incubated at 50 °C for 30 min, followed by centrifuging at 3000 rpm for 10 min. Aliquots of supernatant measuring 0.1 mL were transferred to glass tubes in duplicates, mixed well with 3 mL of GOPOD reagent, and incubated at 50 °C for 20 min. The absorbance was recoded at 510 nm using a reagent blank as a reference. The RS content was calculated according to the manufacturer's instructions. The experiment was conducted with triplicates. The data was presented on a dry weight basis.

2.5. Preparation of Rice Extraction

The cooked rice according to prepare, cook, and cool in each treatment, were finely ground and subjected to water extraction. The rice samples were extracted using a Soxhlet apparatus at 170 °C for 6 h. After extraction, the resulting solutions were concentrated by removing the solvent under reduced pressure using a rotary evaporator. The obtained crude extracts were then dried in a hot-air oven at 50 °C for 12 h prior to further analyses.

2.6. DPPH Radical Scavenging Test

This assay was conducted as described by Pakdeenarong et al. [14]. Preliminary kinetic and optimization tests were conducted to establish assay conditions. Absorbance measurements confirmed that the reaction reached a stable plateau at 30 min, which was used as the incubation time. A 0.1 mM DPPH solution and sample concentrations of 1–500 µg/mL were selected as optimal for generating reliable, linear responses. The radical scavenging capacities of all samples were tested by reacting with the DPPH radical. The 100 µL of rice extraction or standard solution was mixed with 900 µL of 0.1 mM DPPH radical solution. The absorbance of the three replicate mixtures was measured at 515 nm after 30 min of dark reaction using a UV-Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The % inhibition was calculated as follows:

$$\% \text{ Inhibition DPPH radical scavenging} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100 \quad (1)$$

All the chemicals used were obtained from Sigma-Aldrich, St. Louis, MO, USA.

2.7. ABTS Radical Cation Scavenging Activity

The ABTS test was used [14]. The 100 µL solution of all samples was mixed with 900 µL of ABTS^{•+} solution (prepared 0.7 mM ABTS^{•+}, 2.45 mM K₂S₂O₈ in distilled water reacted in darkness for 16 h), and it was allowed to react for 6 min and then measured at A734 nm using UV-Vis spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) in three replicates. Trolox was used as a reference. Radical scavenging was calculated using this equation:

$$\% \text{ Inhibition ABTS radical scavenging} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100 \quad (2)$$

All the chemicals used were obtained from Sigma-Aldrich, MO, USA.

2.8. Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing ability was measured as described by Pakdeenarong et al. [14]. Briefly, FRAP was mixed with ethanol of varied concentrations of all rice flour sample extracts and left for 15 min at 37 °C. The mixtures were measured at 595 nm. Trolox was used as the reference. All the chemicals used were obtained from Sigma-Aldrich, MO, USA.

2.9. Determination of Total Phenolic Content

Total phenolic content (TPC) was assayed as described by Pakdeenarong et al. [14]. Briefly, all samples were mixed with 500 µL of 0.2 N Folin–Ciocalteu reagent and Na₂CO₃, left for 30 min, and then measured at 765 nm. Gallic acid equivalent was used as the reference. All the chemicals used were obtained from Sigma-Aldrich, MO, USA.

2.10. Determination of Total Flavonoid Content

As explained by Pakdeenarong et al. [14], the aluminum chloride assay was conducted to measure total flavonoid concentration (TFC). The mixture of 100 µL of each sample, 500 µL of 5% w/v NaNO₂, and 400 µL of 10% w/v AlCl₃ was reacted for 15 min. The

absorbance was measured in triplicate at 415 nm yielded quercetin equivalent (QE) in mg quercetin dry weight. All the chemicals used were obtained from Sigma-Aldrich, MO, USA.

2.11. In Vitro Prebiotic Effect

Each rice sample (2 g) was included in a carbon-free MRS medium (100 mL) as the sole carbon source at a final concentration of 2.0% (*w/v*). An equivalent concentration of glucose (2%) was incorporated as a positive control, while an additional glucose-free MRS medium containing inulin prebiotic (Krungthepchemi Co., Ltd., Bangkok, Thailand) at 2% *w/v* served as another positive control. All media were autoclaved at 121 °C for 15 min. The concentrations of *Levilactobacillus brevis* TBRC 3003 bacteria (NBRC 108361), *S. thermophilus* and *L. bulgaricus* in each medium were adjusted to log 7.50 CFU/mL, and the *L. brevis* strain was cultivated at 37 °C for 72 h. At 0, 24, 48 and 72 h, each culture sample was taken for numerical bacterial population count.

A conventional plate counting technique was employed for enumeration [15]. In summary, the samples were adequately diluted, and bacterial cells were completely disseminated into individual cells. One hundred microliters of diluted sample solution was inoculated onto the MRS agar plate. Each individual cell proliferated and multiplied, resulting in a colony discernible to the human eye, with each plate containing 30 to 300 colonies. A solitary colony should correspond to individual cells in the initial sample. The colonies were enumerated, and the bacterial count was determined based on the dilution factor and the volume of the inoculum sampled. The results were presented as log CFU/mL.

2.12. Statistical Analysis

A one-way analysis of variance (ANOVA) was conducted using SPSS version 20.0 (IBM Corp., Armonk, NY, USA) to identify significant differences among treatment groups. The significance differences between treatments and the control were determined using Tukey's HSD test ($p < 0.05$).

3. Results

3.1. Effect on Resistant Starch Content

The findings indicated that the RS content of cooked KDML 105, serving as a control, was the lowest at 0.64 ± 0.07 g per 100 g on a dry basis. The KDML 105 rice, when supplemented with extra-virgin coconut oil during cooking in a pressure cooker, yielded an elevated RS content of 1.39 ± 0.08 g per 100 g on a dry basis. Furthermore, the juice of pandan leaves demonstrated the greatest resistant starch content at 2.08 ± 0.06 g per 100 g on a dry basis. The RS content of KDML 105 cooked with butterfly pea flower juice was 1.8 ± 0.21 g per 100 g on a dry basis. This outcome demonstrates that the combination of extra-virgin coconut oil and herbs increase the percentage of resistant starch (Figure 1). The amalgamation of extra-virgin coconut oil and herbal juice significantly affected the antioxidant capacity of KDML 105 rice transformed into resistant starch.

This study showed that extra-virgin coconut oil supplemented with pandan leaf juice enhances resistant starch property. The results agreed with supporting RS3-retrogradation and RS5-amylose–lipid complexation synergy. Composition-aware, accessible input, pressure-cooker-ready route to antioxidant-rich, lower-digestibility rice products are given by these findings.

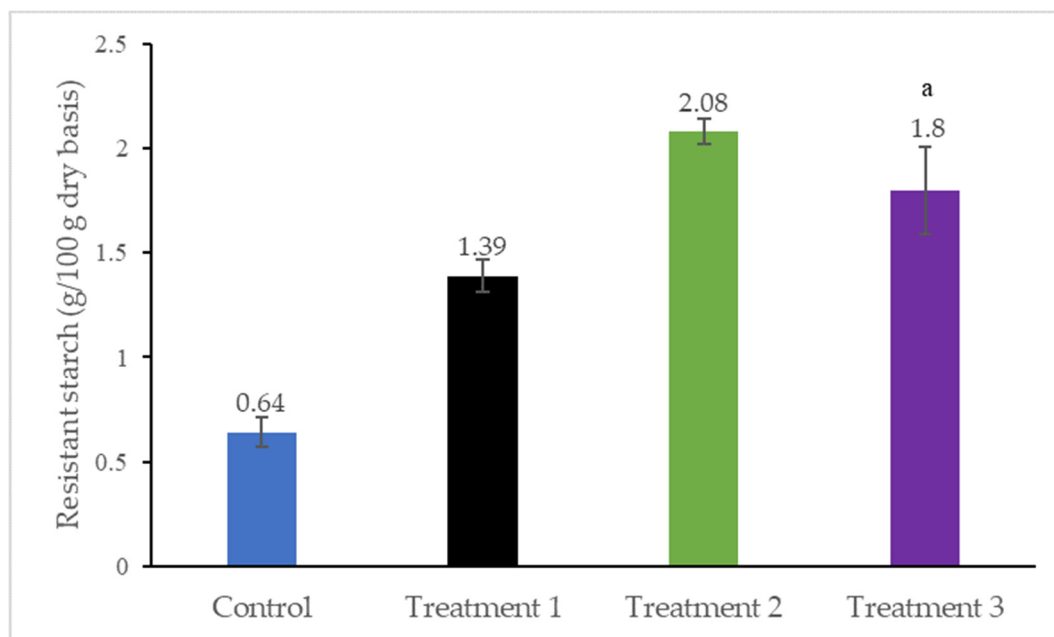


Figure 1. Effect of extra-virgin coconut oil and herbs on antioxidant activity. Values with different letters are significantly different at $p < 0.05$. The results are presented as mean $\pm$ standard deviation. Control = KDML 105 only; Treatment 1 = KDML 105 and extra-virgin coconut oil; Treatment 2 = KDML 105, extra-virgin coconut oil, 2% citric acid and pandan leaves juice; Treatment 3 = KDML 105, extra-virgin coconut oil, 2% citric acid and butterfly pea flower juice.

3.2. Effect on Antioxidant Property Changes

Table 1 summarizes the effects of the different treatments on antioxidant activity and phenolic/flavonoid contents of KDML 105 rice. All treatments showed similar DPPH radical-scavenging activity, with IC_{50} values ranging from 1.58 to 2.01 mg/mL and no significant differences ($p < 0.05$) compared with the control.

Table 1. Effect of RS to increase antioxidant activity.

Samples	IC_{50} DPPH (mg/mL)	IC_{50} ABTS (mg/mL)	FRAP (mg TE/g DW)	TPC (mg GAE/g DW)	TFC (mg QE/g DW)
KDML 105 (Control)	1.72 ± 0.05 ^a	1.13 ± 0.01 ^b	2.82 ± 0.04 ^c	9.10 ± 0.15 ^d	5.38 ± 0.11 ^d
Treatment 1	1.58 ± 0.08 ^a	1.28 ± 0.01 ^a	4.30 ± 0.04 ^a	47.77 ± 0.75 ^a	16.09 ± 0.42 ^a
Treatment 2	2.01 ± 0.16 ^a	0.91 ± 0.03 ^c	0.83 ± 0.03 ^d	14.01 ± 1.75 ^c	6.71 ± 0.16 ^c
Treatment 3	1.72 ± 0.04 ^a	0.54 ± 0.02 ^d	3.63 ± 0.07 ^b	22.41 ± 1.39 ^b	9.79 ± 0.21 ^b

Values with different letters in the columns are significantly different at $p < 0.05$.

In contrast, ABTS^{•+} scavenging, TPC, and TFC were strongly influenced by formulation. Treatment 3 (KDML 105 with extra-virgin coconut oil, 2% citric acid, and butterfly pea juice) exhibited the strongest ABTS^{•+} radical-scavenging activity, with the lowest IC_{50} (0.54 ± 0.02 mg/mL), significantly lower than all other samples. Treatment 2 (pandan juice) showed intermediate ABTS activity (0.91 ± 0.03 mg/mL), while the control and Treatment 1 (extra-virgin coconut oil only) displayed weaker ABTS responses (1.13 ± 0.01 and 1.28 ± 0.01 mg/mL, respectively).

FRAP values did not differ significantly among samples (2.82–4.30 mg TE/g DW). However, phenolic and flavonoid contents increased markedly with supplementation. Treatment 1 had the highest TPC (47.77 ± 0.75 mg GAE/g DW) and TFC (16.09 ± 0.42 mg QE/g DW), followed by Treatment 3, Treatment 2, and the control. Overall, incorporat-

ing extra-virgin coconut oil, with or without herbal juices, substantially enhanced the polyphenol content and ABTS^{•+} scavenging capacity of KDML105 rice.

3.3. Effect on Probiotic Growth

All probiotic strains—*Levilactobacillus brevis* TBRC 3003, *Lactobacillus bulgaricus* TISTR 451, and *Streptococcus thermophilus* TISTR 894—were capable of utilizing the resistant starch (RS)-enriched rice media as a carbon source (Figures 2–4).

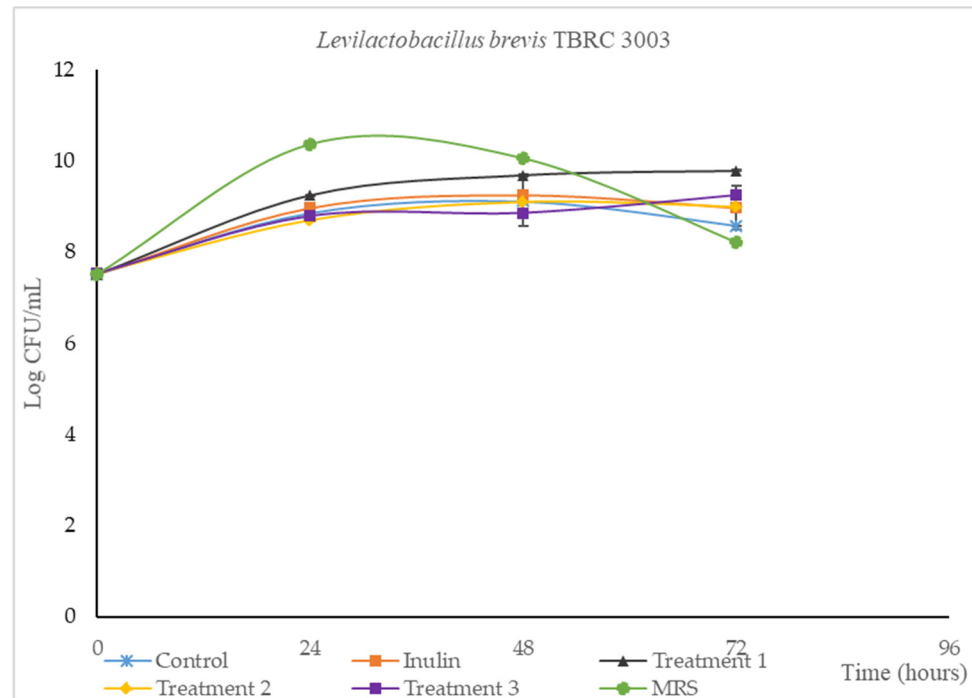


Figure 2. Growth curve of *L. brevis*. The results are presented as mean $\pm$ standard deviation.

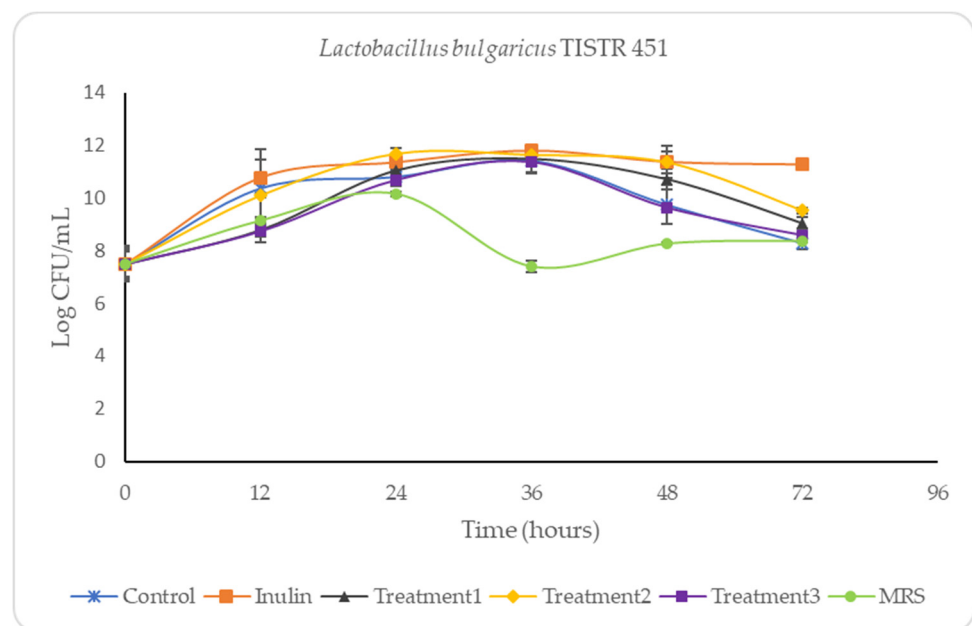


Figure 3. Growth curve of *L. bulgaricus*. The results are presented as mean $\pm$ standard deviation.

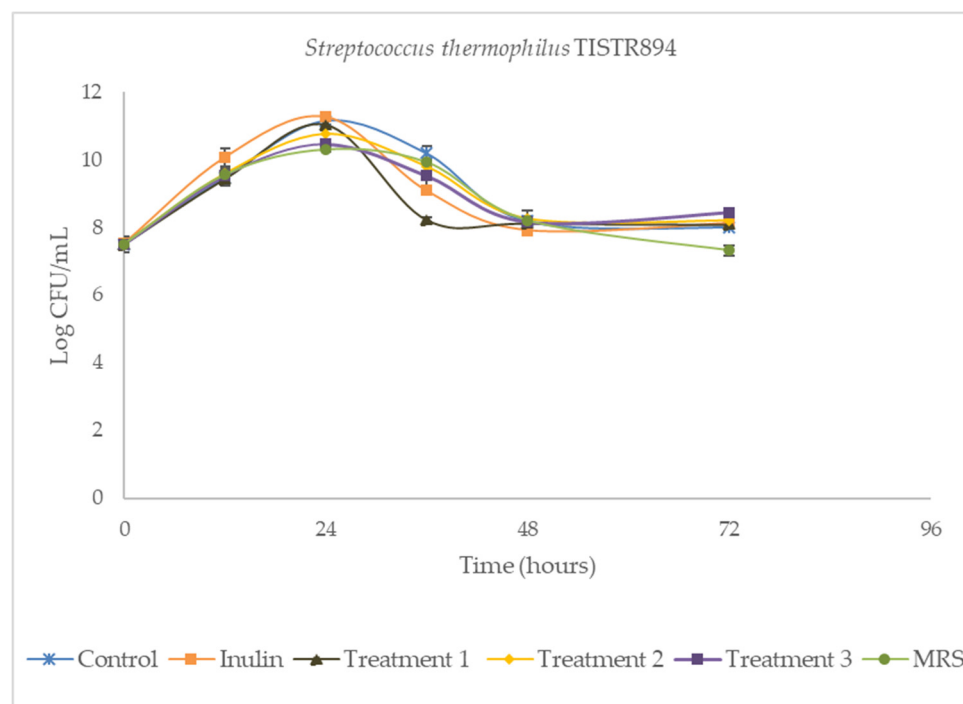


Figure 4. Growth curve of *S. thermophilus*. The results are presented as mean $\pm$ standard deviation.

L. brevis achieved higher mid- to late-phase growth in all RS-rice media compared to the control MRS medium at 48 h, with plate counts confirming this advantage despite noisier OD600 traces due to CO₂ production.

L. bulgaricus reached higher 48 h viable counts in RS-rice media than in MRS. The most substantial growth was observed in the medium containing extra-virgin coconut oil and 2% citric acid mixed with butterfly pea flower juice. This was followed by media containing extra-virgin coconut oil with pandan leaves juice and extra-virgin coconut oil alone. Growth curves in RS-rice showed a short lag phase (0–6 h), a steep log phase (6–24 h), and stable high viability thereafter, whereas growth in MRS plateaued earlier and declined more sharply in the late phase.

S. thermophilus exhibited the most pronounced growth enhancement across all treatments relative to the MRS control. The strain showed a short lag phase and entered an extended log phase (6–30+ h), maintaining stable, high viability through 48 h in the RS-rice matrices.

4. Discussion

This study delineates a clear trade-off between RS maximization and antioxidant potency in KDML 105 rice systems. Consistent with Vidana Gamage et al. [16], who reported that anthocyanin-rich butterfly pea exhibits strong ABTS and FRAP activity due to its highly acylated ternatin pigments, while pandan leaf juice contains more modest levels of extractable phenolics, our results showed lower ABTS^{•+} IC₅₀ values for butterfly pea matrices than for pandan under identical extraction and assay conditions. Although pandan leaves (*P. amaryllifolius*) do possess phenolics and antioxidant activity, their typical TPC and ABTS/DPPH values are lower than those of intensely pigmented botanicals such as butterfly pea when extracted using food-grade methods. Pandan is therefore better characterized as aroma-centric—largely due to 2-acetyl-1-pyrroline—rather than pigment-rich [17].

Among the KDML 105 formulations evaluated, the combination of extra-virgin coconut oil with butterfly pea flower juice (RS3) produced the strongest ABTS activity, whereas

extra-virgin coconut oil alone contributed most prominently to FRAP activity. In contrast, pandan leaf juice produced only modest FRAP values but achieved the highest percentage of RS. Butterfly pea flowers are rich in acylated anthocyanins (ternatins), which create a strongly antioxidant, low-ROS environment. This redox buffering can protect cells during high-rate glycolysis and support extended logarithmic growth phases [18]. However, high polyphenol loads can be neutral or mildly inhibitory to thermophilic bacteria [19].

Poomsa-ad et al. [12] reported elevated RS levels—particularly in high-amylose Sao Hai rice processed using a pressure cooker with extra-virgin coconut oil and 2% citric acid (up to ~6.9% RS)—and demonstrated that herb additions modulate RS formation in KDML 105. In that study, pandan leaf juice ranked higher than butterfly pea flower, gac, and noni juices for RS enhancement. In contrast, the present study prioritized both antioxidant capacity and probiotic performance (*L. brevis*, *L. bulgaricus*, and *S. thermophilus*) rather than RS yield alone.

Across all treatments, *S. thermophilus* benefited the most, followed by *L. bulgaricus*, while *L. brevis* showed the smallest overall response. These differences align with the metabolic and physiological characteristics of each strain. *L. brevis* is an obligately heterofermentative bacterium that prefers glucose, fructose, and maltose but performs best in matrices rich in mixed sugars or pentose-containing plant oligosaccharides. It is also more sensitive to high polyphenol loads and favors moderate redox support combined with diverse carbohydrate sources. Relative to butterfly pea, pandan leaf juice provides a moderate phenolic background together with free amino acids and reducing sugars [20–22]. This matrix supports paced carbohydrate delivery through RS3/RS5 while minimizing polyphenol-related interference, thereby favoring *S. thermophilus* and, to a lesser extent, *L. brevis*. Both strains showed higher 48 h viability in pandan-based systems compared with control media.

By contrast, *L. bulgaricus*—a homofermentative bacterium and rapid acidifier—benefits more strongly from robust antioxidant buffering, such as that provided by butterfly pea anthocyanins. This explains the comparatively smaller growth advantage observed for *L. bulgaricus* in pandan leaf juice RS-cooked rice. *L. bulgaricus* preferentially utilizes glucose and lactose, exhibits very rapid glycolysis, and performs optimally when glucose release is paced to prevent early pH collapse. Strong antioxidant backgrounds and extra-virgin coconut oil-derived lipids further support its metabolism, producing responses comparable to those observed with butterfly pea flower systems.

S. thermophilus, also homofermentative, preferentially metabolizes glucose, lactose, and sucrose, with limited maltose utilization. It efficiently exploits glucose released slowly from malto-oligosaccharides and benefits from steady substrate availability combined with antioxidant protection. Its strong positive response to extra-virgin coconut oil is likely attributable to improved membrane stability. The extra-virgin coconut oil-mediated retrogradation process meters α -1,4-linked malto-oligosaccharides into glucose gradually, supporting sustained growth. Although *L. brevis* can ferment a wide range of sugars, including pentoses and plant-derived oligosaccharides [23], malto-oligo-dominant systems confer it less of a competitive advantage [24].

Extra-virgin coconut oil supplies medium-chain fatty acids that can be incorporated into bacterial membranes. Thermophilic lactic acid bacteria commonly exhibit enhanced acid tolerance and membrane stability when supplied with exogenous fatty acids. In contrast, *L. brevis* shows more variable responses to short-chain fatty acids and may even be slightly sensitive unless these are balanced by plant-derived cofactors [25,26].

Overall, pandan leaf juice favored *S. thermophilus* and *L. brevis* over *L. bulgaricus*. Pandan provides fermentation-friendly phenolics at moderate levels, along with free amino acids and reducing sugars that support early growth without excessive inhibition. Pandan

leaves contain measurable phenolics and flavonoids [27], but at levels substantially lower than strongly pigmented botanicals, alongside free amino acids—particularly glutamate—and reducing sugars such as glucose and fructose [28]. The lower polyphenol burden reduces polyphenol–starch or polyphenol–enzyme binding, which is more pronounced in anthocyanin-rich systems, thereby maintaining RS5 accessibility and minimizing early fermentation stress [29].

S. thermophilus benefits most under conditions of gentle acidification and sufficient amino-nitrogen availability. The pandan RS-rice system creates these conditions by combining moderate phenolics, free amino acids, and lipid support from extra-virgin coconut oil-derived RS5, resulting in smoother pH trajectories and improved late-phase viability [30]. In yogurt systems, cooperative interactions—particularly the supply of amino acids, peptides, and formate—are well known to enhance *S. thermophilus* growth [31].

L. brevis also benefits from slowly released carbohydrates in pandan RS-rice systems and demonstrates good tolerance to plant-based matrices. It can utilize multiple carbon sources simultaneously [32] and frequently performs well in plant fermentations, where slow malto-oligosaccharide release and lactic fermentation improve functional attributes [33]. Collectively, these findings reinforce that moderate-phenolic environments such as pandan leaf juice systems are more compatible with *S. thermophilus* and *L. brevis*, whereas highly antioxidant, anthocyanin-rich matrices preferentially support *L. bulgaricus*.

5. Conclusions

Pressure-cooking KDML 105 rice with extra-virgin coconut oil, 2% citric acid, and pandan leaf or butterfly pea flower juice, followed by cooling and freezing, effectively increased resistant starch formation and tended to enhance probiotic growth, particularly in *S. thermophilus*, *L. bulgaricus*, and *L. brevis*. Because prebiotics are defined by their selective stimulation of beneficial microbes, our use of a fast, acid-sensitive homolactic pair (*S. thermophilus*, *L. bulgaricus*) together with a versatile heterofermenter (*L. brevis*) provided a sensitive model to evaluate whether RS-enriched rice matrices truly favor desirable taxa, and support late-phase viability in ways consistent with health benefits. Overall, RS-engineered KDML 105 rice formulated with extra-virgin coconut oil and Thai herbs shows promise as a ready-to-eat functional food platform with potentially generalizable and extensible synbiotic applications.

6. Patents

Petty patent number 2203002265.

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