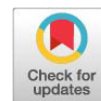


Research Article



Biofunctional Properties and Metabolic Characteristics of Colostrum Yogurt Supplemented with *Smallanthus sonchifolius*

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Abstract | Yoghurt is a popular fermented dairy product and can be used as vehicle to produce functional food that has better health and biofunctionality. Yacon (*Smallanthus sonchifolius*) is rich in fructooligosaccharides, fermentable and phenolic compounds with prebiotic and antioxidant properties. This research investigated the biofunctional characteristics and metabolomic profile of colostrum yogurt (CY) containing different amounts of yacon. A completely randomized design (CRD) was used with four treatments of yacon supplementation (0, 5, 10 and 15%), each treatment with four replicates. Analytical parameters were total lactic acid bacteria (LAB), pH, content of lactic acid, antioxidant activity (IC₅₀), protein content, amino acids composition and profile of proteins by SDS-PAGE. Yacon supplementation increased the lactic acid content, indicating enhanced fermentative activity, while IC₅₀ values significantly (P<0.01) decreased, indicating enhanced antioxidant capacity, particularly at the 15% supplementation level. Amino acid analysis showed a moderately displaced pool of some amino acids without alteration of the nutritional index. SDS-PAGE profiles (10–265 kDa) were similar among colostrum yogurt treatments, with major milk proteins remaining structurally stable across supplementation levels. Total LAB counts and pH values were not significantly affected by yacon supplementation (P > 0.05), indicating that microbial viability and acidification patterns remained stable across treatments. Such findings indicated that supplementation with yacon improves the metabolic and antioxidant properties of colostrum yogurt while preserving microbial viability and protein integrity, thus endorsing its development as a synbiotic, functionally enriched yogurt for a healthy metabolism.

Keywords | Antioxidant activity, Colostrum yogurt, Lactic acid bacteria, Protein profile, Yacon

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INTRODUCTION

Yogurt as one of the most popular fermented milk products, is known due to its nutritional and sensory quality as well as probiotic aspects. It is obtained by the fermentation of milk with Lactic Acid Bacteria (LAB)

such as *Lactobacillus delbrueckii*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which metabolize lactose to produce lactic acid and generate bioactive metabolites beneficial to human health (Yadav *et al.*, 2022; Fazilah *et al.*, 2018). The interest in production of functional yogurt is increasing due to inclusion of bioactive substances and

prebiotic agents in order to enhance the nutritive value and physiological properties (Fazilah *et al.*, 2018; Kumthekar *et al.*, 2021).

Colostrum, the first milk secretion following calving, is rich in proteins, immunoglobulins, growth factors and peptides that play a role in immune system regulation and gut health (Kaplan *et al.*, 2022; Silva *et al.*, 2019). The addition of bovine colostrum to some yogurt formulations has been reported to be enriched with nutrients, microbial balance, and bioactive compounds are beneficial for improving well-being (Silva *et al.*, 2019; Abdel-Ghany and Zaki, 2018; Zhao *et al.*, 2020). However, colostrum enriched yogurt may exhibit distinct biochemical characteristics due to its high protein and bioactive compound content, which can influence fermentation dynamics and functional properties. The incorporation of prebiotic rich plant ingredients may modulate microbial activity, organic acid production, and antioxidant potential, thereby enhancing the metabolic characteristics and overall functional quality of the product (Fazilah *et al.*, 2018; Kumthekar *et al.*, 2021; Rahayu *et al.*, 2024).

Smallanthus sonchifolius (yacon), a tuber originated from the Andes, has been of interest for its fructooligosaccharides (FOS), inulin-type carbohydrates and phenolic composition with prebiotic and antioxidant functions (Fan *et al.*, 2022; Honore *et al.*, 2018). The bioactive compounds present in yacon are known to promote the growth of beneficial bacteria, control lipid metabolism and glucose, and improve metabolic health (Honore *et al.*, 2018; Pereira *et al.*, 2022). When added in dairy matrices, yacon has been found to enhance LAB viability, antioxidant activity and development of secondary metabolites such as lactic acid, amino acids and bioactive peptides (Fazilah *et al.*, 2018; Pereira *et al.*, 2022; Setianingrum *et al.*, 2022). Thus, enriching colostrum-based yogurts with yacon is an innovative way to manufacture a synbiotic functional food using bioactives from animal and plant sources.

Previous studies have reported the incorporation of colostrum into yogurt due to its high protein and bioactive compound content, while the addition of yacon or fructooligosaccharide (FOS) rich ingredients has been shown to enhance probiotic growth and antioxidant properties in fermented dairy products (Krisnaningsih, 2025). However, investigations addressing the combined supplementation of colostrum and *Smallanthus sonchifolius* in yogurt systems remain limited. Therefore, a comprehensive evaluation of microbial viability and biochemical changes during fermentation is necessary to better understand the metabolic characteristics of this complex system. Analytical parameters including total LAB count, pH, organic acid content, antioxidant

activity, protein concentration, amino acid composition, and SDS-PAGE profiling were employed to characterize fermentation-driven metabolic modifications influencing the functional properties of colostrum yogurt.

Thus, the biofunctional properties and metabolomic profile of colostrum yogurt fortified with varying levels of *Smallanthus sonchifolius* was investigated. The results obtained will provide a basis for producing metabolically rich, probiotic dairy products with enhanced nutritional value and antioxidant properties, contributing to the sustainable development of health-focused functional food technology.

MATERIALS AND METHODS

STUDY DESIGN

This study was conducted using a completely randomized design (CRD) with four treatments of *Smallanthus sonchifolius* supplementation (0%, 5%, 10%, and 15%), each performed in four replicates. The evaluated parameters included total lactic acid bacteria (LAB), pH, lactic acid content, antioxidant activity (IC₅₀), protein content, amino acid composition, and protein profile.

PROCESS OF SMALLANTHUS SONCHIFOLIUS PREPARATION

The preparation was prepared with modification based on Ma *et al.* (2021), fresh yacon tubers were washed, peeled, and immersed in 1% (w/v) sodium chloride solution for 3–5 minutes to prevent enzymatic browning. The tubers were then grated using a sterile food processor until a homogeneous pulp was obtained and immediately incorporated into the yogurt formulation.

PROCESS OF YOGURT SUPPLEMENTATION WITH SMALLANTHUS SONCHIFOLIUS

The yogurt was prepared with slight modifications based on Krisnaningsih *et al.* (2025). Fresh milk and day-2 colostrum were mixed at a 1:1 (v/v) ratio. Afterward, 5% (w/v) skim milk powder (based on the total volume of milk and colostrum) was added. Grated *Smallanthus sonchifolius* was incorporated according to treatment levels (0%, 5%, 10%, and 15% w/v) prior to heat treatment. The mixture was then pasteurized at 72 °C for 15 seconds to ensure microbial safety. After cooling to 42 °C, 3% (w/v) starter culture containing *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus* was inoculated into the mixture. The samples were homogenized, transferred into sterile fermentation containers (approximately 100 mL), and incubated at 27 ± 2 °C for 24 hours. Upon completion of fermentation, the yogurt samples were stored at 4 °C for 24 hours to stabilize the gel structure prior to analysis. The overall experimental procedure is illustrated in Figure 1, and the appearance

of colostrum yogurt supplemented with *Smallanthus sonchifolius* is shown in Figure 2.

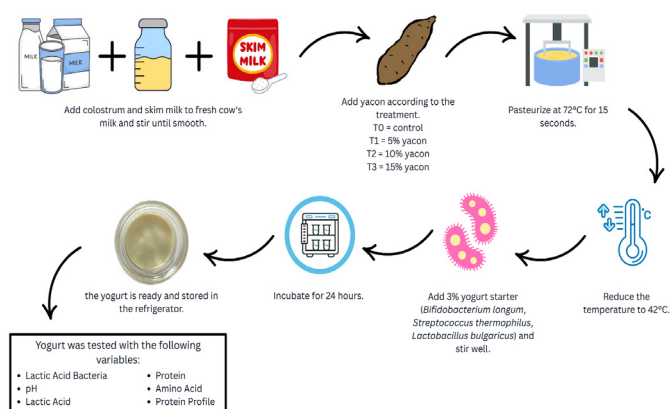


Figure 1: Procedure for the production of colostrum yogurt supplemented with *Smallanthus sonchifolius*.

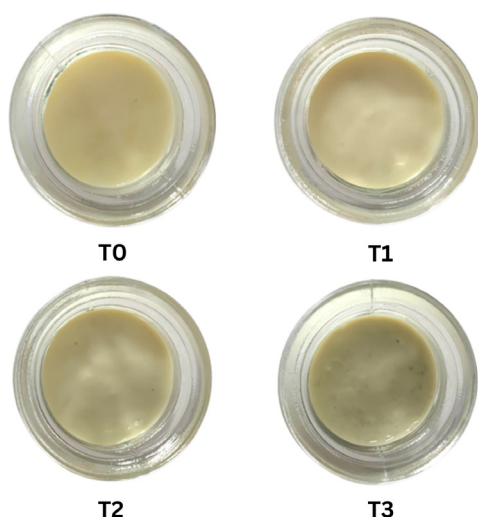


Figure 2: Yogurt Supplemented with *Smallanthus sonchifolius*.

VARIABLES ANALYZED

TOTAL LACTIC ACID BACTERIA (LAB)

LAB were counted by the pour-plate method on MRS agar. A colony count between 30 and 300 colonies per plate was considered valid according to Celik *et al.* (2021).

pH

The pH was determined using a calibrated pH meter with buffer solutions of pH 4 and 7. The pH meter was switched on, and the electrode was rinsed with distilled water. The electrode was then immersed in the sample solution and allowed to stabilize until the pH reading was recorded (Nazziyah *et al.*, 2025).

LACTIC ACID

Lactic acid content was determined using Ultra Performance Liquid Chromatography (UPLC) following the method described by Chai *et al.* (2024) with minor

modifications. Yogurt samples were centrifuged at 10,000 rpm for 10 minutes, and the supernatant was filtered through a 0.22 µm membrane filter prior to analysis. Separation was performed using a UPLC system equipped with an appropriate analytical column, and lactic acid was detected at a retention time of approximately 4 minutes. Quantification was carried out using an external standard calibration curve, and results were expressed as g/kg of yogurt. All analyses were conducted in triplicate.

ANTIOXIDANT IC₅₀

The antioxidant activity was investigated with a minor modification that 2 mL of protein extract was combined with 2 mL of 0.1 mM DPPH solution (Chalid *et al.*, 2021). The solution was shaken well and allowed to incubate in the dark for 30 min, after which its absorbance at 517 nm was recorded by a UV-Vis spectrophotometer. All the D-XYLs were determined in methanol as a blank. The IC₅₀ value was calculated according to the linear regression formulae: $y = ax + b$, where y is 50% inhibition and x expresses the sample concentration of the standard curve for the determination of IC₅₀ values.

PROTEIN

Protein was determined with a minor adjustment that the estimation of degraded protein amount was taken based on formol titration (Khusniati *et al.*, 2021). A sample (10 mL) was treated phenolphthalein and titrated with 0.1 N solution of NaOH. The solution was then added 10 mL of 38% formaldehyde solution and titrated with 0.1 N NaOH standard solution until there turned pink from pale yellow.

AMINO ACID

Amino acid profile of the yogurt was determined by slightly modification that samples one using a Liquid Chromatography Tandem Mass/ Mass Spectrometry System (LCMS/MS, Agilent 6460 Triple Quadropol, USA) (Terzioğlu and Bakirci, 2024).

PROTEIN PROFILE

Electrophoresis was performed according to the method and it was evaluated in terms of inter-protein crosslinking by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) on 4–15% precast gradient gels (Rahayu *et al.*, 2015). Gel calibration was performed using a broad-range (BRM) SDS molecular weight standard, which includes nine proteins spanning the range 6.5–200 kDa. Protein samples (~0.5 mg) were diluted in a sample buffer solution (10 mM Tris-HCl, pH 8.0, with 1 mM EDTA, 25 mg/ml SDS, 50 µl/ml β-mercaptoethanol, 0.1µl/ml bromophenol blue; Merck) and incubated at 40°C for 4 h to unfold the molecules. Following electrophoresis, the gels were stained with Coomassie Blue.

Data generated on total lactic acid bacteria, pH, total acid, antioxidant activity and protein content were tabulated based on the mean and standard deviation of each analysis for ANOVA. If there was a significant ($P \leq 0.05$) difference in mean results, mean separation was carried out using Duncan's Multiple Range Test (DMRT). The amino acid and protein profile data were reported as descriptive.

RESULTS AND DISCUSSION

In the present work, colostrum yogurt supplemented with *Smallanthus sonchifolius* induced a set of consistent biochemical, microbial, and structural changes that acted in unison to improve the functional characteristics of the product. The results showed that yacon acted not only as a prebiotic substrate but also as a metabolic modulator to manipulate the fermentation extent, phenolic activation, amino acid sole kinetics and protein structure in the colostrum yoghurt system. While some of the variables showed independent responses, most were interrelated either directly or indirectly through downstream metabolic pathways of fermentation, reflecting a synergistic interaction between colostrum constituents and bioactive compounds with LAB and LAB-mediated beneficial products from yacon.

LACTIC ACID BACTERIA (LAB), LACTIC ACID AND pH

Table 1 presents the average total lactic acid bacteria in colostrum yogurt added with *Smallanthus sonchifolius*. There was no difference at the level of significance ($P > 0.05$) in the lactic acid bacteria of colostrum yogurt by adding levels *Smallanthus sonchifolius*. LAB count highest was in the control treatment T0 (10.27 ± 0.57 Log CFU/ml) and lowest in T3, at 8.98 ± 0.57 Log CFU/ml. The LAB viability, as measured by total bacterial counts, decreased slightly with increasing supplementation, but there were no statistically significant differences. This decrease did not result in a loss of product quality, since all treatments had counts above 8 Log CFU/mL that can be regarded as adequate for probiotic activity. Another interesting finding was that the decrease in LAB counts

and the increase of lactic acid concentration were reversed, suggesting that the viability of cells of lactic acid bacteria (LAB) were significantly improved by presence of yacon. FOS and free sugars of yacon have been reported to enhance microbiological activities by acting as favourable substrates for fermentation (Fazilah *et al.*, 2018). However, this does not mean it will promote cell numbers, particularly in the presence of matrices containing high protein and immunoglobulin concentrations such as colostrum. Consistently, the increased metabolic output has been previously reported for synbiotic dairy systems (Prasad *et al.*, 2013), and these results have been shown to not match increases in microbial proliferation as high-solid matrices can also exert physical constraints on bacterial replication. The mild decrease of LAB count at the highest supplementation level observed in this study was probably due to a situation where density and viscosity of fermentation medium was increasing rather than inhibition by microorganisms. It was LAB cells became metabolically more active than increased in numbers and that serves as the basis for later observation in lactic acid, antioxidant potential and proteolysis.

The mean level of lactic acid in colostrum yogurt *Smallanthus sonchifolius* is presented in Table 1. The lactic acid content of yogurt showed a significant difference ($P < 0.01$) at different levels of supplementation. The least lactic acid content was observed in T0 (20.08 mg/kg). The highest value of the lactic acid was found in T3 (21.76 mg/kg). The higher values in lactic acid among the treatments indicate that higher levels of *Smallanthus sonchifolius* supplementation increased the proportion of acid production during fermentation. The pH of colostrum yogurt added with *Smallanthus sonchifolius* is presented in Table 1. Supplementation level of *Smallanthus sonchifolius* did not affect ($P > 0.05$) the pH of colostrum yogurt. The control yogurt (T0) had a pH value of 4.15, and the supplemented treatments (T1-T3) had pH values of 4.19-4.18. The pH of all the samples was found to be in the normal range for fermented dairy products, suggesting appropriate fermentation and keeping quality.

Table 1: Physicochemical and microbiological properties of colostrum yogurt supplemented with *Smallanthus sonchifolius*.

Parameters	Treatments			
	T0 $\pm$ SD	T1 $\pm$ SD	T2 $\pm$ SD	T3 $\pm$ SD
Lactic acid bacteria (Log CFU/ml)	10.27 $\pm$ 0.57	9.70 $\pm$ 0.07	9.63 $\pm$ 0.00	8.98 $\pm$ 0.57
pH	4.15 $\pm$ 0.00	4.19 $\pm$ 0.05	4.18 $\pm$ 0.03	4.18 $\pm$ 0.02
Lactic acid (mg/kg)	20.08 $\pm$ 0.04 ^a	21.08 $\pm$ 0.00 ^b	21.48 $\pm$ 0.10 ^c	21.76 $\pm$ 0.02 ^d
Antioxidant IC50 (μ g/mL)	219.165 $\pm$ 1.42 ^d	213.660 $\pm$ 4.92 ^c	194.175 $\pm$ 1.18 ^b	172.365 $\pm$ 1.92 ^a
Protein (%)	6.44 $\pm$ 0.22	6.23 $\pm$ 0.06	5.97 $\pm$ 0.80	5.92 $\pm$ 0.29

Note: T0 = control yogurt (without supplementation), T1 = yogurt supplemented with 5% *Smallanthus sonchifolius*, T2 = yogurt supplemented with 10% *Smallanthus sonchifolius*, T3= yogurt supplemented with 15% *Smallanthus sonchifolius*. Means in a row with different superscripts as ^{a, b} are different significantly ($P < 0.01$).

Progressive and significant increases in lactic acid formation were observed with increasing yacon supplementation, depicting consumption of the carbohydrate fraction from yacon. This is consistent with the known role of FOS and inulin-type fructans in exerting an effect on carbohydrate metabolism pathways in LAB, leading to increased acidogenesis (Honore *et al.*, 2018). The maximum concentration of lactic acid obtained in T3 indicates that the fermentability of substrates had a significant influence on fermentation intensity. Increased acidogenesis also suggests that LAB cells, although in slightly smaller numbers, were working more efficiently metabolically to achieve rapid conversion of available carbohydrates to lactic acid. This accelerated acid production exerted a direct impact not only on the sensorial characteristics of the yogurt, but also on subsequent metabolic pathways (proteolysis and amino acids liberation). Meanwhile, although lactic acid content varied widely among treatments, pH values showed no drastic changes. This stability is due to the buffering action from colostrum, which is rich in casein, minerals and immunoglobulins; these can neutralize metabolites acids and withstand sudden pH changes (Silva *et al.*, 2019). The fact that pH was not altered (in consequence of the greater lactic acid) indicates that the colostrum matrix “spared” the metabolic effect of increased fermentation, preserving its structure. This buffering pattern is common for high-solid dairy systems and is necessary to achieve gel stability during fermentation and storage.

ANTIOXIDANT IC₅₀

Mean antioxidant IC₅₀ values of colostrum yogurt enriched with *Smallanthus sonchifolius* are shown in Table 1. There was significant ($P < 0.01$) variation in antioxidant IC₅₀ values among the supplementation levels. The outcome showed that the highest IC₅₀ value, which indicated the lowest antioxidant effect, was for T0 (219.165 µg/mL), while the lowest IC₅₀ value which implied with highest antioxidant effect, was for *Smallanthus sonchifolius* at position T3 (172.365 µg/mL). These results suggest that yogurt with higher levels of *Smallanthus sonchifolius* supplementation improved the antioxidant capacity. Antioxidant capacity of the yoghurt also increased linearly with greater amounts of yacon supplementation, as IC₅₀ further decreased.

This improvement is one of the most outstanding functional benefits of yacon addition, and can be explained by two concomitant mechanisms. Firstly, yacon is rich in phenolic compounds particularly chlorogenic acid and caffeoylquinic derivatives with natural radical-scavenging activity (Fan *et al.*, 2022). These phenolics can enzymatically transform by LAB at the end of fermentation to generate metabolites with high antioxidant activity. It has been well established that fermentation-induced conversion of plant phenolics can improve the functional characteristics of fortified yogurt systems (Setianingrum *et al.*, 2022).

Second, the association with milk proteins like caseins and whey proteins tends to stabilize phenolic radicals by non-covalent interaction. Protein polyphenol complexes have been described to enhance the antioxidant activity in dairy systems by stabilizing bioactive molecules structurally as well as prevent their losses (Rahayu *et al.*, 2024).

In this work, effects of higher production of lactic acid and antioxidant potential suggests that the more intensity in the fermentation process increased the release, solubilization or activation of phenolic compounds from yacon. Both these mechanisms would account for T3 showing the highest amino acid values, this treatment undergoes the most profound supplementation and the strongest fermentation.

PROTEIN CONTENT

Table 1 shows the protein content of colostrum yogurt supplemented with *Smallanthus sonchifolius*. The protein content of colostrum yogurt was not significantly different ($P > 0.05$) according to the supplementation level of *Smallanthus sonchifolius*. The highest protein content (6.44%) was found in the control treatment T0, and the lowest (5.92%) in T3. These findings suggest that the incorporation of *Smallanthus sonchifolius* up to 15% did not alter the protein constituents of yogurt to a large extent, and overall levels were within an acceptable range for fermented milks. In spite of the decrease in protein content by increasing supplementation, these differences were not significant and values remained within acceptable interval for colostrum yogurt. Small protein decreases could be due to dilution effect from the addition of yacon and possibly light proteolysis during fermentation. Colostrum is rich in casein, whey proteins and bioactive peptides (Kaplan *et al.*, 2022), why the total protein content is maintained even when supplemented with plant material. The invariant protein concentration among treatments also indicates relatively robust buffering capacity and matrix structure during fermentation.

AMINO ACID COMPOSITION

Since the measurements were not repeated, the amino acid data were analyzed and presented descriptively. The results are shown in Table 2. The amino acid profile of yogurt supplemented with *Smallanthus sonchifolius* varied in response to different supplementation levels. Among the individual amino acids, L-aspartic acid and L-histidine increased with supplementation, reaching their highest concentrations in T2. In contrast, essential amino acids such as L-glutamic acid, L-lysine and nonessential amino acid (L-proline) did not change with the increasing supplementation levels. For most of the essential amino acids, slight decreases were recorded from T0 to T3; however, the whole amino acid profile was quite stable. These results suggest that the augmentation of *Smallanthus*

sonchifolius supplementation slightly in the amino acid profile of yogurt, but it did not change its nutritional value.

Table 2: Amino acid composition (mg/kg) of colostrum yogurt supplemented with *Smallanthus sonchifolius*.

Amino acid (mg/kg)	Treatments			
	T0	T1	T2	T3
L-Alanine	2.47	2.24	2.13	2.17
L-Arginine	2.47	2.77	2.52	2.38
L-Aspartic acid	2.93	4.36	4.57	4.53
Glycine	1.55	1.53	1.15	1.20
L-Glutamic acid	12.51	11.21	10.89	11.38
L-Histidine	1.29	1.49	1.69	1.26
L-Isoleucine	2.59	2.49	2.40	2.31
L-Cystine	1.16	1.04	0.99	1.07
L-Leucine	4.90	4.63	4.47	4.28
L-Lysine	12.05	11.75	9.97	9.10
L-Methionine	0.61	0.57	0.54	0.57
L-Tryptophan	0.73	0.74	0.69	0.71
L-Valine	3.98	3.72	3.54	3.48
L-Phenylalanine	1.88	1.78	1.80	1.60
L-Proline	6.39	6.03	5.68	5.65
L-Serine	4.26	4.14	3.89	3.65
L-Threonine	2.86	2.82	2.66	2.56
L-Tyrosine	3.18	3.01	2.92	2.82

Note: T0 = control yogurt (without supplementation), T1 = yogurt supplemented with 5% *Smallanthus sonchifolius*, T2 = yogurt supplemented with 10% *Smallanthus sonchifolius*, T3= yogurt supplemented with 15% *Smallanthus sonchifolius*.

The amino acid profile presented a more complex pattern of adaptations, reinforcing the metabolic consequences of yacon supplementation. Proteolytic enzymes produced by LAB may have contributed to the increased liberation of certain amino acids at moderate supplementation levels (T1 and T2). Aspartic acid and histidine are generally liberated in the first step of proteolysis, which is compatible with the concept of enhanced fermentation intensity being related to the potential degradation of peptides present within the yogurt matrix. In contrast, levels of essential amino acids, such as lysine, leucine and alanine sharply decreased among treatments suggesting their utilization by LAB as nitrogen sources under a high carbohydrate environment. Similarly, when fermentable carbohydrates are plentiful LAB tend to switch the nitrogen metabolism toward cellular maintenance rather than biomass formation provoking a selective utilization of amino acids (Terzioğlu *et al.*, 2024). But they did not change the nutrients' related digestive absorption (and balance).

PROTEIN PROFILE

Protein profile components were not replicated and were therefore presented descriptively. SDS-PAGE results

identified several protein bands in colostrum yogurts treated under all treatments, with sizes of about 10 kDa to 265 kDa. The electrophoregram was presented as Figure 3. The predominant proteins identified were β -lactoglobulin, lactoferrin, bovine serum albumin, caseins (fractions), immunoglobulins, and α -lactalbumin. The molecular weights of the majority of protein bands did not show significant differences with at least one band when *Smallanthus sonchifolius* was added. There was a slight variation for the β -lactoglobulin but with values that decreased at higher levels of supplementation, notably in T3 (133.61 kDa). Molecular weights of lactoferrin and bovine serum albumin were consistently maintained between T0, T1 and T2 with only minor reductions at the highest level of supplementation. Bands of casein as well as α -lactalbumin also exhibited only slight variation in their structure during supplementation with 15% *P. minor* when compared to the original samples.

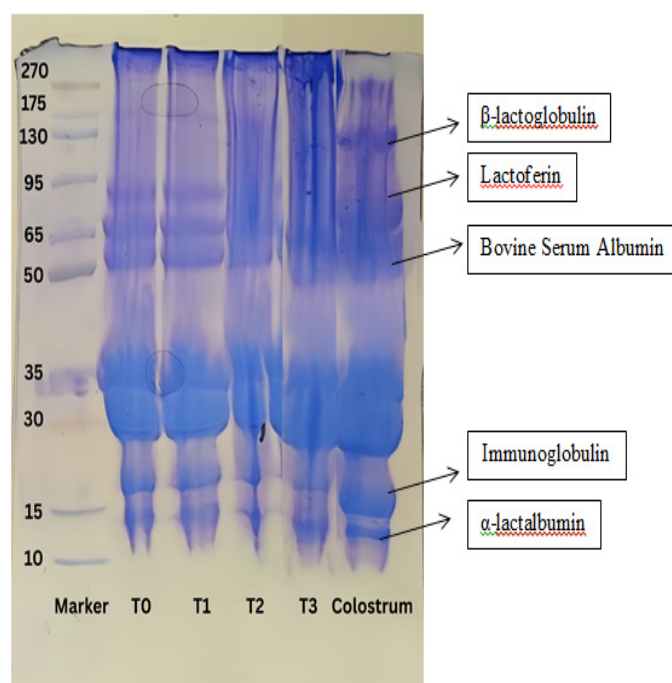


Figure 3: Protein profile of colostrum yogurt supplemented with *Smallanthus sonchifolius*.

Noted: T0= control yogurt (without supplementation), T1 = yogurt supplemented with 5% *Smallanthus sonchifolius*, T2= yogurt supplemented with 10% *Smallanthus sonchifolius*, T3= yogurt supplemented with 15% *Smallanthus sonchifolius*.

SDS PAGE protein profile served as structural evidence for the changes in yogurt and functionally supported the biochemical activities. The slight reductions in the band intensities of β -lactoglobulin and lactoferrin observed at higher supplementation levels may be indicative of small protein polyphenol interactions, which can change the migration behavior under electrophoresis without denaturing proteins (Zhao *et al.*, 2020). These

trends are in line with the increased antioxidant activity at higher supplementation, as protein polyphenol complexes contribute to radical scavenging characteristics. The protection of the casein fractions is especially interesting as these last are the building blocks of yogurt gel microstructure. Their homogeneous gene abundances under all treatments indicate that the structural network of colostrum yogurt remained unaltered despite the biochemical changes during yacon supplementation.

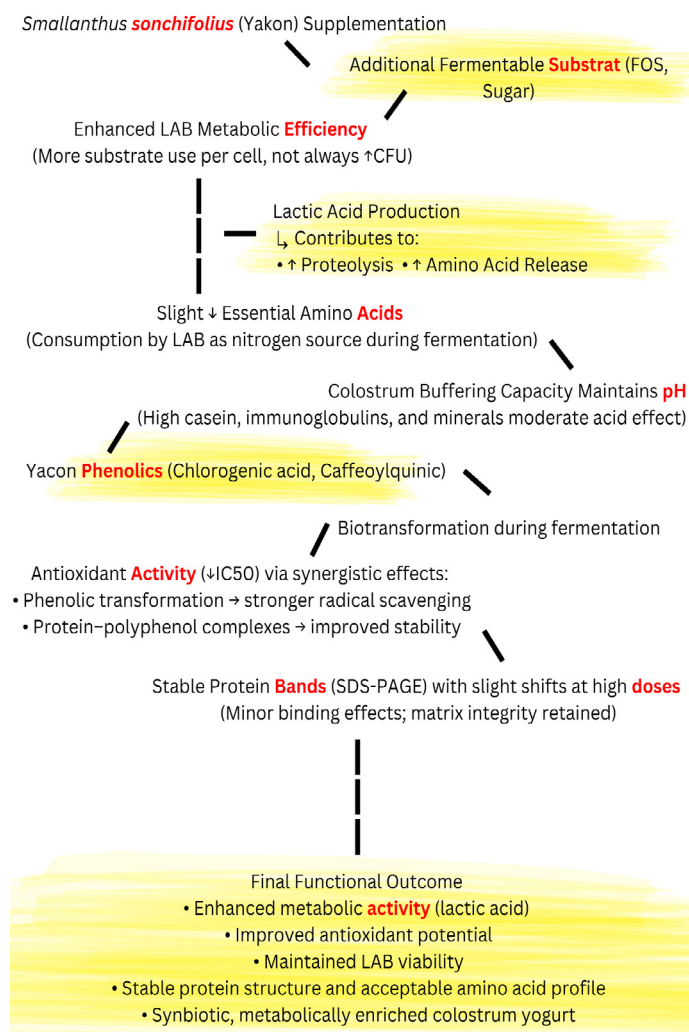


Figure 4: Proposed mechanistic pathway illustrating the effects of *Smallanthus sonchifolius* supplementation on colostrum yogurt fermentation.

Collectively, these data provide a mechanistic framework that connects yacon supplementation with the composition and structure of colostrum yogurt. More available fermentable carbohydrates from yacon promoted LAB metabolic efficacy, resulting in enhanced lactic acid production and selective proteolysis. These metabolic effects as well as the biotransformation of yacon phenolics and protein polyphenol interactions, led to increasing antioxidant activity. However, colostrum buffering capacity guaranteed pH stability and protein matrix integrity. The interplay between these variables demonstrates that yacon

products and colostrum ingredients have a synergistic effect on the functional properties of each other. This mechanistic model implies that *Smallanthus sonchifolius* is not only prebiotic additive, but also a metabolic activator which enhances the nutritional and biofunctional properties of colostrum yogurt, helping to obtain long shelf life, eubiotic and metabolically enriched fermented dairy products. The mechanism to illustrate the effects of *Smallanthus sonchifolius* supplementation on colostrum yogurt fermentation is shown in Figure 4.

CONCLUSION

Supplementation of *Smallanthus sonchifolius* enhanced the biofunctional properties of colostrum yogurt without adversely affecting microbial viability or protein integrity. Increased yacon levels promoted lactic acid production and significantly reduced IC₅₀ values, indicating improved antioxidant capacity. Although minor variations were observed in amino acid composition and protein band intensity, the overall protein profile remained relatively stable across treatments. These findings demonstrated that yacon supplementation up to 15% can improve the metabolic characteristics and antioxidant potential of colostrum yogurt, supporting its development as a functional synbiotic dairy product.

ACKNOWLEDGEMENT

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NOVELTY STATEMENT

This research is novel in combining colostrum yogurt and yacon supplementation to produce a synbiotic dairy product while simultaneously investigating its biofunctional enhancement and metabolomic-related characteristics. The study uniquely demonstrates that yacon can enhance antioxidant and fermentative properties without altering LAB viability or protein structural stability, highlighting its potential as a functional ingredient for advanced synbiotic dairy formulations.

AUTHOR'S CONTRIBUTION

Conceptualization: Premy Puspitawati Rahayu.
Methodology: Premy Puspitawati Rahayu.
Data curation: Dwi Setiawan.
Formal analysis: Ria Dewi Andriani.
Writing-original draft: Premy Puspitawati Rahayu, Dwi Setiawan and Angelica Denta.

Writing-review editing: Noor Syaheera Ibrahim, Lukman Hakim.

GENERATIVE AI AND AI ASSISTED TECHNOLOGY STATEMENT

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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