Impact of using yogurt bacteria co-cultured with probiotics on melatonin content and its derivatives, antioxidant activity, and quality characteristics in cow milk and plant-based milk yogurt

Treechada Utaida

A Thesis Submitted in Partial Fulfillment of Requirements for
degree of Doctor of Philosophy in Food Technology (International Program)
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The examining committee has unanimously approved this Thesis, submitted by Miss Treechada Utaida , as a partial fulfillment of the requirements for the Doctor of Philosophy Food Technology (International Program) at Mahasarakham University

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ABSTRACT

Functional foods designed to improve sleep quality are gaining widespread attention, with melatonin being recognized as a key compound for regulating human circadian rhythms. In the current work, cow milk and plant-based milk yogurts were developed due to their health-promoting properties, which include contributions from yogurt bacteria, probiotics, and the inclusion of melatonin and antioxidants. Although there are many different studies on yogurt produced with probiotic bacteria, this study is the first to explore the effect of using yogurt bacteria co-cultured with probiotics on improving melatonin content and antioxidant activity. The objective of this study was to investigate melatonin, serotonin, and tryptophan contents; antioxidant activity; physical characteristics; and sensory evaluation of yogurt as affected by yogurt bacteria co-cultured with different probiotics and to evaluate the effect of adding tryptophan on enhancing melatonin content, antioxidant properties, and quality aspects during yogurt fermentation. Four different types of yogurt (milk powder and plant-based; soybean, white sesame seed, and chickpea) were prepared using different combinations of yogurt bacteria cultures, including *S. thermophilus, L. delbrueckii* subsp. *bulgaricus*, and two probiotics (*L. acidophilus* and *B. lactis*). The cow milk yogurt (MPBY) and soy milk yogurt (SBBY) fermented by LAB co-culture with both probiotics had higher melatonin and tryptophan contents (MPBY: 3.64 and 5.48 ng/g dw, SBBY: 18.55 and 303.10 ng/g dw, respectively) than unfermented milk (MP: not detected of melatonin and tryptophan 5.00 ng/g dw, SB: melatonin 17.67 ng/g dw and tryptophan 159.07 ng/g dw). The strongest antioxidant activity was evaluated by DPPH, ABTS, and FRAP assays, which were observed in probiotic yogurt inoculated with *L. acidophilus* and *B. lactis*. The color (L*, a*, b*), syneresis (%), and yogurt texture were significantly different ($p \leq 0.05$) by mixed yogurt bacteria cultures and probiotic addition. The FTIR spectrum indicated some new peaks of peptides at the wavelength 1538 cm^{-1}. Furthermore, to increase the content of melatonin and its derivative, the study revealed that adding tryptophan to both cow milk and soy milk at 0.5, 1.0, 1.5, and 2.0% (w/v). The melatonin content was highest in cow milk yogurt (MPBY+tryp 1.5%) and soy milk yogurt (SBBY+tryp 1.5%) compared to unfermented milk. Therefore, during storage, physicochemical properties and bioactive compounds of cow milk and soy milk yogurts were evaluated. The results also demonstrated a 28-day shelf life for the yogurts, during which their antioxidant activity increased throughout the storage. The highest syneresis level occurred after 21 days. The pH value decreased on day 7 compared to the initial day and remained constant until 28 days. Melatonin content of cow milk yogurt was highest on day 28 (1.36 ng/g dw) and day 14 for soy milk yogurt (4.1 ng/g dw). Soy milk yogurt consistently outperformed cow milk yogurt in probiotic counts and ACE inhibitory activity, reflecting its superior bioactive potential. The ACE inhibition of cow milk yogurt was 42.94% to 74.72%, and that of soy milk yogurt was 83.40% to 95.19%, gradually decreasing with time until the final storage. Soy milk yogurt exhibited LAB and probiotic counts of 8.0×10^{4} CFU/g and 8.5×10^{7} CFU/g, respectively, compared to 2.9×10^{4} CFU/g and 1.1×10^{5} CFU/g in cow milk yogurt. These findings underline the potential of soy milk yogurt as a more effective functional food, with its higher melatonin retention, antioxidant properties, and bioactive compounds contributing to its health benefits.

Keyword : Functional food, Antioxidant activity, Tryptophan, Serotonin, Alternative protein, Probiotic

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CHAPTER 1
INTRODUCTION

1.1 Background

Currently, consumers are aware of health monitoring. As a result, there are various functional food products produced to meet the needs of consumers. By 2025, the global functional food industry is estimated to be worth USD 228 million and it will have reached USD 315 million in 2030 (Mane 2022). This information is a good predictor of the growth of the functional food market and also reflects the opportunities in the functional food business. Thailand's functional food products have a high potential to compete with other countries because Thailand has variety functional food raw materials that can be developed in functional food products. Therefore, many functional food products have been developed to meet the needs of functional food consumers. Currently, most functional foods are rich in dietary fiber, antioxidants, vitamins, and minerals. Another interesting group of functional foods is products related to relaxation, sleep, and has high antioxidant activity which has been produced only small amount recently. At present, the elderly population, consumers who work for a limited time, a person who travels long distances across time zones need food that qualifies for sleeping. Functional foods containing a key component including melatonin, serotonin, tryptophan, and bioactive substances, which regulate the body's sleep, wake mechanisms, and substances, antioxidants are needed. Melatonin is a hormone that regulates sleep and wake mechanisms of the body and has an antioxidant activity while serotonin and amino acid tryptophan are precursors in melatonin synthesis. However, information concerning the content of melatonin and melatonin derivatives in fermented milk products especially yogurt is limited. Therefore, the production of functional food products high in melatonin is interesting, especially with the elderly consumers that often have problems with sleeping. The information from the survey on consumer behavior and attitudes towards functional food products in the functional drink and dairy products were fruit juice drinks, fruit powder drinks, herbal tea drinks, yogurt, and mineral water mixed with vitamins. As a result of the overall survey, more than 90% of consumers like to drink functional drinks and have a positive attitude towards functional drinks in terms of the nutritional value that they will receive (Karelakis et al. 2020). Manufacturers have to consider expanding the market by developing a new product to meet the needs of consumers, especially the elderly, to stimulate the functional beverage market for continued growth.

Yogurt is another product with high potential for microbial fermentation to produce fermented milk rich in melatonin, its derivatives and other bioactive compounds. In addition, plant-based yogurt is an alternative for those who are lactose intolerant to cow milk. Another advantage is that it is high in protein and is a suitable substrate for the growth of lactic acid bacteria due to its high content of simple carbohydrates and protein, making it a potential source of prebiotics. However, knowledge related to suitable production processes and type of lactic acid bacteria producing high melatonin and bioactive substances, the effect of high melatonin synthesize probiotics on quality aspects of yogurt, stability, and shelf life is needed is limited. Therefore, this study were conducted to optimize the production process of functional yogurt products rich in melatonin and bioactive compounds through the activity of lactic acid bacteria.

1.2 Objectives of the research

1.1.1 To investigate the effect of LAB and probiotics on chemical and physical properties, melatonin content and its derivatives, and antioxidant activity in cow milk yogurt

1.1.2 To study the impact of LAB and probiotic types on chemical and physical properties, melatonin content and its derivatives, and antioxidant activity in plant-based yogurt

1.1.3 To study the addition of tryptophan to cow milk and plant-based milk yogurt products to increase the content of melatonin and bioactive compounds

1.1.4 To evaluate the effect of storage time on the physicochemical characteristics, melatonin content, antioxidant activity, and ACE inhibitory activity of yogurt

1.3 Expected outcomes

1.3.1 The information about melatonin and its derivatives, and antioxidant activity in yogurt synthesized by different kinds of LAB, and the changes of bioactive compounds in yogurt during the shelf life period were obtained.

1.3.2 Optimum conditions for the production of functional yogurt products containing high melatonin and bioactive compounds from cow milk and plant-based milk were achieved.

1.3.3 The results from this study were provide important information for future development in novel functional yogurt products that are rich in melatonin using effective processing technology to increase melatonin level and bioactive compound contents in functional ingredients.

1.4 Research hypotheses

1.4.1 Different kinds of LAB and probiotic strain may affect the content of melatonin and its derivatives, antioxidant activity, and other bioactive compounds in yogurt.

1.4.2 Melatonin, its derivatives, antioxidant activity, and other bioactive compounds in yogurt can be enhanced from plant-based milk.

1.5 Scope of the research

Phase I: Effect of LAB and probiotics on chemical and physical properties, melatonin content and its derivatives, and antioxidant activity in cow milk yogurt

Cow milk (milk powder) was fermented by LAB and probiotic strains including *Streptocuccus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium lactis*. The content of melatonin, its derivatives, bioactive peptides, other bioactive compounds, antioxidant activity; 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH assay), ABTS free radical scavenging activity assay (ABTS assay), and Ferric reducing antioxidant power (FRAP assay), pH value, titratable acidity and physical properties in yogurt were investigated.

Phase II: Effect of LAB and probiotic types on chemical and physical properties, melatonin content and its derivatives, and antioxidant activity in plant-based yogurt

From the previous researches (Sangsopha, Moongngarm, et al. 2020; Nontasan, Moongngarm, and Chottanom, 2021) soybeans and chickpeas were found to be good sources of protein and high in tryptophan. Their physical characteristics are similar to cow milk. White sesame seeds contain high content of melatonin. Therefore, all 3 types of raw materials were processed into plant-based milk powder. Soybeans, chickpeas, and sesame seeds were prepared into plant-based powder before fermented using LAB and probiotic strains including *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus* and *B. lactis*. The yogurt obtained was analyzed for melatonin content and its derivatives (serotonin and tryptophan) and their chemical properties and the major physical properties.

Phase III: Impact of tryptophan addition on melatonin content and antioxidant activity of production of yogurt from cow milk and soy milk

From Phase I and Phase II, the optimum conditions were selected to study at this Phase by product development of yogurt from cow milk powder and plant-based powder. The yogurt obtained was analyzed for melatonin content and its derivatives (serotonin and tryptophan) and their chemical properties and the major physical properties.

The cow milk was fermented with the addition of tryptophan (melatonin substrate) with 4 levels: 0.5, 1, 1.5 and 2%. Cow milk was added with the LAB chosen from Phase I and yogurt fermentation was done and analyzed for melatonin content and its derivatives (serotonin and tryptophan). The suitable milk mixture with substrate was used to produce melatonin-rich yogurt.

From the optimum proportion of raw materials obtained, the suitable plant-based milk was prepared and then added with melatonin substrate (tryptophan) with 4 levels: 0.5, 1, 1.5 and 2%. Plant-based milk was fermented by selected LAB from Phase II. Yogurt fermentation was performed and analyzed of melatonin content, its derivatives (serotonin and tryptophan) and their chemical properties, which the important physical properties.

Phase IV: Effect of storage time on the physicochemical characteristics, melatonin content, antioxidant activity, and ACE inhibitory activity of yogurt

To study the storage on quality of physicochemical, microbiological properties and bioactive compounds change of yogurt, both cow milk and plant-based milk yogurts. The storage of functional yogurt products at 4°C and sampling for quality analysis were carried out on days 1, 7, 14, 21 and 28.

1.6 Defined word

1.6.1 Melatonin (*N*-acetyl-5-methoxytryptamine) is an indoleamine, biosynthesized from tryptophan in the pineal gland and various organs of animals, and it can exhibit numerous biological functions in human (Aguilera et al. 2015; Rodriguez-Naranjo et al. 2011a).

1.6.2 Bioactive compounds are essential and non-essential compounds that occur in nature and are part of the food chain that can be shown to influence human health (Rodriguez-Naranjo et al. 2011b).

1.6.3 Functional yogurt/bio-yogurt/probiotic yogurt fortified with probiotic strains such as *Bifidobacterium* and *L. acidophilus* that are claimed to have numerous health benefits and should be in adequate numbers (Lourens-Hattingh and Viljoen, 2001; Zeng et al., 2014; Markowiak and Śliżewska, 2017).

CHAPTER 2
LITERATURE REVIEWS

2.1 Melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine) is a neurotransmitter produced by the pineal gland located in the center of the cerebrum which is involved in regulating the cycle of sleep. It is synthesized from tryptophan via serotonin. During the day, the pineal gland produces serotonin, which stimulates awakening and at night it produces melatonin which makes you feel sleepy (Gebhart and Schmidt, 2013). Melatonin is used to treat insomnia by relieving insomnia for shift workers and alleviating jet lag by regulating the human biological clock (Tan et al., 2002; Arnao and Hernández-Ruiz, 2006; Howatson et al., 2012). Furthermore, melatonin also exhibits powerful antioxidant properties by destroying the reaction and also increasing the activity of antioxidant enzymes such as glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase (Aguilera et al. 2015; Rodriguez-Naranjo et al. 2011a). In taking of melatonin through food consumption is beneficial to human functional because melatonin is absorbed when eating foods containing melatonin. Consumption of food rich in melatonin can maintain the plasma melatonin concentration.

2.1.1 Chemical structure of melatonin

Melatonin is a neurological hormone naturally present in the body. First discovered from the bovine pineal gland were purified by indole, which affects the melanin on the frog's skin, lightens the skin color, because the extract from the pineal gland has a chemical formula similar to serotonin. Therefore, the indole substance that bleaches melanin is called melatonin, the chemical name is N-[2-(5-Methoxy-1H-indol-3-yl) ethyl] acetamide or N-acetyl-5-methoxytryptamine. The structure of the melatonin and isomers as shown in Figure 1.

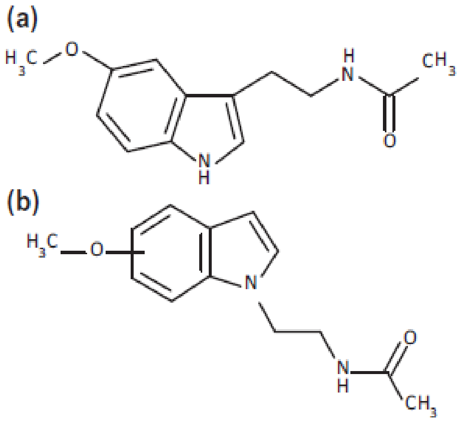


Figure 1 Chemical structure of (a) melatonin and (b) melatonin isomer in food.
Source: Padumanonda et al. (2014)

Melatonin production is therefore stimulated by darkness and secretion is inhibited by light, that is, when there is light. The pineal gland secretes less melatonin without light, more melatonin is produced. Believe that hormone is released according to the 24-hour life cycle or biological rhythm involved in light and darkness (circadian rhythm) such as waking cycle and sleep, seasons mating, the transition from serotonin to melatonin increases at night. To see better at night make the eyes see well in dim spaces or there is little light. Melatonin levels were highest at 2 am in generally healthy children and will rise to the highest at 3 am in the elderly. In addition, the body's production of melatonin decreases with age. This is another answer that scientists believe is another reason why older people are more likely to suffer from insomnia who are young.

2.1.2 Melatonin synthesis

Melatonin is synthesized from tryptophan in which the formation of melatonin. Tryptophan will be changed to -5-hydroxytryptophan with tryptophan-5-hydroxylase. It will change to serotonin and bind to serotonin, coenzyme A that acts as a catalyst for serotonin *N*-acetyltransferase (AANAT). The resulting product is *N*-acetylseratonin before converting to melatonin by acetylserotonin-N- methyltransferase. The first step is the step that determines the enzyme reaction speed. The controls are shown in the Figure 2 and melatonin biosynthetic pathways in plants and humans are shown in the Figure 3.

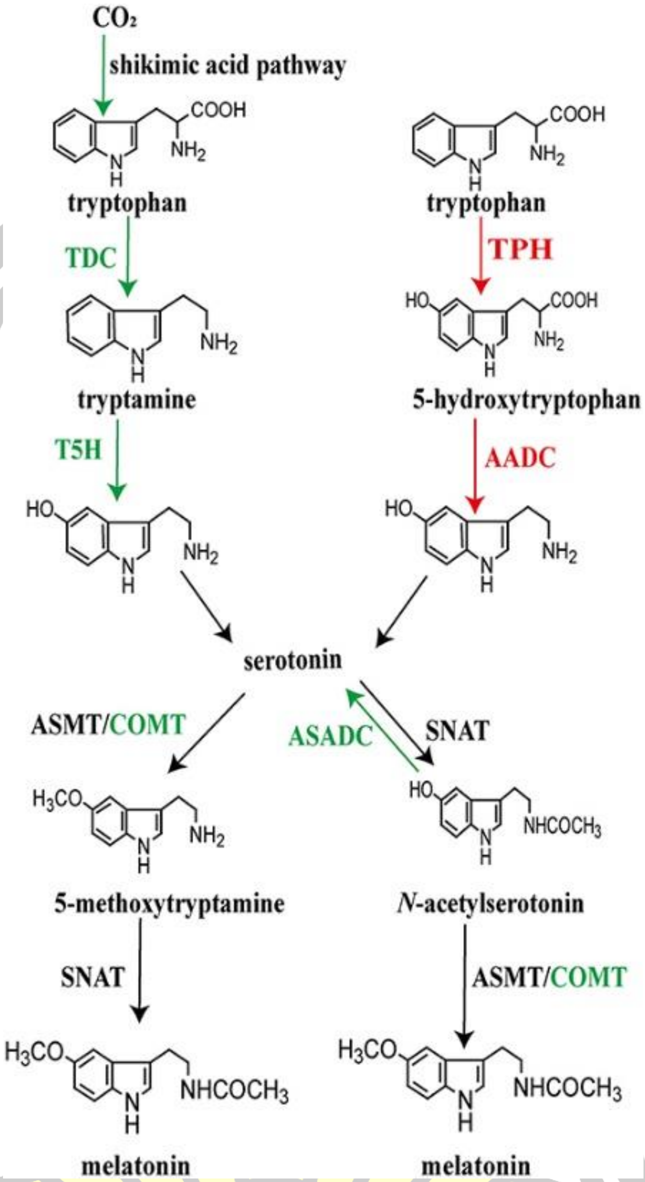


Figure 2 Pathways of melatonin synthesis in different plant and animal.
Source: Zhao et al. (2019)

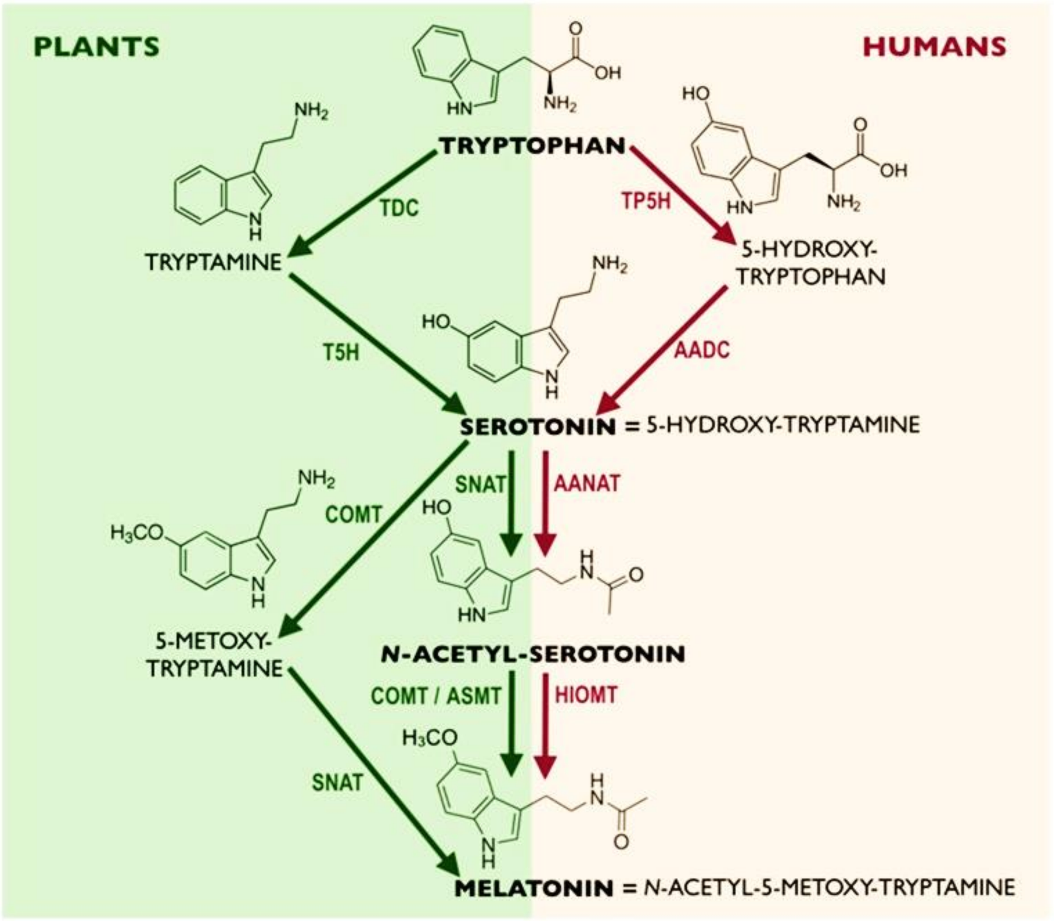


Figure 3 Comparison of melatonin biosynthetic pathways in plants and humans.
Source: Salehi et al. (2019)
AADC—aromatic-l-amino-acid decarboxylase;
AANAT—arylalkylamine N-acetyltransferase;
ASMT—N-acetylserotonin methyltransferase;
COMT—caffeic acid O-methyltransferase;
HIOMT—hydroxyindole-O-methyltransferase;
SNAT—serotonin-N-acetyltransferase; T5H—tryptamine 5-hydroxylase;
TDC—tryptophan decarboxylase; TP5H—tryptophan 5-hydroxylase.

2.1.3 The role and function of melatonin

Melatonin is involved in regulating the body's daily cycle. It is also involved in the reproductive system, nervous system, endocrine system, immune system, antioxidant systems including mechanisms aging of the body both in vitro and in vivo have been reported that melatonin can scavenge free radicals. An antioxidation effect and reduce the destruction of cells which features such this has led to extensive studies of melatonin in the treatment of various diseases such as Alzheimer's disease, Parkinson's disease, cardiovascular disease, and cancer, current knowledge on the use of melatonin in cancer patients. The important role of melatonin can be summarized as shown in Figure 4.

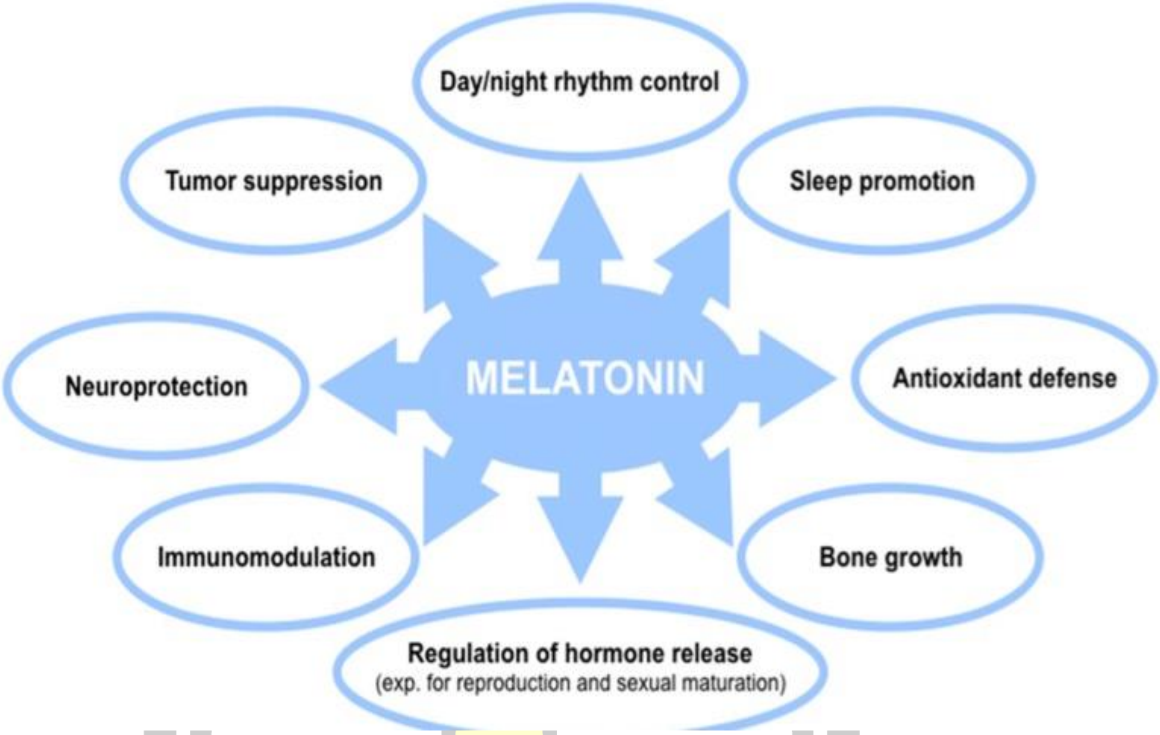


Figure 4 Beneficial functions of melatonin in humans.
Source: Salehi et al. (2019)

2.1.4 Importance role of melatonin in regulating and modifying body systems

Melatonin's mechanism for improving sleep involves modifying the daily cycle and inducing sleepiness, which induces sleep. Reiter et al. (2000) found that volunteers who took melatonin at night slept faster and no symptoms of fatigue or drowsiness after waking, the drunkenness of the time is found in people who fly across time zones and people who work at night, which is drunk at this time is similar to the symptoms that occur with sleep deprivation is will feel confused, forgetful, dizzy, and unable to sleep when you want to sleep caused by interfering with the daily cycle and the body cannot adjust the time such as level melatonin in the body is not related to the time at the destination. Recent data showed that 0.5-5 mg melatonin taken close to the sleep time of the destination (10-12 pm) can reduce the symptoms of motion sickness from flying, believing that the mechanism is due to melatonin's sleepy effects and stimulating the modification of the body's daily cycle system.

1) Radical scavenging activity

Melatonin can scavenge free radicals. Antiradical are important types of radicals, hydroxyl radicals (OH •), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO•), and singlet oxygen (O_2•). Melatonin was able to remove hydroxyl radicals arising from hydrogen peroxide photolysis by ultraviolet rays at 254 nm wavelengths and measuring the presence of hydroxyl radicals by technique spin trapping Electron Paramagnetic Resonance (EPR) using spin trap 5,5-dimethylpyrroline-*N*-oxide (DMPO).

Melatonin, at various concentrations from 1 to 100 µm can reduce the DMPO-OH adduct, or the extraction of hydroxyl radicals into body cells, with an IC50 value lower than the IC50 of glutamine. Thion and mannitol were 5 to 10 times, respectively, indicating that melatonin has a better ability to scavenge hydroxyl radicals than glutathione and mannitol (Allegri et al. 1993). Later, the same and other researchers confirmed melatonin's hydroxyl radical scavenging ability. Whether it is the study of chemical reaction cell studies or even in vivo studies have yielded similar results. Reiter et al. (2000) suggest that studies of hydroxyl radicals by various techniques and methods yielded a reported rate constant of melatonin interactions with various hydroxyl radicals between 1.2×10^{10} to 7.5×10^{10} $M^{-1}s^{-1}$.

Dehydration of hydrogen peroxide, Reiter et al. (2000) demonstrated the efficacy of melatonin in reducing hydrogen peroxide content concerning concentration. Melatonin was also found to remove hydrogen peroxide by conversion to *N*-acetyl-*N*-formyl-5- methoxykynuramine (AFMK) as in Figure 4. From indole ring fracture in melatonin structures due to oxidation, AFMK is also highly effective as a molecule that eliminates ROS, and in the case of co-enzyme enzymes, AFMK was found to be replaceable. N-acetyl-5-methoxykynuramine (AMK) transforms these two steps. It is considered to promote the anti-oxidative activity of melatonin as well. Because both of these substances can eliminate free radicals both. Peroxyl radical removal compared to vitamin E is considered to be inferior to vitamin E especially in reducing lipid peroxidation (Arnao and Hernández-Ruiz 2006).

However, melatonin is still found to help prevent the oxidation of fat in the body. It may be due to the removal of hydroxyl radicals before they react with lipids early in the lipid peroxidation rather than by the peroxyl radicals during the reaction. The direct removal of peroxyl radicals in the body has not been confirmed as shown in Figure 2.5 and the antioxidant cascade of melatonin derivatives in plants as shown in Figure 2.6.

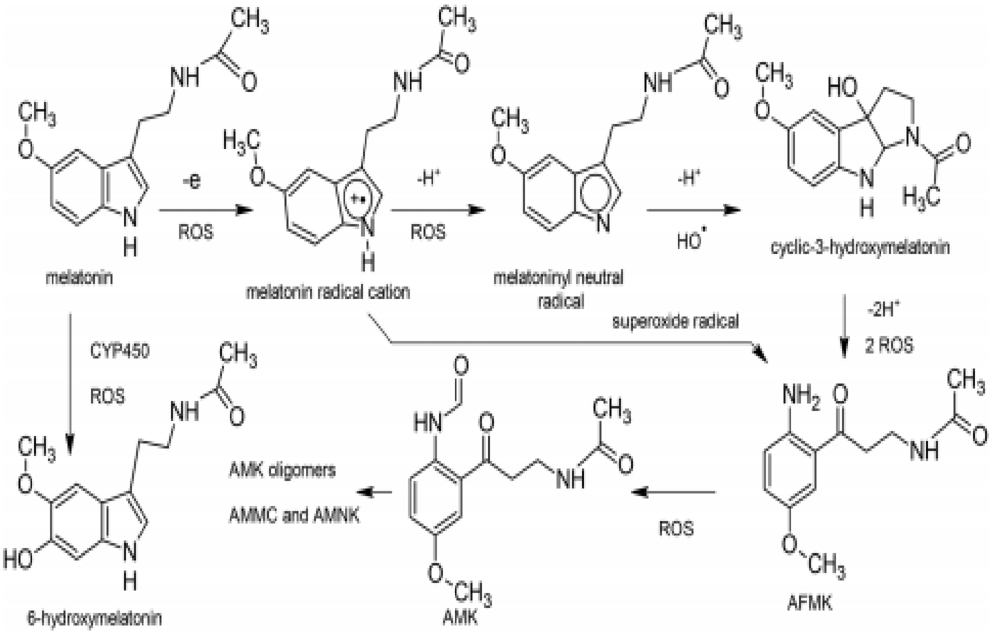


Figure 5 Changing of melatonin structure from antioxidant activity.
Source: Johns and Platts (2014)

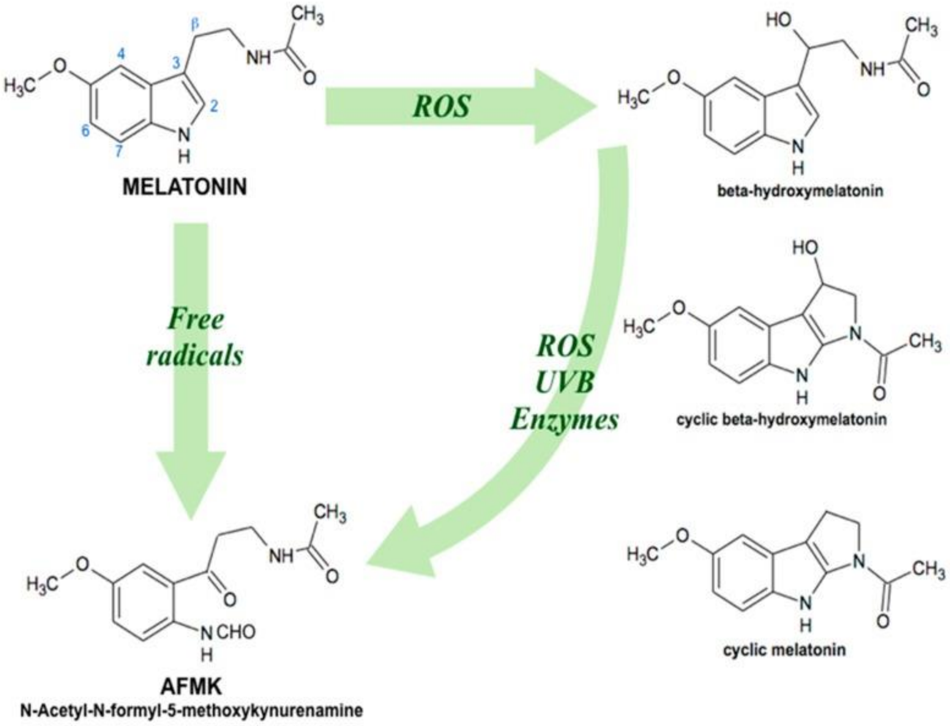


Figure 6 Antioxidant cascade of melatonin derivatives in plants.
Source: Salehi et al. (2019)

2) Working on another mechanism of melatonin that reduces oxidative stress

Melatonin can work to synergize with other anti-oxidants, such as stimulating the production of glutathione. A study report by Reiter et al. (2000) found that melatonin is synergistically vitamin E, Vitamin C, and glutathione. In preventing the oxidation of unsaturated fatty acids although the report did not explain the mechanism the clear function of melatonin. In this case, however, the synergistic effect of melatonin on the efficacy of other anti-oxidants was provided.

2.1.5 Melatonin sources

Most natural sources are found in meat, fish, milk, bananas, peanuts, sesame seeds, dried cherries, dried dates, dried egg whites, spirulina raw, soybeans and pumpkin seeds. From study melatonin in plants it was found that when feeding birds and rabbits to plants containing melatonin, blood melatonin levels were increased. Tonic fortified from plants eaten (BAGHURST and COGHILL n.d.). Tan et al. (2012) and Kocadağli et al. (2014) found that different types of seeds are good sources of melatonin, as shown in Table 1 and melatonin contents in some plant organs (Table 2).

Table 1 Melatonin and melatonin derivatives content in different plants.

Food sample	**Melatonin (pg/g or pg/ml)**	**Melatonin isomer (ng/g or ng/ml)**
Walnut	137.9 ± 27.40	0.3 ± 0.02
Green coffee	39.0 ± 6.50	1.2 ± 0.14
Cacao powder	7.2 ± 0.50	0.4 ± 0.03
Tomato	28.9 ± 4.50	1.6 ± 0.14
Green tea (ml.)	nd	0.3 ± 0.02
Black tea (ml.)	nd	0.3 ± 0.10
Sour cherry	nd	4.6 ± 0.14
Sour cherry concentrate	nd	5.2 ± 0.36
Probiotic yogurt	126.7 ± 9.00	0.9 ± 0.06
Kefir (fermented milk drink) (ml.)	nd	0.6 ± 0.04
Black olive (naturally fermented)	5.3 ± 0.10	0.1 ± 0.01
Bread (crumb)	341.7 ± 29.30	15.7 ± 1.40
Bread (crust)	138.1 ± 23.20	0.4 ± 0.12
Beer (ml.)	94.5 ± 6.70	14.3 ± 0.48
Red wine (ml.)	nd	170.7 ± 29.90

nd: Not detected

Source: Tan et al. (2012) and Kocadağli et al. (2014)

Table 2 Melatonin contents in some plant organs.

Common Name	**Latin Name**	**Organ**	**Melatonin [ng g^{-1} DW/ FW*)**
Coffee robusta	*Coffea canephora Pierr.*	Bean	5800
Coffee arabica	*Coffea arabica* (L.)	Bean	6800
Black pepper	*Piper nigrum* (L.)	Leaf	1093
Wolf berry (goji)	*Lycium barbarum* (L.)	Fruit	530
White radish	*Raphanus sativus* (L.)	Bulb	485
White mustard	*Sinapis alba* (L.)	Seed	189
Black mustard	*Brassica nigra* (L.)	Seed	129
Curcuma	*Curcuma aeruginosa Roxb.*	Root	120
Wolf berry	*Lycium barbarum*	Seed	103
Burmese grape	*Baccaurea ramiflora* Lour.	Leaf	43.2
Fenugreek	*Trigonella foenum-graecum* (L.)	Seed	43
Almond	*Prunus amygdalus* (Batsch)	Seed	39
Sunflower	*Helianthus annuus* (L.)	Seed	29
Fennel	*Foeniculum vulgare* (Gilib.)	Seed	28
Agati	*Sesbania glandiflora* (L.) Desv.	Leaf	26.3
Bitter melon	*Momordica charantia* (L.)	Leaf	21.4
Alfalfa	*Medicago sativum (L.)*	Seed	16
Green cardamom	*Elettaria cardamomum* (White et Maton)	Seed	15
Flax	*Linum usitatissimum* (L.)	Seed	12
Linseed (flax)	*Linum usitatissimum* (L.)	Seed	12
Java bean	*Senna tora* (L.) Roxb.	Leaf	10.5
Sesban	*Sesbania sesban* (L.) Merr.	Leaf	8.7
Anise	*Pimpinela anisum (L.)*	Seed	7
Celery	*Apium graveolens* (L.)	Seed	7
Coriander	*Coriandrum sativum* (L.)	Seed	7
Poppy	*Papaver somniferum* (L.)	Seed	6
Walnut	*Juglans regia* (L.)	Seed	3.5
Milk thistle	*Silybum marianum* (L.)	Seed	2
Sweet cherries	*Prunus avium* (L.)	Fruit	120*
Tart cherries	*Prunus cerasus* (L.)	Fruit	19.5*
Grapevine	*Vitis vinifera* (L.)	Fruit	18*
Cherry	*Prunus cerasus* (L.)	Fruit	18*
Corn	*Zea mays* (L)	Seed	14-53*

Common Name	**Latin Name**	**Organ**	**Melatonin [ng g^{-1} DW/ FW*)**
Cucumber	Cucumis sativus (L)	Seed	11-80*
Strawberry	*Fragaria x ananassa* (Duch.)	Fruit	11.3*
Pomegranate	*Punica granatum* (L.)	Fruit	5.5*
Tall fescue	*Festuca arundinacea*	Seed	5.3*
St. John’s wort	*Hypericum perforatum* (L.)	Flower	4*
Lupine	*Lupinus albus* (L.)	Seed	3.8*
Tomato	*Solanum lycopersicum* (L.)	Fruit	2.5*
Fever few	*Tanacetum parthenium* (L.)	Leaf	2*
St. John’s wort	*Hypericum perforatum* (L.)	Leaf	2*
Oat	*Avena sativa* (L.)	Seed	1.8*
Corn	*Zea mays* (L.)	Seed	1.4*
Grapevine	*Vitis vinifera* (L.)	Fruit	1.2*
Rice	*Oryza sativa japonica* (L.)	Seed	1*

* corresponds to FW. DW, dry weight; FW, fresh weight.
Source: Ravishankar and Ramakrishna (2016)

2.2 Yogurt

Milk is a natural food that contains many nutrients that are essential to functional (Alyaqoubi et al. 2014). But due to the growing trend of milk market value, milk mills must be continuously competitive. Thus resulting in innovation and production of new products that can meet consumer demand for a functional food option. Functional dairy products have been developed that contain nutrients and bioactive substances, good appearance and appearance, which are factors related to consumer preferences (Corbo et al. 2014). It is possible to improve the characterization of products containing nutrients and bioactive compounds (Serafini et al. 2009). However, milk is low in melatonin, with a concentration of 0.004-0.056 ng/mL (Milagres et al. 2014; Valtonen et al. 2005), it may not be sufficient to promote rest and sleep quality. Therefore, it is necessary to improve the production process or processing into other products or with other sources (Figure 7).

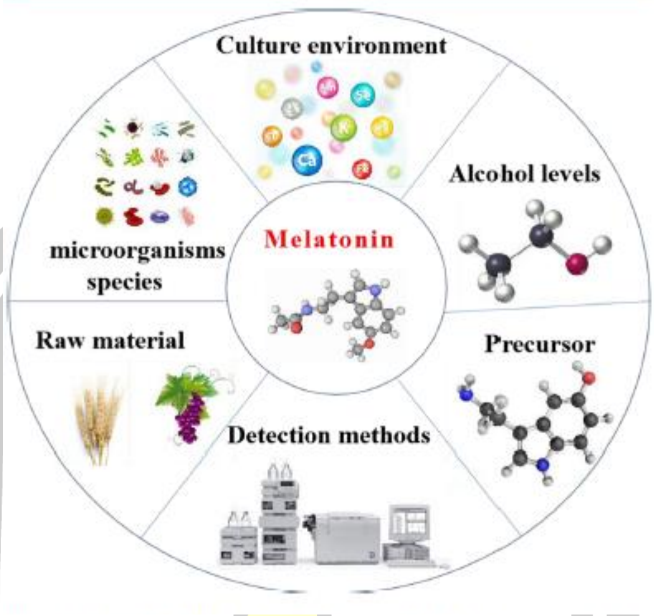


Figure 7 Factor influencing melatonin content in microorganisms.
Source: Que et al. (2020)

Fermented dairy product that one type of high quality fermented food that is highly popular. Because of the free amino acid, lactic acid, galactose, fatty acids, and vitamins (especially B complex vitamins) and good properties such as anti-inflammatory activity (Ano et al. 2015), anti-stress (Yılmaz and Gökmen 2020), improving memory (Yamamura et al., 2009; Liu et al., 2016), neuroprotection and cognitive enhancement (Camfield et al. 2011; Ozawa et al. 2013). Improving lactose intolerance Increased absorption of nutrients (Including minerals) and intestinal-related immune responses lowering cholesterol levels shorten the duration of diarrhea, arrhythmia Ischemia and cancer (Adolfsson, Meydani, and Russell 2004; Buttriss 1997; Flambard 2013).

Fermented milk products (Table 3), such as dahirik, bulgarian yogurt, buttermilk, acidophilus, kefir, kumis, and cheese are produced all over the world using lactic acid bacteria (Shiby and Mishra 2013). Yogurt is known to be rich in functional-promoting bacteria which includes probiotics. It results in better nutrition and functional benefits than unfermented milk (Hoque et al. 2010). It also contains the nutritional and micronutrients level including bioactive substances obtained from fermentation. Therefore, it is more beneficial for functional, it is the starting raw material against functional problems such as type 2 diabetes, cardiovascular disease, etc. (SODINI et al. 2004) and adding certain ingredients to yogurt, especially fruit, will enhance the flavor that will appeal to consumers (Clark and Plotka 2004).

Table 3 Types of fermented milks.

Types of fermented milks	**LAB strains**
Yogurt	Symbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*
Yogurt based on alternative cultures	Cultures of *Streptococcus thermophilus* and all species of *Lactobacillus*
Acidophilus milk	*Lactobacillus acidophilus*
Kefir	Culture prepared from kefir granules, *Lactobacillus kefiri*, species of the genus *Leuconostoc*, *Lactococcus* and *Acetobacter* that grow in a close specific relationship. The kefir granules constitute lactose fermenting yeast (*Kluyveromyces marxianus*) and lactose-free fermenting yeasts (*Saccharomyces unisporus*, *Saccharomyces cerevisiae*, *Saccharomyces exiguus*)
Kumis	*Lactobacillus delbrueckii* subsp. *Bulgaricus*, *Kluyveromyces marxianus*

Source: Shiby and Mishra (2013)

The commercial operation is added after milk fermentation so that microbes are not interrupted. However, the addition of additives during milk fermentation in particular, the initial culture may result in the final product (Amirdivani and Baba 2015). These materials contain phytochemicals that can have a positive effect on the growth and metabolism of bacteria (Ranadheera, Baines, and Adams 2010). Fermented milk products are widely consumed around the world and their consumption rates have increased significantly in recent years and market trends indicate that this will continue to increase. Because these products are beneficial for functional as it has a positive effect on the intestinal microflora and contributing to good functional properties (Bourrie et al., 2016; Chen et al., 2017). The fermentation process increases the nutritional value of many foods and increases the absorption of nutrients. Fermentation of the lactic acid bacteria (LAB) helps digest the lactose thus preventing lactose intolerance (Shiby and Mishra 2013).

The conversion of lactose to lactic acid is an important factor, as well as the formation of other bioactive compounds. Lactic bacteria convert the carbohydrates in milk to oligosaccharides some of which qualify as prebiotics (Granier et al., 2013). In addition to the production of lactic acid, the production of other mixtures by LAB depends on the bacterial strains, fermentation conditions and the fermentation food. The most common strains of LAB used for fermenting milk are *Streptococcus thermophiles*. Usually used in conjunction with Bifidobacteria such as *Bifidobacterium breve* C50, *Bifidobacterium lactis*, *Bifidobacterium longum* and *Bifidobacterium animalis* or with Lactobacilli such as *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus johnsonii lacty*. Fermented milk products have great potential to develop into many innovative functional-promoting and functional foods with beneficial ingredients such as prebiotics or probiotic bacteria. Fermented dairy products like yogurt are the most studied fermented dairy products like kumys, skyr, yakult, and kefir (Bourrie et al., 2016; Chen et al., 2021).

Yogurt is one of the most widely consumed, fermented and very popular dairy products in the world because of its unique flavor and dietary benefits, and produced by the coagulation of milk proteins during fermentation with lactic acid bacteria (LAB) (Masood et al., 2011; Sun-Waterhouse et al., 2013; Xiang et al., 2019). Yogurt contains high content of nutrients, and can be digested and assimilated more easily than fresh milk. (Shao and He 2009). LAB are utilized in yogurt production, including *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium longum*, and *Streptococcus thermophilus*. According to the Food and Agricultural Organization (FAO), yogurt must contain a large number of active and living microorganisms, which play an important role in promoting health, and also an abundant and viable microflora of starter origin at the time of consumption, thus a definition along these lines enshrined in the food laws of many countries, with the minimum counts of viable LAB ranging between 10^6 and 10^8 CFU/mL in yogurt (Borchers et al., 2002). LAB are known to exert beneficial effects on the human body by fostering a balanced intestinal microbiota, enhancing immunity, and reducing blood cholesterol levels (Lee and Lucey, 2010; Masood et al., 2011; Sun-Waterhouse et al., 2013). Moreover, bioactive peptides are formed during yogurt fermentation (Sabeena Farvin et al. 2010), some of which have been found to have antioxidant properties. Although the total antioxidant activity of yogurt is relatively low, purified peptide fractions exert more notable antioxidant effects (Şanlıdere Aloğlu and Öner 2011). Probiotic yogurt, contacting probiotic strains such as *Lactobacillus acidophilus* and *Bifidobacterium bb12 bifidum*, is claimed to offer many health benefits such as improve lactose utilization and inhibit cancer (Drago 2019; Rafter 2003; de Vrese et al. 2001).

2.2.1 Types of yogurt

The types of yogurt can be classified as follows

1) Fat content

Yogurt was made from whole milk unless it was clearly labeled that it was made with reduced fat or skim milk. However, some reports suggested that the amount of total fat in the diet affects the serum cholesterol level. Therefore, it would be better for some people to consume skim milk yogurt instead of whole milk yogurt (Aryana and Olson 2017a). Yogurt can be divided of fat content into four types:

1.1) Non-fat yogurt

Non-fat yogurt or very low fat yogurt or skimmed milk yogurt with lower fat content 0.2-0.5%

1.2) Low-fat yogurt

Low-fat diets increased in popularity in the 1980s and 1990s, and much effort went into producing foods with lower fat contents and improving the flavor and body and texture of foods with lower fat contents so that they more closely resemble their full-fat counterparts. Many low-fat or calorie yogurts were produced and patented about this time, including a low-calorie, low-fat fruit-containing yogurt. More recently, it was shown in a large multiethnic cohort study that a higher intake of saturated milk fat was associated with a lower risk of cardiovascular disease (de Oliveira Otto et al. 2012). Low-fat yogurt or half fat yogurt or semi-skimmed milk yogurt with fat content 0.5-2.0%

1.3) Yogurt

Yogurt or full-fat yogurt or whole milk yogurt with fat content not lower 0.5-2.0%

1.4) High-fat yogurt

High-fat yogurt or cream yogurt that is produced in some countries and may not determine the lowest fat content of yogurt. In Germany, the fat content is not lower than 10%.

2) Consistency

Yogurt can be classified of consistency into two types:

2.1) Set yogurt

Set yogurt (French style yogurt) is a type of yogurt that contains yogurt from a fermentation tank into a cup or container before the milk has a coagulation reaction and let the reaction continue until the inside of the cup forms a curd (Jeong et al. 2018a).

2.2) Stirred yogurt

Stirred yogurt (pre-stirred yogurt or Swiss style yogurt) is a type of yogurt that has been completely fermented in a fermentation tank before being loaded into a cup or container. This type of yogurt is smoother than the set yogurt (Hussein et al. 2020).

3) Flavor

Yogurt can be distributed of flavor into two types:

3.1) Plain yogurt

Natural yogurt is yogurt without added fruit and/or flavor.

3.2) Fruit yogurt

Fruit yogurt or flavored yogurt is yogurt added fruit and/or flavor. Fruit is commonly added to yogurt and has been since 1933. The research stated that brings real fruit mixed in yogurt soaring yogurt sales in Britain. Some of the most common type of fruit added to yogurt including strawberries, blueberries, peaches, raspberries, cherries, oranges, lime and pineapple and many other fruits and fruit flavors (Aryana and Olson 2017a).

4) Shelf-life

Yogurt can be divided of shelf-life into three types (Aryana and Olson 2017a)

4.1) Fresh yogurt

Unheated yogurt (fresh yogurt) is yogurt with shelf life of 16-21 days at refrigerated temperature. Which may be stored for 30-40 days without preservatives and can be stored for 45-60 days by using the same material as well.

4.2) Pasteurized yogurt

Heated yogurt (pasteurized yogurt) that has undergone heat treatment after fermentation is finished at 70ºC for 30-40 seconds to extend shelf life. They can be stored for up to 2-3 months without refrigeration but the heating also defies the yogurt microbes.

4.3) Sterile yogurt

A sterile yogurt that will not degrade within 6 mouth of unrefrigerated storage.

2.2.2 Ingredients in yogurt

The ingredients for yogurt production are as follows.

1) Milk ingredients

Some of the added dairy ingredients that increase the milk solids-not-fat content of yogurt mixes have included skim or whole milk powders, milk protein concentrates, condensed milk, buttermilk powder, whey powder, whey protein concentrates, microparticulated whey protein, and caseinates (sodium, calcium, or sodium-calcium). The reports stated that the addition of condensed skim milk to milk for making yogurt improves yogurt consistency. The addition of milk powder to milk has been used for many years to produce a thicker and firmer yogurt. The researchers stated that up to 0.3% dried whey can replace dried skim milk in manufacturing yogurt, liquid cottage cheese whey protein concentrates could be used in yogurt production (Aryana and Olson 2017a). Saffon et al. (2013) reported that replacing powdered skim milk with heat-denatured aggregates of mixtures of buttermilk concentrate and whey protein concentrate in set-type yogurt modified the textural properties of the resulting yogurt. Bong and Moraru (2014) developed a procedure for manufacturing Greek-style yogurt in which micellar casein concentrate was used to increase the protein content of the milk for making yogurt.

2) Fat content

Yogurt may or may not contain fat. The fat content of yogurt is normally adjusted by varying the amount of added cream, milk, partially skim milk, or skim milk. However, unsalted butter can also be used to increase the yogurt fat content. A butterfat-free yogurt in which an unsaturated fat or oil such as corn oil, cottonseed oil, coconut oil, soybean oil, or other similar oils in amounts of about 1.5 to 6.4% are added has been patented. Historical trends of varying yogurt fat contents are discussed in the various types of yogurt section (Aryana and Olson 2017b).

3) Sweeteners

Most yogurts, other than plain yogurt, are sweetened, usually with sugar. Other sweeteners such as honey can be used, but POPA and USTUNOL (2011) found that the flavor of yogurt sweetened with sucrose was preferred by panelists over yogurts sweetened with either high-fructose corn syrup or honey. Various high intensity sweeteners can be incorporated into yogurt.

The sweeteners fructose, xylitol, sorbitol, cyclamate, and a saccharin and xylitol mixture (use of xylitol in this mixture to cover a disturbing bitter aftertaste from saccharin) could be successfully used in yogurt when added after incubation without markedly deteriorating quality and a method of making yogurt containing pectin and aspartame. The researchers reported that yogurts sweetened with either aspartame or sorbitol were usually preferred over yogurts sweetened with other alternative sweeteners (calcium saccharin, sodium saccharin, fructose, fructose plus monoammonium glycyrrhizinate, acesulfame-K, dihydrochalcone, and sucrose plus monoammonium glycyrrhizinate) but not as desirable as the sucrose-sweetened yogurt control (Aryana and Olson 2017a). When matching the sweetness equivalence of strawberry yogurt containing 11.5% (w/w) sucrose with addition of high-intensity sweeteners to yogurt, REIS et al. (2011) found that more aspartame than sucralose was required. Narayanan et al. (2014) found that levels of 0.7 to 5.5% (w/w) of various commercial stevia sweeteners were appropriate for sweetening naturally flavored vanilla low-fat yogurt.

4) Stabilizers

Stabilizers are often added to yogurt. The report has provided recommendations for usage of stabilizer depending upon preferences in the type of yogurt to be produced. Common stabilizers for use in yogurt and yogurt drinks include modified starch, gelatin, agar, pectin, locust bean gum, xanthan gum, carrageenan, and carboxymethyl cellulose (CMC), used either alone or in combination, depending upon the type of stabilizer (Chandan and Kilara 2013).

5) Yogurt fruits

Fruit for yogurt mixes are mixed and pasteurized fruits and should have a pH of not more than 4.5 to prevent the development of *Clostridium botulinum*. Nuts include fruit or fruit juice and sugar, which is commonly used in sugar and may use corn syrup as well. Including a thickener (stabilizer), flavoring (flavor) and food coloring (color) in the traditional method, it is popular to have a high sugar concentration of about 60%, but there are problems when used in the continuous production process. Therefore, it is used at a lower concentration of about 30-50%. Fruit that can be used can be frozen fruit, canned fruit, or dried fruit such as orange, lemon, pineapple, apple, peach, plum or prune, apricot, strawberry, blueberry, blackberry, cranberry, raspberry and cherry (Aryana and Olson 2017b).

6) Nutritional and functional ingredients

Many nutritional and functional ingredients have been added to yogurt. Because yogurts are not typically vitamin fortified, studies have reported the addition of vitamins (either with or without other added ingredients) including vitamin C, vitamin B12 (by addition of *Propionibacterium shermanii* or 2% yeast extract, vitamins A, folic acid (Aryana 2003), vitamin D3 (Kazmi et al., 2007), and some heart-healthy nutrients (thiamine, riboflavin, niacin, folic acid, manganese, magnesium, and fiber) (Cueva and Aryana 2008). Other examples of adding beneficial ingredients to yogurt during manufacturing include calcium, nanopowdered eggshells (Al Mijan et al., 2014), 7 different types of minerals (iron, magnesium, zinc, manganese, molybdenum, chromium, and selenium) (Achanta et al., 2007), vegetables (cucumber salad, tomato salad, beet salad, and garden salad), nuts (walnuts, hazelnuts, almonds, and pistachios) (Ozturkoglu-Budak et al., 2016), spice oleoresins (cardamom, nutmeg, and cinnamon) (Illupapalayam et al., 2014), fish oil (Estrada et al. 2011; Olson and Aryana 2017), lutein (Aryana et al. 2006), and green tea and green coffee powders (Dönmez et al., 2017; Jeong et al., 2018), and chickpea (Hussein et al. 2020).

2.2.3 Yogurt cultures

The microorganisms used in yogurt (yogurt cultures) are culture concentrates which come in a variety of forms, such as liquids, freeze-dried and deep-frozen, today is popular in the form of frozen. It can be used in the form of frozen yogurt and freeze-dried yogurt inoculum without the need for mother culture and bulk starter, but longer fermentation time.

Although current standard procedures and regulations call for the addition of both *Streptococcus thermophiles* and *Lactobacillus bulgaricus* as the starter culture to be used in the production of yogurt (or possibly yogurt-like products) because of their symbiotic relationship, other cultures or combinations have been suggested over time (Aryana and Olson 2017a). Symbiosis, coexistence promotes mutual prosperity. Although both of these microorganisms were able to grow independently, *Lactobacillus bulgaricus*, which has high proteolytic enzyme activity, produces peptides and amino acids for *Streptococcus thermophiles* growth, while *Streptococcus thermophiles* has high urease activity. Will generate carbon dioxide which helps in the growth of *Lactobacillus bulgaricus*.

Yogurt cultures have an important function, lactic acid production, produces a sour taste with a pH 4.6 and produces a characteristic flavor of yogurt. This particular flavor is mostly derived from the acetaldehyde produced by *Lactobacillus bulgaricus* during fermentation. Other culture may also be used in yogurt, such as bio-active cultures, meaning those that survive and grow in the human gut and have good health effects, including *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus delbrukii ssp bugalicus*, *Streptococcus thermophiles*, *Bifidobacterium lactis*, *Bifidobacterium bifidum* and *Bifidobacterium longum* (Amirdivani and Baba 2015;

Jeong et al. 2018a). However, these culture also fermentation stop at a higher pH, about 4.9-5.0, thus the product is less sour and gives a smoother taste.

Lactic bacteria can also produce tryptamine from tryptophan, however much has not been studied about the role of microbes in melatonin production. *Saccharomyces cerevisiae* is the main strain used for fermentation wines and has been most researched on melatonin synthesis. Research has reported that lactic acid bacteria Bifidobacteria and lactoferrin is involved in body fatigue and sleep. In human clinical studies that the consumption of foods containing Bifidobacteria improves sleep quality due to the secretion of serotonin which is the precursor in melatonin synthesis by *Bifidobacterium* by the mechanism of synthesis as in Figure 8.

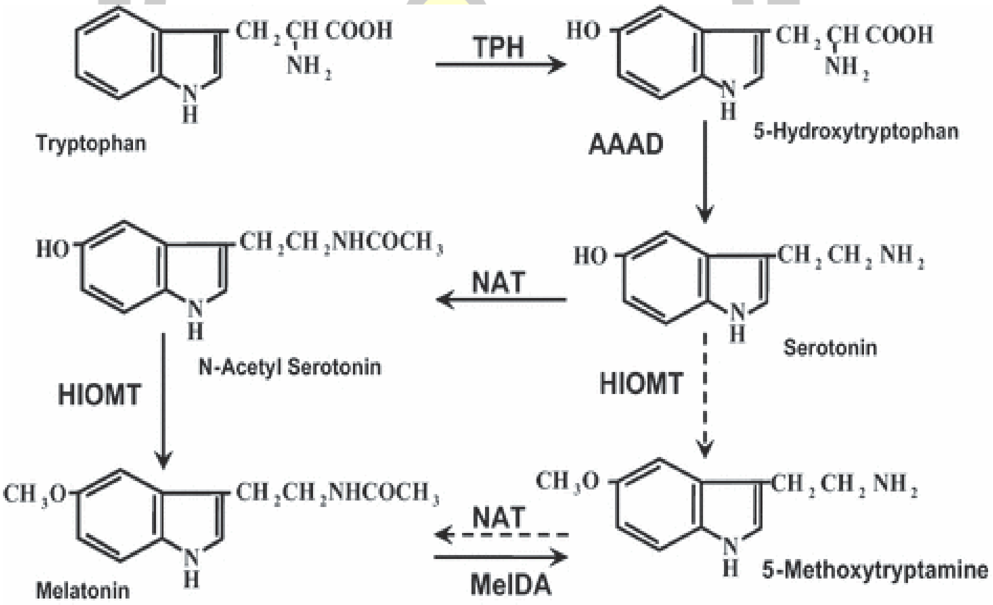


Figure 8 The biosynthetic pathways of melatonin.
Source: Tan et al. (2012)

TPH—tryptophan hydroxylase; AAAD—aromatic amino acid decarboxylase;
NAT—N-acetyltransferase; HIOMT—hydroxyindole-O-methyltransferase;
MelDA—melatonin deacetylase;
the dashed line represents a minor pathway of melatonin biosynthesis.

2.2.4 Production of yogurt

Yogurt production according to the method of Patel et al. (2019); Jeong et al. (2018); Felix da Silva et al. (2017); Amirdivani and Baba (2015) and Yang et al. (2012)

1) Mix preparation

The preparation of ingredients is milk, milk powder, sugar, stabilizer, and other in case of intermittent mixing, it is mixed in the mixing tank. They may be heated to 45–70°C to help the dry ingredients better dissolve. In the case of using continuous mixing method, the dry ingredients will be dissolved in milk or water first or use sugar in liquid form, etc.

2) Heat treatment

For intermittent heating 25–30 min, heat at temperatures above 66°C such as 80–85°C, is used for continuous heating in a heat exchanger. Heat at 90–95°C for 10 min. Heating to aid in dissolving stabilizer and to kill microbes.

In this case, whole milk powder has been reconstituted in distilled water, heated to 90°C for 3 min or 85°C for 30 min and then cooled to 43±1°C, inoculated with 2–3% yogurt starter culture.

3) Homogenization

Homogenization can be defeated before or after heat treatment by homogenizing at temperatures below 60°C, which is commonly used as two stage homogenizer at pressures of 15/4 MPa or 2,500/500 Psi. As it is extinguished to prevent separation of cream during fermentation.

4) Inoculation

Yogurt inoculation is achieved by lowering the temperature of the heat exchanger mixture to a temperature of 1–2°C above the fermentation temperature and then adding the yogurt inoculum and constantly stirring. The freeze-dried yogurt starter culture powder is mixed strains of *Lactobacillus acidophilus* and *Streptococcus thermophiles* at the ratio of 1:1 and activated by dissolving 1 g in pasteurized whole milk and incubating at 43±1°C for 2 h. This activated culture has been used for the yogurt.

5) Fermentation or incubation

All inoculated milk has been placed in a pasteurized plastic cup or pot and then incubated at 42–43°C, the optimum temperature for the growth of lactic acid bacteria, until pH 4.5–4.6 was reached. The yogurt has been cooled to 4°C aiming to stop fermentation.

6) Striking and smoothing

To make the yogurt smooth, it is only used in the case of stirred yogurt, which is achieved by striking the curd that is still warm, spreading out and holding the whey by stirring the yogurt on medium speed for 5–10 min until a homogeneous mixture. The curd is then warmed through a texturizer, which looks like a fine sieve. So that the yogurt is thick and smooth as well as to prevent the separation of the whey. After that, cooling to wait for mixing with fruits.

7) Fruiting

Fruit can be added in the amount of 8–25% of the final product. But generally, it is preferable to add 10-15% in the case of a set yogurt to add fruit to the bottom of the cup before adding the yogurt to the cup. In the case of stirred yogurt, the fruit is mixed with the yogurt before filling it into a bowl.

8) Filling and storage

In the case of stirred yogurt after the yogurt is filled in a cup and sealed. Therefore, it is kept in chilled temperature to wait for further distribution. In the case of set yogurt, it is packed and sealed before the fermentation is complete.

Production of functional yogurt with high melatonin and bioactive compounds that will help promote functional and reduce the risk of disease. Can be eaten in normal people including the sick especially for people who have problems with insomnia or lifestyle changes. This may significantly reduce the risk of disease that may arise or prevent complications that will follow or improve functional clearly. In addition, the production of yogurt has functional properties which contains all the main nutrients is the basis that is necessary for the body. It is also a food product group that increases the function of the body for special purposes. Therefore, various aspects of production process and quality of functional yogurt products containing melatonin and bioactive substances are studied. Including the stability of melatonin in the production process and during storage. It can be used as information to produce or improve the production process of functional food for functional-conscious individuals. It also creates a variety of consumer choices for general consumers. It is also the utilization of existing raw materials to create added value and commercial development in the industrial sector.

2.3 Plant-based yogurt

Plant-based yogurt, also known as dairy-free or vegan yogurt, is a dairy yogurt alternative made from non-dairy ingredients. It has gained popularity among individuals who are lactose intolerant, vegans, or those who prefer to avoid dairy products for various reasons.

2.3.1 Components of plant-based yogurt

The typical components of plant-based yogurt include a primary non-dairy ingredient, which serves as the base for the yogurt. Common base ingredients encompass soy, almond, coconut, cashews, oats, chickpea, sesame seed, and rice.

Soybeans (*Glycine max* L.) are a versatile legume, widely grown for their high nutritional value and industrial applications. Major producers include the United States, Brazil, and China, which account for the majority of global production. Soybeans are used extensively for food, animal feed, and industrial purposes like biofuel. Soybeans are cultivated under a variety of environmental conditions. The plant thrives in well-drained soils with high fertility. Soybeans are a staple crop in many countries, contributing to the global food supply by providing a rich source of protein for both humans and livestock. Soybeans undergo processes like fermentation to improve digestibility and enhance nutritional properties, making them ideal for producing soy-based products like tofu, tempeh, soy milk, and yogurt. Soy milk is widely consumed as a dairy-free alternative due to its high protein content, making it comparable to cow's milk in terms of nutrition. Fermenting soy milk with bacterial cultures (such as *Lactobacillus delbrueckii* and *Streptococcus thermophilus*) produces soy yogurt. This yogurt has a slightly lower fat content than traditional dairy yogurt but provides similar amounts of protein. Additionally, soy yogurt contains probiotics, which promote gut health (Akusu and Wordu 2017; K. Liu 2012).

Chickpeas (*Cicer arietinum* L.), also known as garbanzo beans, are a staple legume, especially in South Asian, Mediterranean, and Middle Eastern diets. They are known for their high protein content and versatility in cooking. Chickpeas are widely grown in countries like India, Turkey, and Australia. They are typically grown in semi-arid regions and have relatively low water requirements compared to other crops, making them a sustainable option in many agricultural systems. Chickpeas are usually harvested once the pods are dry and can be stored for extended periods, making them ideal for large-scale production and trade. Chickpea yogurt is less common than soy yogurt but can be made by fermenting chickpea milk with probiotic cultures. Like soy yogurt, chickpea yogurt can be fortified with additional nutrients and probiotics to enhance its health benefits (Arooj et al. 2021; Sofi et al. 2020).

Sesame (*Sesamum indicum* L.) is an ancient oilseed crop grown for its seeds and oil. It is particularly cultivated in tropical and subtropical regions, including India, China, Sudan, and Ethiopia. Sesame seeds are known for their rich, nutty flavor and high oil content. Sesame is highly drought-resistant, making it suitable for regions with limited rainfall. It is usually grown in well-drained, sandy soils and harvested once the seed pods have matured and dried. The seeds can be used whole or pressed to extract sesame oil, which is widely used in cooking, cosmetics, and traditional medicine. Sesame yogurt can be made by fermenting sesame milk with probiotics. Due to the high fat content, sesame yogurt may have a rich, creamy texture, but its production on a commercial scale is less common compared to soy and almond yogurts (Asghar et al., 2014; Sharma et al., 2021). The chemical composition of soybeans, chickpeas, and sesame seeds are shown in Table 4.

Table 4 The chemical composition of soybeans, chickpeas, and sesame seeds

Component	**Soybeans**	**Chickpeas**	**Sesame Seeds**
Protein	32-44%	19-23%	20%
Fat	12-15% (mainly unsaturated fats)	6% (low, mainly unsaturated fats)	~50% (high, mainly unsaturated fats)
Carbohydrates	Moderate, rich in fiber	Moderate, rich in complex carbohydrates	Low, primarily fiber
Key Compounds	Isoflavones, essential amino acids	Essential amino acids (rich in lysine)	Lignans, calcium, magnesium
Fiber	High (both soluble and insoluble)	High (mainly soluble fiber)	Moderate
Minerals	Iron, calcium, magnesium, potassium	Iron, magnesium, phosphorus	High in calcium, magnesium, phosphorus
Special Nutrients	Omega-3 and omega-6 fatty acids	Low fat, high fiber	Rich in antioxidants (sesamin, sesamolin)

Source: Giri and Mangaraj (2012); Liu, 2012; Akusu and Wordu, 2017; Mehaya et al., 2023

2.3.2 Production of plant-based yogurt

The production of plant-based yogurt is similar to that of traditional dairy yogurt, with some variations to accommodate non-dairy ingredients. The details of the production process as follow:

1) Base ingredient selection and processing

The first critical step in producing plant-based yogurt is the selection and preparation of the base ingredient. Commonly used sources include legumes (e.g., soy, chickpeas, and peanuts), nuts (e.g., almonds and cashews), cereals (e.g., oats), and tropical fruits like coconuts. The base ingredients are soaked, ground, and filtered to create a milk-like substance, which serves as the foundation for yogurt production. Each plant source brings unique nutritional properties and textural qualities to the final product. For example, legumes, particularly soybeans, have gained popularity due to their high protein content and ability to create a creamy texture. As Montemurro et al. (2021) noted, the increased protein levels in legumes make them suitable for mimicking the mouthfeel and creaminess of dairy yogurt. Cereals like oats, on the other hand, contribute a smooth texture but tend to have lower protein content. Additionally, enzymatic and thermal treatments are applied to improve digestibility and reduce anti-nutritional compounds that may interfere with nutrient absorption.

2) Fermentation and culturing process

Fermentation is the cornerstone of yogurt production, where plant-based milk is inoculated with probiotic bacterial strains. The process traditionally relies on lactic acid bacteria such as *Lactobacillus delbrueckii* and *Streptococcus thermophilus* to break down sugars into lactic acid. This process lowers the pH, resulting in a tangy flavor and thickened texture. In plant-based yogurt production, achieving the right fermentation conditions is critical because the sugar content and composition of plant milks differ significantly from cow's milk. Craig and Brothers (2021) explored different bacterial strains and fermentation conditions suitable for plant matrices. For example, soy-based yogurt ferments more easily due to its protein-rich composition, while almond and coconut bases require additional sugars to fuel the fermentation process. Probiotic strains not only contribute to the product flavor and texture but also enhance its functional properties, adding potential health benefits such as improved gut health and immune support. Innovations in this phase include the use of prebiotics to enhance the viability of probiotic cultures. Prebiotics, such as inulin or fructooligosaccharides, are added to plant-based yogurt to promote the growth of beneficial bacteria during fermentation and improve the overall gut health benefits.

3) Fortification for nutritional enhancement

One of the significant challenges in plant-based yogurt production is replicating the nutritional profile of dairy-based yogurt. Plant-based alternatives naturally lack essential nutrients like calcium, vitamin D, and vitamin B12, which are abundant in dairy products. As a result, fortification is necessary to ensure that plant-based yogurts meet dietary requirements for these nutrients. Craig and Brothers (2021) conducted a comprehensive review of the nutritional profiles of commercially available plant-based yogurts, finding that while 45% of products were fortified with calcium, only a small percentage contained adequate levels of vitamin D and B12. The inconsistency in fortification practices can affect the health benefits of plant-based yogurt, especially for individuals following strict plant-based diets or those with dietary restrictions that prevent them from consuming fortified dairy alternatives. To address this, more manufacturers are now fortifying their products with higher levels of these essential micronutrients. Additionally, plant-based yogurts may be fortified with protein to improve their nutritional value, especially when derived from low-protein bases like almond or coconut milk. Advances in food technology have led to the incorporation of plant-derived proteins, such as pea protein, into plant-based yogurt formulations, enhancing both the nutritional profile and textural qualities.

4) Textural and sensory optimization

Replicating the smooth, creamy texture of dairy yogurt in plant-based alternatives remains a major challenge. Plant-based ingredients often have different gelling and emulsifying properties compared to dairy milk, resulting in a yogurt that may be thinner or less creamy than consumers expect. The use of hydrocolloids, such as guar gum, locust bean gum, or pectin, helps improve texture by enhancing the yogurt viscosity and stability. Gupta et al. (2022) emphasized the importance of achieving the right balance of protein content, gel firmness, and consistency to enhance consumer acceptability. Their study on plant-based and dairy yogurts found that consumer preference was closely linked to texture, with firmer, creamier plant-based yogurts receiving higher scores for overall liking. Recent innovations in plant-based yogurt production also include the use of biopolymers and emulsifiers to improve the texture and mouthfeel of products made from lower-fat plant sources, such as oats and almonds. Furthermore, the sensory properties of plant-based yogurts are influenced by the fermentation process, as well as the choice of flavoring and sweetening agents. Studies have shown that while plant-based yogurts often appeal to health-conscious consumers, the high sugar content in many commercial products may detract from their perceived health benefits. Therefore, manufacturers are exploring natural sweeteners and flavor enhancers that maintain taste appeal without significantly increasing sugar levels.

5) Sustainability and Environmental Impact

Another driving force behind the rise of plant-based yogurts is their lower environmental footprint compared to dairy products. The production of plant-based alternatives generally requires fewer natural resources, such as water and land, and results in lower greenhouse gas emissions. According to Gupta et al. (2022) almond-based yogurts have a particularly low carbon footprint due to the sustainability of almond farming in certain regions. However, there are ongoing discussions about the sustainability of some plant ingredients, like almonds and coconuts, which may require large amounts of water for cultivation.

2.3.3 Fortification of plant-based yogurt with probiotics

The fortification of plant-based yogurt with probiotics involves adding beneficial bacteria to the yogurt to enhance its probiotic content. The fortification of plant-based yogurt with probiotics has been a growing area of research due to the increasing consumer demand for functional foods that offer health benefits beyond basic nutrition. This literature review examines the advancements in probiotic fortification of plant-based yogurt, with a focus on the selection of probiotics, their health benefits, and the challenges related to maintaining probiotic viability in non-dairy matrices.

1) Probiotic selection and strain compatibility

Probiotics are live microorganisms that confer health benefits to the host when consumed in adequate amounts, and they are commonly added to yogurt to improve gut health, enhance digestion, and boost immunity. In the context of plant-based yogurt, selecting appropriate probiotic strains is crucial due to the differences in the nutrient composition between dairy and plant-based substrates. Plant-based yogurts often have lower lactose and casein content, which are important for the growth of traditional dairy-associated probiotics like *Lactobacillus delbrueckii* and *Streptococcus thermophilus*. Recent research by Montemurro et al. (2021) highlights the importance of selecting strains that can adapt to plant-based matrices. Probiotic strains such as *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, and *Bifidobacterium animalis* have shown compatibility with plant-based substrates like almond, soy, and coconut milk. These strains are selected for their ability to survive in the non-dairy environment, maintain viability during shelf life, and deliver health benefits to the consumer.

2) Health benefits of probiotic fortification

The fortification of plant-based yogurt with probiotics offers several health benefits, particularly related to gut health and the immune system. Probiotics are known to help balance the gut microbiota, reduce inflammation, and enhance digestive health. According to a study by Rasika et al. (2021), plant-based yogurts fortified with *Lactobacillus* and *Bifidobacterium* strains can positively affect gastrointestinal health, reduce symptoms of irritable bowel syndrome (IBS), and enhance the immune response. In addition to gut health, probiotics in plant-based yogurts may offer benefits related to metabolic health. Ding and Shah (2020) explored how probiotics can lower cholesterol levels and improve glucose metabolism when consumed regularly through yogurt, particularly plant-based alternatives that are low in saturated fats. This is particularly relevant to consumers who choose plant-based yogurts for their potential cardiovascular benefits.

3) Challenges in probiotic viability and shelf life

One of the key challenges in fortifying plant-based yogurt with probiotics is maintaining probiotic viability throughout the product shelf life. Probiotics are sensitive to environmental conditions, including temperature, pH, and oxygen exposure, which can reduce their viability over time. Plant-based matrices often have higher acidity and lower protein content compared to dairy, which can further impact probiotic survival. In their review, Shori (2015) explored various strategies to improve the viability of probiotics in plant-based yogurt. These strategies include microencapsulation, which involves coating probiotic bacteria in a protective layer that shields them from environmental stressors during storage. The use of prebiotics, such as inulin and fructooligosaccharides, has also been shown to enhance the survival and activity of probiotics by promoting their growth during fermentation. Another approach is the optimization of fermentation conditions, such as adjusting the pH and fermentation time to create a more favorable environment for probiotics.

4) Fortification techniques and product innovation

Recent advancements in food technology have led to innovations in the fortification of plant-based yogurts with probiotics (M P et al. 2024). For instance, the co-fermentation of plant-based substrates with probiotics and prebiotics has gained attention as a way to enhance both the nutritional profile and the sensory properties of the yogurt. The synergy between probiotics and prebiotics (referred to as synbiotics) can improve the fermentation process, leading to better texture, flavor, and probiotic stability (Fazilah et al. 2018). Innovative products that use non-traditional probiotic strains and substrates have also emerged. Studies have shown that using novel plant bases like pea or chickpea can result in a yogurt that is both high in protein and a suitable carrier for probiotics. These innovations are particularly appealing to consumers seeking plant-based products with functional health benefits.

The fortification of plant-based yogurt with probiotics has advanced significantly in recent years, with research focusing on selecting compatible probiotic strains, ensuring probiotic viability, and delivering health benefits. Despite challenges such as maintaining probiotic stability in plant-based matrices, strategies like microencapsulation and prebiotic addition are helping to improve the efficacy of probiotic fortification. As consumer interest in plant-based and functional foods grows, continued research into probiotic fortification will likely result in more innovative and nutritionally rich plant-based yogurt products.

2.3.4 Nutritional composition

The nutritional composition of plant-based yogurt has been a focal point of research due to increasing consumer demand for healthier and sustainable alternatives to dairy yogurt. This literature review delves into the key components of plant-based yogurt—macronutrients (proteins, fats, and carbohydrates), micronutrients (vitamins and minerals), and fortification practices—while comparing the nutritional profiles of different plant sources.

1) Macronutrient composition: protein, fat, and carbohydrates

The macronutrient content of plant-based yogurt is highly variable and largely dependent on the plant source used as the base. Protein, an important macronutrient in traditional dairy yogurt, is one area where plant-based alternatives can differ significantly. Soy-based yogurt, for example, tends to have the highest protein content among plant-based options, with about 4-6 g of protein per serving, which is similar to or slightly lower than dairy yogurt. This is due to soybean high natural protein content, making soy yogurt one of the closest substitutes in terms of nutrition. A study by (Ozen et al., 2015) notes that other plant-based yogurts, such as those made from almond, coconut, or cashew, often fall short in protein content, with as little as 1-2 g per serving. This issue, manufacturers have begun incorporating additional plant-based proteins, such as pea protein, into yogurt formulations to boost protein levels. This not only improves the nutritional profile but also enhances the texture and mouthfeel of the product. For instance, a review by Montemurro et al. (2021) highlights how the addition of plant proteins helps plant-based yogurts achieve a more comparable protein level to their dairy counterparts, meeting the nutritional expectations of consumers who prioritize high-protein foods. The fat content in plant-based yogurt is another area that shows considerable variation. Coconut-based yogurts are higher in saturated fat due to the natural fat content of coconuts. This type of yogurt can contain between 4-8 g of fat per serving, with a significant portion being saturated fat. Conversely, almond and oat-based yogurts are typically lower in fat, with a larger proportion of healthy unsaturated fats. According to Hughes and Leitch (2020), almond-based yogurts are rich in monounsaturated fats, which are beneficial for heart health. Carbohydrate levels in plant-based yogurt are generally determined by the base ingredient, with oat and rice-based yogurts containing higher amounts of carbohydrates due to their starch content. Additionally, many commercial plant-based yogurts contain added sugars to enhance taste, which can significantly increase the carbohydrate content. Research by Pham et al., (2024) emphasizes that some plant-based yogurts contain more sugar than their dairy counterparts, raising concerns about their overall health impact. In response, some manufacturers are reducing sugar content or using natural sweeteners like stevia or monk fruit to appeal to health-conscious consumers.

2) Micronutrient composition: vitamins and minerals

The micronutrient content of plant-based yogurt is an area where fortification plays a key role. Unlike dairy yogurt, which is naturally rich in calcium, vitamin D, and vitamin B12, plant-based alternatives often lack these essential nutrients. As a result, manufacturers commonly fortify plant-based yogurts to match or exceed the nutritional profile of dairy yogurt. Calcium fortification is particularly important for plant-based yogurt consumers, as dairy products are a primary source of calcium in many diets. According to Craig and Brothers (2021), approximately 45% of plant-based yogurts are fortified with calcium, bringing them closer to the 250-300 mg of calcium typically found in dairy yogurt per serving. However, the extent of fortification varies, with some products offering much lower levels of calcium, potentially making them less beneficial for bone health. Similarly, vitamin D and vitamin B12 are often added to plant-based yogurts, but the levels are inconsistent across brands. Vitamin D is essential for calcium absorption and bone health, while vitamin B12 is crucial for nerve function and red blood cell production. These nutrients are particularly important for vegans, who may not get enough from their diet alone. Almond-based yogurts, in particular, stand out for their high vitamin E content, an antioxidant that helps protect cells from oxidative damage. On the other hand, soy-based yogurts are a good source of potassium and iron, which are beneficial for cardiovascular health and oxygen transport in the blood. However, the bioavailability of these nutrients can be limited by the presence of anti-nutrients like phytates, which are naturally present in plant foods and can inhibit the absorption of minerals such as calcium and iron. M P et al. (2024) highlight that certain processing methods, such as soaking and fermentation, can help reduce the levels of anti-nutrients, thereby improving nutrient absorption.

3) Fortification and nutritional enhancement

Fortification is a widely adopted practice in the production of plant-based yogurts to compensate for their natural deficiencies in certain essential nutrients. Plamada et al. (2023) discusses how fortification not only improves the nutritional content but also adds value to plant-based yogurt as a functional food. Some manufacturers are now incorporating omega-3 fatty acids into plant-based yogurt made from flax or chia seeds. Omega-3 fatty acids are known for their heart-health benefits, including reducing inflammation and lowering the risk of chronic diseases such as heart disease and stroke. In addition to omega-3 fortification, there has been increasing interest in adding probiotics to plant-based yogurts to enhance their functional health properties. Probiotics, such as *Lactobacillus* and *Bifidobacterium* species, are live bacteria that support gut health and immune function. Research by Craig and Brothers (2021) highlights the rise of synbiotic plant-based yogurts, which combine probiotics with prebiotics—indigestible fibers that promote the growth of beneficial gut bacteria. These fortified yogurts are designed to offer consumers not only the nutritional benefits of plant-based ingredients but also the digestive and immune-boosting effects of probiotics.

4) Comparative nutritional analysis across plant bases

The nutritional composition of plant-based yogurt varies significantly depending on the base used. Soy-based yogurt remains the most nutritionally similar to dairy yogurt, particularly in terms of protein content and essential amino acids. Almond-based yogurt, while lower in protein, offers healthy fats and is rich in vitamin E, making it a good option for those seeking antioxidant benefits. Coconut-based yogurt is higher in saturated fat but is often favored for its creamy texture and taste. Oat-based yogurts have gained popularity due to their creamy texture and relatively balanced nutritional profile, though they tend to be higher in carbohydrates compared to other plant-based yogurts. Research by Dhakal et al. (2024)found that oat-based yogurts, while low in protein, can be fortified with additional nutrients to make them more nutritionally competitive with dairy products.

The nutritional composition of plant-based yogurt is diverse and varies based on the plant source used, processing methods, and fortification practices. While soy-based yogurts offer the most comparable protein levels to dairy, other plant-based options like almond, coconut, and oat-based yogurts provide benefits in terms of healthy fats, vitamins, and minerals. However, fortification is necessary to address deficiencies in calcium, vitamin D, and vitamin B12, making these products more nutritionally complete. As the plant-based yogurt market continues to grow, further innovations in fortification and product development will likely enhance the nutritional profile and appeal of these products.

2.3.5 Health benefits of consuming plant-based yogurt

The health benefits associated with consuming plant-based yogurt have gained significant attention due to the growing popularity of plant-based diets and concerns about dairy intolerances and environmental sustainability. This literature review highlights several key health benefits of plant-based yogurt, including improved digestive health, cardiovascular benefits, and enhanced nutrient intake through fortification.

1) Improved digestive health

One of the most notable health benefits of plant-based yogurt is its positive effect on digestive health, particularly when fortified with probiotics. Probiotics, such as *Lactobacillus* and *Bifidobacterium* strains, are live bacteria that can help balance the gut microbiota, improving digestion and reducing symptoms of digestive disorders like irritable bowel syndrome (IBS) and lactose intolerance. According to a study by Rasika et al. (2021b), plant-based yogurts fortified with probiotics have been shown to support gut health by promoting the growth of beneficial bacteria and inhibiting harmful pathogens. These benefits are similar to those of dairy-based yogurts, but plant-based yogurts may be more suitable for people with lactose intolerance or dairy allergies. In addition to probiotics, the fiber content in certain plant-based yogurts, especially those made from oat or soy, can further enhance digestive health. Fiber acts as a prebiotic, feeding the good bacteria in the gut and promoting regular bowel movements. Montemurro et al. (2021) note that plant-based yogurts enriched with prebiotic fibers like inulin can improve gut function and overall digestive health, offering additional benefits beyond those of dairy yogurt.

2) Cardiovascular health benefits

Plant-based yogurts are also associated with cardiovascular health benefits, primarily due to their lower levels of saturated fats and higher levels of unsaturated fats compared to dairy-based yogurt. Dairy products, particularly those high in saturated fats, have been linked to elevated cholesterol levels and an increased risk of cardiovascular diseases. In contrast, plant-based yogurts made from almond, oat, or soy tend to be lower in saturated fat and rich in heart-healthy unsaturated fats. Research by Hughes and Leitch (2020) indicates that consuming plant-based yogurt can contribute to improved cholesterol profiles, particularly when it is fortified with omega-3 fatty acids. Omega-3s, which are commonly found in flax and chia-based yogurts, have been shown to reduce inflammation, lower blood pressure, and decrease the risk of heart disease. Moreover, plant-based yogurts typically contain lower sodium levels than their dairy counterparts, which is beneficial for maintaining healthy blood pressure levels. Vij et al. (2011) also found that regular consumption of plant-based yogurts, especially those made from soy, can help regulate lipid metabolism, reduce LDL cholesterol levels, and support overall cardiovascular health. This makes plant-based yogurts an attractive option for individuals looking to improve heart health through dietary changes.

3) Weight management and metabolic health

Plant-based yogurts are often lower in calories and fat than traditional dairy yogurts, which can contribute to better weight management. For those looking to maintain or lose weight, the lower calorie content of many plant-based options, combined with their high fiber content, can help increase satiety and reduce overall calorie intake. Craig and Brothers (2021) points out that the high fiber and protein content in soy-based and oat-based yogurts can enhance feelings of fullness, thereby supporting weight management efforts. In particular, the inclusion of prebiotic fibers in plant-based yogurts has been linked to improved glucose metabolism and a reduced risk of developing type 2 diabetes. These metabolic benefits are especially important for individuals with insulin resistance or those at risk of metabolic disorders. Additionally, the lower glycemic index (GI) of certain plant-based yogurts, especially those without added sugars, can help regulate blood sugar levels and prevent spikes, making them a better option for individuals managing diabetes or prediabetes.

4) Nutrient fortification and bone health

While plant-based yogurts may lack certain nutrients naturally found in dairy, such as calcium and vitamin D, fortification practices have significantly improved their nutritional profile. Many plant-based yogurts are now fortified with calcium, vitamin D, and vitamin B12, which are essential for bone health and other bodily functions. Fortified plant-based yogurts can therefore help meet the daily requirements for these nutrients, especially for those who follow a vegan or plant-based diet. According to Craig and Brothers (2021), the addition of calcium and vitamin D to plant-based yogurts has made them a viable alternative to dairy yogurt for maintaining bone health. These fortified products can help prevent bone-related diseases such as osteoporosis, especially in populations at risk of calcium and vitamin D deficiencies. Additionally, vitamin B12, which is typically lacking in plant-based diets, is commonly added to plant-based yogurts, ensuring that consumers meet their recommended daily intake of this essential vitamin.

The health benefits associated with consuming plant-based yogurt include improved digestive health through probiotic and prebiotic content, cardiovascular benefits from lower saturated fats and higher unsaturated fats, and potential support for weight management and metabolic health. Fortification with essential nutrients like calcium, vitamin D, and vitamin B12 makes plant-based yogurt a viable alternative to dairy yogurt, particularly for individuals following vegan or lactose-free diets. As research continues to evolve, plant-based yogurt is likely to play an increasingly important role in promoting both individual and environmental health.

2.3.6 Benefits of probiotics in plant-based yogurt

The incorporation of probiotics into plant-based yogurt has been extensively studied due to the rising popularity of non-dairy alternatives and the recognized health benefits of probiotics for gut health, immune support, and overall wellness. This literature review explores the key benefits of probiotics when added to plant-based yogurt, focusing on their impact on digestive health, immune function, and potential to alleviate symptoms of lactose intolerance and irritable bowel syndrome (IBS).

1) Digestive health and gut microbiota balance

The most widely recognized benefit of probiotics in both dairy and plant-based yogurt is their ability to support digestive health by improving gut microbiota balance. Probiotics are live microorganisms that, when consumed in adequate amounts, confer health benefits, particularly by promoting the growth of beneficial bacteria in the gut. According to Boeck et al. (2021), probiotics such as *Lactobacillus* and *Bifidobacterium* species added to plant-based yogurt can restore the natural balance of gut flora, which is essential for proper digestion and nutrient absorption. This is particularly beneficial for individuals with dysbiosis, a condition where harmful bacteria outnumber beneficial bacteria in the digestive system, leading to issues like bloating, constipation, and diarrhea. Furthermore, plant-based yogurts are often made from ingredients like soy, almond, or oats, which can act as prebiotics—fibers that feed and support the growth of probiotics. When combined, these prebiotics and probiotics (referred to as synbiotics) enhance the efficacy of probiotics in promoting gut health. Craig and Brothers (2021) emphasize that probiotic-enriched plant-based yogurts help maintain a healthy balance of gut microbiota, aiding in the digestion of food and reducing gastrointestinal discomfort.

2) Immune system support

Another significant benefit of probiotics in plant-based yogurt is their role in boosting the immune system. The gut is closely linked to immune function, and a healthy balance of gut bacteria helps regulate immune responses and reduce inflammation. Probiotic consumption has been shown to enhance the production of antibodies and stimulate immune cells, such as T-cells and macrophages, which are essential for fighting infections. Kumar et al. (2023) reports that probiotics added to plant-based yogurt can help improve immune responses by reducing inflammation and enhancing the activity of natural killer cells, which play a critical role in defending the body against viruses and harmful bacteria. The study also notes that individuals who regularly consume probiotic-rich foods, including plant-based yogurts, have a lower risk of upper respiratory infections and other immune-related conditions. Moreover, Craig and Brothers (2021) suggests that probiotics in plant-based yogurt may also modulate gut-associated lymphoid tissue (GALT), which is a key component of the immune system located in the gut. By enhancing the health of GALT, probiotics help strengthen the body's overall immune defense mechanisms.

3) Alleviation of Lactose Intolerance and IBS Symptoms

One of the main reasons for the growing popularity of plant-based yogurt is its suitability for individuals with lactose intolerance, a condition where the body cannot properly digest lactose, the sugar found in dairy products. While plant-based yogurts are naturally free from lactose, the addition of probiotics offers further benefits for individuals with this condition. Probiotics help break down food more efficiently and improve lactose digestion in people with lactose intolerance, even in those who consume small amounts of dairy alongside plant-based foods. Additionally, the presence of probiotics in plant-based yogurt has been shown to alleviate symptoms of irritable bowel syndrome (IBS), a common digestive disorder characterized by bloating, cramping, and changes in bowel movements. Probiotic strains such as *Bifidobacterium animalis* and *Lactobacillus acidophilus*, which are often added to plant-based yogurt, can reduce inflammation in the gut and restore the balance of intestinal bacteria, thereby reducing IBS symptoms. Craig and Brothers (2021) highlights the effectiveness of probiotic-enriched plant-based yogurts in managing IBS symptoms, noting significant improvements in digestion and a reduction in bloating and abdominal pain.

4) Other Potential Health Benefits

In addition to digestive health, immune support, and symptom alleviation for lactose intolerance and IBS, probiotics in plant-based yogurt have been linked to other potential health benefits, such as improving mental health through the gut-brain axis and supporting metabolic health. The gut-brain axis is the bidirectional communication system between the gut and the brain, and emerging research suggests that probiotics may help reduce anxiety and depression by promoting a healthy gut microbiome. Barengolts (2016) emphasize that plant-based yogurts fortified with probiotics may offer these mental health benefits, though more research is needed to fully understand the mechanisms involved. Furthermore, probiotics have been linked to improvements in metabolic health, including better regulation of blood sugar levels and weight management. Some studies suggest that consuming probiotic-rich plant-based yogurt can help improve insulin sensitivity, reduce inflammation, and support healthy weight loss.

The addition of probiotics to plant-based yogurt offers numerous health benefits, particularly for digestive health, immune function, and the management of lactose intolerance and IBS symptoms. By promoting a healthy balance of gut bacteria, probiotics enhance digestion, support immune responses, and reduce gastrointestinal discomfort. Additionally, plant-based yogurts fortified with probiotics provide an attractive option for individuals seeking non-dairy alternatives that support overall health and well-being. The benefits of probiotics in plant-based yogurt are shown in Table 5.

Table 5 The benefits of probiotics in plant-based yogurt.

Health Benefit	**Description**	**Sources**
Digestive Health	Probiotics like *Lactobacillus* and *Bifidobacterium* in plant-based yogurt restore gut flora balance, aiding digestion and reducing issues like bloating and constipation.	Kostic et al. (2023), Chalupa-Krebzdak et al. (2020)
Immune System Support	Probiotics enhance immune responses by increasing the production of antibodies and immune cells, reducing inflammation and the risk of infections.	Sethi et al. (2021), Craig & Brothers (2021)
Alleviation of Lactose Intolerance Symptoms	Probiotics in plant-based yogurt help improve digestion in individuals with lactose intolerance, reducing symptoms like bloating and discomfort.	Chalupa-Krebzdak et al. (2020), Kostic et al. (2023)

Health Benefit	Description	Sources
Relief from IBS Symptoms	Probiotics reduce inflammation and restore gut balance, alleviating IBS symptoms such as bloating, cramping, and irregular bowel movements.	Chalupa-Krebzdak et al. (2020)
Mental Health Benefits	Probiotics may help improve mental health by positively affecting the gut-brain axis, potentially reducing anxiety and depression.	Kostic et al. (2023)
Metabolic Health	Probiotic-rich plant-based yogurts improve insulin sensitivity, support weight management, and may help reduce inflammation.	Kostic et al. (2023), Sethi et al. (2021)

2.4 Probiotic

Probiotics have gained considerable attention recently due to their potential health benefits, particularly in gut health, immune function, and chronic disease prevention. This literature review aims to explore various aspects of probiotics, including their definitions, mechanisms of action, and applications in health and disease management.

2.4.1 Definition and classification of probiotics

Probiotics are live microorganisms, primarily bacteria and yeasts, that provide health benefits to the host when consumed in adequate amounts. According to the definition provided by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (Montemurro et al. 2021). These microorganisms are mainly from the genera Lactobacillus, Bifidobacterium, and Saccharomyces, which are commonly found in fermented foods and dietary supplements (Figure 9).

1) Key probiotic genera

- Lactobacillus: This genus is one of the most studied and widely used in probiotic formulations. Species like *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, and *Lactobacillus casei* are known for their beneficial effects on gut health, including preventing diarrhea and enhancing gut barrier function (Montemurro et al., 2021). For instance, Lactobacillus rhamnosus GG (LGG) is well known for its efficacy in preventing and treating antibiotic-associated diarrhea (Nájera Espinosa et al. 2024).

- Bifidobacterium: Another prominent genus in probiotics, Bifidobacterium species play a key role in modulating the gut microbiota and enhancing digestive health. Bifidobacterium lactis is frequently used in probiotic supplements due to its ability to improve gut health, enhance immune function, and alleviate constipation

(Craig and Brothers 2021). It is commonly found in fermented dairy products and infant formulas.

- Saccharomyces: *Saccharomyces boulardii* is the primary probiotic species in this genus and is widely recognized for its role in treating and preventing diarrhea, particularly in cases of traveler’s diarrhea and antibiotic-associated diarrhea. Unlike bacterial probiotics, *Saccharomyces boulardii* is a yeast, which makes it resilient to antibiotics.

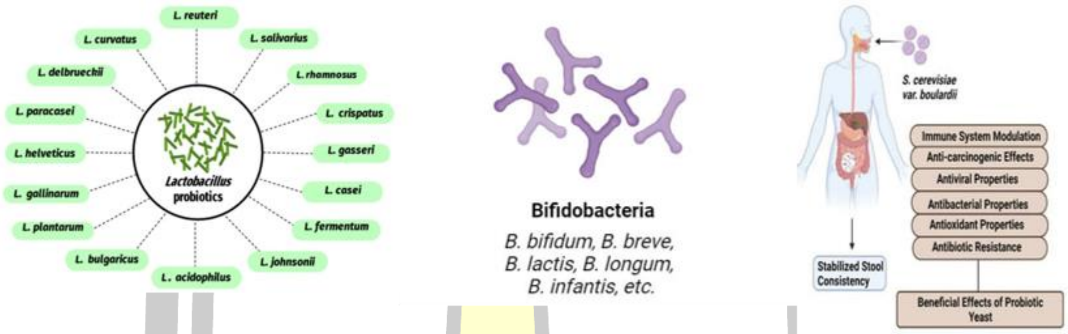


Figure 9 Probiotic strains of the genus Lactobacillus, Bifidobacterium and Saccharomyces.
Source: Abid et al. (2022); Al-Yami et al. (2022)

2) Probiotic strain specific effects

Not all probiotics offer the same benefits; their health effects are strain-specific. *Lactobacillus rhamnosus* GG has demonstrated particular effectiveness in reducing the incidence and duration of diarrhea in children and adults (Montemurro et al. 2021). On the other hand, *Bifidobacterium lactis* HN019 is often associated with immune enhancement and promoting regular bowel movements in healthy adults (Nájera Espinosa et al. 2024). The effectiveness of a probiotic depends on its specific strain, the dose, and the health condition being addressed.

3) Classification based on health benefits

Probiotics are also classified based on their applications (Craig and Brothers 2021; Montemurro et al. 2021; Nájera Espinosa et al. 2024).

- Digestive health: Most probiotics are used to maintain or restore a healthy balance of gut microbiota, which is essential for proper digestion and nutrient absorption. Probiotics such as *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* are commonly used to prevent or treat gastrointestinal issues such as diarrhea, IBS, and constipation.
- Immune modulation: Certain probiotic strains have been shown to enhance immune responses by increasing the production of antibodies and immune cells. Bifidobacterium strains, for instance, are often associated with boosting immune function.

- Mental health: Some studies suggest that probiotics may have a positive impact on mental health through the gut-brain axis, with certain strains potentially alleviating symptoms of anxiety and depression.

2.4.2 Mechanisms of action of probiotics

Probiotics exert their health benefits through several well-established mechanisms. These include competitive exclusion of pathogens, modulation of the immune system, enhancement of gut barrier integrity, and production of bioactive compounds (Craig and Brothers 2021; Montemurro et al. 2021).

1) Competitive exclusion of pathogens

Probiotics help maintain a balanced gut microbiota by outcompeting harmful bacteria for nutrients and adhesion sites in the gastrointestinal tract. This process is known as competitive exclusion. Probiotic bacteria such as Lactobacillus and Bifidobacterium can adhere to the intestinal mucosa, preventing colonization by pathogenic bacteria like *Clostridium difficile*, *Salmonella*, and *Escherichia coli*. Additionally, probiotics secrete compounds like bacteriocins that inhibit the growth of harmful microorganisms.

2) Immune system modulation

One of the most studied benefits of probiotics is their ability to modulate the host’s immune system. Probiotics enhance the production of immune cells, such as macrophages, T-cells, and natural killer cells, and stimulate the production of immunoglobulins, particularly IgA, which plays a crucial role in mucosal immunity. Probiotics can also reduce inflammation by modulating cytokine production and promoting anti-inflammatory responses. For instance, *Bifidobacterium longum* and *Lactobacillus casei* have been shown to reduce pro-inflammatory cytokines such as TNF-α and IL-6 (Markowiak and Śliżewska 2017b).

3) Enhancement of gut barrier integrity

The intestinal epithelial barrier is critical in preventing the translocation of harmful bacteria and toxins into the bloodstream. Probiotics help reinforce the gut barrier by enhancing tight junction integrity between epithelial cells, which prevents the leakage of pathogenic bacteria and their toxins. *Lactobacillus plantarum* and *Lactobacillus rhamnosus* are known to promote gut barrier function by regulating proteins such as occludin and claudin, which are crucial for tight junction maintenance.

4) Production of bioactive compounds

Probiotics produce bioactive substances such as short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, which are essential for gut health. SCFAs provide energy for colonocytes, enhance gut barrier function, and possess anti-inflammatory properties. Additionally, SCFAs lower intestinal pH, creating an unfavorable environment for pathogenic bacteria. Probiotics also produce antimicrobial peptides and hydrogen peroxide, further inhibiting the growth of harmful microorganisms.

5) Regulation of the gut-brain axis

Probiotics are increasingly recognized for their ability to influence the gut-brain axis, which refers to the bidirectional communication between the gastrointestinal tract and the brain. Probiotic strains such as *Lactobacillus rhamnosus* have been shown to produce neuroactive compounds like gamma-aminobutyric acid (GABA), which can affect mood and anxiety. These effects on mental health are thought to occur through the modulation of gut microbiota, which impacts the production of neurotransmitters and the regulation of inflammation.

2.4.3 Probiotics and circadian rhythm

The relationship between probiotics and circadian rhythm is a growing field of interest in research, shedding light on how the gut microbiome and probiotics interact with the body's biological clock. Circadian rhythms govern many physiological processes, including sleep-wake cycles, hormone release, digestion, and metabolism. When the circadian rhythm is disrupted (due to factors like shift work, jet lag, or sleep disorders), it can lead to metabolic disorders, impaired immune responses, and even mental health issues (Figure 10). Probiotics, through their influence on the gut microbiota, can play a crucial role in maintaining and restoring circadian balance (Thaiss et al. 2016; Liu et al. 2020; Matenchuk, Mandhane, and Kozyrskyj 2020; Sivamaruthi et al. 2021).

1) Gut microbiota and the circadian clock

The gut microbiota has its own circadian oscillations, meaning that the composition and activity of the microbial community in the gut change according to the time of day. For instance, the relative abundance of certain bacterial species may increase during the day and decrease at night, aligning with the body metabolic needs during fasting and feeding cycles. When circadian rhythms are disrupted, such as in cases of sleep deprivation or irregular eating schedules, the gut microbiota natural fluctuations are also disturbed. This can lead to an imbalance known as dysbiosis, which has been linked to a variety of health conditions, including obesity, type 2 diabetes, and inflammatory diseases. Probiotic supplementation may help stabilize gut microbiota during circadian misalignment, thereby promoting metabolic and gut health.

2) Impact of probiotics on metabolic health in circadian disruption

Circadian misalignment, like that experienced by shift workers or people who frequently travel across time zones, is associated with negative metabolic outcomes such as increased risk of obesity, insulin resistance, and glucose intolerance. Probiotics may offer a protective effect by restoring healthy microbiota composition and improving metabolic processes during these disruptions. Probiotic strains such as *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Bifidobacterium longum* have been shown to improve glucose metabolism and insulin sensitivity, which are often impaired when circadian rhythms are disrupted. These probiotics may also regulate lipid metabolism, which could help prevent the development of metabolic syndrome under conditions of circadian misalignment.

3) Probiotics and sleep regulation

Circadian rhythms play a critical role in regulating sleep-wake cycles. There is increasing evidence that the gut microbiota, influenced by probiotics, may affect sleep quality and duration. Certain probiotic strains, such as *Lactobacillus brevis* and *Bifidobacterium longum*, produce neurotransmitters like serotonin and GABA, which are essential for sleep regulation. Serotonin, in particular, is a precursor to melatonin, the hormone that helps regulate sleep cycles. By influencing serotonin levels, probiotics can help synchronize the body's sleep-wake cycle, improving sleep quality and duration. Moreover, probiotics may help reduce stress and anxiety, two factors that commonly interfere with sleep and disrupt circadian rhythms.

4) Immune function and circadian rhythms

The immune system operates according to circadian rhythms, with various immune cells showing fluctuating activity throughout the day. Disruption of the circadian clock can impair immune function, making the body more susceptible to infections and inflammation. Probiotics have been shown to enhance immune responses by interacting with circadian-regulated gut microbiota. Probiotics like *Lactobacillus reuteri* and *Bifidobacterium animalis* have been shown to regulate the production of cytokines and other immune molecules in a time-dependent manner. This means that probiotics may help synchronize immune function with the body’s circadian rhythms, improving immune surveillance and response to pathogens, especially in people with disrupted sleep patterns.

5) The gut-brain axis and circadian rhythms

The gut-brain axis, a bidirectional communication network between the gut and the brain, is closely linked to circadian rhythms. Probiotics influence this connection by producing metabolites such as SCFAs and neurotransmitters that affect brain function and behavior. These gut-derived molecules can regulate the body internal clock, influencing mood, cognitive function, and even mental health. Probiotics like *Lactobacillus rhamnosus* have been shown to produce GABA, a neurotransmitter that has calming effects on the nervous system and can help reduce stress and anxiety. By improving gut health and enhancing the gut-brain communication, probiotics may help alleviate circadian-related mental health issues such as depression and anxiety disorders.

The interplay between probiotics and circadian rhythms is an emerging field of study, showing that probiotics may offer significant benefits in maintaining circadian health. Through their ability to modulate the gut microbiota, probiotics can support metabolic health, improve sleep quality, boost immune function, and influence the gut-brain axis in a time-dependent manner. Future research will likely explore the full potential of probiotics in managing circadian-related health disorders, such as metabolic syndrome, insomnia, and mood disorders.

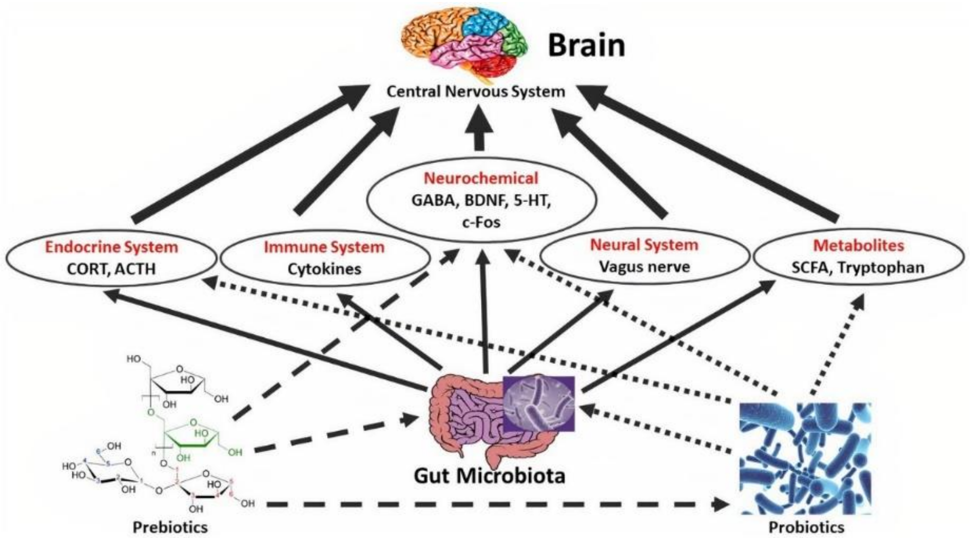


Figure 10 The effect of probiotics on the central nervous system/CNS through the effect on the microbiome-gut-brain axis.
Source: Ansari (2023)

2.4.4 Utilization of probiotics in yogurt

Probiotics, live microorganisms that provide health benefits when consumed in adequate amounts, are commonly utilized in yogurt to enhance its nutritional and health properties. Yogurt is a fermented dairy product traditionally containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, but in recent years, additional probiotic strains like *Lactobacillus acidophilus*, *Bifidobacterium lactis*, and *Lactobacillus rhamnosus* have been incorporated to create probiotic yogurt. These strains have been linked to various health benefits, including improved gut health, enhanced immune function, and better digestion. The utilization of probiotics in yogurt are shown in Table 6.

The utilization of probiotics in yogurt enhances both its nutritional value and health benefits, particularly concerning digestive health, immune function, and metabolic regulation. By incorporating specific probiotic strains, yogurt producers can develop functional foods tailored to improving various health outcomes. The future of probiotic yogurt continues to evolve with new innovations in fortification and strain selection, expanding its potential as a staple health-promoting food product.

Table 6 Utilization of probiotics in yogurt.

Aspect	Details	References
Enhanced Gut Health and Digestion	Probiotic strains like *Lactobacillus acidophilus* and *Bifidobacterium bifidum* promote gut health, improve digestion, and	(Sanders and Marco 2010)

Aspect	Details	References
	reduce symptoms of lactose intolerance and IBS.	
Immune System Modulation	Probiotics such as *Lactobacillus rhamnosus* and *Bifidobacterium lactis* enhance immune function by stimulating immunoglobulin production and modulating cytokine levels.	(Markowiak and Śliżewska 2017b)
Metabolic Health Benefits	Probiotic strains like *Lactobacillus plantarum* and *Bifidobacterium longum* improve insulin sensitivity, regulate blood glucose, and reduce cholesterol levels.	(Punia et al. 2020)
Improved Texture and Flavor	Probiotics, such as *Lactobacillus casei* and *Lactobacillus rhamnosus*, improve yogurt texture and flavor by producing lactic acid and reducing post-acidification.	(Gobbetti et al. 2018; Weese and Martin 2011)
Fortification with Prebiotics	Probiotic yogurt can be fortified with prebiotics, enhancing the growth of probiotics and further supporting gut health and digestion (synbiotic effect).	(Sanders and Marco 2010)

2.5 Bioactive compound

2.5.1 Introduction of bioactive compounds

Bioactive compounds are naturally occurring chemical substances found in various sources like plants, animals, fungi, and microorganisms. Although not classified as essential nutrients, these compounds have been shown to exert significant biological activities, contributing to health benefits and disease prevention. Bioactive compounds include diverse groups such as polyphenols, flavonoids, carotenoids, glucosinolates, alkaloids, and terpenoids. Their ability to interact with biological systems enables them to have antioxidant, anti-inflammatory, antimicrobial, anticancer, and cardioprotective effects. Among the most studied bioactive compounds, polyphenols and carotenoids have received widespread attention for their potent health-promoting properties. Polyphenols, primarily found in fruits, vegetables, tea, and wine, exhibit antioxidant activity by scavenging free radicals, reducing oxidative stress, and protecting cells from damage. Carotenoids, including compounds like beta-carotene, lycopene, and lutein, have been linked to enhanced eye health and a reduced risk of certain cancers. Alkaloids, another class of bioactives, are well-known for their pharmacological properties, including pain relief and antimicrobial activity.

1) Health benefits of bioactive compounds

Bioactive compounds have garnered significant interest in the scientific community due to their ability to prevent and mitigate chronic diseases. The antioxidant properties of polyphenols, for example, have been associated with reducing the risk of cardiovascular diseases by preventing oxidative damage to LDL cholesterol and enhancing vascular function. Recent studies continue to support the notion that diets rich in polyphenol-containing foods are linked to reduced risks of coronary heart disease, stroke, and hypertension. Carotenoids have shown protective effects against certain cancers and age-related eye disorders. For instance, lycopene, predominantly found in tomatoes, has been associated with a lower risk of prostate cancer. Additionally, lutein and zeaxanthin, carotenoids present in leafy greens, play a significant role in maintaining eye health by preventing oxidative stress in the retina and reducing the risk of cataracts and age-related macular degeneration. Bioactive compounds like flavonoids, found in foods such as berries, cocoa, and onions, offer potent cardiovascular benefits. Flavonoids improve endothelial function, reduce inflammation, and modulate blood pressure, contributing to overall heart health. These compounds also possess neuroprotective properties, helping to mitigate age-related cognitive decline and neurodegenerative diseases like Alzheimer’s and Parkinson’s.

2) Mechanisms of cction

The health-promoting effects of bioactive compounds are mediated through various mechanisms. For instance, polyphenols modulate cell signaling pathways related to inflammation, apoptosis (cell death), and antioxidant defense. By regulating enzymes such as NADPH oxidase and nitric oxide synthase, polyphenols reduce the generation of reactive oxygen species (ROS), thereby minimizing oxidative damage and inflammation. Similarly, carotenoids exert their protective effects by quenching singlet oxygen, a reactive oxygen species that contributes to cell damage. Carotenoids also influence gene expression related to oxidative stress and inflammation. For example, beta-carotene can be converted into vitamin A in the body, which plays a critical role in immune function, vision, and skin health. Furthermore, bioactive compounds have been shown to influence gut microbiota composition, promoting a healthy balance of beneficial bacteria. Polyphenols, for example, are metabolized by gut bacteria into bioactive metabolites, which can exert anti-inflammatory and antioxidant effects throughout the body. This interaction between bioactive compounds and gut microbiota has opened new avenues of research into how these compounds contribute to gut health and overall well-being.

3) Bioactive compounds in functional foods

One of the most exciting applications of bioactive compounds is their use in functional foods—foods designed to provide health benefits beyond basic nutrition. The food industry is exploring new methods to extract and stabilize bioactive compounds from natural sources and incorporate them into products such as beverages, cereals, dairy, and snacks. Polyphenol-enriched beverages like green tea, fruit juices, and red wine are marketed for their antioxidant properties, while carotenoid-fortified products aim to improve eye and skin health. Bioactive peptides, derived from protein hydrolysis (especially milk and soy proteins), have also shown promise as functional food ingredients. These peptides can have hypotensive effects, meaning they help to lower blood pressure by inhibiting the enzyme responsible for converting angiotensin I to angiotensin II, a potent vasoconstrictor. Other applications include fortification of foods with omega-3 fatty acids, flavonoids, and glucosinolates for cardiovascular and cancer-prevention benefits.

4) Challenges in bioavailability

Despite their proven health benefits, one of the primary challenges associated with bioactive compounds is their low bioavailability. Bioavailability refers to the fraction of an ingested compound that enters the bloodstream and exerts biological effects. Factors such as the food matrix, digestive stability, metabolism, and absorption efficiency can significantly impact the effectiveness of bioactive compounds in the body. To overcome these challenges, researchers are investigating new methods of enhancing bioavailability, including nanoencapsulation, emulsification, and the use of delivery systems like liposomes. These technologies can protect bioactive compounds from degradation during digestion and improve their absorption in the gut. Future studies are likely to focus on improving the efficacy of bioactive compounds through novel food processing technologies and personalized approaches to nutrition.

Bioactive compounds have emerged as key players in promoting health and preventing chronic diseases. Their antioxidant, anti-inflammatory, and cardioprotective properties make them valuable for functional foods and nutraceuticals. Despite challenges related to bioavailability, advances in food technology and personalized nutrition approaches may enhance the effectiveness of bioactive compounds in the future (Milenkovic et al. 2017; Chai et al. 2018; Câmara et al. 2020; Shrinet et al. 2021)

2.5.2 Bioactive compounds in yogurt

Yogurt is a nutrient-rich fermented dairy product that is widely consumed due to its health benefits. One of the key reasons for its health-promoting properties is the presence of bioactive compounds. These bioactive components include probiotics, peptides, polyphenols (in fortified variants), vitamins, and minerals, which work synergistically to support overall well-being. As research on functional foods has expanded, yogurt has become a significant focus for the delivery of bioactive compounds. Research has been increasing interest in exploring and optimizing the bioactive potential of yogurt to deliver targeted health benefits (Fernández-Mar et al. 2012; Hafeez et al. 2014; Fraga et al. 2019; Mediza Romero et al. 2021; Chelladhurai et al. 2023).

1) Bioactive peptides in yogurt

Bioactive peptides, generated from the hydrolysis of milk proteins during fermentation, have been recognized for their diverse biological activities. These peptides possess antihypertensive, antimicrobial, antioxidant, and immune-modulating properties. During yogurt fermentation, bacterial starter cultures like *Lactobacillus* and *Streptococcus thermophilus* hydrolyze casein and whey proteins into smaller peptide fragments that exhibit specific bioactivities. The most prominent health benefit associated with these peptides is their ability to inhibit the angiotensin-converting enzyme (ACE), which helps to manage high blood pressure. The studies have highlighted how yogurt-based peptides act as ACE inhibitors, contributing to cardiovascular health by reducing hypertension. Additionally, bioactive peptides can modulate immune responses and possess anti-inflammatory properties, which help in managing conditions like inflammatory bowel disease (IBD). The fermentation process also generates opioid-like peptides from casein, which may regulate gut motility and have beneficial effects on digestive health.

2) Probiotics as bioactive components in yogurt

Probiotics, the live beneficial microorganisms present in yogurt, are among the most extensively researched bioactive components. Probiotic strains such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* contribute to enhanced gut health, improved digestion, and strengthened immunity. Probiotics function by balancing gut microbiota, modulating the immune response, and exerting anti-inflammatory effects. According to (Fernández-Mar et al. 2012), consuming probiotic-rich yogurt can positively influence gut microbiota composition, enhancing microbial diversity and promoting the growth of beneficial bacteria. Regular consumption of probiotic yogurt has also been shown to alleviate symptoms of lactose intolerance, irritable bowel syndrome (IBS), and other gastrointestinal disorders. Furthermore, probiotics may protect against infections by enhancing the body's immune defenses, particularly by increasing the production of antibodies and improving the activity of natural killer cells.

3) Polyphenol-fortified yogurt

Polyphenols, known for their potent antioxidant properties, are a class of bioactive compounds that have been incorporated into yogurt formulations to boost health benefits. Polyphenols from natural sources such as fruits, teas, and vegetables can be added to yogurt, making it a functional food with enhanced antioxidant capacity. Polyphenol-enriched yogurts not only provide protection against oxidative stress but also offer cardiovascular and neuroprotective benefits. In a study by Mediza Romero et al. 2021), yogurt fortified with polyphenols from green tea exhibited increased antioxidant activity compared to non-fortified variants. The polyphenols in yogurt helped to neutralize free radicals, reducing oxidative damage to cells, which is a key factor in aging and disease development. Additionally, the synergistic relationship between probiotics and polyphenols in yogurt enhances the stability and bioavailability of polyphenols during digestion. This suggests that polyphenol-fortified yogurts may offer a more effective delivery method for these compounds compared to other foods. However, polyphenol fortification presents certain challenges, particularly with regard to flavor, as some polyphenols impart a bitter taste. Researchers are working on overcoming these sensory limitations by modifying yogurt production methods and using encapsulation technologies to mask undesirable flavors.

4) Vitamins and other fortifications

Yogurt can also be fortified with essential vitamins and minerals to further enhance its bioactive properties. Vitamin D and vitamin E fortifications, for instance, have been studied extensively due to their roles in immune function, bone health, and antioxidant activity. Vitamin D-fortified yogurt has been shown to improve bone mineral density and reduce the risk of fractures, particularly in older populations and individuals with vitamin D deficiencies. Additionally, omega-3 fatty acids have been added to yogurt to support heart health. Omega-3-enriched yogurt products have been linked to reduced blood triglyceride levels, improved heart rate variability, and decreased inflammation. Similarly, yogurt fortified with plant sterols helps to reduce cholesterol absorption in the intestines, which is beneficial for individuals with high cholesterol levels.

5) Mechanisms of action and health implications

The bioactive compounds in yogurt work through several mechanisms to confer health benefits. For example, probiotics support digestive health by increasing the population of beneficial gut bacteria, which outcompete harmful pathogens. They also produce short-chain fatty acids (SCFAs) such as butyrate, which have anti-inflammatory effects in the gut and support the integrity of the gut lining. Bioactive peptides exert their effects by interacting with enzymes and receptors in the body. For instance, peptides that inhibit ACE help regulate blood pressure by reducing the conversion of angiotensin I to angiotensin II, a molecule that constricts blood vessels. Antioxidants such as polyphenols scavenge reactive oxygen species (ROS) and prevent oxidative damage to cells, which is a critical factor in preventing chronic diseases like cancer and cardiovascular conditions.

6) Challenges and future directions

Despite the clear health benefits of bioactive compounds in yogurt, challenges remain in terms of bioavailability, stability, and sensory characteristics. Probiotics, for instance, must survive the acidic environment of the stomach to reach the intestines, where they exert their beneficial effects. Similarly, polyphenols may be unstable during storage and processing, leading to reduced efficacy. To address these challenges, researchers are exploring novel food processing techniques and delivery systems such as microencapsulation and nanoencapsulation to enhance the stability and bioavailability of bioactive compounds in yogurt. These technologies protect the active ingredients from degradation and improve their absorption in the digestive tract. The development of personalized functional yogurts based on individual health needs and genetic profiles is a growing area of research. Advances in food technology and nutrigenomics could enable the creation of customized yogurt products designed to deliver specific bioactive compounds that target individual health conditions, such as cardiovascular disease, diabetes, or cognitive decline.

Bioactive compounds in yogurt, including peptides, probiotics, polyphenols, and fortified nutrients, play a pivotal role in promoting health. The health benefits of these compounds extend from improving cardiovascular and digestive health to boosting immune function and protecting against oxidative stress. Advances in food technology and research will continue to enhance the potential of yogurt as a functional food, paving the way for more effective delivery of bioactive compounds and broader health applications.

2.6 Antioxidant activity

2.6.1 Introduction of antioxidant activity

Antioxidants are compounds that neutralize reactive oxygen species (ROS) and free radicals, protecting cells from oxidative damage. The body's oxidative stress arises when there is an imbalance between the production of free radicals and the capacity of the body's antioxidant defenses. This stress is a key factor in the development of chronic diseases such as cancer, cardiovascular diseases, neurodegenerative disorders, and aging. Antioxidants, therefore, play a pivotal role in promoting health and preventing various diseases. In the last decade, research into antioxidant activity has intensified, especially focusing on natural sources of antioxidants such as polyphenols, flavonoids, carotenoids, and vitamins in foods (Bratic and Larsson 2013; Skrovankova 2015; Wilson 2017; Fraga et al. 2019; Chung et al. 2022).

1) Mechanisms of antioxidant action

The antioxidant activity of a compound is defined by its ability to scavenge free radicals, chelate metal ions, or inhibit oxidative enzymes. Primary antioxidants neutralize free radicals directly by donating electrons, whereas secondary antioxidants prevent oxidative stress by chelating metal ions or inhibiting pro-oxidant enzymes such as lipoxygenase. These mechanisms work to maintain the balance between free radicals and antioxidants in the body, thereby reducing oxidative stress and preventing cellular damage. For instance, polyphenols are widely studied for their antioxidant activity. They act by donating hydrogen atoms or electrons to neutralize free radicals, thus preventing the initiation of oxidation. Flavonoids, a subclass of polyphenols, exhibit antioxidant properties by stabilizing ROS and inhibiting oxidation reactions, while carotenoids such as lycopene quench singlet oxygen molecules, thereby mitigating oxidative stress.

2) Antioxidants in food sources

Plant-based foods are a rich source of antioxidants, including fruits, vegetables, grains, herbs, and teas. Among these, polyphenols and flavonoids have been most extensively studied. Foods like berries, citrus fruits, cocoa, tea, and red wine are known for their high polyphenol content, which contributes to their antioxidant capacity. These compounds are often linked with the prevention of diseases such as cardiovascular disorders and cancers. Research by Sona Skrovankova et al. (2015) showed that the polyphenols found in berries exhibit potent antioxidant activity and can improve vascular function, reduce blood pressure, and lower the risk of heart diseases.

Additionally, flavonoids found in tea, particularly green tea, have been shown to reduce oxidative stress, which is a key mechanism in preventing neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease. In recent years, there has been increasing interest in incorporating antioxidants into functional foods and beverages to boost their health benefits. Functional foods are those that provide health benefits beyond their basic nutritional value. Several food industries have focused on fortifying products like yogurt, juices, and snacks with antioxidants to enhance their health-promoting properties. Functional beverages, such as green tea, fruit juices, and antioxidant-rich smoothies, have gained popularity due to their high polyphenol content and potential health benefits. For instance, green tea, which is rich in catechins (a type of flavonoid), has been shown to reduce oxidative stress and inflammation, contributing to its reputation as a powerful antioxidant beverage. Moreover, polyphenol-enriched yogurts and fortified cereals have been shown to improve antioxidant status in consumers, potentially protecting against oxidative damage

3) Antioxidants in human health

Research has emphasized the critical role that antioxidants play in mitigating oxidative stress, thereby protecting against the onset of chronic diseases. Numerous studies have demonstrated that dietary antioxidants can reduce the risk of cancer, cardiovascular diseases, and neurodegenerative disorders by preventing oxidative DNA damage, lipid peroxidation, and inflammation. A study by (Chung et al. 2022) found that individuals with a high dietary intake of antioxidants, such as vitamins C and E, had lower rates of cardiovascular disease and improved endothelial function. These findings suggest that antioxidant-rich diets, including fruits, vegetables, and whole grains, have a cardioprotective effect. Similarly, antioxidants such as carotenoids (e.g., beta-carotene and lutein) have been linked to improved eye health, reducing the risk of age-related macular degeneration and cataracts.

4) Antioxidants and aging

Oxidative stress is closely linked with aging, as the accumulation of free radicals over time can damage cells, proteins, and DNA, contributing to age-related diseases and the aging process itself. Antioxidants have been studied for their potential to slow the aging process by neutralizing oxidative damage. Antioxidants such as resveratrol (found in red wine) and curcumin (found in turmeric) have been particularly noted for their anti-aging properties. Bratic and Larsson (2013) highlighted the role of antioxidants in improving mitochondrial function, which is critical in slowing down cellular aging. The study found that antioxidant supplementation could reduce age-related oxidative damage, improving overall vitality and reducing the risk of neurodegenerative diseases such as Alzheimer’s. The potential of antioxidants in anti-aging skincare has also been explored, with vitamins C and E often used in topical treatments to prevent oxidative damage to the skin caused by ultraviolet (UV) radiation.

Antioxidants are crucial in maintaining health by neutralizing free radicals and reducing oxidative stress. They play a vital role in preventing chronic diseases, aging, and inflammation. Dietary sources of antioxidants, especially from plant-based foods, are essential for promoting health and preventing oxidative damage. However, challenges related to bioavailability and stability persist, necessitating further research into more effective delivery systems and personalized dietary approaches to optimize antioxidant intake.

2.6.2 Analysis of antioxidant activity

Antioxidant activity is crucial in preventing cellular damage caused by oxidative stress. Oxidative stress results from an imbalance between reactive oxygen species (ROS) and the body's ability to neutralize them with antioxidants. Antioxidants scavenge free radicals, prevent lipid peroxidation, and inhibit oxidative DNA damage, thus playing a protective role in diseases such as cancer, cardiovascular disease, and neurodegenerative disorders. The numerous studies have refined analytical methods for determining antioxidant activity, especially focusing on natural compounds found in food, medicinal plants, and nutraceuticals (Bratic and Larsson 2013; Skrovankova 2015; Wilson 2017; Fraga et al. 2019; Chung et al. 2022)..

1) Standard In vitro antioxidant assays

In vitro assays are widely used due to their simplicity, cost-effectiveness, and speed in determining the antioxidant potential of compounds.

1.1) DPPH Radical Scavenging Assay**:** The DPPH assay measures the ability of an antioxidant to reduce DPPH radicals, which change color upon interaction with antioxidants. This method is popular due to its simplicity but is limited to certain types of antioxidants that can reduce DPPH radicals.

1.2) ABTS Radical Cation Decolorization Assay**:** The ABTS assay generates a blue-green radical cation that decolorizes in the presence of an antioxidant. Unlike the DPPH assay, ABTS can be used to measure both lipophilic and hydrophilic antioxidants, making it more versatile in different food matrices.

1.3) Ferric Reducing Antioxidant Power (FRAP**)**: FRAP measures the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by antioxidants. This assay is particularly suited for phenolic compounds, which can donate electrons to reduce ferric ions**.**

1.4) Oxygen Radical Absorbance Capacity (ORAC)**:** ORAC quantifies the antioxidant's capacity to neutralize peroxyl radicals, offering an advantage over other assays as it simulates physiological conditions better. ORAC is often used to measure antioxidants in food and supplements, such as polyphenols in berries, teas, and wines

2) Advanced analytical techniques

While conventional assays remain widely used, advancements in analytical technology have significantly enhanced the accuracy and depth of antioxidant analysis.

2.1) High-Performance Liquid Chromatography (HPLC)**:** HPLC, often coupled with spectrophotometric detectors or mass spectrometry (HPLC-MS), allows for the separation and quantification of individual antioxidants in complex mixtures such as plant extracts and food. This technique is commonly used to analyze polyphenols, flavonoids, and carotenoids, providing detailed insight into the specific antioxidant content of foods.

2.2) Electron Spin Resonance (ESR) Spectroscopy**:** ESR spectroscopy directly measures the interaction between antioxidants and free radicals by detecting the unpaired electrons. This method provides precise measurements of antioxidant activity, particularly in terms of scavenging specific radicals such as hydroxyl radicals and superoxide anions.

2.3) Ultra-Performance Liquid Chromatography (UPLC**)**: UPLC is an advanced version of HPLC with higher sensitivity and resolution, allowing faster and more accurate separation of antioxidants. UPLC is particularly useful for analyzing phenolic compounds in small quantities of plant or food samples.

3) In vivo and Ex vivo models

In vitro assays, while useful, do not fully account for the complexities of biological systems. In vivo and ex vivo models provide more realistic insights into the antioxidant behavior within organisms, including the bioavailability, absorption, and metabolism of antioxidant compounds.

3.1) Animal models: Rats and mice are commonly used in in vivo studies to test the antioxidant properties of compounds. Antioxidant activity is often assessed by measuring biomarkers of oxidative stress, such as malondialdehyde (MDA), glutathione levels, and enzymatic activity of superoxide dismutase (SOD) and catalase. These studies provide insight into how antioxidants work to protect tissues from oxidative damage.

3.2) Human studies**:** Human clinical trials are increasingly used to assess the antioxidant effects of functional foods and supplements. Studies often measure changes in blood markers of oxidative stress, such as 8-hydroxy-2'-deoxyguanosine (8-OHdG), which indicates oxidative DNA damage, and protein carbonyls, which signal oxidative protein damage.

3.3) Ex vivo methods: Ex vivo methods involve analyzing biological samples (e.g., blood, tissue) obtained from living organisms to study antioxidant activity outside the body. This method is useful for understanding antioxidant effects without the complexities of whole-organism studies.

4) Applications in food and pharmaceutical industries

The analysis of antioxidant activity is essential for food and pharmaceutical industries, particularly in the development of functional foods, supplements, and therapeutic agents.

4.1) Functional Foods: Antioxidant-rich foods, such as fruits, vegetables, teas, and wines, are increasingly marketed for their health benefits. Functional foods are designed to go beyond basic nutrition by providing health-promoting bioactive compounds. For instance, polyphenols in berries and flavonoids in tea have been shown to reduce oxidative stress, lowering the risk of chronic diseases.

4.2**)** Pharmaceuticals**:** In the pharmaceutical industry, antioxidants are used to develop drugs and therapies to combat diseases linked to oxidative stress. Antioxidant drugs, such as N-acetylcysteine (NAC) and vitamin E, are utilized for their ability to neutralize free radicals and mitigate the oxidative damage that contributes to diseases such as Alzheimer's, Parkinson's, and cardiovascular disorders.

4.3) Cosmetics**:** The cosmetic industry has also adopted antioxidants like vitamin C, vitamin E, and resveratrol into skincare products to prevent skin damage from UV radiation and environmental pollutants. These antioxidants work by neutralizing free radicals that cause premature aging and skin cancer.

5) Bioavailability and Metabolism of Antioxidants

Bioavailability remains a critical factor in determining the actual health benefits of antioxidants. Many antioxidant compounds, especially polyphenols, have poor bioavailability, meaning they are not easily absorbed or metabolized in the body. Recent research has focused on improving the delivery and stability of antioxidants through nanoencapsulation, emulsification, and other food processing technologies.

As antioxidant research progresses, there is a growing interest in personalized nutrition and targeted antioxidant therapies. Advances in nutrigenomics and metabolomics allow researchers to tailor antioxidant interventions based on individual genetic profiles and metabolic conditions. This precision nutrition approach holds potential for maximizing the health benefits of antioxidants in preventing disease and promoting longevity. The analysis of antioxidant activity has evolved significantly, with advanced techniques complementing traditional assays to provide a more comprehensive understanding of how antioxidants function. As interest in antioxidants continues to grow, their application in functional foods, pharmaceuticals, and cosmetics will likely expand. Future research will focus on improving the bioavailability of antioxidants and developing personalized antioxidant strategies to optimize health outcomes.

2.6.3 Antioxidant activity in yogurt

Yogurt has been widely recognized not only for its probiotic benefits but also for its rich content of bioactive compounds that contribute to its antioxidant activity. These compounds, which include vitamins, peptides, and phenolic compounds, can play a critical role in preventing oxidative stress, a process linked to the development of various chronic diseases such as cardiovascular disease, diabetes, and cancer. This review focuses on the antioxidant activity in yogurt. It covers the sources of antioxidants, the methods used to assess antioxidant activity, and the impact of yogurt antioxidant potential on health. The highlights key aspects of antioxidant activity in yogurt, with a brief overview, are presented in Table 7.

Table 7 The key aspects of antioxidant activity in yogurt.

Aspect	**Details**
Sources of antioxidants in yogurt	Dairy components: Vitamins A, C, E, selenium, and zinc contribute to antioxidant activity. Fermentation by LAB: Bioactive peptides, exopolysaccharides (EPS) produced during fermentation enhance antioxidant capacity. Fortification: Fruits (e.g., berries), herbs, and green tea extracts are rich in polyphenols, boosting antioxidant activity. Plant-Based Yogurt: Antioxidant-rich plant-based options (e.g., soy, almond) with added functional ingredients (e.g., spirulina) also contribute.
Methods to assess antioxidant activity	DPPH assay: Measures the radical scavenging ability of yogurt and is enhanced by fruit fortification. ABTS assay: Evaluates yogurt’s ability to reduce the ABTS radical cation, increased by herbal extracts. FRAP assay: Assesses the ferric-reducing power, used to measure antioxidant potential of fortified yogurts. Chemical analysis (HPLC, GC-MS): Identifies and quantifies individual antioxidants (e.g., flavonoids, phenolic acids).
Health Benefits of Antioxidants in Yogurt	Oxidative Stress Reduction: Regular consumption of antioxidant-rich yogurt lowers oxidative stress markers. Anti-inflammatory Effects: Yogurt’s antioxidants help reduce inflammation, linked to improved gut health. Gut Health and Immune Support: Probiotics and antioxidants in yogurt synergize to enhance gut barrier function and immune modulation.
Innovations in Antioxidant Enhancement	Superfood Fortification: Adding chia seeds, spirulina, and turmeric improves the antioxidant profile of yogurt.

Aspect	**Details**
	Microencapsulation: Encapsulation of polyphenols or other antioxidants protects them during processing and improves bioavailability. Strain Selection: Certain probiotic strains (e.g., *Lactobacillus plantarum*) enhance the antioxidant properties of yogurt.

Source: (Şanlıdere Aloğlu and Öner 2011; Dabija et al. 2018; Dinkçi et al. 2021; Stobiecka et al. 2022)

CHAPTER 3

METHODOLOGY

The aims of this work were to investigate the effect of yogurt bacteria and probiotic on melatonin, melatonin derivative content, and antioxidant activity of cow milk yogurt and plant-based milk yogurt, to evaluate the effect of tryptophan addition on improving melatonin and antioxidant activity of yogurt, and to study the shelf life and quality of physicochemical, microbiological and bioactive compounds changes of functional yogurt were studied. Thus, the experimental design was below.

3.1 Experimental plan
3.2 Instruments and equipment
3.3 Chemicals and reagents
3.4 Materials
3.5 Methods
3.6 Statistical analysis

3.1 Experimental plan

The experimental plan was divided into four phases as follows:

Phase I: Effect of LAB and probiotics on chemical and physical properties, melatonin content and its derivatives, and antioxidant activity in cow milk yogurt

Phase II: Effect of LAB and probiotic types on chemical and physical properties, melatonin content and its derivatives, and antioxidant activity in plant-based yogurt

Phase III: Impact of tryptophan addition on melatonin content and antioxidant activity of production of yogurt from cow milk and soy milk

Phase IV: Effect of storage time on the physicochemical characteristics, melatonin content, antioxidant activity, and ACE inhibitory activity of yogurt

The experimental flame work of this study as in Figure 11.

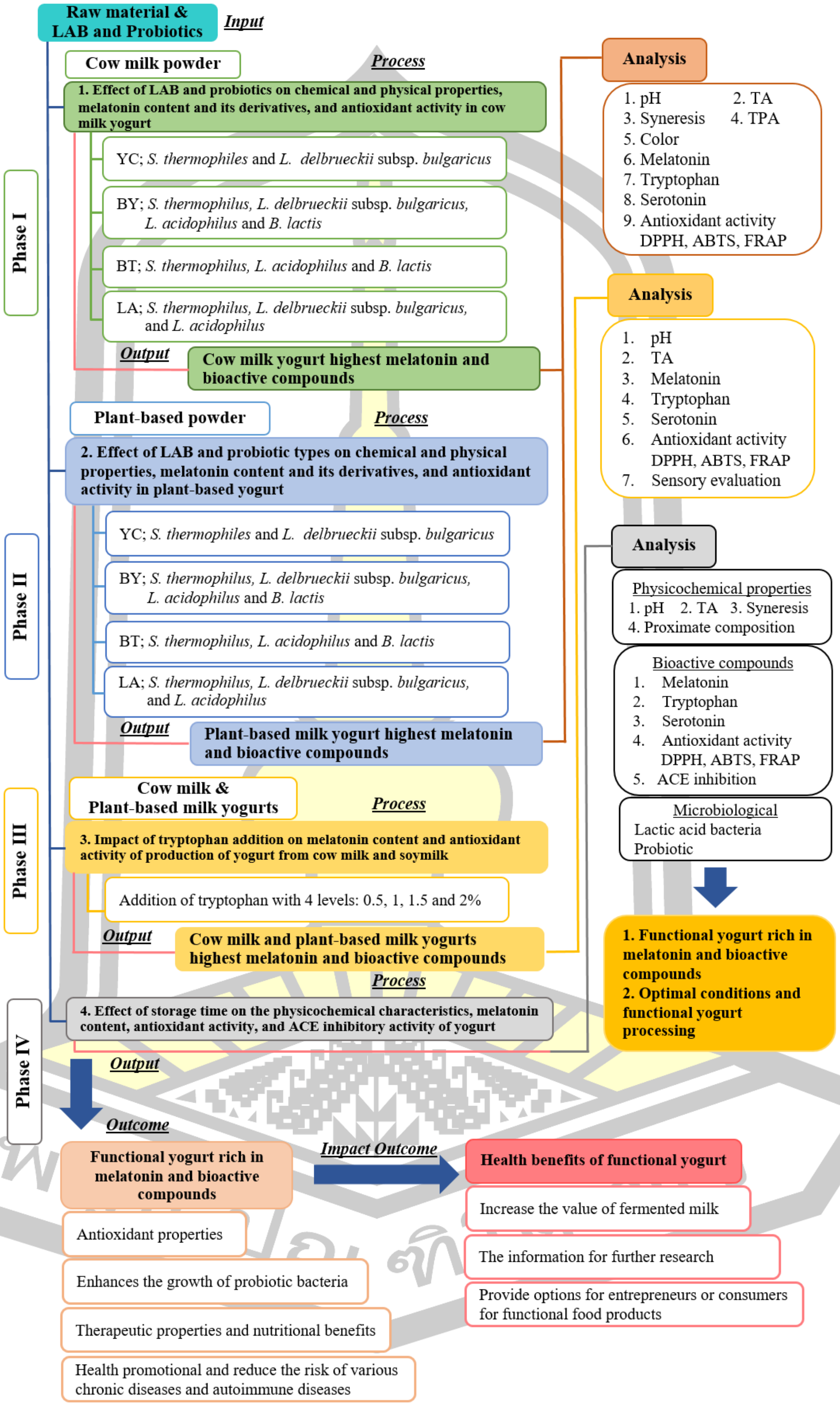


Figure 11 Experimental flame work.

This study can be divided into four phases.

3.1.1 Phase I: Effect of LAB and probiotics on chemical and physical properties, melatonin content and its derivatives, and antioxidant activity in cow milk yogurt

Cow milk (milk powder) was fermented by LAB and probiotic strains including *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus* and *B. lactis*. The content of melatonin, its derivatives, bioactive peptides, other bioactive compounds, antioxidant activity; DPPH, ABTS, and FRAP assays, pH value, titratable acidity and physical properties in yogurt were investigated. LAB and probiotic giving the highest potential for the synthesis of melatonin and its derivatives in yogurt. The suitable milk was used to produce melatonin-rich yogurt (Figure 12).

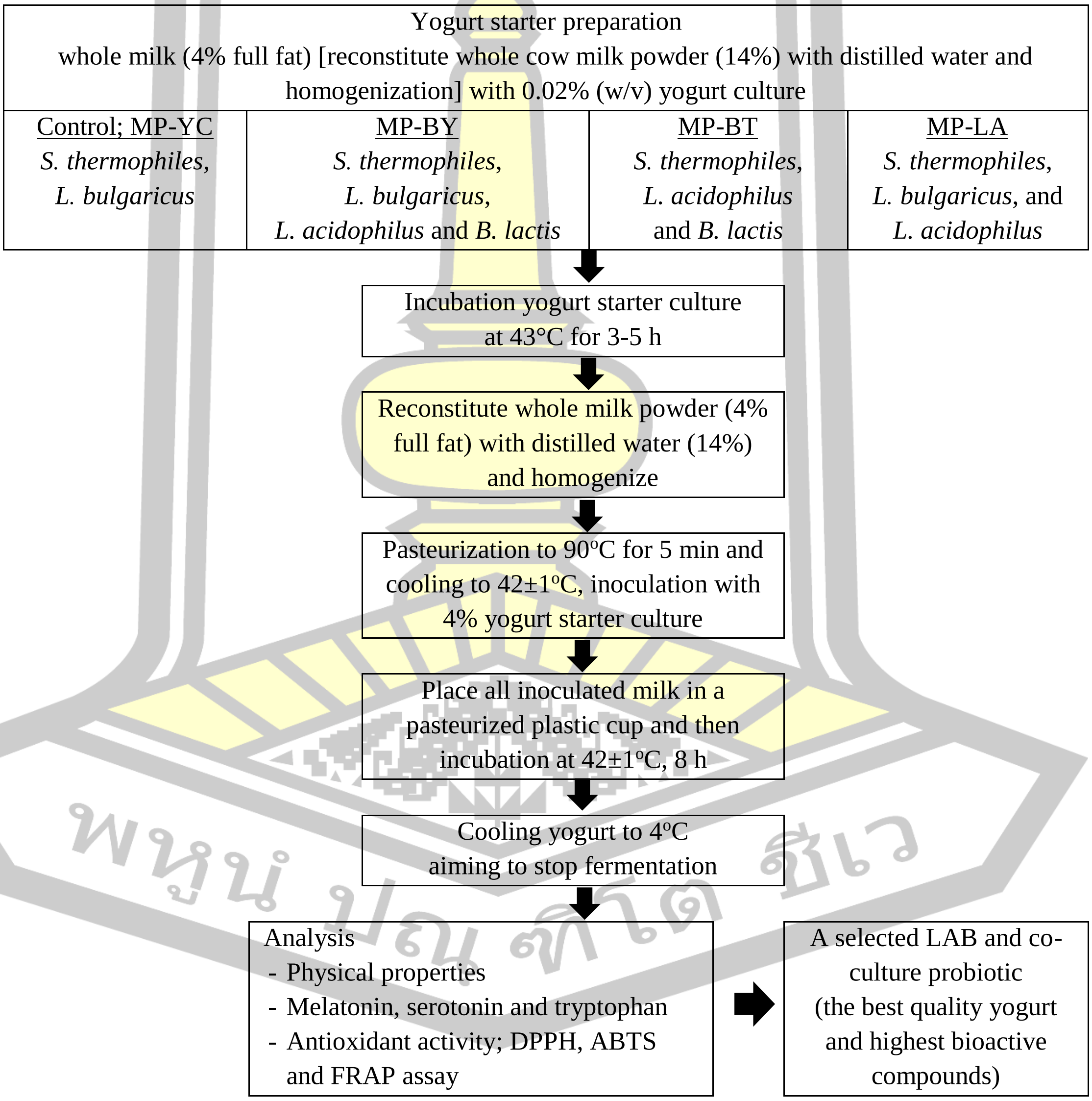


Figure 12 Schematic flow chart of study design for Phase I.

3.1.2 Phase II: Effect of LAB and probiotic types on chemical and physical properties, melatonin content and its derivatives, and antioxidant activity in plant-based yogurt

From the previous researches (Sangsopha et al. 2020; Nontasan et al. 2022) soybean and chickpea were found to be good sources of protein and high in tryptophan. Their physical characteristics are similar to cow milk. White sesame seeds contain high content of melatonin. Therefore, all 3 types of raw materials were processed into plant-based milk powder. Soybean powder, chickpea powder, and white sesame seed were prepared into plant-based milk before fermented using by LAB and probiotic strains including *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus* and *B. lactis*. The yogurt obtained was analyzed for melatonin content and its derivatives (serotonin and tryptophan) and their chemical properties and the major physical properties (Figure 13).

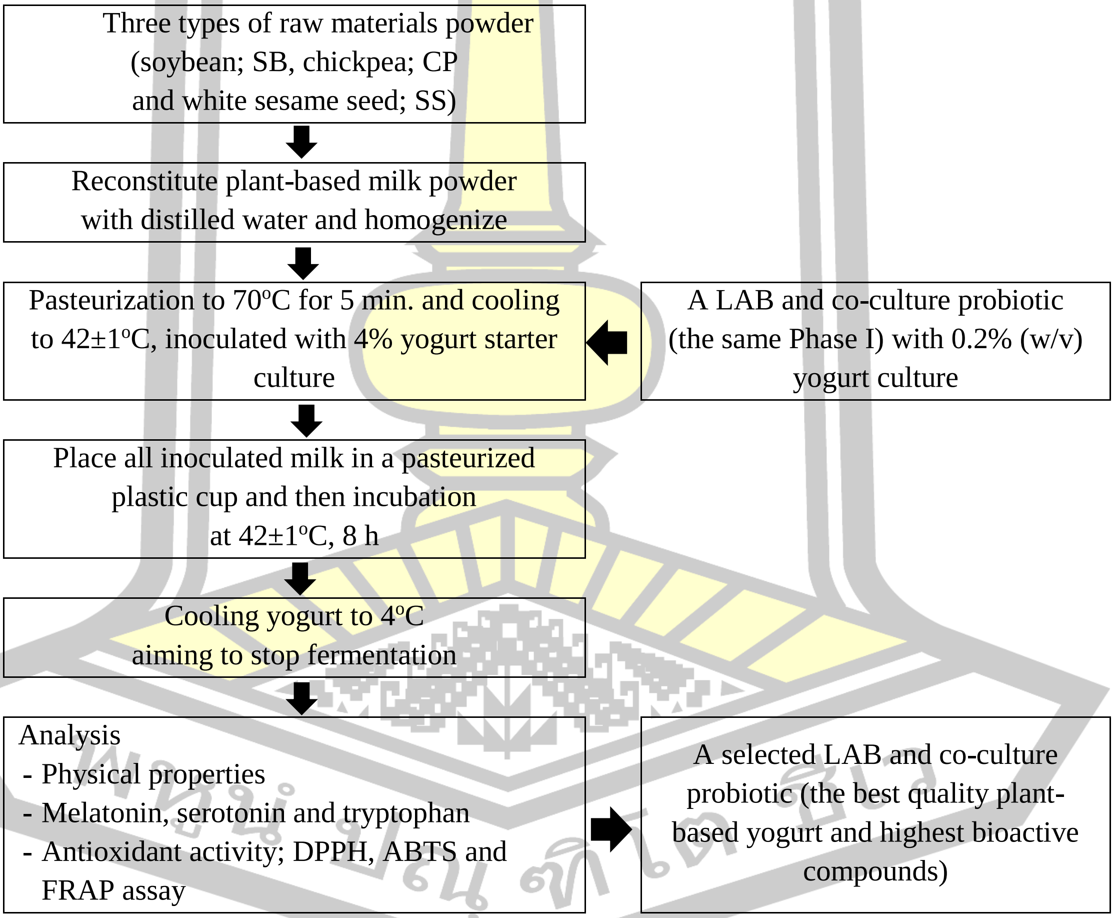


Figure 13 Schematic flow chart of study design for Phase II.

3.1.3 Phase III: Impact of tryptophan addition on melatonin content and antioxidant activity of production of yogurt from cow milk and soy milk

From Phase I and Phase II, the optimum conditions were selected to study at this Phase by product development of yogurt from cow milk powder and plant-based powder. The yogurt obtained was analyzed for melatonin content and its derivatives (serotonin and tryptophan) and their chemical properties (Figure 14).

The cow milk was fermented with the addition of tryptophan (melatonin substrate) with 4 levels; 0.5, 1, 1.5 and 2%. Cow milk was added with the LAB chosen from Phase I and yogurt fermentation was done and analyzed for melatonin content and its derivatives (serotonin and tryptophan). The suitable milk mixture with substrate was used to produce melatonin-rich yogurt.

From the optimum proportion of raw materials obtained, the suitable plant-based milk was prepared and then added with melatonin substrate (tryptophan) with 4 levels; 0.5, 1, 1.5 and 2%. Plant-based milk was fermented by selected LAB from Phase II. Yogurt fermentation was performed and analyzed of melatonin content, its derivatives (serotonin and tryptophan) and their chemical properties.

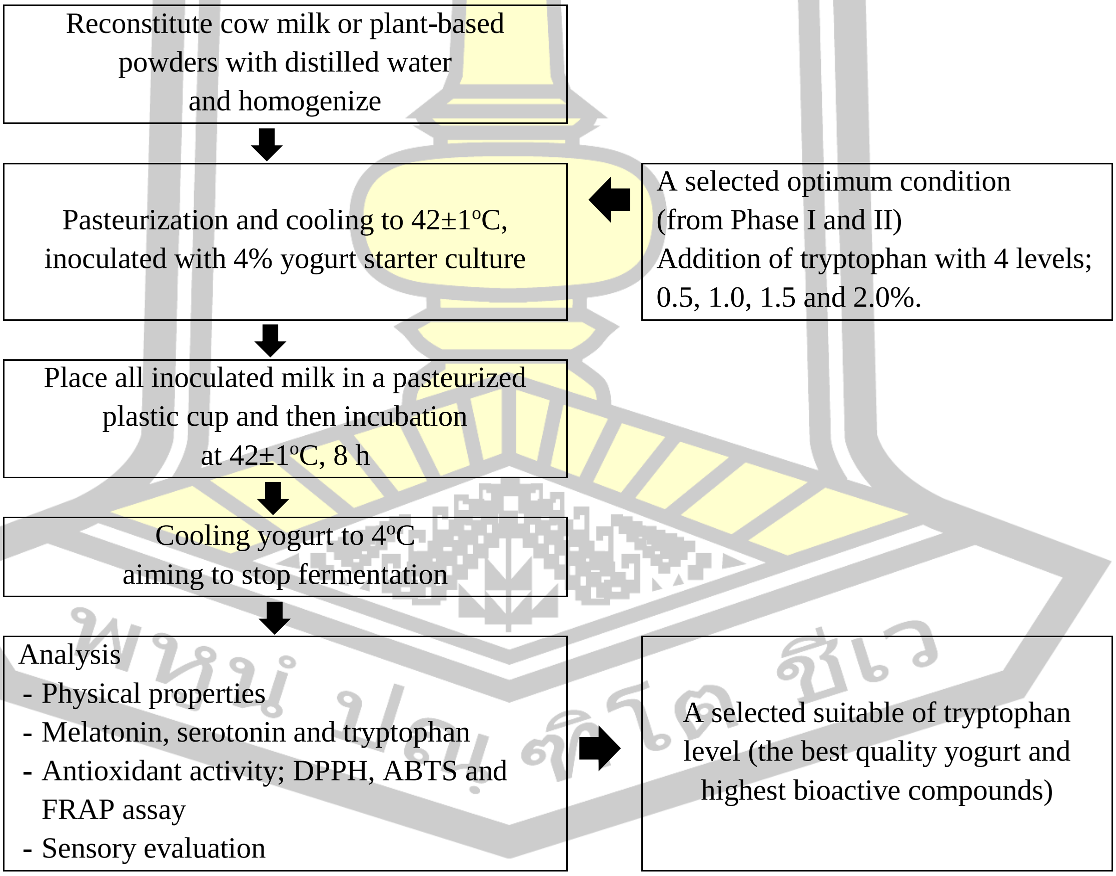


Figure 14 Schematic flow chart of study design for Phase III.

3.1.4 Phase IV: Effect of storage time on the physicochemical characteristics, melatonin content, antioxidant activity, and ACE inhibitory activity of yogurt

To study the storage on quality of physicochemical, microbiological properties and bioactive compounds changes of yogurt, both cow milk and plant-based milk yogurts. The storage of functional yogurt products at 4°C and sampling for quality analysis were carried out on days 1, 7, 14, 21 and 28 (Figure 15).

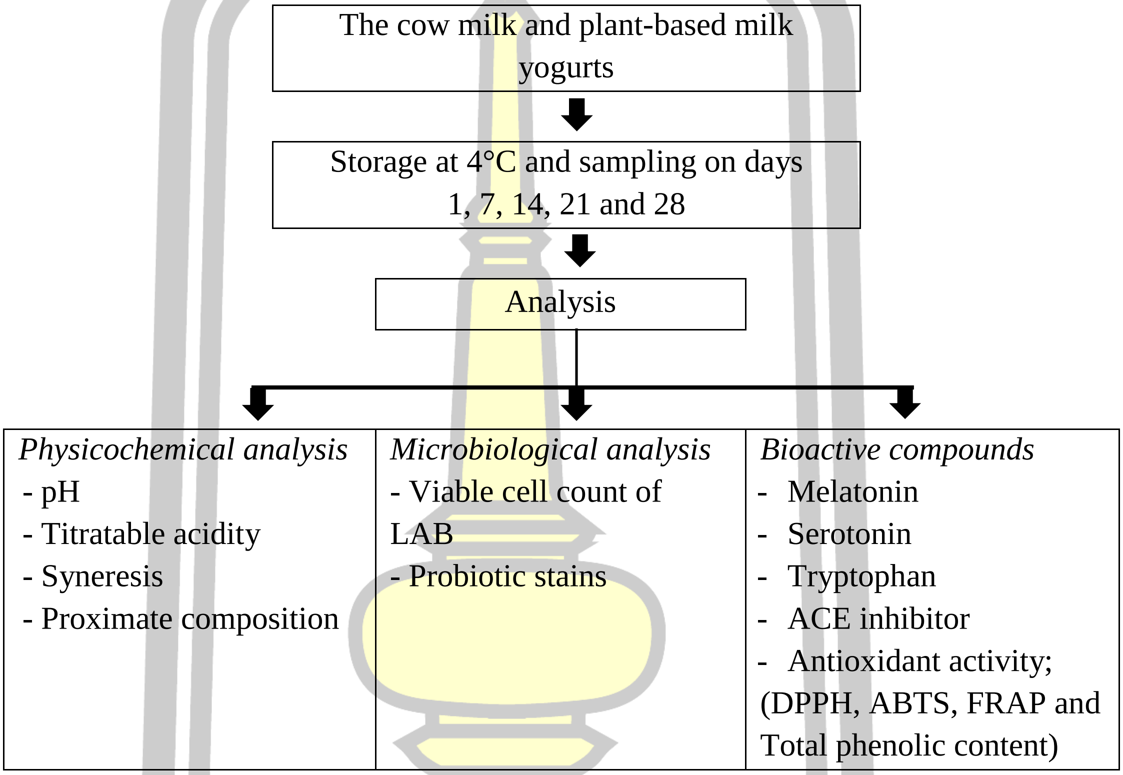


Figure 15 Schematic flow chart of study design for Phase IV.

3.2 Instruments and equipment

The instruments and equipment were used in the experiments are given in Table 8.

Table 8 Instruments and equipment used.

List	Instrument/Equipment	Details
1	Analytical Balances	Presica 25A. Switzerland
2	Blender machine	MX-900M, Panasonic
3	Centrifuge	Universal 320R, Germany
4	Chroma meter	Minolta CR-300, Japan
5	C18 column (5um, 4.6 mm × 150 mm) for HPLC	Agilent Zobax SB-C18 on an Agilent 1100, Santa Clara, CA USA
6	Freeze Dryer	Heto Power Dry, PL3000, Freeze Dryer, Czech
7	Fourier transform infrared (FTIR) spectroscopy	PerkinElmer, USA
8	Hot air oven	Binder, Germany
9	LC-MS/MS	Shimadzu 20ADS, Japan
10	Micro plate reader	Germany
11	pH meter	Fiveeasy FE20, Mettler Toledo
12	Sep-Pak C18 Solid Phase Extraction (SPE) cartridge	Waters, USA
13	Shaking Incubator	Model LSI-1005R. Lab Tech,Korea
14	Texture analyzer	TA-XT plus, UK
15	Vortex mixer	Hamony, Japan
16	Ultrasonic bath	Powersonic 420, Korea
17	Other equipment such syringe fillers, filter papers, laboratory glassware etc.	-

3.3 Chemicals and reagents

All chemicals and reagents were used in the experiments are given below (Table 9).

Table 9 Chemicals and reagents used.

List	Chemical/Reagents	Types/Grades	Details
1	2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)	analytical	Fluka Chemical Co. (Buchs, Switzerland)
2	2,2-diphenyl-1-picrylhydrazyl (DPPH)	analytical	Fluka Chemical Co. (Buchs, Switzerland)
3	2, 4, 6-Tris(2-pyridyl)-s-triazine (TPTZ)	analytical	Fluka Chemical Co. (Buchs, Switzerland)
4	ACE Kit-WST (water-soluble Tetrazolium)	analytical	DOJINDO Laboratories (Subsidiary, USA)
5	Acetone nitrile	HPLC	BDH (Poole, UK)
6	Ascorbic acid	analytical	KEMAUS (N.S.W., Australia)
7	Bifidobacterium Agar	analytical	HIMEDIA Laboratories Pvt. Ltd. (Mumbai, India)
8	Ethanol	analytical	BDH (Poole, UK)
9	Folin–Ciocalteu's reagent	analytical	Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA)
10	Formic acid (98%)	analytical	LOBA Chemie Pvt. Ltd. (Mumbai, India)
11	Glacial acetic acid	analytical	RCI Labscan Ltd. (Thailand)
12	Hydrochloric	analytical	Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA)
13	Iron (II) sulfate 7-hydrate (Ferrous sulfate standard)	analytical	Ajax Finechem Pty Ltd. (Australia and New Zealand)
14	Iron (III) chloride 6-hydrate (Ferric chloride)	analytical	BDH (Poole, UK)
15	Lactobacillus MRS Agar	analytical	HIMEDIA Laboratories Pvt. Ltd. (Mumbai, India)
16	Lactobacillus MRS Broth	analytical	HIMEDIA Laboratories Pvt. Ltd. (Mumbai, India)
17	L-Cysteine 99%	Biochemistry	LOBA Chemie Pvt. Ltd. (Mumbai, India)
18	L-Tryptophan standard (≥98%)	HPLC	Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA)
19	Melatonin standard (≥99.5%)	HPLC	Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA)
20	Methanol	HPLC	BDH (Poole, UK)
21	Methanol	analytical	BDH (Poole, UK)
22	Phenolphthalein	analytical	Merck (Germany)
23	Phosphate buffer saline	analytical	Univar, USA

List	Chemical/Reagents	Types/Grades	Details
24	Potassium persulfate (98%)	analytical	LOBA Chemie Pvt. Ltd. (Mumbai, India)
25	Serotonin standard	analytical	Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA)
26	Sodium acetate	analytical	Univar, USA
27	Sodium hydroxide (98%)	analytical	LOBA Chemie Pvt. Ltd. (Mumbai, India)
28	Other regents	analytical	-

3.4 Materials

All materials were used in the experiments are given below.

3.4.1 Four types of LAB and probiotic strain were purchased from Chr. Hansen Ltd. (Hoersholm, Denmark) as Freeze dried-direct vat set yogurts. The detail shown in Table 10.

Table 10 Types of LABs and probiotic strains.

List	LAB and probiotic strains	Code
1	*Streptococcus thermophilus* *Lactobacillus delbrueckii* subsp. *bulgaricus*	YC-380
2	*Streptococcus thermophilus* *Lactobacillus delbrueckii* subsp. *bulgaricus* *Bifidobacterium animalis* subsp. *lactis* *Lactobacillus acidophilus*	ABY-3
3	*Streptococcus thermophilus* *Bifidobacterium animalis* subsp. *lactis* *Lactobacillus acidophilus*	ABT-5
4	*Lactobacillus acidophilus*	LA-5

3.4.2 Whole milk powder (4% full fat) was purchased from local supermarkets in Maha Sarakham Province, Thailand.

3.4.3 Plant-based samples were purchased from supermarkets in Maha Sarakham Province, Thailand. All samples were guaranteed by GMP and HACCP standard certification. All samples were dried to powder, with shelf life of 4-6 months stored at refrigerator and consisted of soybean powder (*Glycine max* (L.) Merrill), chickpea powder (*Cicer arietinum* L.) and white sesame seed (*Sesamum indicum* L.).

3.5 Methods

Phase I: Effect of LAB and probiotics on chemical and physical properties, melatonin content and its derivatives, and antioxidant activity in cow milk yogurt

1.1) Fermentation of yogurt

Yogurt was produced according to the method described by Yang et al. (2012); Amirdivani and Baba (2015); Felix da Silva et al. (2017); Jeong et al. (2018); Patel et al. (2019).

Yogurt starter preparation: Weigh 140 g of whole milk powder (4% full fat) to ensure that the total solids content is approximately 14%. Gradually add distilled water to the milk powder while stirring to fully reconstitute the milk. Ensure the final volume is 1 liter. Then, the reconstituted milk was homogenized using a homogenizer to ensure an even distribution of fat and milk solids, pasteurized to 90^{o}C for 5 min and then cooled to 42 ± 1^{o}C. Weigh out 0.02% (w/v) of the respective yogurt cultures 1) YC-380 (*S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus)*, (2) ABY-3 (*S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *B. animalis* subsp. *lactis* and *L. acidophilus*), (3) ABT-5 (*S. thermophilus*, *B. animalis* subsp. *lactis* and *L. acidophilus*), and (4) YC-380+LA-5 (*L. acidophilus*). This corresponds to 0.2 grams of starter culture for every liter of milk. Add the selected starter culture to the pasteurized milk. Gently stir the mixture to evenly distribute the culture. Then all inoculated milk were placed in a pasteurized plastic cup and incubated at 42 ± 1^{o}C to 3–5 h (pH typically between 4.5 and 4.6). Once the yogurt had formed, stop the fermentation by cooling the yogurt to 4°C.

Yogurt production: Whole milk powder (4% full fat) was reconstituted in distilled water to achieve a total solids content of 14%, homogenized using a homogenizer, pasteurized to 90^{o}C for 5 min and then rapidly cooled to 42 ± 1^{o}C, inoculated with 4% yogurt starter culture. The inoculated milk was placed in a pasteurized plastic cup and incubated at 42 ± 1^{o}C to 8 h. The yogurt was sampled for analysis every hour and cooled to 4^{o}C aiming to stop fermentation. After each sampling, the yogurts were frozen at -30°C for a minimum of 12 h and subsequently freeze-dried at -55°C for 48 h, or until the a_w reached a value between 0.3 and 0.5. The freeze-dried yogurt samples were then stored in aluminum foil bags and analyzed for their melatonin and its derivatives content, antioxidant activity, and FTIR.

1.2) Physical and chemical analysis

1.2.1) Titratable acidity (g of lactic acid 100/g) with sodium hydroxide (0.1 M) was used to neutralize the acids contained in the yogurt using phenolphthalein as an indicator (Yang et al. 2012; Felix da Silva et al. 2017). The titratable acidity was calculated as follows:

$$\text{g of lactic acid 100 g} - \text{1 of yogurt} = \frac{\text{volume of sodium hydroxide (mL)} \times \text{molar of sodium hydroxide} \times 0.9}{\text{yogurt sample weight (g)}}$$

1.2.2) The pH values of the yogurt were measured using a pH meter. Samples were taken from the incubator hourly until 8 h and allowed to cool to room temperature (25±2°C) before measuring the pH.

1.2.3) Yogurt syneresis was analyzed with slight modifications (Chang et al., 2018). Yogurt (10.xx g) was centrifuged at 600 × g for 6 min at 4°C. The clear serum having separated from the yogurt was poured off and weighed. Syneresis was expressed in grams of whey lost and the syneresis was calculated using the following formula:

$$\text{Syneresis (\%)} = \frac{[\text{supernatant (g)} \times 100]}{\text{sample weigh (g)}}$$

1.2.4) Color analysis was performed using a chroma meter expressed as the Hunter system L*, a* and b* coordinates (Pranil et al., 2021).

1.2.5) Yogurt textural attributes were measured by a texture analyzer (Yang et al. 2012) and the yogurt temperature was at 4±1°C. Texture profile analysis was applied with a cylinder probe (P/20 diameter 20 mm) using a pre-test speed of 1 mm/s, post-test speed of 5 mm/s, test speed of 5 mm/s, and distance of 30 mm.

1.3) Determination of melatonin, tryptophan and serotonin

Analysis of melatonin, free tryptophan and serotonin in yogurt products were adapted according to the method by (Sangsopha et al. 2019, 2020)

1.3.1) Sample extraction

The yogurt sample (accurately 2.5 g) was dissolved in 20 mL of 80% methanol and mixed by sonication in an ultrasonic bath for 30 min in the dark. Then, the mixture was shaken by a shaking incubator at 200 × g, 4°C for 16 h and centrifuged at 5,000 × g at 4°C for 5 min. The obtained extract was purified using a Sep-Pak C18 Solid Phase Extraction (SPE) cartridge following Pranil et al. (2021). The purified extract obtained was then passed through a 0.22 μm syringe filter into an amber glass vial before LC-MS/MS analysis.

1.3.2) LC–MS/MS analysis of melatonin and its derivatives

A Shimadzu 20ADS Liquid Chromatograph coupled with a Shimadzu 8030 Mass Spectrometer operated in electrospray ionization mode (ESI) was used to determine melatonin, serotonin and tryptophan. An InertSustain® C18 column (2.1 mm × 150 mm, 3.5 μm) was the stationary phase, while the mobile phase was 0.45% formic acid in HPLC grade water (solvent A) and acetonitrile (solvent B). Flow regulation (time, solvent A:solvent B) was as follows: 0-5 min, 80:20%; 5-6 min, 50:50% to 0:100%; 6-9 min, 0:100%; and 9-10 min, 80:20% with a flow rate of 0.25

mL/min and 2 µL injection volume. A standard curve of external standards was plotted and used to quantify melatonin, tryptophan, and serotonin contents, with results indicated as ng/g dry basis (Pranil et al. 2021).

1.4) Determination of antioxidant activity

The antioxidant activity of yogurt were measured according to (Skrovankova et al. 2015; Kim et al. 2020; Pranil et al. 2020).

1.4.1) DPPH free radical scavenging activity

The DPPH radical scavenging assay was performed on the chosen strains adjusted to an OD600 of about 1.0 with phosphate-buffered saline (PBS, pH 7.4) was added to 0.05 mM DPPH solution (1:2 v/v) and mixed together. The mixtures were left to stand at room temperature for 30 min in the dark. The control reaction was arranged using ethanol added to the DPPH solution. The absorbance of each mixture was measured at 517 nm. Each sample assay was performed in triplicate. The results were compared with those of ascorbic acid (10 and 100 µg/mL), and the antioxidant activity was calculated using the following formula:

$$\text{Scavenging effect (\%)} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \times 100$$

where As is the absorbance of the test sample, and Ac is the absorbance of the control at 517 nm.

1.4.2) ABTS radical scavenging activity

The incubation was measured by mixing 7 mM of ABTS with 2.45 mM potassium persulfate (1:1 v/v) and leaving the mixture at room temperature in the dark for 24 h. After that, the ABTS solution was diluted with distilled water until the initial absorbance value of 0.7±0.005 at 734 nm was reached. Then, 170 µL of the selected strain samples and 30 µL of ABTS solution were mixed and incubated for 10 min at room temperature. The absorbance of the mixture was performed at 734 nm. The scavenging rate was calculated as follows:

$$\text{Scavenging rate (\%)} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \times 100$$

where As is the absorbance of the test sample, and Ac is the absorbance of the control at 734 nm.

1.4.3) Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) was assessed according to methods described by Shabboo Amirdivani and Ahmad Salihin Hj Baba (2015);

Lucas et al. (2006). The 20 µL of yogurt extracts, appropriate dilutions, was mixed with 900 µL of the freshly prepared FRAP reagent. This buffer was prepared by mixing 300 mM/L acetate buffer (pH 3.6), 20 mM/L $FeCl_3$, 10 mM 2,4,6- tris (2-pyridyl)-s-triazine in 30 mM/L (ferrictripyridyltriazine; TPTZ) with 40 mM HCl in the ratio of 10:1:1. Incubation of mixtures at 37°C for 10 min, centrifugation (1400 × g, 2 min) and absorbance reading (593 nm) of the blue TPTZ complex formed with reduced ferrous ions at against a blank sample. The results was calculated from a standard scale of $FeSO_4.7H_2O$ and expressed as mg ferrous sulfate/g sample as follows:

$$\mathrm{FRAP} = \frac{\mathrm{CV}}{\mathrm{S}}$$

where C is the concentration of FRAP from standard curve (g/mL), V is the volume of sample prepare (mL), and S is the sample (g).

1.5) Fourier transform infrared (FTIR) spectroscopy measurement

The variations in functional components of the cow milk yogurt were determined by FTIR spectroscopy and recorded using a Frontier and Spotlight 200i System (Lin et al. 2020). The sample powder was maintained at room temperature and 2 mg amounts were pressed into pellets (1–2 mm thick films). FTIR spectra were obtained for wave numbers from 4000 cm^{-1} to 400 cm^{-1}, with a 4 cm^{-1} spectral resolution summarizing 8 scans.

Phase II: Effect of LAB and probiotic types on chemical and physical properties, melatonin content and its derivatives, and antioxidant activity in plant-based yogurt

2.1) Fermentation of plant-based yogurt

Plant-based yogurt fermentation were produced according to the method described by Yang et al. 2012; Amirdivani and Baba 2015; Felix da Silva et al. 2017; Jeong et al. 2018; Patel et al. 2019; Hussein et al. 2020.

Plant-based yogurt starter preparation: Weigh 120 g of plant powder, slowly add distilled water to the plant powder, and 2% sugar was added while stirring to fully reconstitute the plant powder. Ensure the final volume was 1 liter. Then, the reconstituted plant-based milk was homogenized using a homogenizer, pasteurized to 70°C for 5 min and then cooled to 42±1°C. Weigh out 0.2% (w/v) of the respective yogurt cultures 1) YC-380 (*S. thermophilus, L. delbrueckii* subsp. *bulgaricus),* (2) ABY-3 (*S. thermophilus, L. delbrueckii* subsp. *bulgaricus, B. animalis* subsp. *lactis* and *L. acidophilus*), (3) ABT-5 (*S. thermophilus, B. animalis* subsp. *lactis* and *L. acidophilus*), and (4) YC-380+LA-5 (*L. acidophilus*). Add the selected starter culture to the pasteurized milk. Then all inoculated plant-based milk were placed in a pasteurized plastic cup and incubated at 42±1^{o}C to 4–6 h (pH typically between 4.5 and 4.6). Once the yogurt had formed, stop the fermentation by cooling the yogurt to 4°C.

Plant-based yogurt production: Plant-based milk powder was reconstituted in distilled water and added 2% of sugar to achieve a total solids content of 5–6%, homogenized using a homogenizer, pasteurized to 70^{o}C for 5 min and then rapidly cooled to 42±1^{o}C, inoculated with 4% yogurt starter culture. The inoculated plant-based milk was placed in a pasteurized plastic cup and incubated at 42±1^{o}C to 8 h. The plant-based yogurt was sampled for analysis every 2 h and cooled to 4^{o}C aiming to stop fermentation. After each sampling, the plant-based yogurts were frozen at -30°C for a minimum of 12 h and subsequently freeze-dried at -55°C for 72 h, or until the a_w reached a value 0.3-0.5. The freeze-dried plant-based yogurt samples were then stored in aluminum foil bags and analyzed for their melatonin and its derivatives content, antioxidant activity, and FTIR.

2.2) Physical and chemical analysis

2.2.1) Titratable acidity (g of lactic acid 100/g) with sodium hydroxide (0.1 M) was used to neutralize the acids contained in the plant-based yogurt using phenolphthalein as an indicator. The titratable acidity was calculated as follows:

$$\text{g of lactic acid 100 g} - \text{1 of yogurt}$$
$$= \frac{\text{volume of sodium hydroxide (mL)} \times \text{molar of sodium hydroxide} \times 0.9}{\text{yogurt sample weight (g)}}$$

2.2.2) The pH values of the plant-based yogurt were measured using a pH meter. Samples were taken from the incubator hourly until 8 h and allowed to cool to room temperature (25±2°C) before measuring the pH.

2.2.3) Plant-based yogurt syneresis was analyzed with slight modifications. Plant-based yogurt (10.xx g) was centrifuged at 600 × g for 6 min at 4°C. The clear serum having separated from the plant-based yogurt was poured off and weighed. Syneresis was expressed in grams of whey lost and the syneresis was calculated using the following formula:

$$\text{Syneresis (\%)} = \frac{\text{supernatant (g)} \times 100}{\text{sample weigh (g)}}$$

2.2.4) Color analysis was performed using a chroma meter expressed as the Hunter system L*, a* and b* coordinates.

2.2.5) Plant-based yogurt textural attributes were measured by a texture analyzer (Yang et al. 2012) and the plant-based yogurt temperature was at 4±1°C. Texture profile analysis was applied with a cylinder probe (P/20 diameter 20 mm) using a pre-test speed of 1 mm/s, post-test speed of 5 mm/s, test speed of 5 mm/s, and distance of 30 mm.

2.3) Determination of melatonin, tryptophan and serotonin

2.3.1) Sample extraction

The plant-based yogurt sample (accurately 2.5 g) was dissolved in 20 mL of 80% methanol and mixed by sonication in an ultrasonic bath for 30 min in the dark. Then, the mixture was shaken by a shaking incubator at 200 × g, 4°C for 16 h and centrifuged at 5,000 × g at 4°C for 5 min. The obtained extract was purified using a Sep-Pak C18 Solid Phase Extraction (SPE) cartridge following Pranil et al. (2021). The purified extract obtained was then passed through a 0.22 µm syringe filter into an amber glass vial before LC-MS/MS analysis.

2.3.2) LC–MS/MS analysis of melatonin and its derivatives

A Shimadzu 20ADS Liquid Chromatograph coupled with a Shimadzu 8030 Mass Spectrometer operated in electrospray ionization mode (ESI) was used to determine melatonin, serotonin and tryptophan. An InertSustain® C18 column (2.1 mm × 150 mm, 3.5 µm) was the stationary phase, while the mobile phase was 0.45% formic acid in HPLC grade water (solvent A) and acetonitrile (solvent B). Flow regulation (time, solvent A:solvent B) was as follows: 0-5 min, 80:20%; 5-6 min, 50:50% to 0:100%; 6-9 min, 0:100%; and 9-10 min, 80:20% with a flow rate of 0.25 mL/min and 2 µL injection volume. A standard curve of external standards was plotted and used to quantify melatonin, tryptophan, and serotonin contents, with results indicated as ng/g dry basis (Pranil et al. 2021).

2.4) Determination of antioxidant activity

2.4.1) DPPH free radical scavenging activity

The DPPH radical scavenging assay was performed on the chosen strains adjusted to an OD600 of about 1.0 with phosphate-buffered saline (PBS, pH 7.4) was added to 0.05 mM DPPH solution (1:2 v/v) and mixed together. The mixtures were left to stand at room temperature for 30 min in the dark. The control reaction was arranged using ethanol added to the DPPH solution. The absorbance of each mixture was measured at 517 nm. Each sample assay was performed in triplicate. The results were compared with those of ascorbic acid (10 and 100 µg/mL), and the antioxidant activity was calculated using the following formula:

$$\text{Scavenging effect (\%)} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \times 100$$

where As is the absorbance of the test sample, and Ac is the absorbance of the control at 517 nm.

2.4.2) ABTS radical scavenging activity

The incubation was measured by mixing 7 mM of ABTS with 2.45 mM potassium persulfate (1:1 v/v) and leaving the mixture at room temperature in the dark for 24 h. After that, the ABTS solution was diluted with distilled water until the initial absorbance value of 0.7±0.005 at 734 nm was reached. Then, 170 µL of the selected strain samples and 30 µL of ABTS solution were mixed and incubated for 10 min at room temperature. The absorbance of the mixture was performed at 734 nm. The scavenging rate was calculated as follows:

$$\text{Scavenging rate (\%)} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \times 100$$

where As is the absorbance of the test sample, and Ac is the absorbance of the control at 734 nm.

2.4.3) Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) was assessed according to methods described by Shabboo Amirdivani and Ahmad Salihin Hj Baba (2015); Lucas et al. (2006). The 20 µL of yogurt extracts, appropriate dilutions, was mixed with 900 µL of the freshly prepared FRAP reagent. This buffer was prepared by mixing 300 mM/L acetate buffer (pH 3.6), 20 mM/L $FeCl_3$, 10 mM 2,4,6- tris (2-pyridyl)-s-triazine in 30 mM/L (ferrictripyridyltriazine; TPTZ) with 40 mM HCl in the ratio of 10:1:1. Incubation of mixtures at 37°C for 10 min, centrifugation (1400 × g, 2 min) and absorbance reading (593 nm) of the blue TPTZ complex formed with reduced ferrous ions at against a blank sample. The results was calculated from a standard scale of $FeSO_4.7H_2O$ and expressed as mg ferrous sulfate/g sample as follows:

$$\text{FRAP} = \frac{\text{CV}}{\text{S}}$$

where C is the concentration of FRAP from standard curve (g/mL), V is the volume of sample prepare (mL), and S is the sample (g).

2.5) Fourier transform infrared (FTIR) spectroscopy measurement

The variations in functional components of the cow milk yogurt were determined by FTIR spectroscopy and recorded using a Frontier and Spotlight 200i System (Lin et al. 2020). The sample powder was maintained at room temperature and 2 mg amounts were pressed into pellets (1–2 mm thick films). FTIR spectra were obtained for wave numbers from 4000 cm^{-1} to 400 cm^{-1}, with a 4 cm^{-1} spectral resolution summarizing 8 scans.

Phase III: Impact of tryptophan addition on melatonin content and antioxidant activity of production of yogurt from cow milk and soy milk

3.1) The optimum conditions from Phase I and II were selected to study in Phase III by product developing of cow milk and soy milk yogurt. Whole milk powder was used to prepare yogurt formulations. The soy milk yogurt was made by adding 2% of sugar (Hussein et al. 2020).

3.2) The yogurt production: Mixing well for all the ingredients were heated at 90°C for 5 min then cooled to 42±1°C. The LAB and probiotics were inoculated at 42±1°C then added tryptophan with 4 levels; 0.5, 1.0, 1.5 and 2.0%. All treatment were fermented until 8 h and sampled for analysis every 4 h and cooled to 4°C for physical properties analysis. The yogurts were frozen at -30°C for a minimum of 12 h and subsequently freeze-dried at -55°C for 48 (cow milk yogurt) and 72 h (soy milk yogurt), or until the a_w reached a value 0.3-0.5. The freeze-dried yogurt samples were then stored in aluminum foil bags and analyzed for their melatonin and its derivatives content, and antioxidant activity.

3.3) The yogurt obtained was analyzed for melatonin content and its derivatives (serotonin and tryptophan), antioxidant activity and their physical properties as shown in Phase I.

3.4) Sensory evaluation

Consumer preference of yogurt samples: The panelists are individuals both male and female, at least 18 years old were rated their preference of appearance, color, odor, flavor, texture (flexibility), and overall preferences using a 9-point hedonic scale with 9 being like extremely until 1 is dislike extremely (Felix da Silva et al. 2017). The consumer preference was performed by randomly coding three numbers in each sample in random order. The samples were presented to each panelist differently, served in white plastic cups, does not exceed 10°C, tested in order from left to right, and within 3 day of yogurt production. Then the score of each sample preference with the sample code and the panelist was rinsed their mouth with water during each test (Yang et al. 2012).

Phase IV: Effect of storage time on the physicochemical characteristics, melatonin content, antioxidant activity, and ACE inhibitory activity of yogurt

To study the storage on quality of physicochemical, microbiological properties and bioactive compounds change of yogurt, both cow milk and soy milk yogurts. The storage of functional yogurt products at 4°C and sampling for quality analysis were carried out on days 1, 7, 14, 21 and 28. The titratable acidity, pH, and syneresis of yogurt samples were analyzed, while other analyses were performed by spray freezing yogurt samples every 7 days and then analyzed.

4.1) Physical, Chemical, and Physicochemical analysis

4.1.1) Titratable acidity (g of lactic acid 100/g) with sodium hydroxide (0.1 M) was used to neutralize the acids contained in the cow milk and soy milk yogurts using phenolphthalein as an indicator. The titratable acidity was calculated as follows:

$$\begin{aligned}&\text{g of lactic acid 100 g} - 1 \text{ of yogurt}\\&= \frac{\text{volume of sodium hydroxide (mL)} \times \text{molar of sodium hydroxide} \times 0.9}{\text{yogurt sample weight (g)}}\end{aligned}$$

4.1.2) The pH values of the cow milk and soy milk yogurts were measured using a pH meter. Samples were taken from the incubator hourly until 8 h and allowed to cool to room temperature (25±2°C) before measuring the pH.

4.1.3) Cow milk and soy milk yogurts syneresis was analyzed with slight modifications. The yogurt (10.xx g) was centrifuged at 600 × g for 6 min at 4°C. The clear serum having separated from the yogurt was poured off and weighed. Syneresis was expressed in grams of whey lost and the syneresis was calculated using the following formula:

$$\text{Syneresis (\%)} = \frac{\text{supernatant (g)} \times 100}{\text{sample weigh (g)}}$$

4.1.3) Proximate composition analysis of yogurt refers to the basic nutritional components of the yogurt, including moisture, protein, fat, carbohydrates (primarily lactose), and ash (mineral content) according to the method described by Moraru and Biliaderis 2016; Yilmaz-Ersan et al. 2016; Alakali et al. 2018.

4.2) Determination of melatonin, tryptophan and serotonin

4.2.1) Sample extraction

The yogurt sample (accurately 2.5 g) was dissolved in 20 mL of 80% methanol and mixed by sonication in an ultrasonic bath for 30 min in the dark. Then, the mixture was shaken by a shaking incubator at 200 × g, 4°C for 16 h and centrifuged at 5,000 × g at 4°C for 5 min. The obtained extract was purified using a Sep-Pak C18 Solid Phase Extraction (SPE) cartridge following Pranil et al. (2020); Pranil et al. (2021); Nontasan et al. (2022). The purified extract obtained was then passed through a 0.22 µm syringe filter into an amber glass vial before LC-MS/MS analysis.

4.2.2) LC–MS/MS analysis of melatonin and its derivatives

A Shimadzu 20ADS Liquid Chromatograph coupled with a Shimadzu 8030 Mass Spectrometer operated in electrospray ionization mode (ESI) was used to determine melatonin, serotonin and tryptophan. An InertSustain® C18 column (2.1 mm × 150 mm, 3.5 µm) was the stationary phase, while the mobile phase was 0.45% formic acid in HPLC grade water (solvent A) and acetonitrile (solvent B). Flow regulation (time, solvent A:solvent B) was as follows: 0-5 min, 80:20%; 5-6 min, 50:50% to 0:100%; 6-9 min, 0:100%; and 9-10 min, 80:20% with a flow rate of 0.25 mL/min and 2 μL injection volume. A standard curve of external standards was plotted and used to quantify melatonin, tryptophan, and serotonin contents, with results indicated as ng/g dry basis (Pranil et al. 2021; Nontasan et al. 2022).

4.3) Determination of antioxidant activity

4.3.1) DPPH free radical scavenging activity

The DPPH radical scavenging assay was performed on the chosen strains adjusted to an OD600 of about 1.0 with phosphate-buffered saline (PBS, pH 7.4) was added to 0.05 mM DPPH solution (1:2 v/v) and mixed together. The mixtures were left to stand at room temperature for 30 min in the dark. The control reaction was arranged using ethanol added to the DPPH solution. The absorbance of each mixture was measured at 517 nm. Each sample assay was performed in triplicate. The results were compared with those of ascorbic acid (10 and 100 μg/mL), and the antioxidant activity was calculated using the following formula:

$$\text{Scavenging effect (\%)} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \times 100$$

where As is the absorbance of the test sample, and Ac is the absorbance of the control at 517 nm.

4.3.2) ABTS radical scavenging activity

The incubation was measured by mixing 7 mM of ABTS with 2.45 mM potassium persulfate (1:1 v/v) and leaving the mixture at room temperature in the dark for 24 h. After that, the ABTS solution was diluted with distilled water until the initial absorbance value of 0.7±0.005 at 734 nm was reached. Then, 170 μL of the selected strain samples and 30 μL of ABTS solution were mixed and incubated for 10 min at room temperature. The absorbance of the mixture was performed at 734 nm. The scavenging rate was calculated as follows:

$$\text{Scavenging rate (\%)} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \times 100$$

where As is the absorbance of the test sample, and Ac is the absorbance of the control at 734 nm.

4.3.3) Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) was assessed according to methods described by Amirdivani and Baba (2015). The 20 µL of yogurt extracts, appropriate dilutions, was mixed with 900 µL of the freshly prepared FRAP reagent. This buffer was prepared by mixing 300 mM/L acetate buffer (pH 3.6), 20 mM/L $FeCl_3$, 10 mM 2,4,6- tris (2-pyridyl)-s-triazine in 30 mM/L (ferrictripyridyltriazine; TPTZ) with 40 mM HCl in the ratio of 10:1:1. Incubation of mixtures at 37°C for 10 min, centrifugation (1400 × g, 2 min) and absorbance reading (593 nm) of the blue TPTZ complex formed with reduced ferrous ions at against a blank sample. The results was calculated from a standard scale of $FeSO_4.7H_2O$ and expressed as mg ferrous sulfate/g sample as follows:

$$\mathrm{FRAP} = \frac{\mathrm{CV}}{\mathrm{S}}$$

where C is the concentration of FRAP from standard curve (g/mL), V is the volume of sample prepare (mL), and S is the sample (g).

4.3.4) Total phenolic content (TPC)

The total phenolic content analysis was described by Chen et al. 2021; Mehmood et al. 2022; Cumbane et al. 2024 utilizes the Folin-Ciocalteu spectrophotometric technique. In brief, 50 µL of sample extract (10 mg/mL) was combined with 50 µL of Folin-Ciocatleu reagent (1:5 DI water) and vortexed. After that, 80 µL of 7.5% sodium carbonate (Na_2CO_3) was added, and the mixture was incubated, typically for 30 min at 45°C. Followed by incubation, the absorbance was measured at 750 nm using a UV-Visible spectrophotometer. The TPC was then calculated using a calibration curve based on gallic acid standards, and reported as mg GAE/g dry weight of material.

4.4) Determination of ACE-inhibition activity

ACE inhibition activity was performed with the ACE Kit-WST; Donkor et al. 2012; Alauddin et al. 2015; Chen et al. 2021; Kariyawasam et al. 2021 with slight adjustments.

4.4.1) Sample extraction: A sample of approximately twenty-five grams of cow milk and soy milk yogurts each batch was centrifuged for 15 min at 4°C at 4000 × g. After collecting the supernatant, 10 M NaOH was used to adjust the pH to 8.3. Following that, the suspension was centrifuged at 7000 × g for 10 min at 4 °C. Using a freeze drier, the supernatant was collected once more and freeze dried for 24 h. Three milliliters of deionized water were used to dissolve about 0.25 g of the lyophilized powder (lyophilized yogurt sample).

4.4.2) ACE inhibition: For the method, the enzyme working solution and indicator working solution were prepared according to the ACE Kit-WST technical manual. 20 µL of lyophilized yogurt sample solution was added to each sample well of a 96-well microplate and 20 µL and 40 µL deionized water for the blank 1 and blank 2 wells, respectively. Then, 20 µL of substrate buffer to each well followed by 20 µL of enzyme working solution to each sample well and blank 1 well. The microplate was incubated at 37°C for 60 min. Next, 200 µL of indicator working solution were added to each well and incubated at room temperature (25±1°C) for 10 min. The absorbance was read at 450 nm using a microplate reader. The results were shown as a percentage of ACE inhibition activity (confirmation of the presence or absence of ACE inhibition in Figure 16) can be calculated from the equation:

$$\text{ACE inhibition rate (\%)} = \frac{[Ablank\ 1\ -\ Asample]}{Ablank\ 1\ -\ Ablank\ 2} \times 100$$

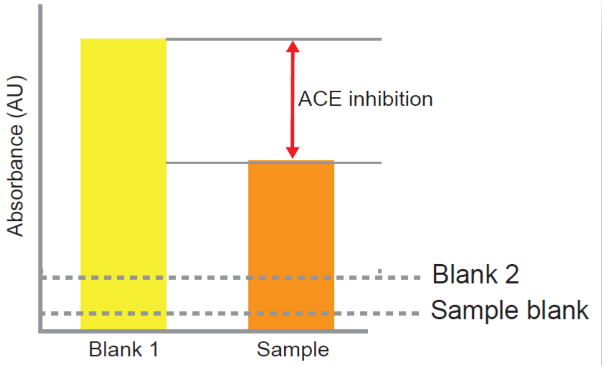


Figure 16 Confirmation of the presence or absence of ACE inhibition

4.5) Microbiology analysis

The yogurt microbiological analysis was produced according to the method described by ISO 15214:1998 (E); Felix da Silva et al. 2017; Jeong et al. 2018. The LAB counts were carried out in triplicate on days 1, 7, 14, 21 and 28. The 450 mL of 0.1% sterile peptonized water was added to 50 g of yogurt sample, blended with a stomacher for 2 min (the sample was diluted 10^{-1}), and diluted to the appropriate dilution. Then, the diluted sample 1 mL of each dilution was pipetted into a Petri dish in duplicate, and poured 45±1°C MRS agar into the Petri dish, immediately mixed the sample and medium by pour plate technique, and incubated at 30±1°C for 72±3 h with the dish inverted. The number of colonies were counted between 15–300 per dish, the results were recorded, and selected 5 colonies to perform a gram stain and catalase test. The results were reported as CFU/g.

The strain of *B. lactis* viable cells count (ISO 29981:2010; IDF 220:2010 and PCR technique) was done with De Man, Rogosa Sharp MRS-IM culture media added glucose, dicloxacillin solution of lithium chloride and cysteine hydrochloride. The plates were incubated in anaerobic jars containing anaerobac generator (Probac) at 37^{o}C for 72 h. For *L. acidophilus* was used De Man, Rogosa Sharp MRS-IM with maltose (ISO 15214:1998 and PCR technique). Pour plate technique was used and incubated inverted in anaerobic jars containing anaerobac generator at 37^{o}C for 72 h. The viable cells count were reported as CFU/g

3.6 Statistical analysis

Phase I, II, III and IV: Each treatment in all experiments were performed in triplicate. The results were expressed as mean and standard deviations. The statistical software, SPSS statistics version 21 was employed to analyze the data. Data was subjected to a one-way analysis of variance (one-way ANOVA) in a completely randomized design, and Duncan's multiple range test at $p \leq 0.05$ was used to test the significant difference between mean values.

CHAPTER 4

RESULTS AND DISCUSSION

Results were reported with discussion as follows:

4.1 Symbols used for data analysis

The symbols used for data analysis and to express the results were as follows:

$\bar{x}$ = Mean
SD = Standard deviation
p = Probability
n = Number of replications

4.2 Overall of results

Results were expressed as follows:

Phase I (Objective 1): Effect of LAB and probiotics on chemical and physical properties, melatonin content and its derivatives, and antioxidant activity in cow milk yogurt

1. Production of yogurt from cow milk
2. Chemical properties analysis of cow milk yogurt
    2.1 pH value
    2.2 %Titratable acidity
3. Physical properties analysis of cow milk yogurt
    3.1 Texture
    3.2 Syneresis
    3.3 Color value
4. Antioxidant activity
    4.1 DPPH
    4.2 ABTS
    4.3 FRAP
5. Fourier transform infrared (FTIR)
6. Melatonin, tryptophan and serotonin contents

Phase II (Objective 2): Effect of LAB and probiotic types on chemical and physical properties, melatonin content and its derivatives, and antioxidant activity in plant-based yogurt

1. Production of yogurt from plant-based milk
2. Chemical properties analysis of plant-based milk yogurt
    2.1 pH value
    2.2 %Titratable acidity
3. Physical properties analysis of cow milk yogurt

  - 3.1 Texture
  - 3.2 Syneresis
  - 3.3 Color value

4. Antioxidant activity
   - 4.1 DPPH
   - 4.2 ABTS
   - 4.3 FRAP
5. Fourier transform infrared (FTIR)
6. Melatonin, tryptophan and serotonin contents

Phase III (Objective 3): Impact of tryptophan addition on melatonin content and antioxidant activity of production of yogurt from cow milk and soy milk

1. Chemical properties analysis of cow milk yogurt
   - 1.1 pH value
   - 1.2 %Titratable acidity
2. Antioxidant activity
   - 2.1 DPPH
   - 2.2 ABTS
   - 2.3 FRAP
3. Melatonin, tryptophan and serotonin contents
4. Sensory evaluation

Phase IV (Objective 4): Effect of storage time on the physicochemical characteristics, melatonin content, antioxidant activity, and ACE inhibitory activity of yogurt

1. Production of yogurt from cow milk and soy milk were added tryptophan at 1.5%
2. Chemical properties analysis of yogurt
   - 2.1 pH value
   - 2.2 %Titratable acidity
3. Physical properties analysis of cow milk yogurt
   - 3.1 Syneresis
4. Antioxidant activity
   - 4.1 DPPH
   - 4.2 ABTS
   - 4.3 FRAP
   - 4.4 Total phenolic content
5. Melatonin, tryptophan and serotonin contents
6. ACE inhibition
7. Proximate composition
8. Viable cell count of LAB and probiotic stains

4.3 Results and discussion

4.3.1 Effect of LAB and probiotics on chemical and physical properties, melatonin content and its derivatives, and antioxidant activity in cow milk yogurt

4.3.1.1 Change of pH and titratable acidity (TA) during yogurt fermentation

Changes in the pH values of yogurts fermented by YC and YC with probiotics are shown in Figure 17a. The pH value during yogurt fermentation gradually decreased with increasing fermentation time. The pH of milk at the beginning of the experiment before the starter cultures were added ranged between 6.65 and 6.67. When starter cultures were added pH decrease varied from 6.41 to 6.48. At the end of 8 h of fermentation, pH values of yogurt MP-YC, MP-BY, MP-BT, and MP-LA decreased from 6.41, 6.45, 6.41, 6.48 to 4.11, 4.31, 4.32 and 4.31, respectively. Fermentation using a four-strain mixture of YC and two probiotics (MP-BY) or three strains (MP-BT and MP-LA) or 2 strains (MP-YC) had no significant ($p \leq 0.05$) effect on the rate of pH decrease over time. The pH value and TA reached the desired value after 4 h; however, this study aimed to evaluate the influence of co-culture YC with probiotics on melatonin, antioxidant activity, and yogurt quality characteristics. Therefore, fermentation time was prolonged until 8 h, the period normally used for regular yogurt fermentation, to allow the milk to curd, develop flavor, and produce some bioactive compounds.

The TA progressively increased, whereas the pH slowly decreased over fermentation time. The TA of yogurt produced from MP-YC, MP-BY, MP-BT and MP-LA increased significantly from 0.19, 0.20, 0.21, and 0.18% to 1.07, 0.83, 0.85, and 0.78%, respectively (Figure 17b). Yogurt fermented by YC and probiotic cultures, MP-YC, MP-BY, and MP-BT required 3 h to reach the desired pH (approximately 4.5), whereas yogurt obtained from MP-LA took 4 h to reach 4.48 (Figure 17a). The decrease in pH and increase in TA were caused by lactose fermentation and the release of lactic acid by YC and probiotics. Increasing acidity and declining pH values were not significantly different in yogurt obtained from different YC mixtures and probiotics, concurring with Câmara et al. (2020). All yogurt samples were added with *S. thermophilus* and *L. bulgaricus*, the conventional yogurt cultures, while changes in pH and TA were attributed to *L. bulgaricus*, the main acid producer (A. Y. and Tamime 2007).

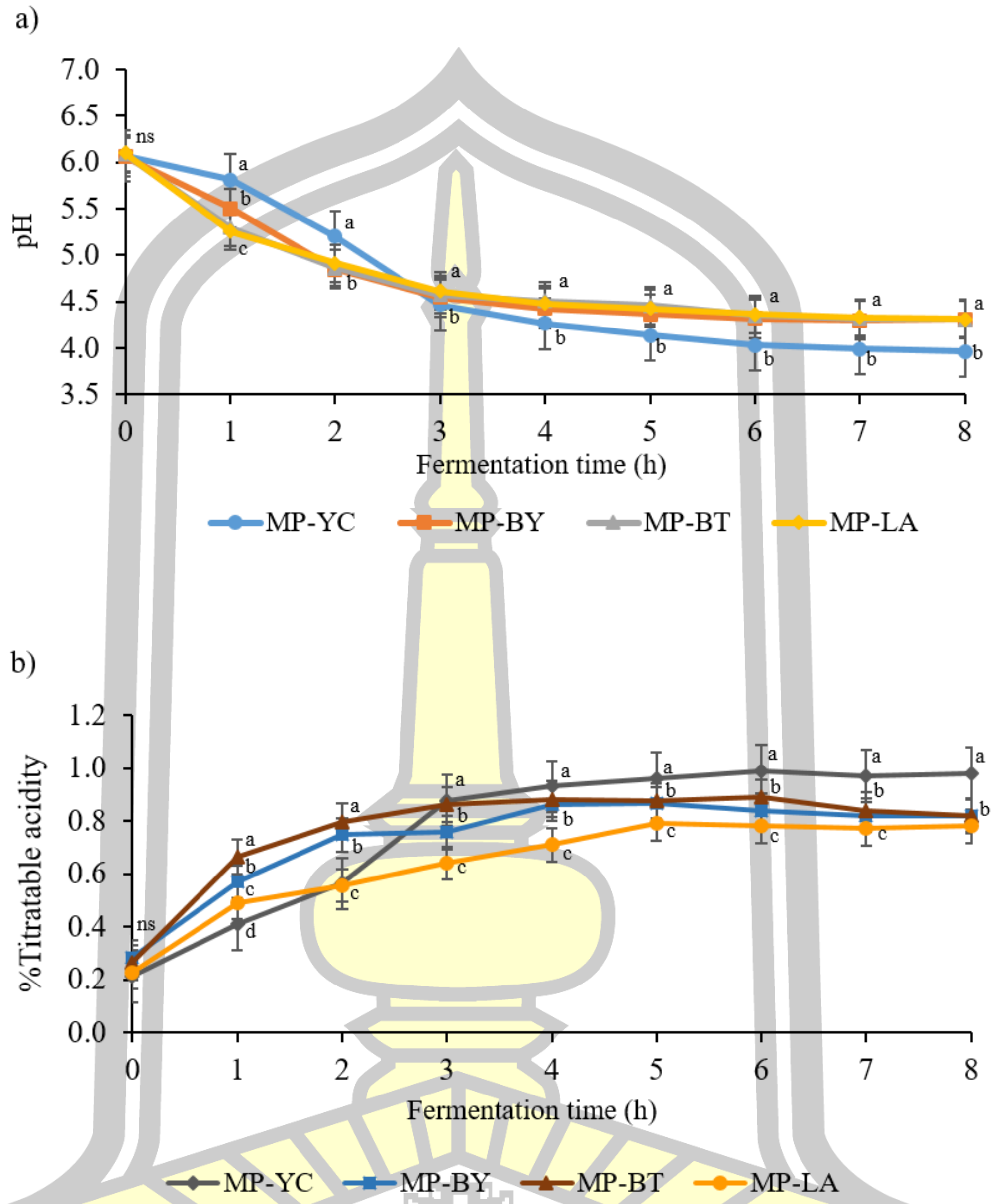


Figure 17 pH value (a) and titratable acidity (b) during yogurt fermentation. Lowercase letters comparing fermentation time with different superscripts are significantly different ($p \leq 0.05$).

4.3.1.2 Effect of yogurt bacteria and probiotic type on texture of yogurt

TPA parameters as firmness, consistency and cohesiveness of yogurt samples are presented in Table 11 Firmness values of all yogurt samples were not significantly different, ranging from 3.79 to 8.06 N. Changes in texture characteristics during yogurt fermentation were seen after incubation at 42°C for 2 h. Firmness or hardness is an important yogurt property that controls the quality and acceptability of a product. Adequate firmness without syneresis is essential for good yogurt quality. Yogurt jelly-like texture results from the three-dimensional network structure of milk proteins induced by decreasing pH and increasing acidity during milk fermentation. Firmness values of yogurt samples in this study were comparable with results reported by Yilmaz-Ersan et al. (2016). They found that yogurt prepared from reconstituted skim milk powder had firmness of 267.70 g (or 2.62 N) which was higher than the average value of industrial yogurt (1.55 N). The firmness of fermented milk products is impacted by many factors including the starter culture, total soluble solid and protein content of the product (Papaioannou et al. 2022). Higher hardness of yogurt was related to longer incubation time, while lower yogurt incubation time adversely impacted the textural quality of the yogurt (Sah et al. 2016). Cohesiveness values of all yogurt samples were not significantly different ($p \leq 0.05$), ranging between 1.39 and 1.59 N. Our results were slightly higher than previously reported by Paulo Vieira et al. (2021) who found that the cohesiveness value of natural yogurt was 0.29 N (30.29 g approximately). One explanation for the similar behavior of this parameter in all yogurts is that they have comparable proximate milk protein content after preparation from whole milk powder. The consistency of the samples was also not significantly high in yogurts with added prebiotic compared with the control at the end of fermentation (8 h).

4.3.1.3 Effect of yogurt bacteria and probiotic type on syneresis

Syneresis or whey separation is a textural defect in yogurt affected by coagulum fracture due to low protein and fat contents and high mineral content. In this study, yogurt syneresis values were significantly different ($p > 0.05$) after fermentation for 8 h (Table 11). Lowest syneresis values were found in MP-YC and MP-LA (17.60 and 14.82%, respectively), corresponding to the higher firmness of MP-YC and MP-LA than MP-BY and MP-BT. These results concurred with Almada-Corral et al. (2023) who found that fermented milk prepared using multi-strain probiotics had better texture and nutrition than milk produced using single-strain probiotics.

4.3.1.4 Effect of yogurt bacteria and probiotic type on color

Color values of the yogurts are shown in Table 11 The type of bacteria and probiotic combination had no effect on the color values. This result concurred with Ścibisz et al. (2019) who reported color values of yogurt ranging from 74.06 to 77.94 for lightness (L*), -3.01 to -3.14 for redness (a*) and 11.18 to 10.42 for yellowness (b*). The whiteness in milk results from the presence of colloidal particles such as milk lipid globules and casein micelles which scatter light in the visible spectrum. The yogurt samples had negative a* values indicating greenness and positive b* values indicating yellowness due to the presence of beta-carotene and other carotenoids in dairy products. The color of yogurt varies depending on many factors but mainly depends on the pigments in the cow feed.

Table 11 Texture profile analysis, syneresis and color values of yogurts fermented by different mixed cultures at 8 h of fermentation.

Treatment	**Firmness (N)**	**Consistency (N.sec)**	**Cohesiveness (N)**	**Index of viscosity (N.sec)**	**Syneresis (%)**	**Color**		
						L*	**a***	**b***
MP-YC	$8.06{\pm}0.69^{a}$	8.67±0.82	1.51±0.10	2.35±0.08	$17.60{\pm}0.46^{b}$	74.06±0.45	-3.01±0.04	10.42±0.07
MP-BY	$3.79{\pm}0.49^{b}$	9.16±0.22	1.59±0.18	2.64±0.25	$22.01{\pm}1.20^{c}$	77.94±0.74	-3.11±0.03	11.01±0.06
MP-BT	$4.20{\pm}0.32^{b}$	8.18±1.08	1.39±0.08	2.41±0.40	$24.31{\pm}1.30^{c}$	74.86±3.91	-3.14±0.19	11.18±0.32
MP-LA	$7.12{\pm}1.22^{a}$	9.19±0.27	1.58±0.27	2.40±0.17	$14.82{\pm}0.80^{a}$	75.48±0.61	-3.02±0.02	10.78±0.02

Lowercase letters within columns with different superscripts are significantly different ($p \leq 0.05$).

4.3.1.5 Effect of yogurt bacteria and probiotics on antioxidant activity of yogurt

The antioxidant activities (IC_{50}) of yogurt fermented with YC and YC combined with one or two probiotics evaluated by DPPH and ABTS radical scavenging and FRAP are shown in Figure 18a, b and c, respectively. The smaller IC_{50}, value the greater the scavenging rate. The DPPH antioxidant activity of all yogurt samples significantly improved after fermentation for 4 and 8 h ($p > 0.05$). The IC_{50} values of MP-YC and MP-LA yogurt initially slightly increased but decreased after fermenting for 8 h. The MP-YC, MP-BY and MP-BT yogurt samples showed significantly stronger DPPH radical scavenging at 8 h, with IC_{50} values 44.61, 39.72, and 42.90 mg/mL, respectively than the MP-LA yogurt (52.11 mg/mL) ($p > 0.05$). The IC_{50} of ascorbic acid on DPPH scavenging with the greatest antioxidant activity (8.24 mg/mL) is also shown for comparison with the yogurt samples.

The antioxidant properties of yogurt using the ABTS radical scavenging method revealed that yogurt fermented with different mixed cultures and during the first four hours of fermentation time had significantly different IC_{50} values ($p > 0.05$). However, after 6 to 8 h of fermentation the antioxidant activity of all samples gradually improved but was not significantly different with IC_{50} values of 13.96, 14.38, and 15.12 mg/mL for MP-YC, MP-BY and MP-BT yogurt, respectively whereas MP-LA had the lowest antioxidant activity at 8 h (IC_{50} = 16.03 mg/mL). The highest IC_{50} value was observed in MP-BT (13.96 mg/mL), while the IC_{50} of ascorbic acid as the antioxidant standard was 9.14 mg/mL.

The FRAP assay measures the ability to reduce Fe^{3+}-TPTZ (ferric tripyridyltriazine) iron complexes of antioxidants. MP-BY and MP-BT yogurts were significantly more potent for reducing ferric to ferrous ions compared to plain yogurt MP-YC. The FRAP values of all yogurt samples slightly increased after fermenting for 2 h and were then stable between 2 and 8 h of fermentation. The FRAP values of MP-YC, MP-BY, MP-BT, and MP-LA at 8 h fermentation were 1147.23, 1192.37, 1183.19, and 1179.64 µm $FeSO_4$/g dw, respectively and not significantly different ($p \leq 0.05$). Antioxidant compounds increased during fermentation. This finding was supported by Shori et al. (2022) who found that total phenolic and flavonoid contents of yogurt fermented by *L. casei* ATCC 393 increased and these compounds expressed antioxidant properties. Probiotics can chelate positively charged minerals such as iron, zinc, copper, cobalt, and manganese. The chelating capacity of probiotic strains is due to the physiological chelators that exist in the intracellular cell-free extract of probiotics (Wang et al. 2017). YC and probiotics contain antioxidant enzymes including superoxide dismutase that catalyzes the decomposition of superoxide free radicals into hydrogen peroxide and water. This enzyme acts as a regulator of free radical formation (Heydari et al. 2022).

Studies have shown that specific probiotic strains, such as *L. acidophilus* and *B. animalis* subsp. *lactis*, also play a pivotal role in boosting antioxidant activity, not only by directly scavenging free radicals but also by enhancing the body's endogenous antioxidant defenses through increased production of antioxidant enzymes like superoxide dismutase and catalase (Wang et al. 2017). Additionally, research demonstrates that such probiotic combinations can significantly improve the yogurt antioxidant potential, largely due to increased levels of bioactive peptides and fermented metabolites with strong radical-scavenging activity (Ali et al. 2022).

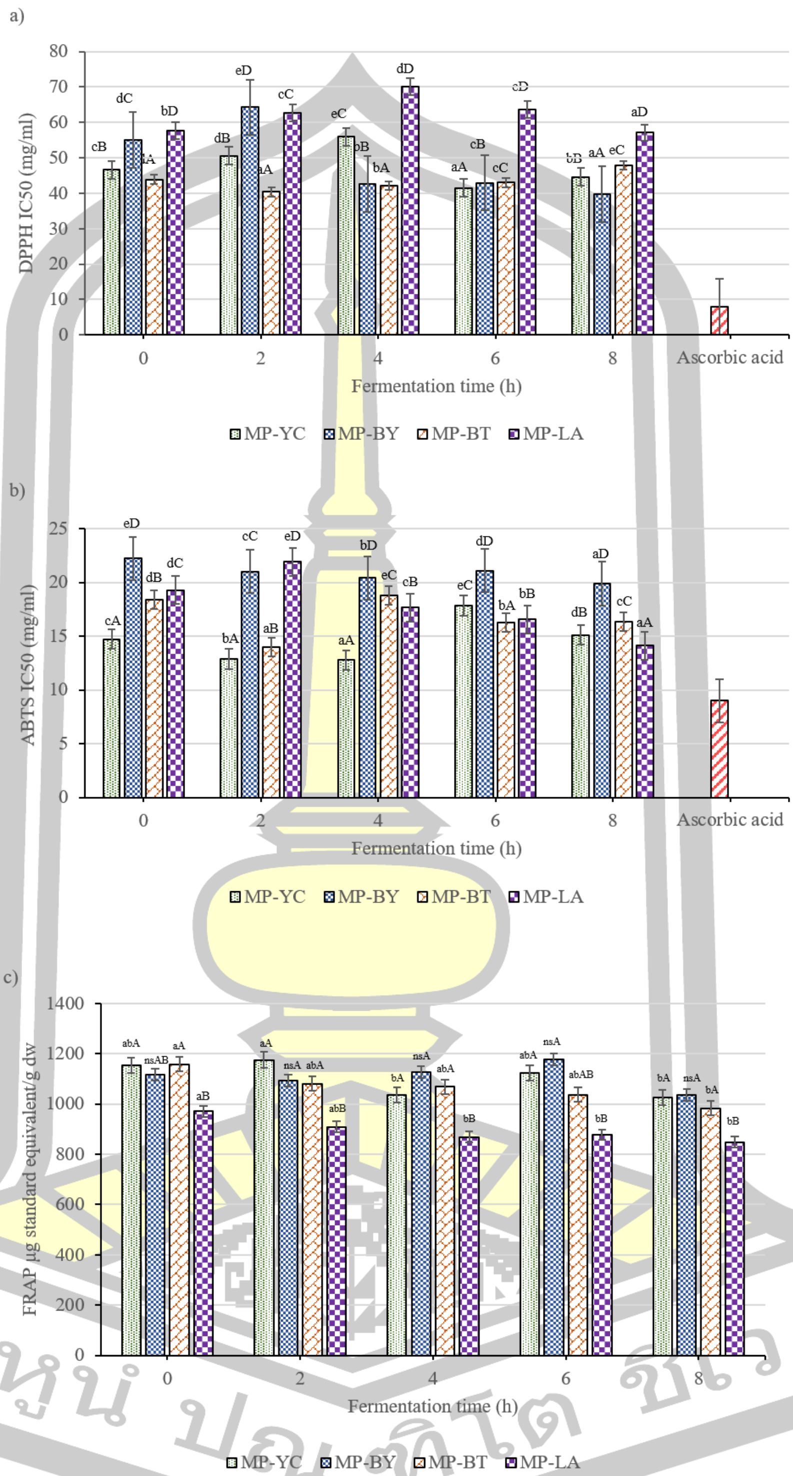


Figure 18 Yogurt antioxidant activity by DPPH (a), ABTS (b), and FRAP (c) fermented by different mixed cultures.

Capital letters comparing yogurt bacteria and probiotic types and lowercase letters comparing fermentation time with different superscripts are significantly different ($p \leq 0.05$).

4.3.1.6 Effect of yogurt bacteria and probiotics on FTIR of yogurt

The FTIR spectra of yogurt samples produced using traditional starter cultures showed well-defined bands in the protein regions, particularly around 1650 cm⁻¹ (Amide I) and 1540 cm⁻¹ (Amide II) (Figure 19). These peaks were consistent with the presence of β-sheet and α-helix structures, which are typical of dairy proteins such as casein and whey. The intensity of these bands provides insight into protein denaturation and the formation of new protein matrices during fermentation (Papadopoulou et al. 2021). Additionally, the carbohydrate region (1200–1000 cm⁻¹) exhibited pronounced peaks, indicative of lactose hydrolysis and conversion into lactic acid by *L. bulgaricus* and *S. thermophilus*. The reduction in lactose signals, coupled with the rise in bands associated with lactic acid, demonstrates successful fermentation (Saji et al. 2024).

The incorporation of probiotic strains, such as *L. acidophilus* and *B. lactis*, led to notable shifts in the FTIR spectra, particularly in the carbohydrate and protein regions. In comparison to traditional yogurt, probiotic yogurts exhibited lower intensities in the Amide I and Amide II bands, suggesting altered protein interaction and potential changes in the protein network structure (Papadopoulou et al. 2021). This is likely due to the unique metabolic activities of probiotic bacteria, which may result in different degrees of proteolysis or acidification during fermentation. Moreover, the carbohydrate-related peaks (1200–1000 cm⁻¹) in probiotic yogurts (MP-BY, MP-BT, and MP-LA) showed a higher degree of intensity, implying enhanced breakdown of lactose and potentially higher production of exopolysaccharides (EPS). EPS is known to improve the textural properties of yogurt, contributing to a creamier mouthfeel. These polysaccharides also have potential prebiotic effects, further increasing the health benefits of probiotic yogurts. Comparing the FTIR spectra of yogurt with and without probiotics reveals several key differences. Probiotic yogurts typically exhibit higher absorption in the lipid region (3000–2800 cm⁻¹), suggesting greater retention or even enhanced synthesis of fatty acids by certain probiotic strains. This can contribute to a more complex flavor profile and improved nutritional value of the yogurt.

The FTIR analysis of yogurt provides insights into the chemical structure and molecular interactions during fermentation. For instance, it identifies changes in functional groups associated with proteins, lipids, and carbohydrates, which are critical for texture, flavor, and nutritional quality. FTIR has been used to monitor the transformation of tryptophan into bioactive melatonin derivatives, which can enhance the antioxidant properties of yogurt. These compounds interact with free radicals, reducing oxidative stress and improving the product's health benefits. The melatonin content in yogurt, especially when fermented with specific probiotic strains, significantly contributes to its antioxidant activity. Melatonin acts as a potent antioxidant, scavenging free radicals such as reactive oxygen and nitrogen species. Studies indicate that fermentation enhances the bioavailability of melatonin and its derivatives, which can be detected via FTIR and biochemical assays. These changes were influenced by microbial activity, fermentation time, and yogurt matrix composition, affecting both the nutritional and functional properties of the yogurt. For instance, melatonin's ability to protect against oxidative stress is highlighted in recent studies, showing its role in mitigating molecular damage and promoting health. Incorporating probiotics and optimizing fermentation processes can further boost melatonin levels and antioxidant activity in yogurt, making it a functional food with potential health benefits (Joseph et al. 2024; Monteiro et al. 2024; Zhao et al. 2019).

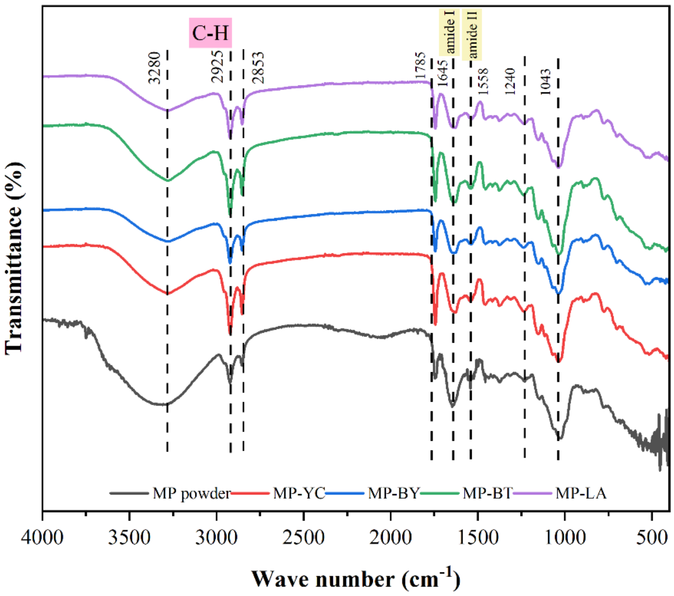


Figure 19 FTIR spectrum of cow milk yogurts at 8 h fermentation.

4.3.1.7 Impacts of yogurt bacteria and probiotic combinations on melatonin, serotonin and tryptophan contents

Changes in melatonin and its metabolite concentration including serotonin and tryptophan in yogurts fermented with YC mixed with probiotics are shown in Table 12. Concentrations of melatonin, serotonin, and tryptophan in whole milk powder were measured before inoculation with the starter cultures. Results revealed tryptophan as the only metabolite that could be detected (5.00 µg/g dw) in milk powder used as raw material for yogurt preparation. Melatonin and serotonin were not detected because whole milk contains amounts below the detection limit used in this study. At 0 h of fermentation, when different combinations of starter cultures were transferred into the pasteurized reconstituted milk, the initial melatonin and tryptophan concentrations increased. However, only melatonin in MP-BY yogurt was observed (3.31 ng/g dw). Melatonin content increased significantly ($p \leq 0.05$) after fermentation for 4 and 8 h, with highest melatonin level observed in MP-BY (four strain mixture) (6.67 ng/g), whereas melatonin levels in MP-LA, MP-BT and MP-YC were

3.54, 3.56, and 2.16 ng/g, respectively. Melatonin content in yogurt fermented by YC mixed with probiotics was higher than recorded in previous reports in both raw milk and yogurt. Sangsopha et al. (2020) reported melatonin content in raw cow milk at only 0.03 ng/mL, while melatonin content in plain yogurt at 0.13 ± 0.01 ng/mL was reported by Kocadağlı et al. (2014) and 0.126 ng/mL in probiotic yogurt studied by Que et al. (2020).

In this study, melatonin concentration increased by 3 folds in yogurt fermented with YC mixed with two probiotics compared to yogurt fermented with YC at only 2.16 ng/g. When both *B. animalis* subsp. *lactis* and *L. acidophilus* were involved in the mixed culture as the four-strain mixture (MP-BY), melatonin was detected at 0 h and then increased until the highest value at 8 h. By contrast, when only YC (two strain mixture, MP-YC) was used or YC was combined with *B. animalis* subsp. *lactis* (three strain mixture, MP-BT) or *L. acidophilus* (three strain mixture, MP-LA), melatonin content was found to be lower than MP-BY. There was a synergistic relationship between YC and probiotics, with both *B. animalis* subsp. *lactis* and *L. acidophilus* resulting in higher melatonin production.

Serotonin content was also determined but not detected in all samples because the serotonin amount was below the limit of detection in this study. This finding was supported by Allegri et al. (1993) who reported that 5-hydroxytryptophan, the precursor of serotonin in the serotonin pathway, was found in low concentrations in yogurt.

Non-protein tryptophan or free tryptophan content in yogurt prepared from YC and different probiotics is presented in Table 4.2. Yogurt fermentation increased free tryptophan concentration compared with unfermented milk (5.00 µg/g). At 0 h, after the starter culture was added to the milk, tryptophan contents were 5.40, 5.25, 6.15 and 8.08 µg/g for MP-YC, MP-BY, MP-BT and MP-LA, respectively. After fermenting for 4 and 8 h, the tryptophan contents significantly improved in all samples, varying from 4.73 to 10.74 µg/g. Results concurred with Yılmaz and Gökmen (2020) who reported that free tryptophan levels in commercial yogurts ranged from 3.2 to 13.4 mg/Kg dw. Changes in free amino acid concentrations were due to the different bacteria applied in yogurt preparation because free amino acid levels are affected by different strains and interactions between the bacteria cultures involved in yogurt fermentation (Allegri et al. 1993). One serving of 130 g of set yogurt contained 630 ng of melatonin and 1300 µg of free tryptophan providing beneficial impacts to health. The probiotic yogurt product had high melatonin content compared to melatonin-rich food products such as melatonin-rich milk powder (80 pg/g of powder) (Salehi et al. 2019). This is the first report on the improvement of melatonin content in yogurt by mixing YC and probiotics.

Research studies have shown that probiotic strains, including *L. acidophilus* and *B. animalis* subsp. *lactis*, have a significant effect on increasing tryptophan in yogurt, which in turn increases melatonin production (Neves Casarotti et al. 2020). Moreover, the fermentation process facilitated by yogurt cultures, including *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, has been shown to influence tryptophan metabolism, further enhancing melatonin content. The synergistic effects of probiotic strains when combined with traditional yogurt bacteria can optimize these bioactive compounds, creating a functional food with enhanced health benefits (Sharifi-Rad et al. 2020). For instance, combining *L. acidophilus* with *B. longum* has been reported to increase melatonin concentrations in yogurt, which contributes to both its sleep-regulating and antioxidant capacities (Wang et al. 2019). Consuming melatonin and tryptophan-enriched yogurt offers significant health benefits, particularly by supporting sleep regulation and reducing oxidative stress. Melatonin plays a crucial role in managing the body circadian rhythm and promoting restful sleep, making it especially beneficial for individuals facing sleep disturbances or irregular sleep patterns. Tryptophan, as a precursor to melatonin and serotonin, further supports mood regulation and cognitive function, enhancing the overall impact of these nutrients on mental health (Gao et al. 2020). Additionally, melatonin powerful antioxidant properties help protect cells from oxidative stress, which is linked to aging and chronic diseases such as cardiovascular disease and neurodegeneration. Regular intake of melatonin and tryptophan-enriched yogurt could, therefore, serve as a functional food that not only improves sleep quality but also promotes cellular health and mental well-being, making it a promising dietary intervention for overall human health (Masood et al. 2011).

Table 12 Melatonin, serotonin and tryptophan contents of yogurt at different fermentation times compared with milk powder.

Treatment	**Melatonin (ng/g dw)**			**Tryptophan (µg/g dw)**			**Serotonin**		
	0	**4**	**8**	**0**	**4**	**8**	**0**	**4**	**8**
Milk powder	ND	(-)	(-)	5.00±0.94^{B}	(-)	(-)	ND	(-)	(-)
MP-YC	ND	ND	2.16±0.03^{C}	5.40±0.^{12bB}	10.74±0.66^{aA}	10.17±0.27^{a}	ND	ND	ND
MP-BY	3.31±0.01^{b}	3.63±0.05^{aA}	6.67±0.21^{aA}	5.25±0.01^{bB}	4.73±0.54^{bD}	9.25±0.11^{a}	ND	ND	ND
MP-BT	ND	ND	3.56±0.12^{B}	6.15±0.68^{bB}	7.97±0.08^{aC}	8.52±0.83^{a}	ND	ND	ND
MP-LA	ND	3.37±0.01^{bB}	3.54±0.10^{aB}	8.08±0.53^{bA}	8.76±0.46^{abB}	9.22±0.66^{a}	ND	ND	ND

Capital letters within columns and lowercase letters within a row for each sample of melatonin and its derivative contents with different superscripts are significantly different ($p \leq 0.05$), ND represents an amount that was not detected, and (-) represents an amount that was not determined.

4.3.2 Effect of LAB and probiotic types on chemical and physical properties, melatonin content and its derivatives, and antioxidant activity in plant-based yogurt

4.3.2.1 The pH and titratable acidity during plant-based yogurt fermentation

The pH value of plant-based milk yogurt (Figure 20) that soybean, chickpea and white sesame yogurts had similar results, showings a decreased pH value with the fermentation time increased. All of plant-based milk yogurt took 4-7 h to obtain the desired pH of 4.5-4.6. The pH value in yogurt, soy milk and LAB combination with probiotics were decreased sequentially until 8 h, with all samples having a similar decrease in pH. It can be seen that each treatment had a high pH. The pH during fermentation gradually decreased from 6.26 to 4.5–4.6 (desired pH) in 6 h for SB-YC while yogurt fermented using both YC and probiotics (SB-BY, SB-BT, and SB-LA) reached the desired pH in 4 to 5 h. In the 8 h of fermentation, SB-BY had the lowest acidity. The change in pH value of all chickpea yogurt samples shows similar reducing trends. The CP-LA yogurt fermented with yogurt bacteria combined with *L. acidophilus* took over 6 h to achieve the desired pH (4.6), while a regular chickpea yogurt (CP-YC) and other samples (CP-BY, CP-BT) took 3-4 h. The pH results of white sesame milk yogurts showed that the pH values generally decreased over time in all experiments, except for the yogurt sample fermented with *Lactobacillus acidophilus* (SS-LA). The final of fermentation (8 h) was found to be 3.80 (SS-LA).

The titratable acidity of plant-based milk yogurts (Figure 21) found that the %TA were increased with increasing fermentation time. LAB-fermented soy milk and chickpea yogurts had a higher %TA than yogurt co-cultured with probiotics. While, white sesame yogurt had the opposite effect. The TA of the soy milk yogurts increased with fermentation time from 0.20 to 0.99% at 0 and 8 h. SB-BY (0.21–0.99%) and SB-LA (0.20–0.93%) had higher TA than yogurt fermented by SB-YC (0.23–0.75%) and SB-BT (0.24–0.74%). However, the results showed that LAB and probiotic strains produce an increased amount of lactic acid as the fermentation period increases, which decreases the pH of the entire sample. The amount of lactic acid in soy milk yogurt co-cultured with probiotics increased continuously throughout the fermentation until 8 h, the SB-BY gave the highest amount of lactic acid (0.99%). Similar to white sesame yogurts had the %TA as 4.38% in SS-BT.

The titratable acidity of the chickpea yogurts was found that %TA increased with the fermentation. LAB-fermented chickpea yogurt had a higher %TA than yogurt co-cultured with probiotics. However, results showed that the LAB strain and probiotics produced lactic acid. The amount increased as the fermentation time increased, which caused the pH value of all samples to decrease. Chickpea yogurt exhibited a pH range of 4.2 to 4.5 across different fermentation times and chickpea concentrations. The acidity, expressed as lactic acid equivalent, ranged from 0.8% to 1.2% (v/v), indicating typical yogurt acidity levels (Jeong et al. 2018b; Macit et al. 2019).

Considering the correlation between pH and lactic acid content in plant-based milk yogurt with LAB and probiotics, it was observed that as the fermentation time extended, the pH value decreased compared to earlier stages of fermentation. The co-culture of LAB and probiotic strains led to a continuous increase in lactic acid production as fermentation time increased, resulting in a relatively stable pH value within the first 0-5 h of yogurt fermentation. This pH variation is influenced by several factors, including bacterial lactic acid production, the incorporation of flavorings, and other food additives, all contributing to increased acidity. Acidification is crucial in food preservation through fermentation, as the metabolic activity of bacteria accumulates acid, lowering the pH and inhibiting the growth of spoilage bacteria (Amirdivani and Baba 2015). The pH values in this study concurred with Grasso et al. (2020) who reported that the pH values in commercial soy milk yogurts ranged from 3.99 to 4.56. This pH range can be attributed to soy milk compositions, yogurt bacteria, and the ingredients used in yogurt production. Soy milk yogurt fermented by *Leuconostoc mesenteroides* and *Bifidobacterium plantarum* had initial pH values ranging between 6.83 and 6.92, which decreased significantly to 4.41–4.57 at 24 h (Kim and Han 2019). Specific pH ranges are required as they influence the action of certain gelling agents in soy milk yogurt. The increase in TA resulted from the accumulation of lactic acid and other organic acids produced through fermentation of the starter culture. The TA values of the yogurts were 0.45–0.55% and 0.74–0.99% at 0 h and 8 h fermentation, respectively and comparable to results reported by Grasso et al. (2020) for commercial soy milk yogurts.

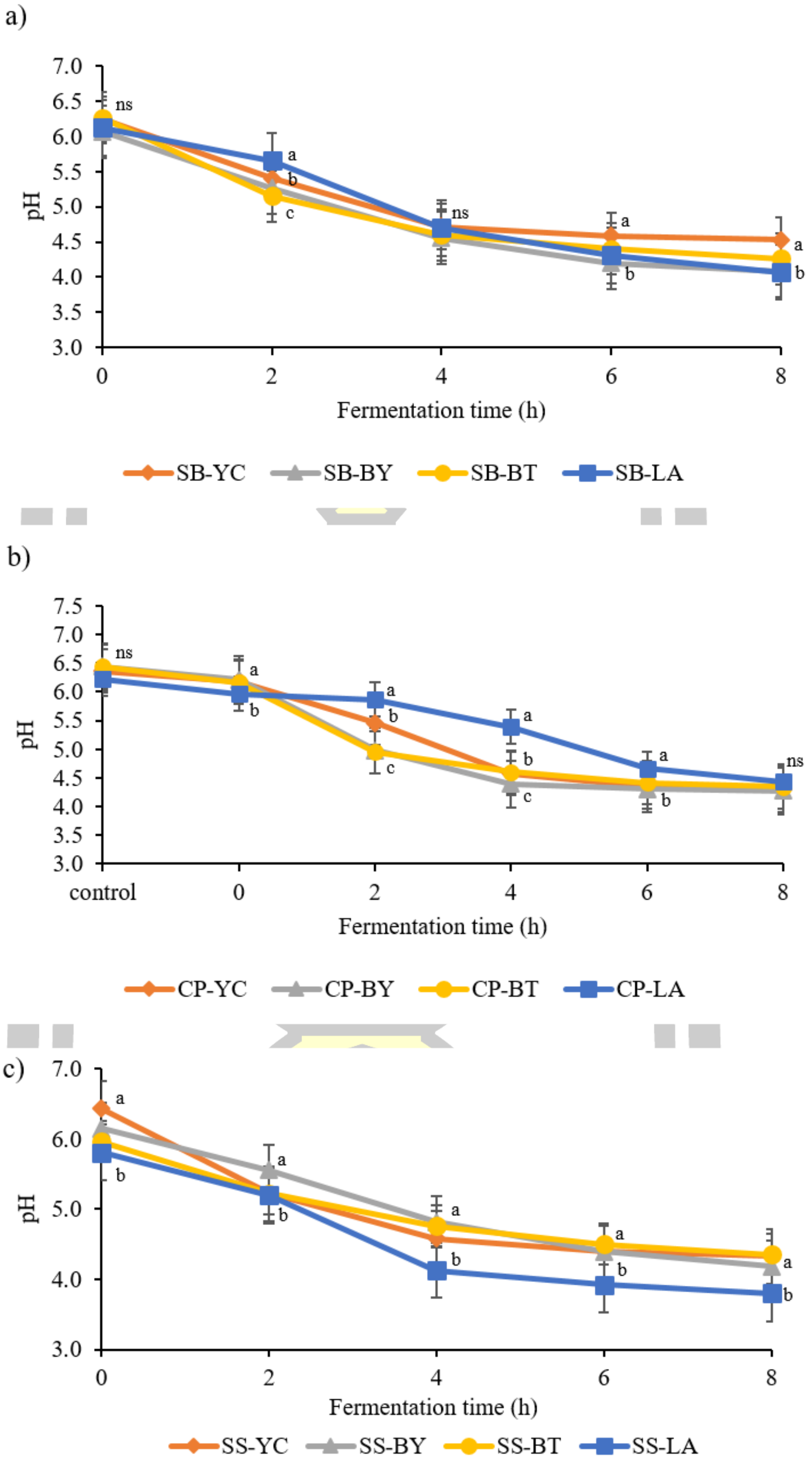


Figure 20 pH value during fermentation of plant-based milk yogurt. Soy milk yogurt (a), chickpea yogurt (b) and white sesame seed yogurt (c) Lowercase letters comparing fermentation time with different superscripts are significantly different ($p \leq 0.05$).

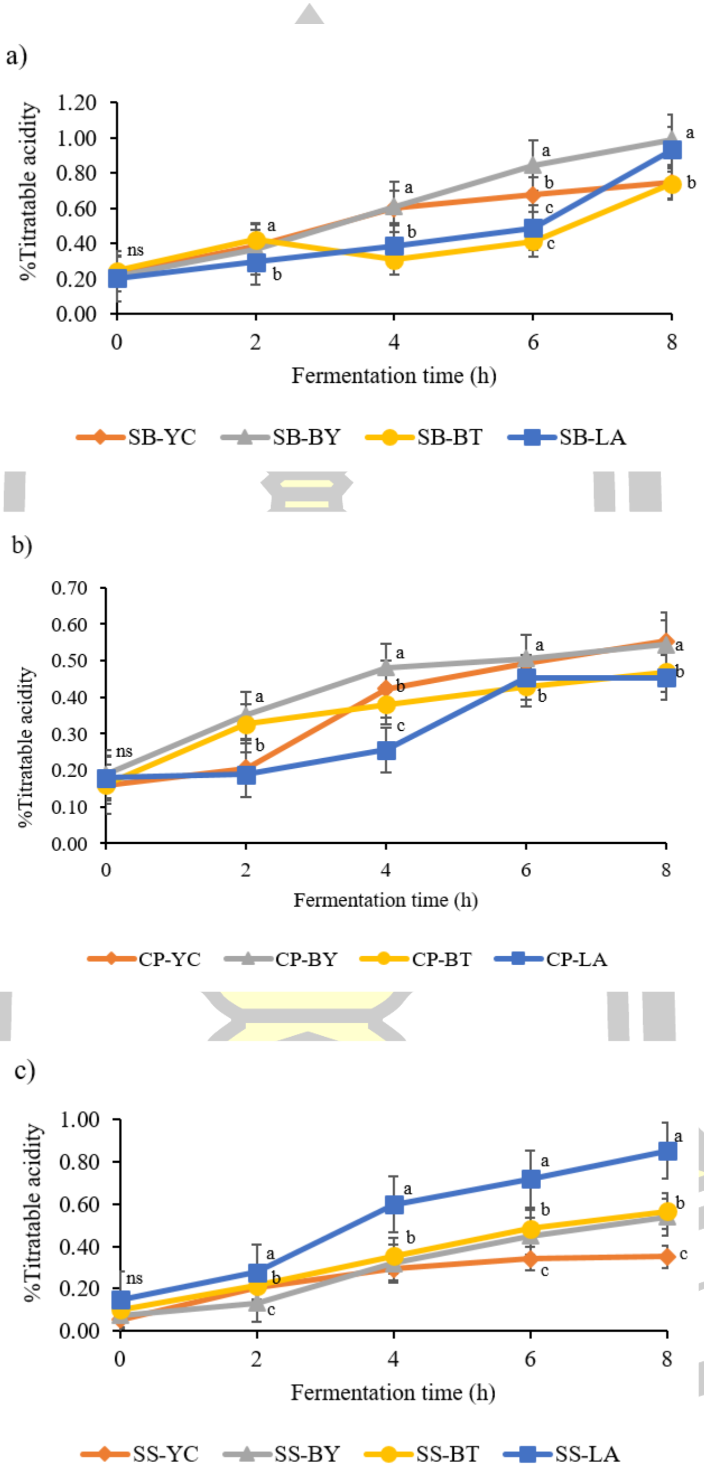


Figure 21 The titrated acidity during fermentation of plant-based milk yogurt. Soy milk yogurt (a), chickpea yogurt (b) and white sesame seed yogurt (c) Lowercase letters comparing fermentation time with different superscripts are significantly different ($p \leq 0.05$).

4.3.2.2 Effect of the yogurt bacteria and probiotic types on plant-based yogurt texture

Texture profile analysis of plant-based yogurts as soy milk and chickpea yogurt (Table 13 and 14, respectively) that addition of LAB and LAB co-culture with probiotics show that the texture were significant difference with the control (0 h). The noticeable in chickpea yogurt had a clear liquid that separates at the start time of the fermentation. Furthermore, the white sesame yogurt does not curd form like other plant-based yogurt samples. Therefore, texture characteristics were not tested.

The analysis of the physical characteristics of soy milk yogurt exposed to bacteria containing LAB and probiotics revealed that there were significant differences in TPA across all treatments ($p \leq 0.05$). The firmness, consistency and index of viscosity of the SB-BY differed from that of the SB-BT and SB-LA. However, the cohesiveness values for SB-BY and SB-BT were comparable ($p > 0.05$). Furthermore, soy milk yogurt enriched with probiotics (SB-BY, SB-BT, and SB-LA) had a significantly smoother consistency than plain yogurt (SB-YC). Soy milk yogurts have lower protein content and differences in the coagulation properties of the proteins reduces consistency compared with milk yogurt without adding thickener, especially at low pH, which affects the visual appearance of these products (Harper et al. 2022).

Changes in the texture attributes of chickpea yogurt during fermentation for 0-8 h revealed that both the fermentation duration and the type of bacteria had an impact on the texture profile analysis of firmness, consistency, and cohesiveness ($p \leq 0.05$). The chickpea yogurt supplemented with probiotics (CP-BY, CP-BT and CP-LA) exhibited differences in these characteristics compared to yogurt with only bacterial culture (CP-YC), particularly at 0 and 8 h. Texture analysis indicated that chickpea yogurt exhibited a smooth and creamy texture, similar to conventional yogurt, with no significant differences in texture parameters such as firmness and cohesiveness (Werning et al. 2022).

4.3.2.3 Effect of yogurt bacteria and probiotics on syneresis of plant-based yogurt

In this study, the effect of syneresis properties of plant-based yogurts (Table 13, 14, 15) were significantly different ($p \leq 0.05$) for all treatments with increased fermentation time compared to that start time (0 h) and used by the LAB in combination with different probiotics also showed significantly different syneresis that the white sesame yogurt was highest. After fermentation for 8 h, lowest syneresis values were found in SB-BT, CP-BY and SS-LA (30.08, 36.58 and 64.93%, respectively). These results concurred with Soni et al. (2020) who found that fermented milk prepared using multi-strain probiotics had better texture and nutrition than milk produced using single-strain probiotics.

The syneresis properties of soy milk yogurts were affected by the yogurt bacteria in combination with different probiotics. The highest syneresis (40.92%) was found in SB-BY. While, at 0 h, no syneresis was observed, which could be attributed to the yogurt being freshly made, with the gel structure still intact and fully hydrated. The absence of liquid separation at this point indicates a well-formed gel network that holds water effectively (Yang et al. 2012; Mei et al. 2016). Syneresis, the separation of whey protein on the surface of yogurt, is a significant and noticeable defect that can negatively impact consumer acceptance of the product (Erkaya-Kotan 2020). The percentage of syneresis of soy milk yogurts ranged between 30.08 and 40.92%. Cui, Chang, and Nannapaneni (2021) reported that the syneresis in soy milk yogurt fermented by yogurt starters alone (YFL-901, consisting of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*) was significantly higher compared to those made with a combination of yogurt starters and probiotics like *L. rhamnosus* (LGG), *B. animalis* subsp. *lactis* BB-12, and *L. acidophilus* La-5 which most soy milk yogurt products showed only a slight increase in syneresis. These findings suggest that the addition of probiotics enhanced the water-holding capacity of the yogurt, reducing whey formation significantly. Probiotics like BB, LA, and LGG are known to produce exopolysaccharides, which improve the yogurt texture by interacting with free water in a gel-like structure, thus helping to reduce syneresis.

In addition, the results of syneresis properties of chickpea yogurts were significantly different for all treatments used by the LAB in combination with different probiotics. CP-BY has the lowest percentage of syneresis, i.e. less liquid separation than other samples, which was considered suitable. Chickpea yogurt had a clear liquid that separates at the start of fermentation. However, at 0 h, no syneresis was observed in CP-LA and SS-BY, probably due to the same reason as SB-BY

4.3.2.4 Effect of yogurt bacteria and probiotics on color of plant-based yogurt

The results of color analysis during the fermentation of plant-based yogurts (Table 13, 14, 15) found that the duration of increased fermentation resulted in L*, a*, b* were significantly different ($p \leq 0.05$) compared to the start time. The plant-based yogurt used by the LAB in combination with different probiotics also showed significantly different color values. The highest L* value was white sesame yogurt, a* value was chickpea yogurt, and b* value was soy yogurt. Furthermore, the type of bacteria and probiotic combination had an effect on the color values ($p \leq 0.05$). The color of yogurt varies depending on many factors but mainly depends on the pigments in the raw material.

The color values of soy milk yogurt were yellowish and showed significantly different ($p \leq 0.05$) color values when fermented by different cultures. The type of probiotic bacteria had an effect on the L*, a* and b* ($p \leq 0.05$) whereas soy milk yogurt with added probiotics gives brightness (75.15) was observed in SB-BY, while the highest a* (0.23) and b* (15.41) values were found in SB-LA. There was significant difference between the green and yellow color value compared to LAB yogurt, except the brightness of the SB-BY. While, the chickpea yogurts were dark yellow. The chickpea yogurt samples used by the LAB in combination with various probiotics also showed significantly different ($p \leq 0.05$) color values. The highest L*, a*, and b* values were CP-LA. Color analysis demonstrated that chickpea yogurt exhibited a creamy white color, similar to conventional yogurt, with no significant differences observed in color parameters such as L*, a*, and b* values (Hussein et al. 2020; Plamada et al. 2023). The SS-BY also differs from the control sample (SS-YC) in terms of brightness, redness, and yellowness values.

Color values are essential for qualitative assessments of food quality. The visual assessment of food color closely corresponds with consumer or taster evaluations, helping to establish standard criteria for comparing instrumental measurements (AMSA 2012). The brightness (L*) of yogurt is related to the particle size of the fat and protein globules. This affects light reflection and scattering ability. The size of these particles is greatly influenced by the choice of unit operation and the processing parameters used. Soy milk yogurt showed the b* in yellow and results agreed with Grasso et al. (2020). The a* (red-green axis) values were negative for all yogurts. The soy milk containing 100% extract had the lowest brightness value (L = 53.40).

Table 13 Texture profile analysis, syneresis and color values of soy milk yogurts fermented by different mixed cultures at 8 h of fermentation.

Treatment	Firmness (N)	Consistency (N.sec)	Cohesiveness (N)	Index of viscosity (N.sec)	Syneresis (%)	Color		
						L*	a*	b*
SB-YC	$24.23±3.37^{b}$	$65.14±8.63^{a}$	$2.49±2.52^{b}$	$0.89±0.40^{c}$	$36.30±0.47^{b}$	$74.27±0.52^{b}$	$-0.43±012^{b}$	$13.83±0.85^{b}$
SB-BY	$40.51±2.55^{a}$	$19.65±1.01^{c}$	$6.48±1.18^{a}$	$2.22±0.19^{b}$	$40.92±1.28^{b}$	$75.15±0.42^{a}$	$-0.01±0.03^{a}$	$12.95±0.01^{b}$
SB-BT	$17.96±1.91^{c}$	$37.33±3.82^{b}$	$4.07±0.15^{ab}$	$2.65±0.15^{a}$	$30.08±0.77^{a}$	$74.07±0.66^{b}$	$0.08±002^{a}$	$12.85±0.39^{b}$
SB-LA	$26.1±0.50^{b}$	$4.22±0.14^{d}$	$0.58±0.13^{c}$	$0.45±0.08^{d}$	$34.24±0.51^{a}$	$71.45±0.38^{c}$	$0.23±0.13^{b}$	$15.41±0.46^{a}$

Lowercase letters within a column with different superscripts are significantly different ($p \leq 0.05$).

Table 14 Texture profile analysis, syneresis and color values of chickpea yogurts fermented by different mixed cultures at 8 h of fermentation.

Treatment	Firmness (N)	Consistency (N.sec)	Cohesiveness (N)	Index of viscosity (N.sec)	Syneresis (%)	Color		
						L*	a*	b*
CP-YC	$0.17±0.01^{c}$	$0.24±0.00^{b}$	$0.05±0.00^{b}$	$0.08±0.00^{a}$	$42.77±0.25^{b}$	$73.81±0.02^{b}$	$-2.33±0.01^{c}$	$17.10±0.20^{c}$
CP-BY	$0.28±0.01^{b}$	$0.40±0.01^{a}$	$0.10±0.00^{a}$	$0.06±0.00^{b}$	$36.58±0.84^{a}$	$68.67±0.17^{c}$	$-2.38±0.03^{bc}$	$17.27±0.04^{c}$
CP-BT	$0.22±0.01^{c}$	$0.27±0.02^{b}$	$0.05±0.00^{b}$	$0.00±0.01^{d}$	$52.94±0.49^{c}$	$73.57±1.18^{b}$	$-2.45±0.17^{ab}$	$19.15±0.52^{b}$
CP-LA	$0.30±0.01^{a}$	$0.35±0.02^{a}$	$0.07±0.01^{b}$	$0.02±0.00^{c}$	$42.77±0.25^{b}$	$75.07±1.27^{a}$	$-2.51±0.02^{a}$	$20.86±0.73^{a}$

Lowercase letters within a column with different superscripts are significantly different ($p \leq 0.05$).

Table 15 The syneresis and color value of white sesame yogurts fermented by different mixed cultures at 8 h of fermentation.

Treatment	Syneresis (%)	Color		
		L*	a*	b*
SS-YC	74.99±0.28^{b}	73.81±0.02^{b}	-2.33±0.01^{c}	17.10±0.20^{c}
SS-BY	67.37±0.92^{a}	68.67±0.17^{c}	-2.38±0.03^{bc}	17.27±0.04^{c}
SS-BT	66.67±1.63^{a}	73.57±1.18^{b}	-2.45±0.17^{ab}	19.15±0.52^{b}
SS-LA	64.93±2.80^{a}	75.07±1.27^{a}	-2.51±0.02^{a}	20.86±0.73^{a}

Lowercase letters within a column with different superscripts are significantly different ($p \leq 0.05$).

4.3.2.5 Effect of yogurt bacteria and probiotics on antioxidant activity of plant-based yogurt

The yogurt from plant-based milks exhibited antioxidant capacities as measured by DPPH assay as shown in Figure 22. SB-BY and CP-BY showed the highest IC_{50} DPPH radical scavenging activity of each raw material. While, white sesame yogurt was SS-LA. The DPPH activities of SB-BY, SB-BT, and SB-LA were 9.03, 13.59, and 10.69 mg/mL and significantly higher than the control yogurt, SB-YC. The DPPH IC_{50} values of chickpea yogurt, CP-YC, CP-BY, CP-BT, and CP-LA were 23.20, 24.12, 29.65, and 28.17 mg/mL, respectively. CP-BY showed the highest IC_{50} DPPH compared with yogurt co-cultured with probiotics ($p \leq 0.05$). Chickpea yogurt extracts demonstrated significant antioxidant activity in the DPPH assay, with a calculated IC_{50} value of 315 ± 12 µg/mL. This indicates the concentration of chickpea yogurt extract required to scavenge 50% of DPPH radicals (Arnao and Hernández-Ruiz 2006; Chung et al. 2022). However, the antioxidant activity of soy milk yogurt improved notably with fermentation, particularly at 4 and 6 h, where the IC_{50} values were at their lowest for some strains. By 8 h, the IC_{50} values either stabilized or showed a slight increase (indicating reduced activity), the activity remained higher compared to the unfermented samples at 0 h. This enhancement in antioxidant activity is attributed to the breakdown of complex compounds into bioactive components, such as phenolics and flavonoids, facilitated by microbial activity. These bioactive compounds are highly effective in scavenging free radicals like DPPH. The peak activity were observed at 4–6 h likely reflects optimal enzymatic activity during fermentation. Fermentation of soy milk, which naturally contains high levels of isoflavones and phenolic compounds, tends to release more bioactive compounds, resulting in the most significant improvement. Additionally, different microbial strains exhibit varying enzymatic potentials, influencing the breakdown of macromolecules and the release of antioxidants. For instance, SB-YC, as a yogurt bacteria culture with broad enzymatic activity, demonstrated intermediate antioxidant activity. In contrast, the antioxidant activity of chickpea yogurt showed significant improvement, especially with CP-BY and CP-LA at 2 and 4 h. Between 6 and 8 h, the IC_{50} values remained relatively constant or slightly increased. Both BY and LA consistently exhibited superior activity during the mid-fermentation stage (4 h). The moderate improvement in chickpea yogurt may be attributed to its protein and polyphenol content, which become more bioavailable after fermentation. LA (likely *L. acidophillus*) is particularly effective, as it produces bioactive peptides and releases phenolic compounds. Prolonged fermentation, however, can lead to the degradation of antioxidant compounds or their consumption by microorganisms, resulting in a decline in antioxidant activity at later stages. In addition, the previous researches reported the combination use of probiotics with yogurt starters resulted in increased antioxidant activity (Sah et al. 2016) and the antioxidant activity of yogurt was increased during storage compared to day 0 (Kariyawasam et al. 2021).

On the contrary, plant-based yogurts showed antioxidant capacity as measured by the ABTS assay (Figure 23). SB-LA and SS-BY showed the highest IC_{50} ABTS scavenging activity of each raw material. However, CP-BY still showed the highest ($p \leq 0.05$) antioxidant activity via IC_{50} ABTS. The values are shown as follows; CP-YC, CP-BY, CP-BT, and CP-LA were 7.90, 3.04, 12.31, and 20.23 mg/mL, respectively. In the ABTS assay, chickpea yogurt extracts also showed potent antioxidant activity, with an IC_{50} value of 280 ± 10 µg/mL. This value represents the concentration of chickpea yogurt extract necessary to inhibit 50% of ABTS radicals (Fernández-Mar et al. 2012; Al-Sheraji et al. 2013; S. Chen et al. 2021).

Examination of the antioxidant properties of FRAP (Figure 24) revealed that all plant-based yogurt had antioxidant potential, especially for probiotic *L. acidophillus* fermented samples. The inhibition value was the best of the three raw materials (SB-LA, CP-LA and SS-LA). The FRAP values of SB-YC, SB-BY, SB-BT, and SB-LA were 2331.04, 1928.01, 2468.92, and 2577.86 FRAP µg$FeSO_4$equivalent/g dw, respectively. Yogurt containing probiotics had higher FRAP values compared to the control. The FRAP values in the sample initially decreased. At the end of the fermentation, the highest FRAP value was observed in SB-LA yogurt ($p \leq 0.05$). As for chickpea yogurt; CP-YC, CP-BY, CP-BT, and CP-LA were 1968.78, 2011.65, 1901.51, and 2288.92 FRAP µg standard equivalent/g dw, respectively. The best inhibition value of yogurt as CP-LA ($p \leq 0.05$). The FRAP assay revealed the ferric reducing antioxidant power of chickpea yogurt extracts. At a concentration of 500 µg/mL, chickpea yogurt exhibited a FRAP value of 0.75 ± 0.03 mM Fe(II)/g, indicating its ability to reduce ferric ions (Saleh et al. 2020; Ganeshan et al. 2024).

The results of the three antioxidant activity assays (DPPH, ABTS, and FRAP) showed that adding probiotic cultures in combination with yogurt cultures increased the antioxidant activity which the greatest activity was found in SB-LA yogurt. The results of this study agreed with previous studies. The combined use of probiotics with yogurt starters resulted in increased antioxidant activity (Sah et al. 2016) during storage compared to day 0 (Kariyawasam et al. 2021). Changes in DPPH, FIC (ferrous ion chelating), and FRAP radical scavenging activities were recorded in fermented soybean and almond milk and combinations using different starter cultures (*L. rhamnosus* ATCC 7469, *L. plantarum* ATCC 14917, *L. casei* ATCC 393, and *L. acidophilus* ATCC 4356) during 21 days of storage. All samples showed a higher percentage of scavenging activity than the corresponding control, and samples prepared in the same treatment without using the starting culture. Fermented almond milk by *L. casei*, *L. acidophilus*, and *L. plantarum* had higher scavenging activity than *L. rhamnosus* on the 1st day. The antioxidant activity in *Lycium barbarum* yogurt also increased during the first 2 weeks of storage and was significantly higher than plain yogurt (Shori et al. 2022b). The results indicate that chickpea yogurt was a rich source of antioxidants, as evidenced by its low IC_{50} values in DPPH and ABTS assays and significant FRAP value. These findings underscore the potential health benefits of consuming chickpea-based products due to their antioxidant properties (Werning et al. 2022).

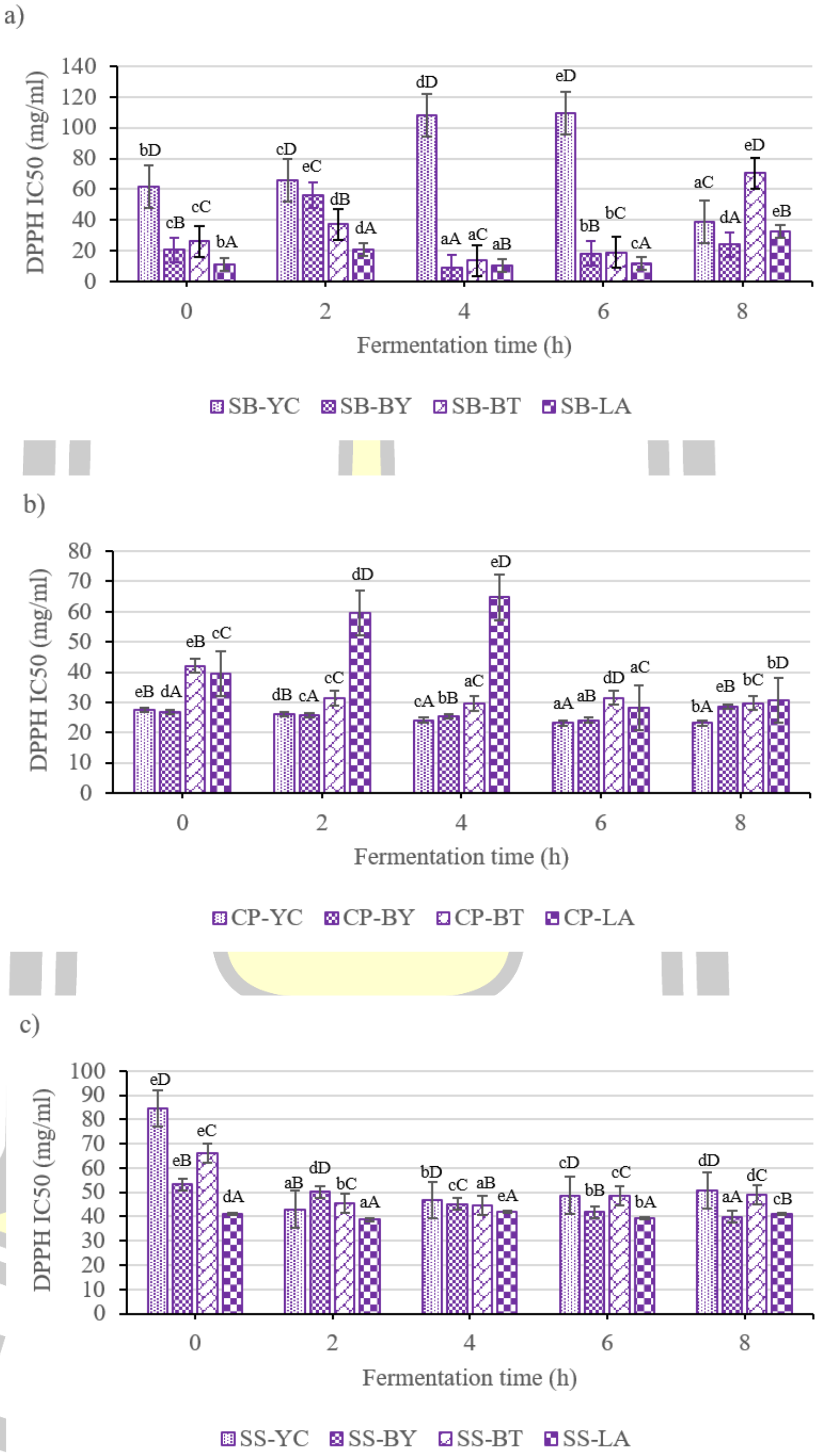


Figure 22 Antioxidant activity by DPPH measurement of plant-based milk yogurt. Soy milk yogurt (a), chickpea yogurt (b) and white sesame seed yogurt (c)
Capital letters comparing yogurt bacteria and probiotic types and lowercase letters comparing fermentation time with different superscripts are significantly different ($p \leq 0.05$).

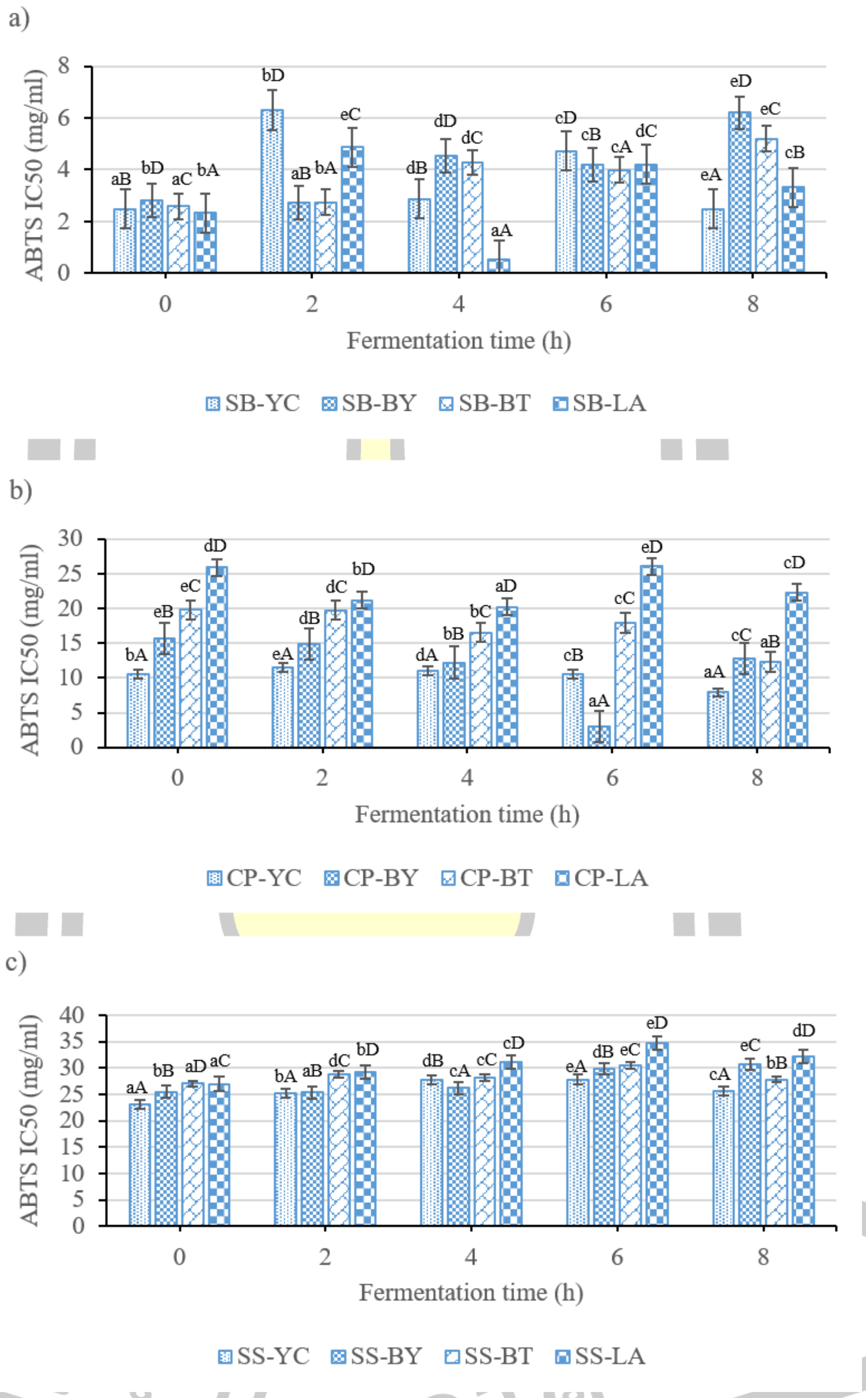


Figure 23 Antioxidant activity by ABTS measurement of plant-based milk yogurt. Soy milk yogurt (a), chickpea yogurt (b) and white sesame seed yogurt (c)
Capital letters comparing yogurt bacteria and probiotic types and lowercase letters comparing fermentation time with different superscripts are significantly different (*p* ≤ 0.05).

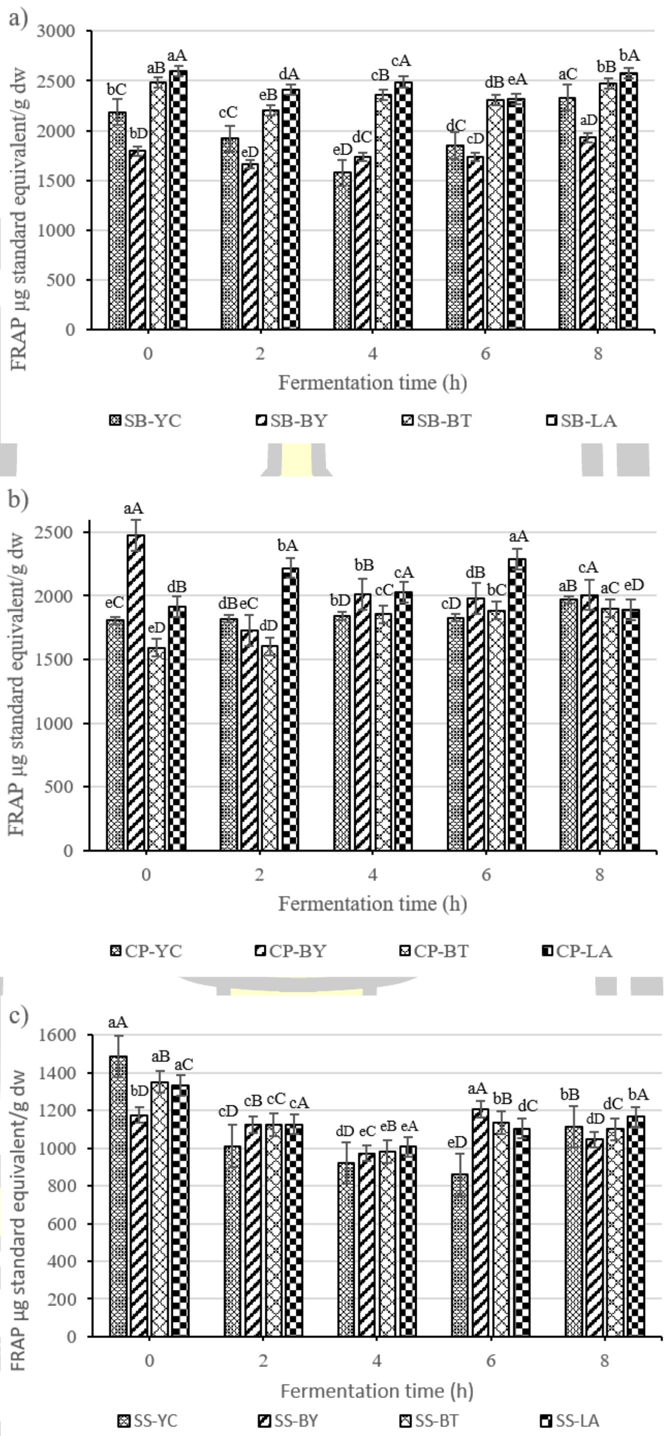


Figure 24 Antioxidant activity by FRAP assay of plant-based milk yogurt.
Soy milk yogurt (a), chickpea yogurt (b) and white sesame seed yogurt (c)
Capital letters comparing yogurt bacteria and probiotic types and lowercase letters comparing fermentation time with different superscripts are significantly different ($p \leq 0.05$).

4.3.2.6 Effect of yogurt bacteria and probiotics on FTIR of plant-based yogurt

FTIR analysis was conducted to assess the changes in chemical profile compositions of plant-based yogurts during fermentation. FTIR spectral data in the range 4,000–400 cm^{-1} collected from probiotic and control yogurt are shown in Figure 25. All soy milk yogurt samples had FTIR spectrum at 3200–2800, 1700–1600, and 1550–1249 cm^{-1}. Soybean flour as a raw material shows more than 30 peaks while the number of peaks decreased during yogurt fermentation and showed similar patterns of FTIR spectra because the yogurt bacteria and probiotic cultures applied in this study fermented soy milk similarly. The chickpea yogurt samples had FTIR spectrum at 3200–2800, 1700–1600, and 1550–1240 cm^{-1} as well. While, white sesame yogurt samples had FTIR spectrum at 3200–2800, 1700–1600, 1550–1249, and 1000–900 cm^{-1}. Indicates polysaccharide structures and lactose content, which is relevant for monitoring fermentation. The deconvolution of amine bands was constituted by at least four spectra at 3200–2800, 1650, 1538 and 1240 cm^{-1} corresponding to amine bands of the N–H (amino group) stretching vibrations (Wang et al. 2013). The peak at 1640 cm^{-1} was assigned to water (O–H) and amide I band (80% C=O stretch, 10% C–N stretch, and 10% N–H bending) and 1550 cm^{-1} (amide II band, 40% C–N stretching and 60%N–H bending vibration) (Papadopoulou et al. 2021). The primary locations of fatty acid peaks are approximately 2960, 2929, and 1740 cm^{-1} (Manolache et al. 2013). The asymmetric and symmetric CH_2 stretching modes were associated with the peaks located at 2928 and 2860 cm^{-1}, respectively (Patra et al. 2022). According to Greulich et al. (2024), protein is typically detected by the absorbance of amide I (1600–1700 cm^{-1}), which is caused by the amide's stretching of the C=O bond; amide II (1510–1570 cm^{-1}), which is caused by the N–H bonds' bending vibration; and amide III (1350–1200 cm^{-1}), which is caused by the combination of N–H and C–N stretching in the plane. A study's amide I, amide II, and amide III each had peaks in Figure 3 located at 1640, 1538, and 1240 cm^{-1}, respectively. The high sensitivity of the amide I band has been used to explain secondary protein structures but further deconvolution methods are required to separate the peaks (Zeng et al. 2014).

FTIR analysis plays a crucial role in evaluating the structural and functional transformations in plant-based yogurts, particularly during fermentation. It detects molecular changes in proteins, lipids, and carbohydrates, which impact yogurt texture, flavor, and health benefits. Peaks in the amide regions of FTIR spectra reflect protein breakdown, releasing bioactive peptides that enhance antioxidant activity. Similarly, the analysis identifies increased phenolic compounds, known for their free radical scavenging properties, released during microbial activity. Melatonin, synthesized from tryptophan through microbial pathways, is a potent antioxidant. Its content and bioavailability increase significantly during optimal fermentation, contributing to enhanced antioxidant properties of the yogurt. The specific microbial strains used and the fermentation conditions play a significant role in maximizing melatonin production and improving yogurt functionality. The combination of FTIR findings and melatonin content underscores the potential of plant-based yogurts as functional foods with health-promoting benefits, particularly in reducing oxidative stress (Joseph et al. 2024; Monteiro et al. 2024; Zhao et al. 2019).

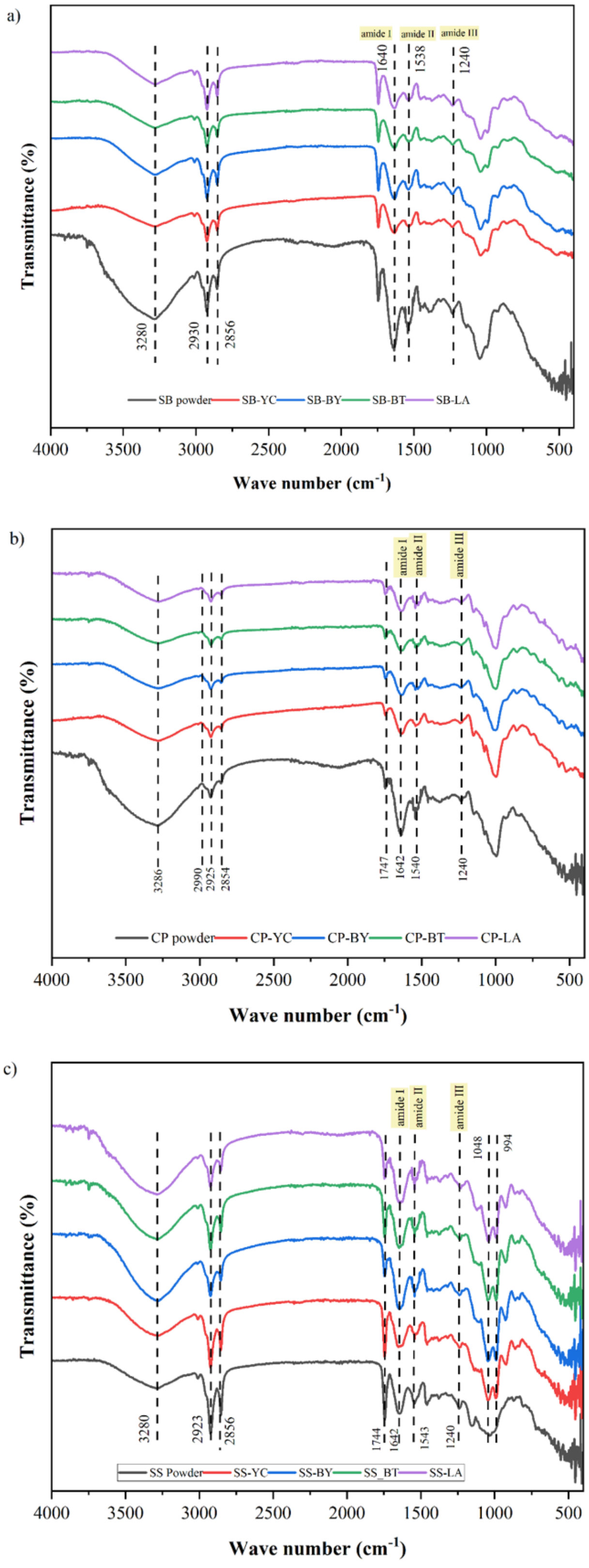


Figure 25 FTIR spectrum of plant-based yogurts at 8 h fermentation. Soy milk yogurt (a), chickpea yogurt (b) and white sesame seed yogurt (c)

4.3.2.7 Impacts of yogurt bacteria and probiotic combinations on melatonin, serotonin and tryptophan contents of plant-based yogurt

The results of the LC-MS/MS analysis of the soy milk and chickpea yogurt showed tryptophan content in all samples (Table 16 and 17, respectively). In addition, melatonin content was also found in SB-BY and SB-BT of the soy milk yogurt samples, whereas that of chickpea yogurt was found in the CP-BT and CP-LA samples. Although white sesame yogurt has a good antioxidant value but with the initial property of not causing curd form like other samples of plant-based yogurt. Therefore, melatonin content and its derivatives were not analyzed. However, it may be developed into drinking yogurt or adding stabilizers to develop other food products.

Melatonin, serotonin, and tryptophan content in soy milk yogurt are shown in Table 4.11. Yogurt fermented with different types of bacteria and probiotic cultures gave diverse melatonin content. Melatonin was detected in SB-BY yogurt at fermentation times of 4 and 8 h at levels of 12.83 and 21.20 ng/g, respectively while melatonin in SB-YC and SB-LA was detected after fermentation for 8 h (23.51 ng/g and 11.86 ng/g DW, respectively). The melatonin content found in SB-BY and SB-YC was not significantly different. These results indicated that soy milk yogurt fermented with yogurt bacteria and yogurt bacteria co-cultured with two types of probiotics gave higher melatonin production than probiotic cultures alone. The tryptophan content in soybean flour was 159.07 ng/g. The tryptophan content in SB-YC and SB-BY yogurt increased at 4 h (175.33 and 303.10 ng/g, respectively) and then decreased at 8 h of the fermentation process ($p \leq 0.05$), showing the opposite effect to SB-BT. The SB-LA tryptophan content increased with fermentation at 4 and 8 h (292.75 and 396.18 ng/g, respectively). Juhnevica-Radenkova et al. (2020) reported that the availability of tryptophan is an important factor in determining the concentration of indolic compound products i.e. melatonin. Yogurt bacteria and probiotics may use tryptophan to produce melatonin isomers during malolactic fermentation. Peuhkuri et al. (2012) provided information showing that tryptophan is an essential amino acid in the body that produces serotonin and melatonin. In this study, serotonin was undetectable in both raw materials and soy milk-based yogurt while Nontasan et al. (2022) reported that serotonin content in germinated soybean, germination under normal and salt stress conditions ranged between 37 and 57 ng/g. The melatonin and tryptophan levels found in soy milk yogurt fermented by YC mixed with *L. acidophilus* and *B. lactis* were higher than in soy milk yogurt fermented by SB-BT. Kocadağlı et al. (2014) explained that food samples such as bread and beer fermented with yeast contained relatively high levels of melatonin compared to other food samples. Low concentrations of melatonin were found in probiotic yogurt (0.9 ng/g) while yeast-fermented foods contain more melatonin than bacterial-fermented foods. Yogurt fermented with *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1 has also been evaluated for its sleep-promoting effects. Self-reported sleep quality improved in the yogurt group compared to the control group. The yogurt group also reported improved perceptions of general health and vitality (St-Onge et al. 2023). Moreover, pasteurized milk containing 3.90% soy milk powder, 4.50% mulberry leaf tea, and 89.40% raw milk developed by Sangsopha et al. (2019) contained melatonin (1.49 ng/mL) and free tryptophan (0.26 µg/mL).

The LC-MS/MS analysis of the melatonin and its derivative contents in chickpea yogurt are detailed in Table 4.12. Chickpea yogurts showed tryptophan content was 44.65–711.85 µg/g dw. The difference samples showed melatonin content 0.42 and 2.05 ng/g dw. This shows the sample of the CP-LA and CP-BT, respectively at 4 h of fermentation, whereas chickpea yogurt was found in the CP-BT (2.05 ng/g dw) and CP-LA (0.42–1.98 ng/g dw). The statistical analysis showed significant differences ($p \leq 0.05$) between the samples. The chickpea yogurt fermented by LAB co-culture with probiotics had a higher melatonin content than unfermented chickpea milk. The highest content of melatonin and tryptophan and the strongest antioxidant activity evaluated by DPPH, ABTS and FRAP assay was observed in probiotic yogurt (CP-BT, CP-LA, and CP-BY) inoculated with *L. acidophilus* and *B. lactis.*

Table 16 Melatonin, serotonoin, and tryptophan contents of soy milk yogurt under different fermentation time compared with unfermented soybean powder.

Treatment	Melatonin (ng/g dw)			Tryptophan (ng/g dw)			Serotonin		
	0	**4**	**8**	**0**	**4**	**8**	**0**	**4**	**8**
Soybean powder	17.67±5.15	(-)	(-)	159.07±0.45	(-)	(-)	ND	(-)	(-)
SB-YC	1.26±0.19^{b}	ND	23.51±0.02^{aA}	128.69±0.55^{A}	175.33±0.75^{B}	57.52±0.13^{C}	ND	ND	ND
SB-BY	ND	12.83±1.72	21.20±0.86^{AB}	27.66±0.98^{bB}	303.10±0.59^{aA}	76.73±0.94^{bC}	ND	ND	ND
SB-BT	ND	ND	ND	103.70±0.99^{aA}	32.76±0.81^{bC}	101.05±0.57^{aBC}	ND	ND	ND
SB-LA	ND	ND	11.86±0.10^{B}	41.49±0.14^{cB}	292.75±0.19^{bA}	396.18±0.11^{aA}	ND	ND	ND

Capital letters within the columns and lowercase letters within a row for each sample of melatonin and its derivative contents, with different superscripts are significantly different ($p \leq 0.05$), ND represents an amount that was not detected, and (-) represents an amount that was not determined.

Table 17 Melatonin, serotonoin, and tryptophan contents of chickpea milk yogurt under different fermentation time compared with unfermented chickpea powder.

Treatment	**Melatonin (ng/g dw)**			**Tryptophan (ng/g dw)**			**Serotonin**		
	0	**4**	**8**	**0**	**4**	**8**	**0**	**4**	**8**
Chickpea powder	ND	(-)	(-)	460.67±0.12	(-)	(-)	ND	(-)	(-)
CP-YC	ND	ND	ND	91.33±0.14^{bC}	268.29±0.72^{aB}	201.09±0.84^{abB}	ND	ND	ND
CP-BY	ND	ND	ND	147.07±0.36^{bBC}	87.26±0.29^{cC}	615.06±0.21^{aA}	ND	ND	ND
CP-BT	ND	2.05±1.47^{A}	ND	315.12±0.15^{aA}	87.11±0.31^{bC}	44.65±0.88^{bC}	ND	ND	ND
CP-LA	1.98±0.43^{a}	0.42±0.53^{bAB}	1.41±0.17^{a}	248.37±0.11^{bAB}	711.85±0.50^{aA}	103.92±0.19^{bBC}	ND	ND	ND

Capital letters within the columns and lowercase letters within a row for each sample of melatonin and its derivative contents, with different superscripts are significantly different ($p \leq 0.05$), ND represents an amount that was not detected, and (-) represents an amount that was not determined.

4.3.3 Impact of tryptophan addition on melatonin content and antioxidant activity of production of yogurt from cow milk and soy milk

The type of LAB and probiotic strains were chosen that can be produce important substances, the next step was studied the substrate addition, including tryptophan as 4 level (0.5, 1.0, 1.5 and 2.0% of milk) affect the production of melatonin and its derivatives and bioactive compound.

4.3.3.1 The pH and titratable acidity during fermentation of cow milk yogurts adding tryptophan

The addition of tryptophan to cow milk yogurt slightly affected pH and titratable acidity (TA) during fermentation (Figure 26). Both the control and yogurts supplemented with 0.5%, 1.0%, and 1.5% tryptophan exhibited a steady decline in pH from 6.06 to around 4.14 by the end of fermentation. However, yogurt supplemented with 2% tryptophan showed a higher final pH (4.97) and lower TA (0.57%) compared to the control yogurt (1.11%). These findings suggest that lower tryptophan concentrations may enhance acid production, while higher concentrations could inhibit it, though fermentation time remained largely unaffected.

The slight differences in pH and TA in the tryptophan-supplemented yogurts suggest that tryptophan could enhance the metabolic activity of lactic acid bacteria (LAB), potentially boosting acid production (Correia and Vale 2022). Tryptophan’s role in microbial metabolism, particularly in pathways related to amino acid breakdown, may explain this effect (Naibaho et al. 2022). These results align with other studies showing that amino acids can enhance fermentation. Additionally, the impact of tryptophan on yogurt flavor and texture requires further investigation, as it could influence proteolysis and release flavor-enhancing peptides.

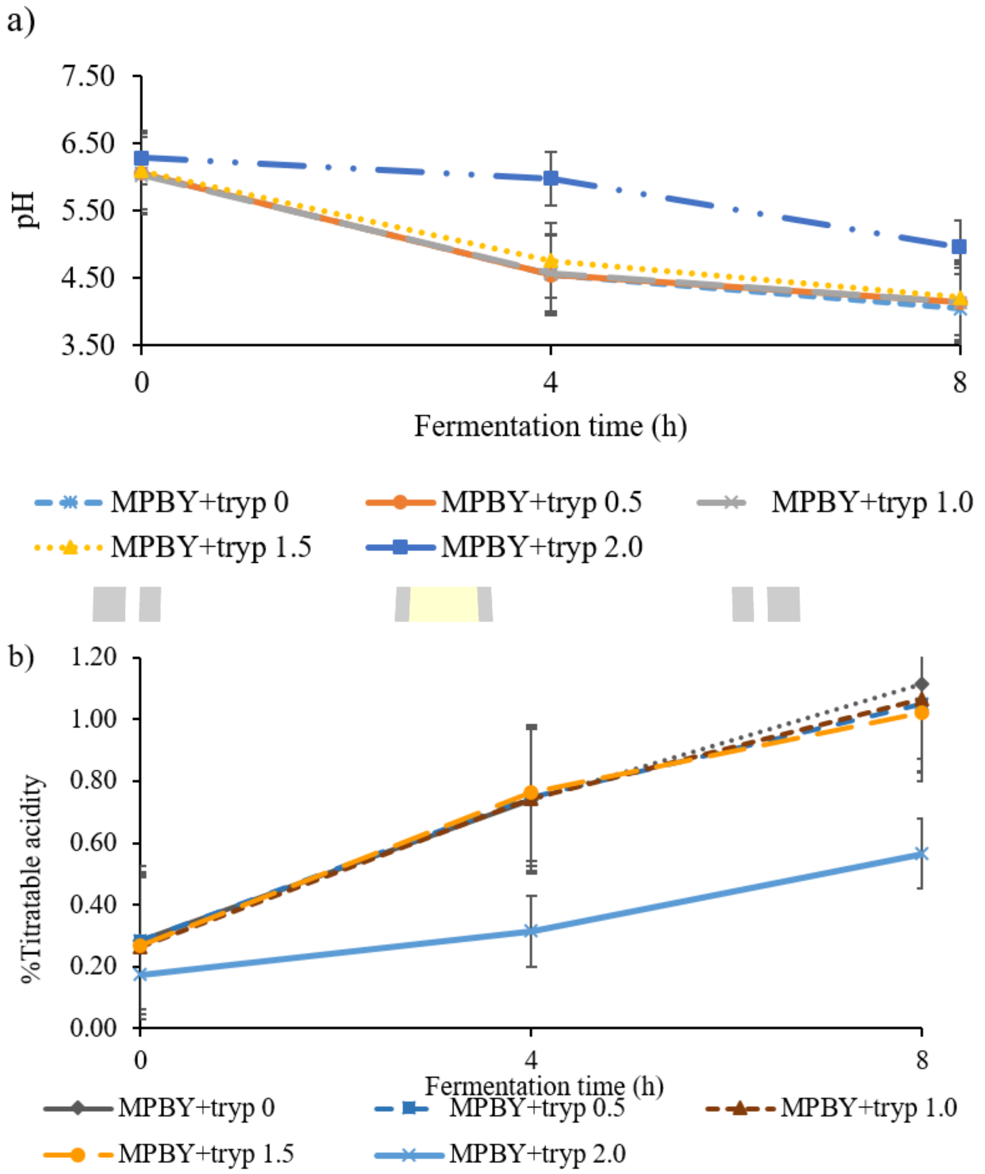


Figure 26 pH value (a) and titrated acidity (b) during fermentation of cow milk yogurts adding tryptophan.

4.3.3.2 Effect of yogurt bacteria and probiotic combinations and adding tryptophan on antioxidant activity

The antioxidant activities of cow milk yogurt were evaluated using DPPH, ABTS, and FRAP assays, and the effect of yogurt bacteria and probiotic combinations, along with the addition of tryptophan, was investigated (Figure 27). The DPPH IC_{50} values indicated that at 0 h, the antioxidant activity was highest in the control (MPBY+tryp 0%) and lowest in samples with higher tryptophan content, indicating that adding tryptophan initially reduced antioxidant capacity. While, at 4 h, the DPPH IC_{50} values increased, particularly in the MPBY+tryp 0.5% sample, reflecting lower antioxidant activity during early fermentation. The fermentation process may have caused temporary breakdown of antioxidants. However, at 8 h, the DPPH IC_{50} values decreased significantly across all samples, showing improved antioxidant activity. The sample with 2.0% tryptophan had the highest antioxidant capacity at this stage, suggesting that longer fermentation enhances antioxidant properties due to the production of bioactive compounds. In addition, MPBY+tryp 1.5% also provided constant DPPH IC_{50} values throughout the fermentation, indicating stable antioxidant efficacy. The antioxidant capacity as measured by the FRAP assay followed a similar trend, with the MPBY+tryp 1.5% sample demonstrating the highest antioxidant properties among all tested combinations. The higher ferric reducing ability observed suggests that tryptophan at this concentration enhances antioxidant potential. In addition, the ABTS radical scavenging activity was highest in the MPBY+tryp 1.5% sample, indicating that higher tryptophan concentrations may be more effective in scavenging ABTS. Thus, different tryptophan concentrations had different effects on the antioxidant properties of yogurt, with MPBY+tryp 1.5% showing the best performance in all assays, indicating that moderate tryptophan concentrations could enhance antioxidant bioavailability or stimulate antioxidant production during fermentation.

This is consistent with findings from other studies that highlight the role of tryptophan in enhancing antioxidant properties through its involvement in metabolic pathways that generate antioxidant compounds (Kim and Jeong 2016). Additionally, the presence of multiple probiotic strains (MP-BY) could synergize with tryptophan, further boosting the antioxidant properties by increasing the production of bioactive peptides and metabolites that antioxidants (Raynal-Ljutovac 2016). The involvement of probiotics is critical, as probiotics are known to produce bioactive compounds with antioxidant effects during fermentation. The combination of probiotics and tryptophan appears to enhance the antioxidant potential by promoting the generation of these compounds. Similar effects have been observed in other studies where the combination of amino acids and probiotics improved the health-promoting properties of fermented dairy products (Nagpal and Yadav 2017; Zhu 2018).

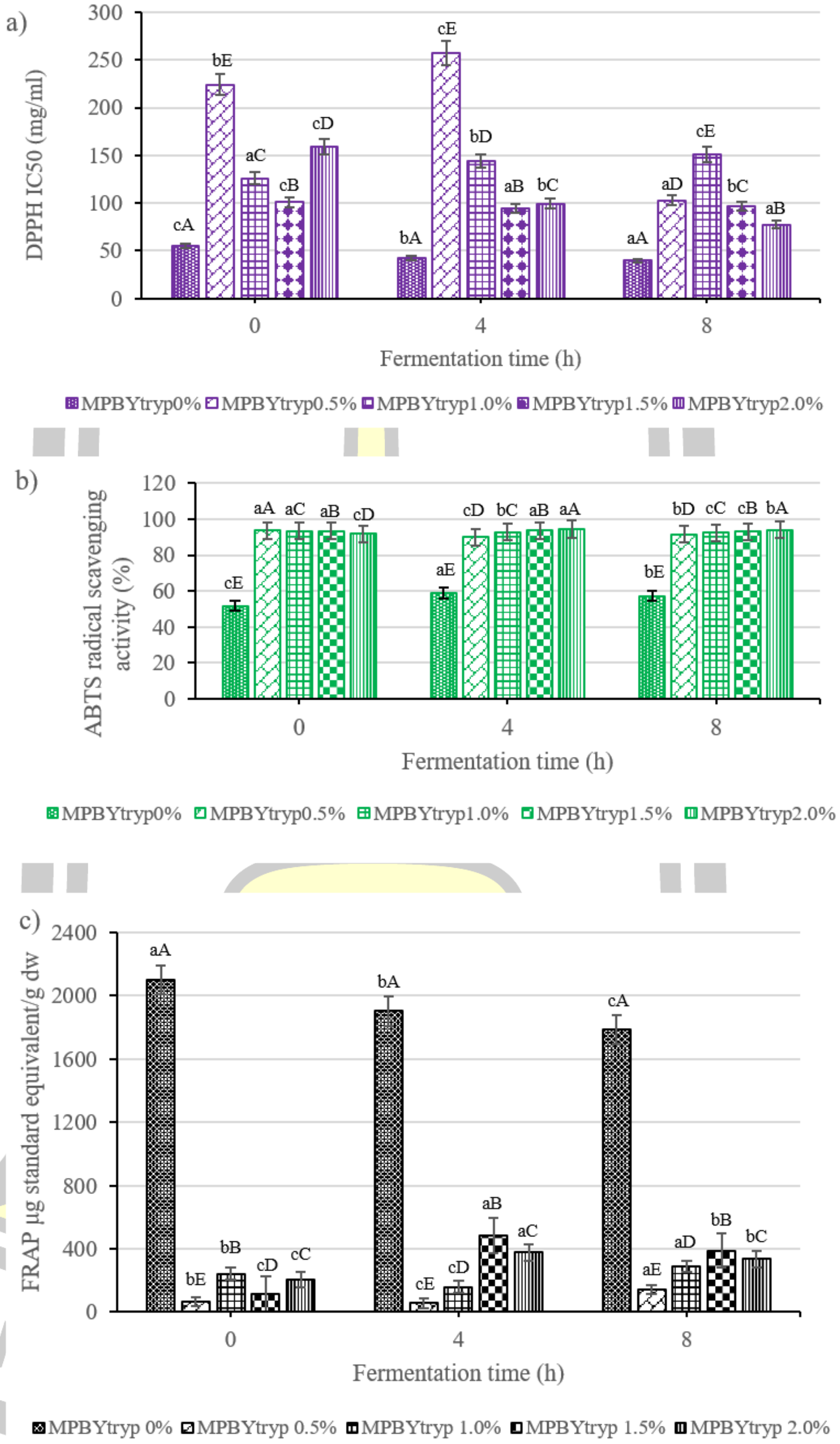


Figure 27 Antioxidant activity of extracted from cow milk yogurts adding tryptophan measured using DPPH IC$_{50}$ (a), ABTS redical scavenging (b) and FRAP assay (c). Capital letters comparing yogurt bacteria and probiotic types and lowercase letters comparing fermentation time with different superscripts are significantly different ($p \leq 0.05$).

4.3.3.3 Effect of yogurt bacteria and probiotic combinations and adding tryptophan on melatonin, serotonin and tryptophan contents

Changes in the concentration of melatonin and its derivatives, including serotonin and tryptophan in yogurts fermented with YC in combination with probiotics at various levels of tryptophan, are shown in Table 18. The results showed that tryptophan and serotonin content could be detected (28.57-305,497 and 8.00-11.47 ng/g dw, respectively) in yogurts with various levels of added tryptophan. Melatonin can be detected from the beginning of the fermentation process, and tryptophan (MPBY+1.5% tryptophan) was added at 1.5%. Furthermore, melatonin content became detectable after 8 h of fermentation at the 0.5% (MPBY+tryp 0.5%) and 2.0% (MPBY+tryp 2.0%) levels of added tryptophan, which could be attributed to the generation of melatonin amounts below the detection limit used in this study.

The addition of tryptophan increased the total tryptophan content in all supplemented yogurt samples. The highest tryptophan concentration was observed in the yogurt fortified with 2% tryptophan (MPBY+tryp 2.0%), followed by lower concentrations in the 0.5%, 1.0%, and 1.5% tryptophan-supplemented samples. Serotonin levels increased significantly in yogurt samples containing probiotics and tryptophan, with MPBY+tryp 1.0% showing the highest serotonin content. The combination of yogurt bacteria and probiotics likely enhanced the conversion of tryptophan into serotonin, aided by microbial activity during fermentation. The melatonin content also rose in tryptophan-supplemented yogurts, with the MPBY+tryp 1.5% sample displaying the highest melatonin levels. The increased production of melatonin suggests that the addition of tryptophan, combined with the activity of probiotics, supports the synthesis of melatonin during fermentation. Therefore, the addition of tryptophan, particularly in combination with probiotics, enhanced the production of serotonin and melatonin, with different tryptophan concentrations yielding varied effects on their levels.

The results suggest that tryptophan supplementation in yogurt, alongside the presence of probiotic strains, significantly influences the production of melatonin and serotonin. Tryptophan is a well-known precursor for serotonin and melatonin biosynthesis. The observed increase in serotonin and melatonin levels in tryptophan-added yogurt could be attributed to the enhanced availability of tryptophan for metabolic conversion during fermentation. The highest serotonin content in the MPBY+tryp 1.0% sample and melatonin in MPBY+tryp 1.5% suggests that certain tryptophan concentrations optimize this conversion. Previous studies confirm that the presence of specific probiotics can enhance these metabolic pathways, thereby increasing serotonin and melatonin production (Marco and Tachon 2013b). Probiotic strains such as *Lactobacillus* and *Bifidobacterium* have been shown to influence the production of bioactive compounds through their interaction with amino acids like tryptophan. These microbes may enhance the conversion of tryptophan to serotonin and melatonin, potentially by facilitating enzymatic reactions involved in the metabolic pathways. The yogurt containing multiple probiotic strains (MP-BY) likely benefits from this synergistic effect, which is supported by studies showing that probiotic combinations can improve the bioavailability and metabolic use of tryptophan (Hashikura et al. 2023). However, not all microbial strains are equally effective at converting tryptophan into melatonin or serotonin, leading to variations in their detectable levels. Tryptophan may serve alternative roles in microbial metabolism, such as being utilized for growth or converted into other by-products like indole derivatives, which can reduce melatonin yield. Additionally, melatonin and serotonin may interact with proteins or other components in milk powder or yogurt, becoming bound within the matrix and challenging their detection using standard analytical methods. During shorter fermentation periods (2–4 h), tryptophan levels may remain relatively high as microbes actively metabolize it, coinciding with increased synthesis of serotonin and melatonin. While, extended fermentation (6–8 h) can lead to the degradation or microbial consumption of these compounds, ultimately resulting in reduced detectable levels. This suggests that balancing fermentation duration is critical to optimize the retention and bioavailability of melatonin and serotonin in yogurt formulations. There may be other environmental factors that affect tryptophan conversion, including: exposure to oxygen and light, as melatonin and serotonin are sensitive to oxidation and UV light, leading to possible degradation during or after fermentation and storage conditions, as inappropriate storage temperature and post-fermentation oxygen exposure can further destabilize these biocomponents (Contreras-Correa et al. 2023; Pyo, Kwon, and Jung 2024).

Table 18 Melatonin, serotonoin, and tryptophan contents of cow milk yogurt adding tryptophan under different fermentation time compared with milk powder.

Treatment	**Melatonin (ng/g dw)**			**Tryptophan (ng/g dw)**			**Serotonin (ng/g dw)**		
	0	**4**	**8**	**0**	**4**	**8**	**0**	**4**	**8**
Milk powder	ND	(-)	(-)	5.00±0.94	(-)	(-)	ND	(-)	(-)
MPBY+tryp0	ND	ND	ND	551.70 ± 0.04^{aD}	28.57 ± 0.04^{cD}	188.74 ± 0.24^{b}	8.14 ± 0.00^{aC}	8.13 ± 0.00^{aE}	8.08 ± 0.00^{bD}
MPBY+tryp0.5	ND	ND	0.13 ± 0.03^{C}	17707.67 ± 0.31^{aC}	2110.71 ± 2.91^{bD}	2582.54 ± 0.14^{b}	8.31 ± 0.00^{bC}	8.35 ± 0.00^{aD}	8.22 ± 0.00^{cC}
MPBY+tryp1.0	ND	ND	ND	157318.49 ± 0.18^{aB}	150923.44 ± 1.66^{bC}	146944.44 ± 0.05^{c}	11.47 ± 0.27^{aA}	9.15 ± 0.03^{bB}	8.34 ± 0.00^{cB}
MPBY+tryp1.5	2.12 ± 0.13^{a}	0.51 ± 0.14^{b}	1.82 ± 0.33^{aB}	184435.71 ± 0.12^{A}	182292.37 ± 0.30^{B}	176544.46±0.24	10.67 ± 0.27^{aB}	9.73 ± 0.03^{bA}	10.75 ± 0.07^{aA}
MPBY+tryp2.0	ND	ND	2.33 ± 0.22^{A}	11912.17 ± 0.36^{CD}	305462.52 ± 0.16^{A}	305497.69±0.18	8.08 ± 0.00^{bC}	8.83 ± 0.06^{aC}	8.00 ± 0.00^{bE}

Capital letters within the columns and lowercase letters within a row for each sample of melatonin and its derivative contents, with different superscripts are significantly different ($p \leq 0.05$), ND represents an amount that was not detected, and (-) represents an amount that was not determined.

4.3.3.4 The pH and titratable acidity during fermentation of soy milk yogurt adding tryptophan

The effect of adding tryptophan on the pH (Figure 28a) and titratable acidity (Figure 28b) during the fermentation of soy milk yogurt was evaluated, with different tryptophan concentrations (0.5, 1.0, 1.5, and 2.0%) combined with LAB and probiotic co-cultures. The pH of soy milk yogurt were show a decrease in the fermentation time was increased. Soy milk yogurt added with 1.5% and 2.0% tryptophan took 8 h to reach pH values of 4.44 and 4.46, respectively. The slower drop in pH at higher tryptophan concentrations indicates that tryptophan may delay acid production during fermentation, likely due to its buffering effect or influence on microbial metabolism. The slower reduction in pH observed in soy milk yogurt with higher tryptophan concentrations (1.5% and 2.0%) suggests that tryptophan may slow down the acidification process. Tryptophan could act as a buffering agent, delaying the production or release of acids by LAB and probiotics. This buffering effect has been observed in other studies where amino acid supplementation in yogurt reduced the rate of pH decline, especially when used in combination with probiotics (Muniandy et al., 2017). The extended fermentation time needed to reach a final pH of 4.44 and 4.46, compared to LAB soy milk yogurt, may also be linked to the metabolic effects of tryptophan. Some studies have shown that tryptophan may influence microbial metabolism by providing an additional energy source or by altering the production of metabolic byproducts like organic acids (Salvucci 2016).

The titratable acidity increased over time, reflecting the accumulation of organic acids during fermentation. However, soy milk yogurt added with 1.5% and 2.0% tryptophan were showed lower TA compared to the LAB yogurt, indicating reduced acid production in the presence of tryptophan. Even though acidity increased with fermentation time, the presence of tryptophan in combination with probiotics reduced the rate of acidification compared to the LAB soy milk yogurt, suggesting that tryptophan influences the fermentation process by altering the acid production pathway. The lower titratable acidity in the tryptophan-added soy milk yogurts (1.5% and 2.0%) suggests that tryptophan might reduce the rate of acid production. This could be due to an inhibitory effect on certain LAB strains responsible for lactic acid production or a shift in microbial metabolism towards non-acidic byproducts. A similar effect has been observed in dairy yogurts where amino acids, including tryptophan, altered the fermentation profile by decreasing acid output (Damin 2009). The inclusion of probiotics in the fermentation process may also play a role in modulating acidity. Probiotics are known to have a slower fermentation process compared to traditional LAB strains, which could explain the reduced titratable acidity in the soy milk yogurt supplemented with probiotics and tryptophan. Additionally, the combination of LAB and probiotics may create a more balanced microbial ecosystem that limits excessive acid production, preserving the overall yogurt quality (Serrano et al. 2019).

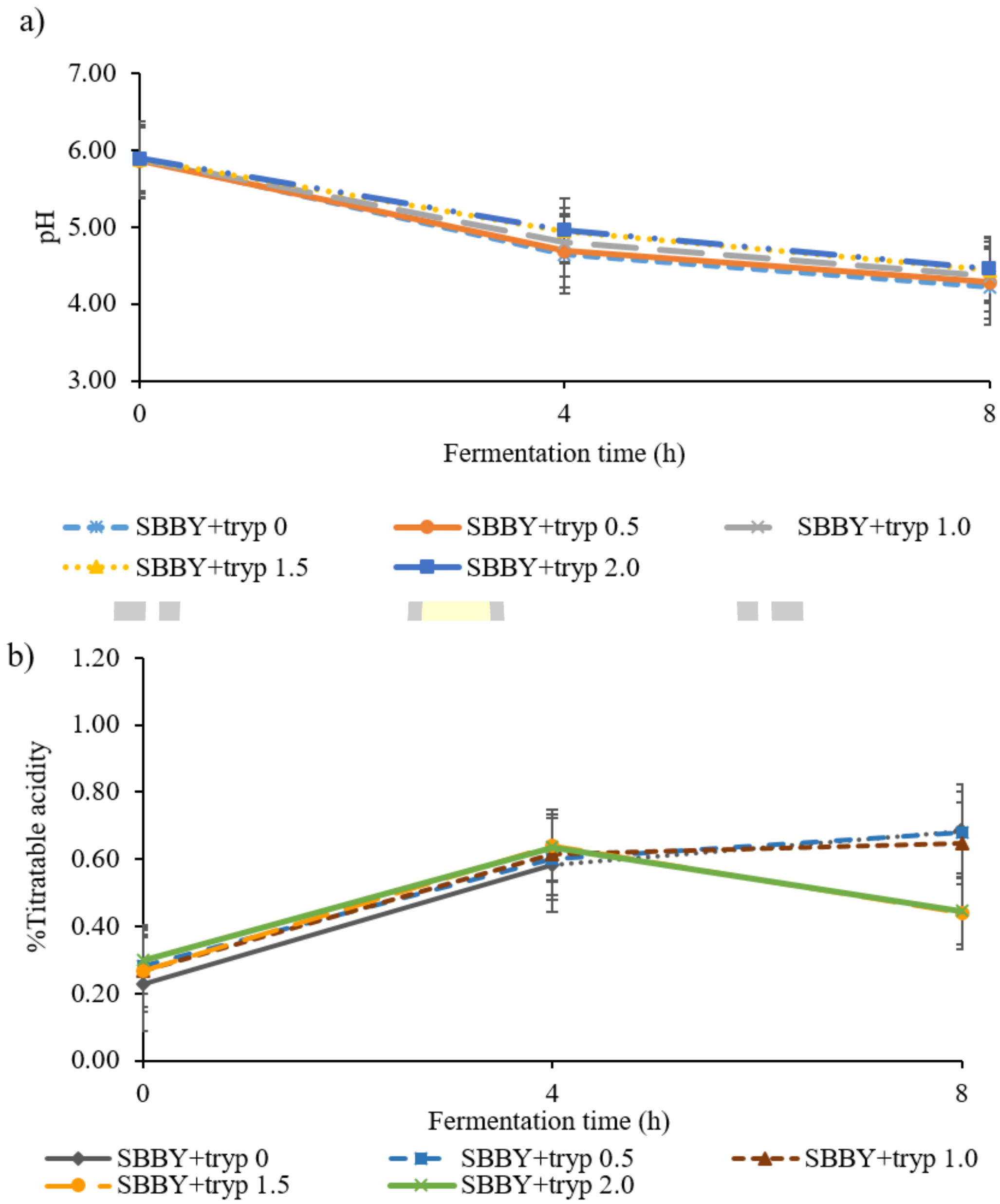


Figure 28 pH value (a) and titrated acidity (b) during fermentation of soy milk yogurts adding tryptophan.

4.3.3.5 Effect of yogurt bacteria and probiotic combinations and adding tryptophan on antioxidant activity of soy milk yogurt

The effect of adding tryptophan, along with combinations of LAB and probiotics, on the antioxidant activity of soy milk yogurt was measured using ABTS, DPPH, and FRAP assays (Figure 29). The highest ABTS radical scavenging activity was observed in soy milk yogurt added with 0.5% tryptophan in SBBY. The DPPH IC_{50} radical scavenging activity indicated that the SBBY+tryp 0.5% sample had the best antioxidant capacity in this assay, demonstrating lower IC_{50} values compared to other formulations. This indicates that 0.5% tryptophan supplementation provided stronger free radical scavenging. The FRAP assay showed that soy milk yogurt added with 2.0% tryptophan (SBBY+tryp 2.0%) exhibited the highest antioxidant properties, indicating the highest potential for reducing oxidative stress.

The results demonstrate that tryptophan addition, combined with LAB and probiotics, significantly enhances the antioxidant capacity of soy milk yogurt. The variation in antioxidant activity across the ABTS, DPPH, and FRAP assays suggests that different concentrations of tryptophan have differential effects on antioxidant mechanisms. The ABTS radical scavenging activity observed in SBBY+tryp 0.5% suggests that lower concentrations of tryptophan are more effective at neutralizing ABTS radicals. This could be due to the specific interactions between tryptophan and the radicals measured in the ABTS assay. Previous studies have shown that certain amino acids, including tryptophan, can enhance the scavenging of specific radicals by acting as electron donors or enhancing the production of antioxidant peptides during fermentation (Makri 2019). The SBBY+tryp 0.5% and SBBY+tryp 2.0% sample showed the best antioxidant capacity in both the DPPH IC_{50} and FRAP assay, respectively. This indicates that at higher concentrations, tryptophan may improve the overall radical scavenging ability and ferric reducing power of soy milk yogurt. Tryptophan, as a precursor to bioactive compounds like serotonin and melatonin, can participate in metabolic pathways that produce strong antioxidants, potentially explaining the enhanced DPPH and FRAP results. Additionally, the combination of LAB and probiotics may further enhance these effects, as probiotics can produce peptides and metabolites with antioxidant properties during fermentation (Nagai 2016).

The combination of *S. thermophilus*, *L. bulgaricus*, *B. lactis*, and *L. acidophilus* (SBBY) likely plays a crucial role in boosting antioxidant activity in soy milk yogurt. Probiotics are known to enhance the production of bioactive compounds with antioxidant properties, such as exopolysaccharides and short-chain fatty acids, during fermentation. The synergistic effect of probiotics and tryptophan may lead to increased production of these compounds, contributing to the observed improvements in antioxidant activity. Similar results have been observed in other studies where probiotics enhanced the bioavailability of amino acids, thereby improving the antioxidant potential of fermented products (Peng et al. 2020).

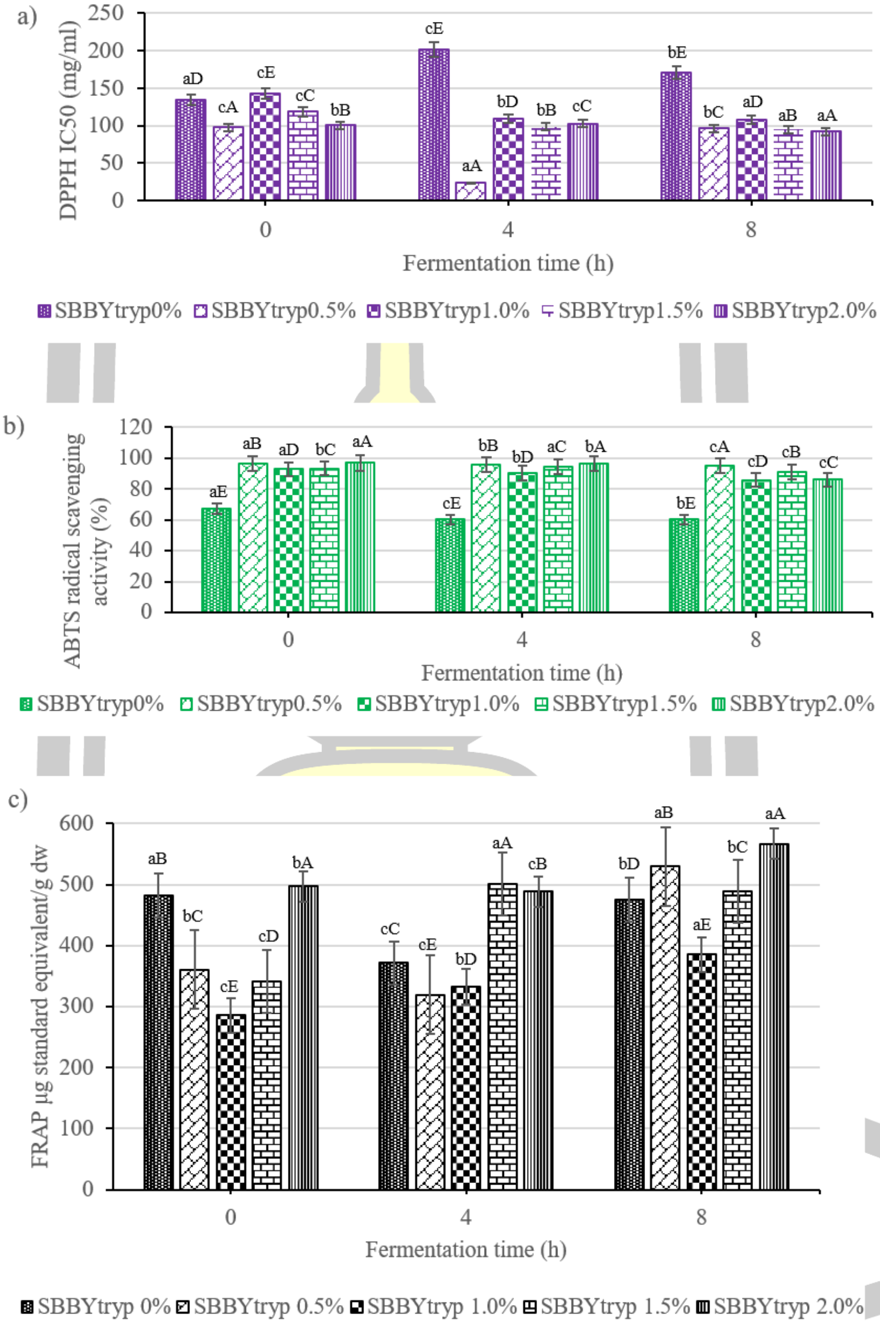


Figure 29 Antioxidant activity of extracted from soy milk yogurts adding tryptophan measured using DPPH IC_{50} (a), ABTS (b) and FRAP assays (c).

Capital letters comparing yogurt bacteria and probiotic types and lowercase letters comparing fermentation time with different superscripts are significantly different ($p \leq 0.05$).

4.3.3.6 Effect of yogurt bacteria and probiotic combinations and adding tryptophan on melatonin, serotonin and tryptophan contents of soy milk yogurt

Changes in the content of melatonin and its derivatives, including serotonin and tryptophan in soy milk yogurts fermented with YC in combination with two probiotics at various levels of tryptophan, are shown in Table 19. Tryptophan levels were ranged from 48.10 to 10303.21 ng/g dw in soy milk yogurts added with various tryptophan concentrations. The highest tryptophan content was observed in soy yogurt with 2.0% added tryptophan (SBBY+tryp 2.0%). Serotonin was detected in the range of 7.78 to 12.91 ng/g dw across all samples, with the highest levels seen in yogurts with 1.5% and 2.0% tryptophan (SBBY+tryp 1.5% and SBBY+tryp 2.0%). Melatonin was detected only in soy milk yogurt added with higher levels of tryptophan. At 1.5% tryptophan (SBBY+tryp 1.5%), melatonin was detected at 4 and 8 h of fermentation. In the 2.0% tryptophan yogurt (SBBY+tryp 2.0%), melatonin was measured from the start of fermentation up to 4 h, indicating earlier melatonin production at higher tryptophan levels.

The results highlight the influence of tryptophan addition on the production of bioactive compounds, including serotonin and melatonin, during the fermentation of soy milk yogurt with LAB and probiotics. The production of these compounds is dose-dependent, with higher tryptophan concentrations leading to increased serotonin and earlier melatonin production. The increase in tryptophan content with higher supplementation levels was expected, as tryptophan was directly added to the soy milk yogurt. The range of 48.10 to 10303.21 ng/g dw confirms that the supplementation was effective, and the higher concentrations (particularly at 2.0%) likely provide sufficient substrate for the biosynthesis of bioactive compounds such as serotonin and melatonin. This is consistent with studies showing that exogenous tryptophan supplementation in fermented products can boost the availability of this essential amino acid, which is a precursor for several metabolic pathways (Marco and Tachon 2013a).

The detection of serotonin in all samples, with concentrations ranging from 7.78 to 12.91 ng/g dw, suggests that the fermentation process, aided by probiotics, effectively converts tryptophan into serotonin. The higher serotonin content in SBBY+tryp 1.5% and SBBY+tryp 2.0% yogurts indicates that greater tryptophan availability enhances serotonin biosynthesis. This supports findings from other research, which indicates that probiotics, particularly *L. acidophilus* and *B. lactis*, can boost the production of serotonin by promoting the conversion of tryptophan during fermentation (Desbonnet et al. 2008). Melatonin production was detected only in yogurts supplemented with higher tryptophan levels (1.5% and 2.0%), and its timing depended on the concentration of added tryptophan. In SBBY+tryp 1.5%, melatonin was detected only after 4 h of fermentation, suggesting that the melatonin biosynthesis pathway requires a threshold level of tryptophan or an extended fermentation period. In contrast, in SBBY+tryp 2.0%, melatonin was produced much earlier, appearing from the start of fermentation up to 4 h, likely due to the higher tryptophan availability. This early melatonin production suggests that higher tryptophan levels accelerate the metabolic pathways responsible for melatonin synthesis. This observation aligns with previous studies that shown a direct relationship between tryptophan supplementation and melatonin production in fermented foods (Martín-Cabrejas et al. 2017).

During fermentation, the levels of tryptophan, melatonin, and serotonin in soy milk yogurt change due to microbial activity and metabolic processes. Tryptophan is actively consumed by probiotics for conversion into melatonin and serotonin or diverted into other metabolic pathways, such as indole derivatives or kynurenine. Additionally, melatonin and serotonin may bind to soy proteins or other matrix components, reducing their detectability. Extended fermentation times (beyond 8 h) often lead to degradation of these compounds due to oxidative stress or enzymatic activity. Soybean powder, used as a reference, contains high tryptophan levels but lacks microbial conversion processes, resulting in steady tryptophan levels without melatonin or serotonin synthesis. Optimizing fermentation time (4–6 h) and using specific probiotics can enhance the production and stability of bioactive compounds in soy milk yogurt (L. Wang et al. 2024; Xiao et al. 2023). Sejbuk, Siebieszuk, and Witkowska (2024) highlight the role of dietary components such as tryptophan in the production of sleep-regulating metabolites like melatonin and serotonin. It emphasizes the role of plant-based foods in enhancing these metabolites due to their rich antioxidant and polyphenol content, which supports stability during storage. The findings suggest that plant-based matrices, such as soy, better facilitate melatonin production compared to animal-based systems like cow milk. Arslan et al. (2024) have studied connects plant-based nutrition with higher levels of tryptophan and its derivatives, showcasing the superior efficiency of soy-based systems in producing and stabilizing melatonin and serotonin during storage. The antioxidants in soy-based matrices are key contributors to maintaining bioactivity.

Table 19 Melatonin, serotonoin, and tryptophan contents of soy milk yogurt adding tryptophan under different fermentation time compared with soybean powder.

Treatment	**Melatonin (ng/g dw)**			**Tryptophan (ng/g dw)**			**Serotonin (ng/g dw)**		
	0	**4**	**8**	**0**	**4**	**8**	**0**	**4**	**8**
Soybean powder	17.67±5.15	(-)	(-)	159.07±0.45	(-)	(-)	ND	(-)	(-)
SBBY+tryp0	0.52±0.25	ND	ND	99.35±0.17^{aE}	63.83±0.11^{abE}	48.10±0.02^{bE}	8.48±0.00^{aC}	7.90±0.00^{cD}	8.21±0.00^{bA}
SBBY+tryp0.5	ND	ND	ND	2882.80±0.40^{aD}	2289.63±1.21^{bD}	1961.79±0.43^{cD}	8.17±0.00^{aC}	8.15±0.00^{aB}	7.85±0.00^{bC}
SBBY+tryp1.0	ND	ND	ND	6978.84±0.82^{aB}	3381.37±1.36^{bC}	2992.71±1.14^{cC}	8.28±0.00^{bC}	8.30±0.00^{aA}	7.81±0.00^{cCD}
SBBY+tryp1.5	ND	0.52±0.29	0.48±0.21	3152.87±0.38^{cC}	4472.74±0.39^{bB}	7618.62±0.76^{aA}	12.91±0.40^{aA}	7.92±0.00^{bC}	8.12±0.00^{bB}
SBBY+tryp2.0	6.38±0.08	0.52±0.22	ND	10303.21±1.78^{aA}	5855.65±1.24^{bA}	5100.58±0.27^{cB}	9.20±0.13^{aB}	7.82±0.00^{bE}	7.78±0.00^{bD}

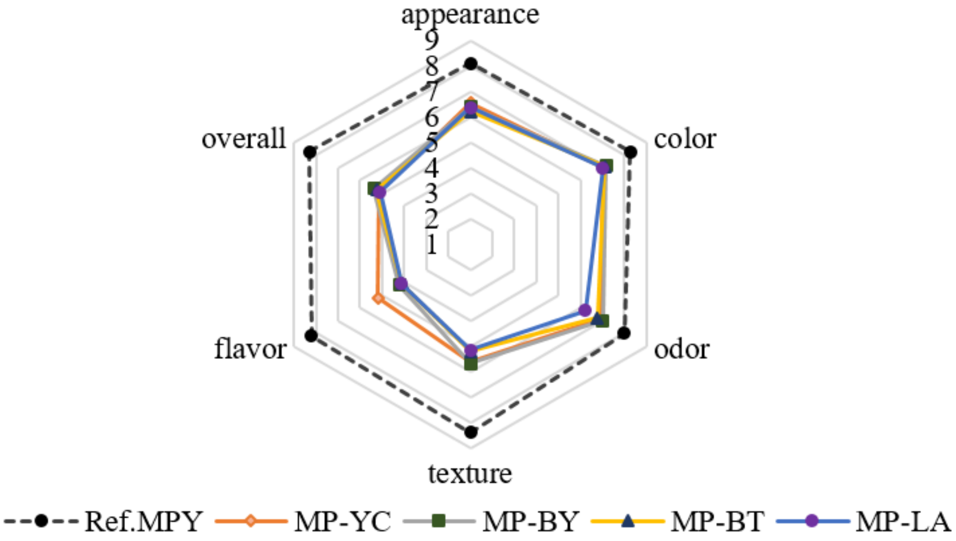
Capital letters within the columns and lowercase letters within a row for each sample of melatonin and its derivative contents, with different superscripts are significantly different ($p \leq 0.05$), ND represents an amount that was not detected, and (-) represents an amount that was not determined.

4.3.3.7 The sensory evaluation of cow milk and soy milk yogurts

Sensory evaluation of the cow milk and soy milk yogurt samples was evaluated by 50 volunteers aged 20-30 years, with results shown in Figure 30. The sensory evaluation highlights significant differences between the commercial sample (Ref. MPY) and the non-probiotic (MP-YC) and probiotic-supplemented yogurts (MP-BY, MP-BT, and MP-LA). The difference of probiotics had an impact on the sensory attributes of the yogurts. With a 5.38 overall score, MP-BY achieved rather well in terms of appearance (6.40), color (7.18), odor (7.00) and texture (5.69). The inclusion of probiotics may change the flavor and consistency of regular yogurt, since all probiotic samples had lower taste and texture scores, with an especially mild taste in every category. Although probiotic yogurt provides health benefits, its sensory attributes, especially taste and texture, need to be improved to satisfy consumers who prefer regular yogurt.

The SB-YC, SB-BT, and SB-BY samples had higher values in appearance, color, texture, flavor, and acceptability than SB-LA. The appearance and color characteristics of SB-YC, SB-BY, and SB-BT were not significantly different ($p > 0.05$) from commercial yogurt. For overall acceptability, SB-YC, SB-BY, SB-BT, and SB-LA were also not significantly different ($p > 0.05$). The panelists rated the preference of SB-LA the lowest compared with the other groups of probiotics, possibly due to the dark color and low consistency with the brightness (L*) and consistency values analysis. Sensory evaluation of soy milk yogurt fermented by yogurt bacteria co-culture with probiotics (SB-BT) revealed the highest scores in yogurt fermented by *S. thermophilus* co-cultured with *L. acidophilus* and *B. lactis*. Kamarinou et al. (2023) reported that the expression of various flavors in fermented foods depended on different probiotic strains, with *L. rhamnosus* SP3 showing fruity flavors in cheese. The type of bacteria used in yogurt fermentation affects public sensory acceptance.

a)



b)

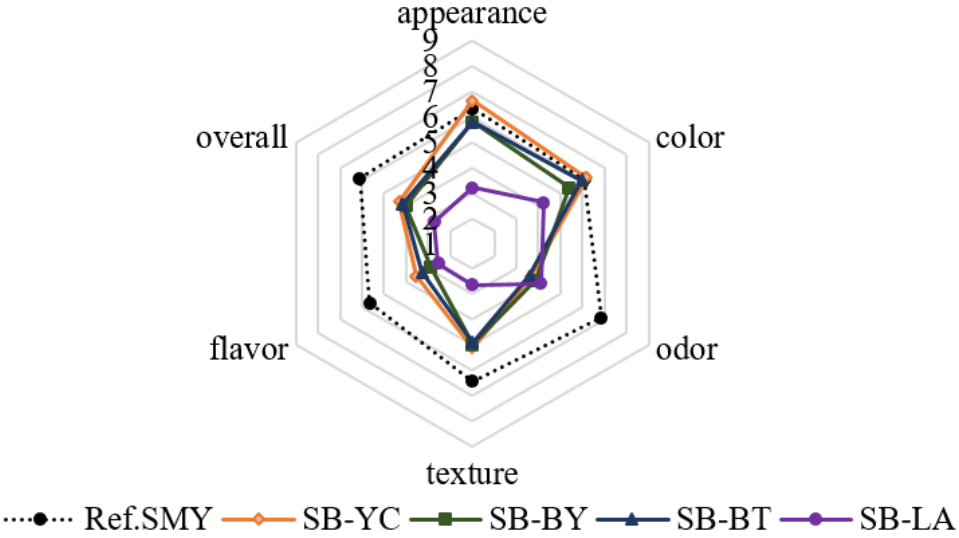


Figure 30 Sensory evaluation of cow milk (a) and soy milk (b) yogurts fermented with different LAB and probiotics.

The sensory evaluation of cow milk yogurt and soy milk yogurt was conducted based on their fermentation with LAB and probiotics, tryptophan addition, along with the effects of antioxidant activity, and melatonin content. Specifically, MPBY+trp1.5% and SBBY+trp1.5% were selected for sensory analysis. The results revealed that the addition of tryptophan influenced the yogurt taste, imparting a salty flavor, and the fermented yogurt exhibited a bitter aftertaste (data not shown). To address these issues, yogurt formulations without tryptophan addition were preferred. Furthermore, the textural properties of soy milk yogurt were significantly enhanced by preparing the soy milk through filtration before mixing it with yogurt cultures, followed by further incubation.

The results of the sensory evaluation (Figure 31) showed that cow milk yogurt was moderately liked by participants, with an appearance, color, and odor (7.14, 7.48, and 7.38, respectively). It scored for texture 1 (visual texture: the smoothness of the yogurt), texture 2 (taste-tested texture: the smoothness of the yogurt), flavor, and overall acceptance received 6.22, 6.16, 6.04, and 6.10, respectively indicating that while the product was well-received, there was still room for improvement, particularly in flavor. In contrast, soy milk yogurt received much lower scores. The overall acceptance score was 4.12, with appearance rated at 6.02 and color at 5.70. Odor (4.38), texture (4.04–5.24), and flavor (4.08) were rated unfavorably, indicating that participants were generally dissatisfied with the sensory qualities of the soy-based yogurt. These findings highlight that while cow milk yogurt remains popular due to its superior sensory properties, soy milk yogurt needs significant improvement, especially in terms of odor, flavor, and texture, to enhance consumer appeal and acceptance.

The sensory evaluation results demonstrated a clear distinction in consumer preference between cow milk yogurt and soy milk yogurt. Cow milk yogurt was moderately liked, with relatively high scores for appearance, color, and odor, indicating its sensory qualities are well-accepted. However, slightly lower ratings for flavor and texture suggest areas for improvement to enhance consumer satisfaction further. Soy milk yogurt, on the other hand, received significantly lower ratings across all sensory attributes, particularly for odor, flavor, and texture. These findings highlight common challenges associated with soy-based yogurt, such as its characteristic beany flavor and less creamy texture, which can deter consumer acceptance. The low scores in texture may also be attributed to differences in the gel network formation between soy and dairy proteins, where soy milk lacks the casein content essential for creating the smooth, creamy texture typical of cow milk yogurt. The unfavorable odor scores may stem from volatile compounds produced during soy milk fermentation. To improve the sensory appeal of soy milk yogurt, strategies such as enzymatic treatments to reduce beany flavors, the use of flavor enhancers, and textural modifications through hydrocolloids or protein fortification could be explored. Additionally, optimizing fermentation conditions and using specialized starter cultures may mitigate the offputting sensory attributes while enhancing overall acceptance. These improvements are essential to making soy milk yogurt a more competitive alternative to cow milk yogurt in the market, particularly for consumers seeking plant-based options. The findings underscore the importance of addressing sensory limitations to broaden consumer acceptance of soy milk yogurt while maintaining its nutritional and health benefits. Future studies focusing on tailored processing and formulation could significantly enhance its sensory qualities, aligning it more closely with consumer expectations (Fatima and Hekmat 2020; Gupta et al. 2022).

In the addition, Dhakal et al. (2023) have studied approaches to reduce the disadvantages of plant-based yogurts, such as soft structure, syneresis reaction, off-flavors and anti-nutritional components. The physical and sensory properties of plant-based yogurts are largely determined by the unit operation used and the type of plant-based milk used in the formulation. Soaking and blanching legumes, grains, and nuts before extraction with water produces yogurts that are firmer, stronger, and more viscous. Off-flavors can be reduced by roasting, pH adjustment, chemical deodorization, or enzyme treatment. The flavor can be improved by adding probiotic lactic acid bacteria and ultrasonication. Fermentation with lactic acid bacteria increases mineral absorption and reduces alpha-galactosidase. Otolowo et al. (2022) have studied the substitution of cow's milk with soy milk in yogurt production by evaluating the sensory qualities of the yogurt and identifying challenges of soy yogurt, including different tastes and textures, which may harm consumer perception. The majority of the sensory attributes assessed (range 8.00–8.01) were found in all samples (except in yogurt made from 100% soy milk). These studies show the beneficial effects of using soy milk as a substitute for cow's milk at all levels. Therefore, lactose intolerant and vegetarians can find alternatives to 100% soy yogurt and enjoy the health benefits of yogurt.

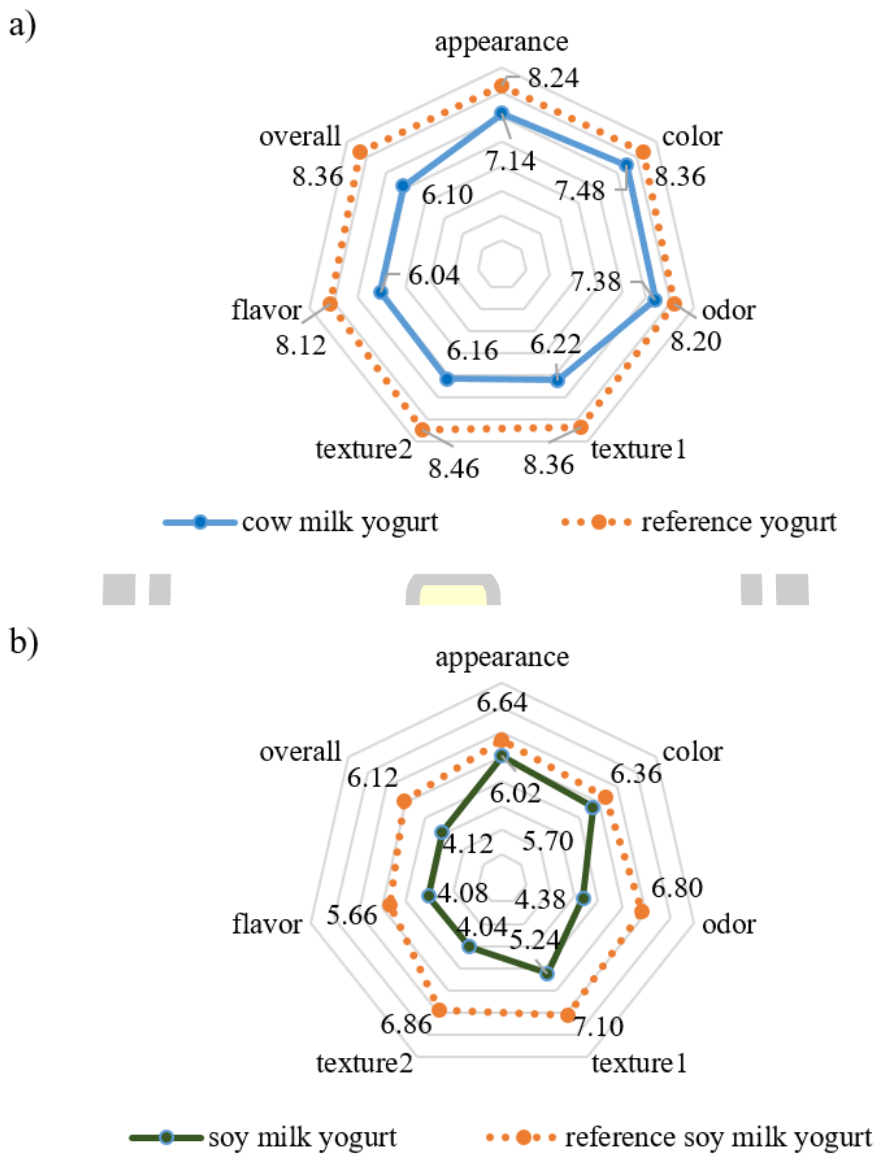


Figure 31 Sensory evaluation of cow milk (a) and soy milk (b) yogurts.

4.3.4 Effect of storage time on the physicochemical characteristics, melatonin content, antioxidant activity, and ACE inhibitory activity of yogurt

The 1.5% tryptophan level was chosen to study the shelf life of both types of yogurt. This is because the highest production of melatonin and its derivatives were produced in yogurt during the fermentation period. Although, in the case of soy milk yogurt, was second in antioxidant efficiency.

4.3.4.1 Change of pH and titratable acidity during yogurt storage

The study evaluated the changes in pH and TA during the storage of cow milk and soy milk yogurts over a period of time. Both cow milk and soy milk yogurts exhibited a decrease in pH on the 7th day of storage compared to the initial day. However, after this drop, the pH levels stabilized and remained relatively constant throughout the remaining storage period (Figure 32a and 33a). The TA of both yogurt types increased by the 7th day of storage compared to the initial day. After this rise, the TA levels remained stable and constant until the end of the storage period (Figure 32b and 33b).

The changes in pH and TA during storage are common in fermented dairy and plant-based products like cow and soy milk yogurts due to ongoing metabolic activity by LAB and probiotics. The decrease in pH during the first 7 days of storage was attributed to the continued fermentation process, where LAB and probiotics convert lactose (in cow milk yogurt) and sugars (in soy milk yogurt) into lactic acid. The relatively stable pH after this initial drop indicates that the microbial activity slows down significantly once a certain acidity level is reached (Granato et al. 2010). This is consistent with previous studies, which suggest that pH stabilization occurs once the bacteria reach a stationary phase in fermentation. The increase in TA on the 7th day of storage aligns with the rise in lactic acid production due to fermentation. After this initial increase, the stability in TA can be explained by the completion of sugar fermentation, meaning that further acid production is minimal (Mohd-Zaki et al. 2016). The constant TA during the later stages of storage suggests that the product has reached a stable state in terms of acidity, making it less prone to further biochemical changes. While both cow milk and soy milk yogurts exhibited similar trends in pH decrease and TA increase, the extent of these changes may differ due to the distinct compositions of the two types of milk. Cow milk contains lactose, while soy milk has different protein and carbohydrate sources, which can influence the fermentation process. Soy milk yogurt may also present a less pronounced acidity compared to cow milk yogurt due to its lower buffering capacity.

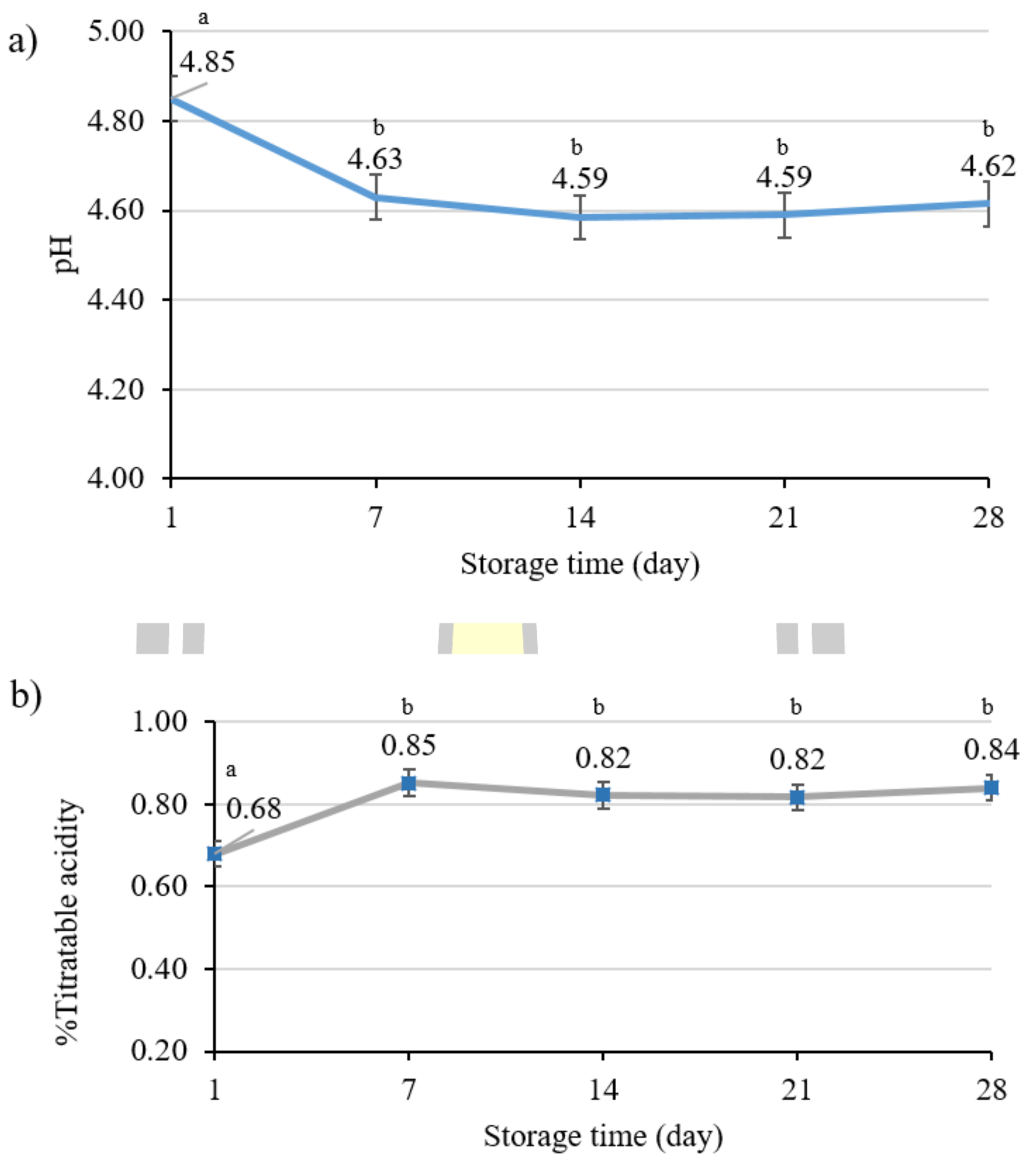


Figure 32 pH value (a) and titratable acidity (b) of cow milk yogurts during storage. Different lowercase letters indicate significantly different ($p \leq 0.05$).

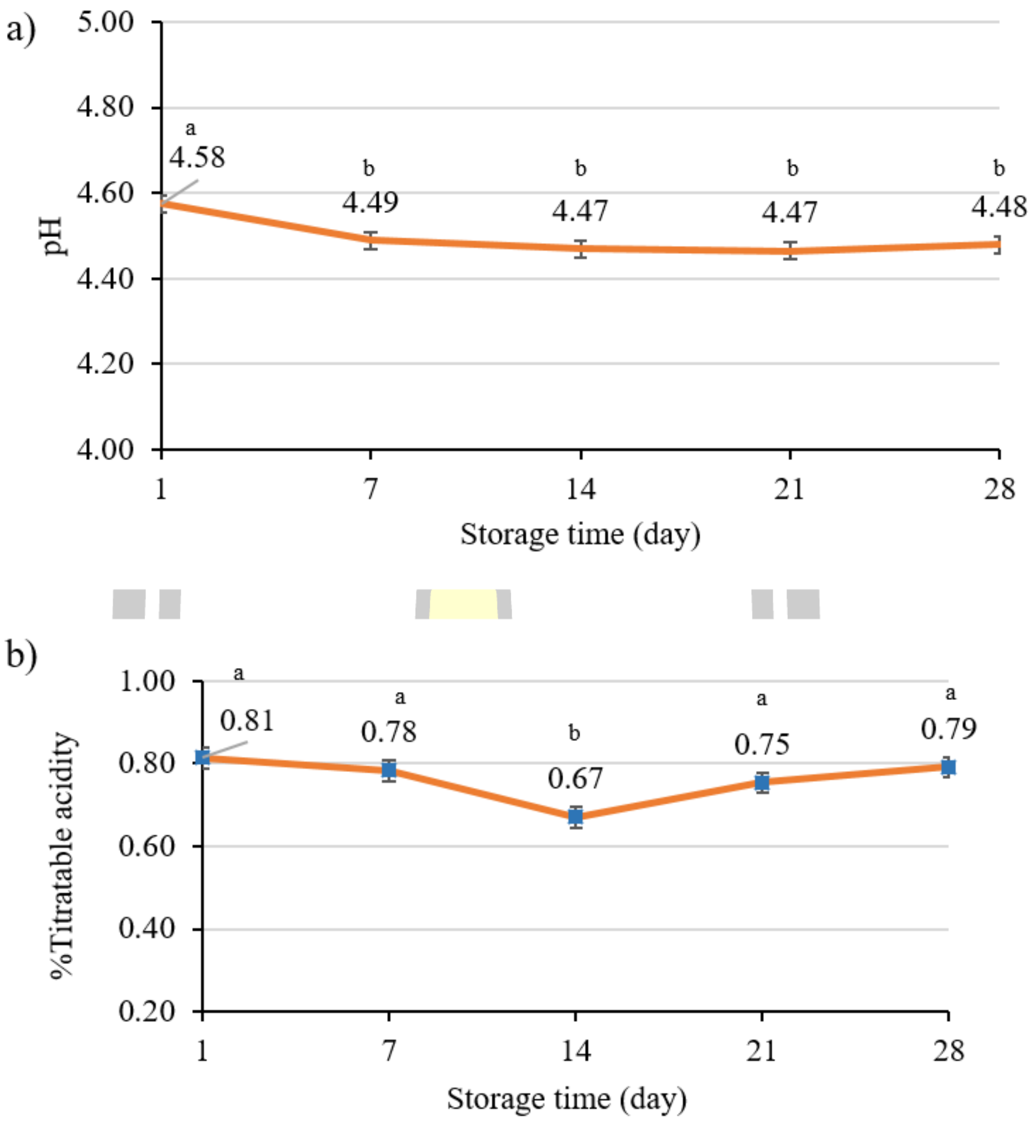


Figure 33 pH value (a) and titratable acidity (b) of soy milk yogurts during storage. Different lowercase letters indicate significantly different ($p \leq 0.05$).

4.3.4.2 Effect of yogurt storage on syneresis

The study evaluated the effect of storage duration on syneresis of both yogurt types over a period of 28 days. The results revealed a significant increase ($p \leq 0.05$) in syneresis in both yogurt types as storage time progressed (Table 25). The cow milk yogurt exhibited minimal syneresis on day 21. While, the soy milk yogurt on day 1 showed only minimal syneresis, indicating good structural integrity and water retention. However, from day 7 onwards, the water separation became more noticeable in sample, with a marked increase in syneresis by day 14. The most significant water separation was observed on the 21^{st} day of storage. Both types of yogurt showed a clear trend of increasing syneresis reaction with time, with significantly more water dissociated at day 21, indicating that yogurt with lower dissociated water content may have better structural stability in terms of syneresis reaction during long-term storage.

The increase in syneresis observed in both yogurt types during storage can be attributed to changes in the protein matrix and gel structure as time progresses. During storage, the protein network may become less capable of retaining water, leading to a breakdown of the gel and subsequent release of water (syneresis). Several studies have demonstrated that syneresis tends to increase with extended storage due to ongoing structural changes in the yogurt matrix, such as protein rearrangement or the relaxation of the gel network (Giannoglou et al. 2020). The significant difference in syneresis between the two yogurt types may be due to differences in their composition, such as fat content, protein concentration, and the presence of stabilizers. Yogurt which exhibited lower levels of syneresis, may contain stabilizers or higher protein content, which can improve water-holding capacity by creating a denser protein network (Fernández-García and Salazar 2021). The syneresis (whey separation) of cow milk yogurt and soy milk yogurt varies depending on the formulation, processing methods, and storage conditions. Typically, syneresis was measured as the percentage of whey separated from the total weight of the yogurt sample over a specified period. For cow's milk yogurt, standard syneresis ranges from 30–50%. Higher values suggest poor water-binding capacity, which may result from weak protein interactions or an unbalanced formulation. However, optimizing conditions and using stabilizers such as gelatin or pectin can lower syneresis to 20–30%. In comparison, soy milk yogurt generally exhibits higher syneresis, with standard values between 40–60%. This is due to its lower protein content, differences in protein structure, and a less robust gel network. Nonetheless, filtration of soy milk and the incorporation of stabilizers like carrageenan or guar gum can effectively reduce syneresis to 30–40% (Hashemi Gahruie et al. 2015). The inclusion of certain additives, such as hydrocolloids, has also been shown to reduce syneresis by strengthening the gel structure, thereby minimizing water separation over time (Mohammadi-Gouraji et al. 2021). The increase in syneresis over time highlights the importance of optimizing yogurt formulation and storage conditions to minimize water separation and maintain product quality. Extended storage, as observed in this study, can negatively affect yogurt texture and consumer perception. Therefore, manufacturers should consider formulation adjustments or the use of stabilizers to reduce syneresis during shelf life.

Table 20 Syneresis during storage of cow milk and soy milk yogurts.

Storege time (day)	**Syneresis (%)**	
	cow milk yogurts	**soy milk yogurts**
1	15.42±0.51^{a}	25 .14±0.95^{a}
7	15.08±0.57^{a}	27.52±1.21^{a}
14	15.78±1.00^{a}	28.39±1.31^{a}
21	18.19±1.53^{b}	31.67±0.72^{b}
28	16.09±1.13^{b}	30.64±2.54^{b}

Lowercase letters within a column with different superscripts are significantly different ($p \leq 0.05$).

4.3.4.3 Effect of yogurt storage on melatonin, serotonin and tryptophan contents

The concentration of melatonin and its derivatives, including serotonin and tryptophan in cow milk yogurt fermented with YC in combination with two probiotics at 1.5% tryptophan levels during storage, are shown in Table 26. The results showed that tryptophan content could be detected (190.06–369.48 µg/g dw) in yogurts with various days of storage. While, serotonin content was detected at 7th and 28th day of the storage (16.19 and 15.19 ng/g dw at, respectively). Melatonin was detectable at first day and 28th day of the storage (1.03 and 1.36 ng/g dw, respectively). Melatonin, serotonin, and tryptophan concentration in cow milk yogurt fermented with YC and two probiotics at 1.5% tryptophan levels showed significant variations ($p \leq 0.05$) throughout storage. The data indicate that while tryptophan was consistently present throughout the storage period, both serotonin and melatonin exhibited specific detection patterns at different storage intervals. The tryptophan content (ranging from 190.06 to 369.48 µg/g dw) was detectable across all storage days, suggesting that tryptophan, as an amino acid precursor to serotonin and melatonin, remains relatively stable in yogurt over time. This stability could be attributed to the inherent resilience of amino acids under common storage conditions, which are less susceptible to degradation compared to their derived bioactive compounds (Bortolini et al. 2021). Additionally, the presence of probiotics in the yogurt may help maintain tryptophan levels, as certain probiotic strains have been shown to enhance the retention of essential amino acids during fermentation and storage (Sharma et al. 2020). Serotonin, in contrast, was only detectable on the 7th and 28th days of storage, with concentrations of 16.19 and 15.19 ng/g dw, respectively. The detection of serotonin at these specific time points could indicate an initial synthesis of serotonin by probiotic strains during the early stages of storage, followed by a gradual decline, potentially due to oxidative degradation or enzymatic breakdown. The ability of probiotics to produce serotonin has been observed in previous studies, though the synthesis and stability of serotonin in dairy matrices can be affected by factors such as pH, temperature, and the presence of reactive oxygen species during storage (Ouwehand and Salminen 2019). Interestingly, melatonin was detectable on the first and 28th days of storage, with concentrations of 1.03 and 1.36 ng/g dw, respectively. The increase in melatonin by the 28th day could suggest that probiotic activity continues to influence melatonin biosynthesis late into the storage period. This trend might be due to prolonged fermentation or probiotic metabolism, which may continue to convert tryptophan into melatonin over time. The relatively small increase in melatonin concentration could also point to a stabilization effect of the yogurt matrix, preventing rapid degradation (Santos et al. 2020). Furthermore, melatonin's antioxidant properties may offer some protection against its own degradation during storage, which could explain its persistence in the yogurt even after 28 days. The findings demonstrate that the interaction between tryptophan and probiotics plays a key role in the biosynthesis and stability of serotonin and melatonin in yogurt during storage. The use of probiotics, in combination with tryptophan fortification, appears to support the sustained presence of these bioactive compounds, although the stability of serotonin seems more susceptible to storage-related factors. This insight is particularly important for developing functional yogurts aimed at delivering health-promoting bioactive compounds, as optimizing storage conditions and probiotic combinations could enhance the retention of serotonin and melatonin over time.

Changes in the concentration of melatonin and its derivatives, including serotonin and tryptophan in soy milk yogurts fermented with YC in combination with two probiotics at 1.5% tryptophan levels during storage, are shown in Table 27. The results showed that tryptophan and serotonin content could be detected (356.67–396.07 µg/g dw and 15.61–22.31 ng/g dw, respectively) in soy milk yogurts with various days of storage. Melatonin was detectable at 14th and 21st day of the storage (4.10 and 0.38 ng/g dw, respectively). The results revealed notable fluctuations in these bioactive compounds during storage. The data demonstrated that while tryptophan and serotonin were consistently detectable throughout the storage period, melatonin was only present on days 14 and 21, with a decline observed in its concentration toward the end of the storage period. Tryptophan levels (356.67–396.07 µg/g dw) remained relatively stable over time, suggesting that the soy milk yogurt matrix, in conjunction with the probiotic fermentation, supports the retention of tryptophan during storage. Tryptophan stability can be attributed to its role as an essential amino acid and its ability to withstand enzymatic and oxidative changes under typical storage conditions (Bortolini et al. 2021). Serotonin concentrations ranged between 15.61 and 22.31 ng/g dw across the storage period, indicating ongoing tryptophan metabolism by probiotics. The consistent detection of serotonin reflects the active conversion of tryptophan into serotonin during fermentation. However, slight variations in serotonin levels could be attributed to factors such as pH changes, temperature fluctuations, and oxygen exposure, which can affect serotonin stability in soy milk-based yogurts (Morsy et al. 2021). The sustained serotonin levels suggest that the probiotics used in this formulation are capable of maintaining serotonin production during storage, which is a valuable feature for functional foods aiming to deliver serotonin-related health benefits. Melatonin was only detectable on days 14 and 21, with concentrations of 4.10 ng/g dw and 0.38 ng/g dw, respectively. The initial increase in melatonin content by day 14 suggests that probiotic activity may have promoted melatonin biosynthesis from tryptophan during early to mid-storage. However, the significant decline in melatonin by day 21 indicates its susceptibility to degradation as storage time progresses. Melatonin, being sensitive to oxidative stress and light exposure, may undergo degradation if not adequately protected by packaging or storage conditions (Santos et al. 2020). Additionally, melatonin's rapid decline could be linked to the specific metabolic activities of the probiotic strains, which may cease melatonin production after a certain period or under specific environmental conditions. These findings highlight that the probiotic fermentation process in soy milk yogurt can effectively maintain tryptophan and serotonin concentrations over time, but melatonin stability is more challenging to preserve, especially during extended storage. Optimizing storage conditions, such as improving packaging to minimize oxygen exposure or adding natural antioxidants, may help reduce the degradation of melatonin and prolong its availability in fermented products (García-Burgos et al. 2020). The ability to retain bioactive compounds like serotonin and melatonin in soy milk yogurt presents potential for creating functional plant-based yogurts aimed at promoting mood and sleep-related health benefits. However, further improvements in formulation and storage techniques are necessary to enhance the retention of melatonin and its derivatives over longer periods.

Comparison of melatonin and its derivatives in cow milk and soy milk yogurts during storage. Initially, soy milk yogurts naturally contain higher levels of tryptophan and exhibit better conversion properties during fermentation, whereas melatonin levels of cow milk yogurts are limited by lower levels of tryptophan. The degradation during storage showed that both types of yogurts deteriorated over time, but soy milk yogurts retained more melatonin due to their high antioxidant matrix, which prevented oxidative degradation. Cow milk yogurts lost more melatonin during storage, partly because melatonin was bound to casein, reducing its absorption. In addition, melatonin derivatives of soy milk yogurts retained higher levels of derivatives, such as *N*-acetylserotonin, during storage, because the soy matrix allowed better enzyme conversion and stability. In cow milk yogurts, derivative formation was limited by matrix interactions and lower substrate availability. Furthermore, the main influencing factors of soy milk yogurts benefit from the high antioxidant content (isoflavones) and looser protein matrix, which stabilizes melatonin and derivatives. In general, the probiotic strains in soy milk yogurt are more efficient at converting tryptophan to melatonin and its derivatives. Thus, soy milk yogurt is more efficient than cow milk yogurt at retaining and maintaining melatonin during storage, making it a better health food option for delivering bioactive melatonin. Proper storage (refrigeration, protection from light and oxygen) is important for maintaining melatonin levels in both types (Fatima and Hekmat 2020; Yakum et al. 2022).

Table 21 Melatonin, serotonin, and tryptophan contents of cow milk yogurts during storage time.

Treatment	Melatonin (ng/g dw)	Tryptophan (µg/g dw)	Serotonin (ng/g dw)
Milk powder	ND	5.00 ± 0.94	ND
MPBY+tryp1.5 day 1	1.03 ± 0.07^{b}	190.06 ± 1.37^{e}	ND
MPBY+tryp1.5 day 7	ND	366.22 ± 0.09^{b}	16.19 ± 0.09^{a}
MPBY+tryp1.5 day 14	ND	369.48 ± 0.22^{a}	ND
MPBY+tryp1.5 day 21	ND	350.21 ± 0.01^{d}	ND
MPBY+tryp1.5 day 28	1.36 ± 0.04^{a}	351.61 ± 0.02^{c}	15.19 ± 0.08^{b}

Lowercase letters within the column with different superscripts are significantly different ($p \leq 0.05$) and ND represents an amount that was not detected.

Table 22 Melatonin, serotonoin, and tryptophan contents of soy milk yogurts during storage time.

Treatment	Melatonin (ng/g dw)	Tryptophan (µg/g dw)	Serotonin (ng/g dw)
Soybean powder	17.67 ± 5.15^{a}	159.07 ± 0.45^{d}	ND
SBBY+tryp1.5 day 1	ND	394.59 ± 7.00^{a}	16.01 ± 0.05^{b}
SBBY+tryp1.5 day 7	ND	396.07 ± 6.87^{a}	21.08 ± 0.45^{a}
SBBY+tryp1.5 day 14	4.10 ± 0.02^{b}	376.75 ± 6.90^{b}	16.23 ± 0.09^{b}
SBBY+tryp1.5 day 21	0.38 ± 0.01^{c}	375.98 ± 0.67^{b}	22.31 ± 0.44^{a}
SBBY+tryp1.5 day 28	ND	356.67 ± 0.83^{c}	15.61 ± 0.10^{b}

Lowercase letters within the column with different superscripts are significantly different ($p \leq 0.05$) and ND represents an amount that was not detected.

4.3.4.4 Effect of yogurt storage on antioxidant activity of yogurts

The antioxidant activity result of cow milk yogurt during storage (Figure 34) found that the DPPH IC_{50} antioxidant properties of yogurt was significantly ($p \leq 0.05$) improved after storage for 21st day. While, examination of the antioxidant properties of ABTS and FRAP value revealed that the antioxidant efficacy was the best at 28th day. The results of the antioxidant activity in cow milk yogurt during storage, as assessed by DPPH, ABTS, and FRAP assays, revealed significant improvements over time, particularly towards the later stages of storage. The DPPH assay showed a notable increase in antioxidant activity by the 21st day of storage, while ABTS and FRAP values peaked at 28 days, indicating enhanced antioxidant efficacy. In addition, the previous researches repoted the antioxidant activity of yogurt was increased during storage compared to day 0 (Kariyawasam et al. 2021).

The improvement in DPPH antioxidant activity by day 21 could be attributed to the continued metabolic activities of probiotic bacteria present in the yogurt. During fermentation and storage, these probiotics may produce bioactive compounds, including peptides, phenolics, and organic acids, which contribute to the yogurt antioxidant properties (Shori et al. 2022a). The release of peptides from milk proteins, especially those with antioxidant potential, could account for the observed increase in DPPH scavenging activity. These peptides act as free radical scavengers by donating hydrogen atoms or electrons to neutralize reactive oxygen species (ROS) and free radicals, thereby improving the yogurt's overall antioxidant capacity. The further enhancement of antioxidant properties, as indicated by ABTS and FRAP values on day 28, suggests that longer storage times allow for the accumulation of additional antioxidant compounds or the development of synergistic interactions among bioactive molecules. The ABTS assay, which measures the ability to neutralize cation radicals, and the FRAP assay, which evaluates the reduction of ferric ions to ferrous ions, both indicate that the antioxidant potential of yogurt continues to evolve over time. The fermentation process and subsequent storage likely promote the release of antioxidant compounds, such as vitamins, phenolic acids, and peptides, which contribute to this increased activity (Sah et al. 2016). Moreover, the role of probiotics in enhancing antioxidant activity should not be overlooked. Certain probiotic strains, including *Lactobacillus* and *Bifidobacterium*, have been shown to produce exopolysaccharides (EPS) with antioxidant properties, as well as influence the bioavailability of vitamins and other compounds that function as antioxidants (Kumar et al., 2015). These EPS and bioactive metabolites accumulate during fermentation and storage, further contributing to the observed improvements in ABTS and FRAP values by Day 28. The progressive increase in antioxidant activity across all assays suggests that storage, rather than diminishing the yogurt's nutritional value, may actually enhance its functional properties. However, it is important to consider that extended storage beyond 28 days could lead to declines in yogurt quality due to proteolysis or the degradation of bioactive compounds. Therefore, further studies are warranted to determine the optimal balance between maximizing antioxidant benefits and maintaining yogurt overall sensory and nutritional quality. These findings are particularly valuable for yogurt manufacturers aiming to market functional dairy products with enhanced health benefits. Understanding the dynamics of antioxidant activity during storage can help optimize shelf life and ensure that consumers receive products with peak bioactive potential.

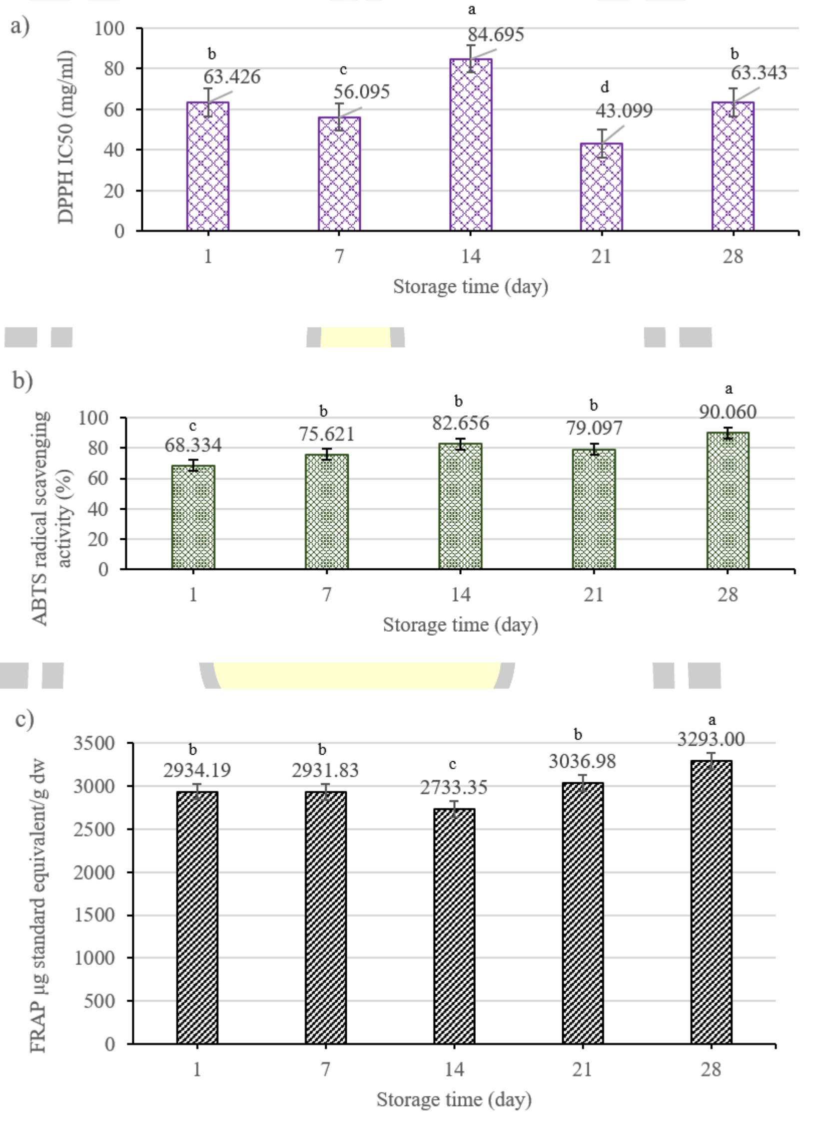


Figure 34 Antioxidant activity of extracted from cow milk yogurt during storage time measured using DPPH IC_{50} (a), ABTS radical scavenging (b) and FRAP assay (c). Different lowercase letters indicate significantly different ($p \leq 0.05$).

The antioxidant capacity of soy milk yogurt during storage (Figure 35), as measured by DPPH, ABTS, and FRAP assays, showed varying trends in activity over time, reflecting changes in the yogurt bioactive components. On the first day of storage, the DPPH IC50 value indicated the highest radical scavenging activity, while ABTS activity peaked on day 21, and FRAP showed the greatest antioxidant potential on day 7. The strong DPPH radical scavenging activity on day 1 suggests that the freshly prepared soy milk yogurt contained a high concentration of bioactive compounds, such as isoflavones, peptides, and phenolic acids, which are known for their antioxidant properties. Soy milk is rich in isoflavones, compounds that can act as free radical scavengers, contributing to the yogurt initial high DPPH antioxidant capacity (Bortolini et al. 2021). However, over time, the decline in DPPH activity could be due to the degradation or conversion of these compounds during storage, reducing their effectiveness in neutralizing free radicals.

The ABTS assay, which measures the ability to neutralize cation radicals, revealed peak activity on day 21, indicating that antioxidant compounds continued to evolve during storage. The increased ABTS activity at this stage could be attributed to the progressive breakdown of proteins and other macromolecules in the soy matrix, leading to the release of smaller peptides and phenolic compounds with improved radical scavenging properties. Probiotics used in the fermentation of soy milk yogurt may also enhance antioxidant activity by producing exopolysaccharides and metabolites with radical-scavenging abilities (Morsy et al. 2021). This highlights the dynamic nature of antioxidant activity in soy-based yogurt, where some bioactive compounds may increase in potency during storage due to ongoing fermentation processes.

Interestingly, the FRAP assay showed the highest antioxidant potential on day 7, which could be linked to the presence of reducing agents that can convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). This suggests that by the first week of storage, soy milk yogurt may contain optimal levels of compounds like peptides, reducing sugars, and other polyphenols that enhance its antioxidant potential (Sah et al. 2016). The subsequent decline in FRAP values after day 7 could indicate either the consumption or degradation of these reducing agents as storage progresses. The differences in the timing of peak antioxidant activity across the three assays underscore the complexity of the antioxidant mechanisms in soy milk yogurt.

Each method measures different aspects of antioxidant function that DPPH focuses on hydrogen-donating ability, ABTS assesses electron transfer capacity, and FRAP evaluates reducing power. The varying results over time suggest that different antioxidant compounds dominate at different stages of storage. For instance, early-stage antioxidants such as isoflavones may contribute to DPPH activity, while peptides and smaller metabolites released later may enhance ABTS and FRAP activities. These findings are significant for optimizing the functional benefits of soy milk yogurt as a source of antioxidants. Depending on the desired antioxidant properties, manufacturers may need to adjust fermentation conditions or storage times to maximize specific antioxidant benefits. Moreover, understanding the evolving nature of antioxidant activity in plant-based yogurt could help in designing products with enhanced health benefits that maintain their efficacy throughout their shelf life.

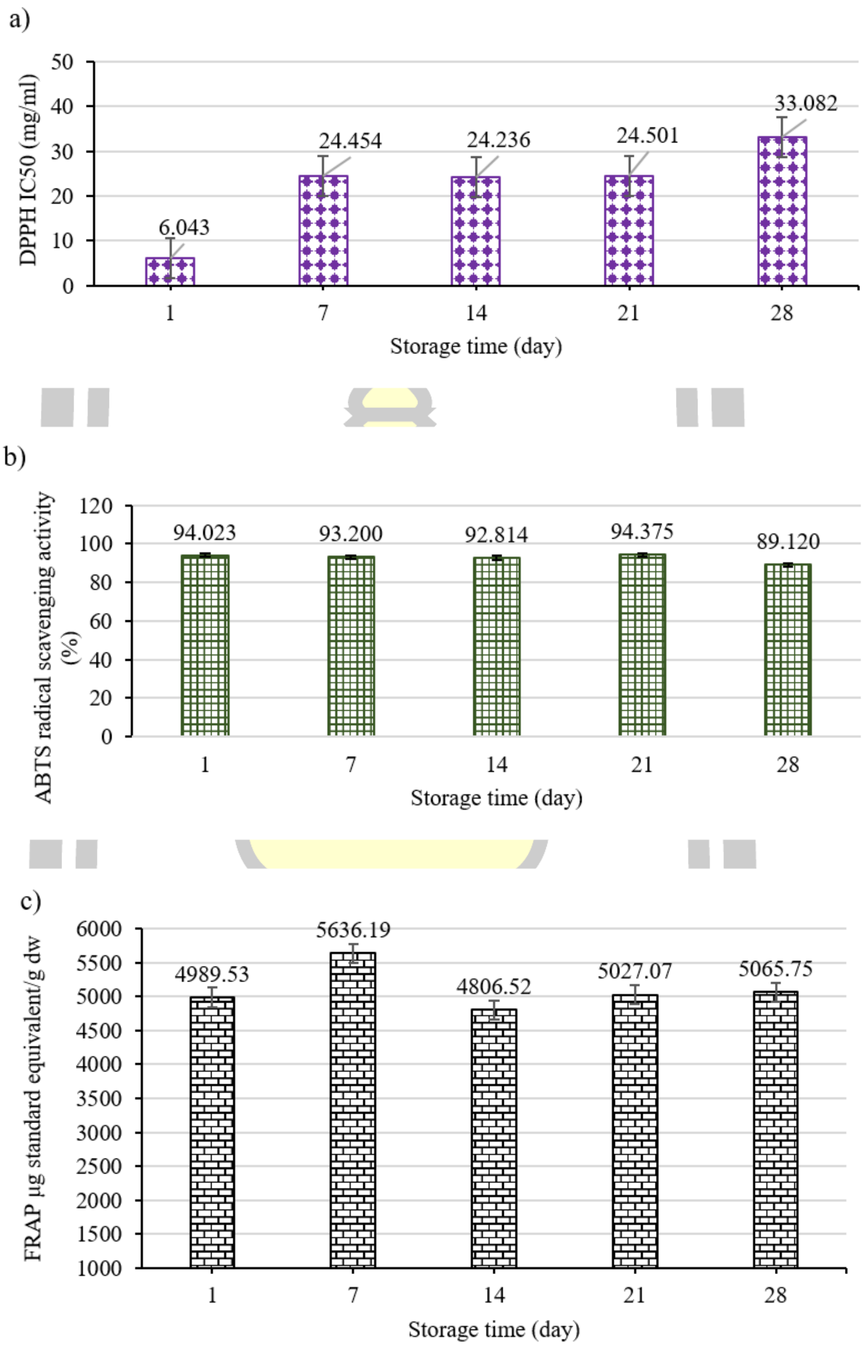


Figure 35 Antioxidant activity of extracted from soy milk yogurt during storage time measured using DPPH IC_{50} (a), ABTS radical scavenging (b) and FRAP assay (c). Different lowercase letters indicate significantly different ($p \leq 0.05$).

The total phenolic content (TPC) of cow milk yogurt and soy milk yogurt during storage was measured and expressed in mg gallic acid equivalents (GAE) per gram dry weight (DW) (Figure 36). The cow milk yogurt (Figure 4.20a), the TPC ranged from 1.467 to 1.485 mg GAE/g DW throughout the 28 days of storage. The TPC was relatively stable, with only slight variations, and the highest value (1.485 mg GAE/g DW) was recorded on day 21. For soy milk yogurt (Figure 4.20b), the TPC showed more fluctuations compared to cow milk yogurt, ranging from 1.597 to 1.628 mg GAE/g DW. The highest value (1.628 mg GAE/g DW) was observed on day 14, while the lowest value (1.597 mg GAE/g DW) occurred on day 7. After 28 days, the TPC reached 1.607 mg GAE/g DW, close to the initial value on Day 1 (1.616 mg GAE/g DW).

The TPC of both cow milk yogurt and soy milk yogurt demonstrated relatively stable profiles during storage, although some variation was observed, especially in soy milk yogurt. Phenolic compounds play an essential role in the antioxidant properties of fermented foods, and their retention over time can significantly impact the health benefits of yogurt products. For cow milk yogurt, the TPC remained relatively constant, with only minor fluctuations observed across the storage period. The small increase in TPC on day 21 suggests that ongoing fermentation may have promoted the release or synthesis of phenolic compounds, which are known to be influenced by microbial activity (Sah et al. 2016). Probiotic fermentation can lead to the liberation of bound phenolics from milk proteins, enhancing their bioavailability and antioxidant properties (Al-Sheraji et al. 2013). The minor decrease by day 28 might reflect slight degradation of phenolic compounds due to factors such as oxidation or enzymatic breakdown, which can occur during extended storage. In contrast, the soy milk yogurt displayed more pronounced changes in TPC throughout the storage period, with a peak at day 14. Soy milk is inherently rich in phenolic compounds, particularly isoflavones, which may account for the higher baseline TPC compared to cow milk yogurt (Bortolini et al. 2021). The increase in TPC observed on day 14 could be a result of enhanced microbial activity and the hydrolysis of complex phenolic compounds, making them more available during the middle stages of storage. The decline after day 14 may be due to the degradation of phenolic compounds or changes in their structure over time (Morsy et al. 2021). However, by day 28, the TPC in soy milk yogurt remained higher than that of cow milk yogurt, indicating that soy milk yogurt retains more phenolics even after extended storage. The differences between cow milk and soy milk yogurt can be attributed to their distinct matrices and the types of phenolic compounds present. Soy milk contains higher levels of isoflavones and flavonoids, which are more abundant than the phenolics found in cow milk (Pradeep and Manoj 2021). Additionally, the metabolic activity of probiotics may differ in the two types of yogurt, influencing how phenolic compounds are synthesized or broken down over time. These findings suggest that soy milk yogurt may offer superior phenolic retention and, consequently, better antioxidant potential over time compared to cow milk yogurt. However, further optimization of fermentation conditions and storage practices could help maintain or even enhance the phenolic content in both types of yogurt, maximizing their functional health benefits.

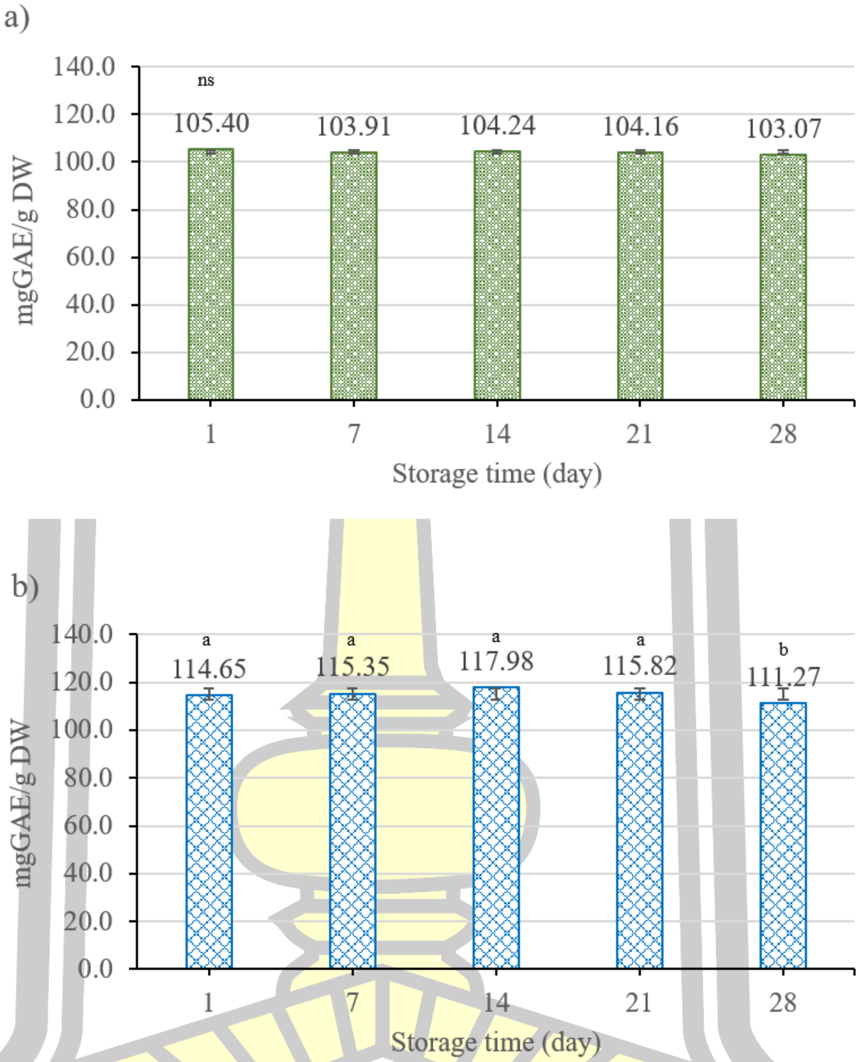


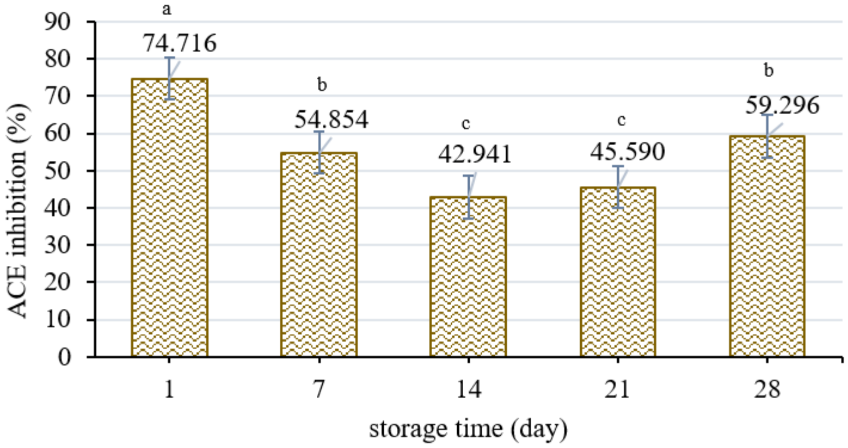
Figure 36 Total phenolic content of cow milk (a) and soy milk (b) yogurts during storage time.
ns represent an amount that non-significantly different ($p >$ 0.05) and different lowercase letters indicate significantly different ($p \leq$ 0.05).

4.3.4.5 Effect of yogurt storage on ACE inhibition

The effect of storage on the angiotensin-converting enzyme (ACE) inhibitory activity of cow milk yogurt and soy milk yogurt over 28 days (Figure 37). Results indicated that the ACE inhibition activity of both types of yogurt was significantly affected by storage duration. For cow milk yogurt, the ACE inhibitory activity decreased significantly by the 14th day of storage compared to the initial day. After this point, there was a slight increase by the end of the storage period (day 28), although this change was not statistically significant on the 7th day of storage ($p > 0.05$). On the other hand, soy milk yogurt exhibited the highest ACE inhibitory activity from the initial day up to day 14, after which the activity decreased significantly by the final storage day. The analysis showed that soy milk yogurt maintained high ACE inhibitory activity throughout the storage period, particularly during the first 14 days ($p \leq 0.05$).

The findings of this study highlight the influence of storage conditions on the bioactive properties of fermented dairy and non-dairy products, specifically ACE inhibition, which is relevant for cardiovascular health due to its role in blood pressure regulation. The significant decrease in ACE inhibitory activity by day 14 in both types of yogurt is consistent with previous studies, which suggest that proteolytic activity and the degradation of bioactive peptides occur during storage, resulting in reduced ACE inhibition (Donkor et al. 2012; Gobbetti et al. 2018). The initial higher ACE inhibitory activity in soy milk yogurt compared to cow milk yogurt may be attributed to the differences in protein content and peptide release during fermentation. Soy proteins are known to release peptides with potent ACE inhibitory activity, particularly during the early stages of fermentation (Agil et al. 2013). However, the significant decline in ACE inhibition in soy milk yogurt after day 14 suggests that further proteolysis or degradation of bioactive peptides occurs with extended storage (Nguyen et al. 2014). The slight increase in ACE inhibitory activity in cow milk yogurt toward the end of the storage period, though not statistically significant, could be due to the formation of small peptides as a result of continued proteolysis (Amadoro et al. 2021). Nonetheless, the lack of significant changes between day 14 and 28 suggests that the major decline in ACE inhibition occurs during the first two weeks of storage, after which the inhibitory activity plateaus.

a) MP-BY lyophilized



b) SB-BY lyophilized

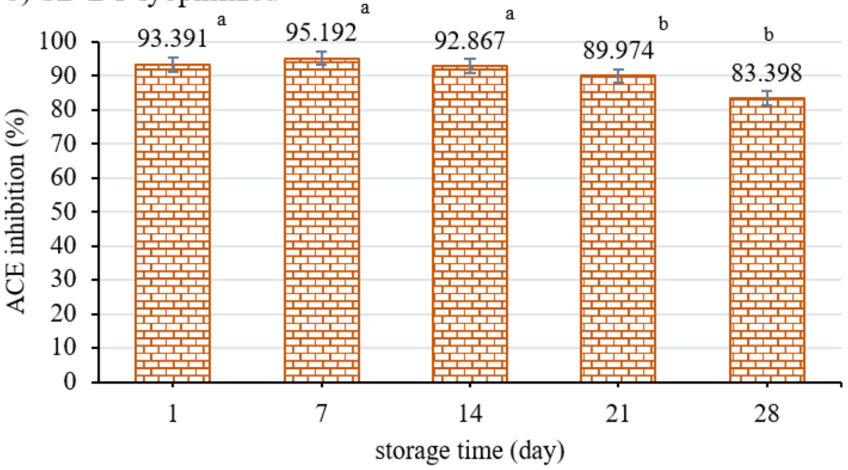


Figure 37 ACE inhibition of cow milk (a) and soy milk (b) yogurts during storage time.

Different lowercase letters indicate significantly different ($p \leq 0.05$).

4.3.4.6 Effect of yogurt storage on proximate composition of yogurts

The effect of storage on the proximate composition of cow milk yogurt (Table 28) and soy milk yogurt (Table 29) was evaluated over a 28-day period. The proximate analysis showed significant differences in all parameters, including moisture, protein, fat, and carbohydrate content, for both types of yogurt during storage, except for the ash content in cow milk yogurt, which remained relatively stable throughout the storage period ($p > 0.05$). Notably, the fat content in cow milk yogurt increased dramatically during storage, with a 6–7-fold increase from the initial value by the end of the storage period. In contrast, soy milk yogurt exhibited a decline in fat content, decreasing by approximately 1-fold by the final day of storage compared to its initial amount. This inverse trend between the two types of yogurt indicates different mechanisms of fat metabolism or separation during storage. The changes in moisture content also followed a similar trend, with cow milk yogurt showing a decrease and soy milk yogurt maintaining more stable moisture levels throughout the storage period.

The results of this study highlight the dynamic changes that occur in the proximate composition of yogurt during storage, and these changes can significantly affect the nutritional quality and texture of the product. The increase in fat content in cow milk yogurt could be due to the concentration effect caused by water loss or syneresis, a common phenomenon where liquid separates from the yogurt gel matrix during storage. This separation can lead to a higher relative fat concentration as moisture content decreases (Abd El-Gawad and Ahmed 2011). The stability of ash content in cow milk yogurt suggests that mineral content is relatively unaffected by storage conditions, which aligns with previous findings that ash (mineral) composition tends to be stable in dairy products over time (Donkor et al. 2012). However, the significant decline in fat content in soy milk yogurt could be attributed to different lipid metabolism pathways in plant-based yogurts compared to animal-based yogurts. Soy proteins may interact with lipids differently during fermentation and storage, leading to lipid oxidation or separation (McClements and Gumus 2016). Additionally, the decrease in fat content in soy milk yogurt may be a result of enzymatic activity or microbial fermentation that metabolizes lipids during storage. This effect is more pronounced in non-dairy yogurts where lipid-protein interactions differ from those in milk-based products (Salmerón 2017). The higher initial fat content in cow milk yogurt and its subsequent increase during storage suggests that water loss through syneresis plays a more dominant role in milk-based products than in plant-based yogurts, which may retain moisture better due to different gelation properties (Mok et al. 2020).

Table 23 Proximate composition of cow milk yogurts during storage time.

Storage time (day)	**Moisture (%)**	**Protein (%)**	**Fat (%)**	**Ash^{ns} (%)**	**Fiber (%)**	**Carbohydrate (%)**
Milk powder	$1.29±0.11^{c}$	$31.35±0.99^{b}$	$3.71±0.014^{c}$	4.99±0.72	$11.40±0.09^{b}$	$47.27±1.52^{a}$
1	$2.08±0.01^{c}$	$33.32±0.14^{a}$	$21.69±1.55^{a}$	4.29±0.08	$12.89±0.08^{a}$	$25.74±1.58^{b}$
7	$3.15±0.07^{bc}$	$33.50±0.05^{a}$	$19.27±0.05^{b}$	4.96±1.35	$9.58±0.26^{c}$	$29.54±1.67^{b}$
14	$5.56±1.99^{a}$	$32.78±0.10^{a}$	$18.40±0.67^{b}$	5.20±0.24	$11.12±0.48^{b}$	$26.93±1.84^{b}$
21	$4.74±0.04^{ab}$	$33.56±0.37^{a}$	$19.24±0.96^{b}$	5.33±0.43	$10.66±0.14^{bc}$	$26.45±1.86^{b}$
28	$4.57±0.16^{ab}$	$33.30±0.32^{a}$	$20.55±0.73^{ab}$	5.62±0.64	$9.86±1.01^{c}$	$26.12±1.58^{b}$

Lowercase letters within the column with different superscripts are significantly different ($p \leq 0.05$).

Table 24 Proximate composition of soy milk yogurts during storage time.

Storage time (day)	**Moisture (%)**	**Protein (%)**	**Fat (%)**	**Ash (%)**	**Fiber (%)**	**Carbohydrate (%)**
soybean powder	$4.79±0.05^{a}$	$45.56±0.13^{b}$	$23.46±0.35^{a}$	$5.64±0.28^{a}$	$7.85±0.34^{a}$	$12.72±1.05^{c}$
1	$2.09±0.01^{e}$	$46.83±0.45^{a}$	$15.87±1.01^{bc}$	$3.98±0.20^{b}$	$4.72±0.188^{b}$	$26.523±1.07^{ab}$
7	$2.34±0.03^{cd}$	$46.57±0.37^{ab}$	$16.98±1.18^{b}$	$4.58±0.51^{ab}$	$4.75±0.65^{b}$	$24.77±1.37^{b}$
14	$2.29±0.04^{d}$	$46.73±0.58^{ab}$	$13.83±0.62^{d}$	$4.53±0.39^{ab}$	$4.61±0.19^{b}$	$28.03±0.95^{a}$
21	$2.43±0.02^{bc}$	$47.27±0.02^{a}$	$16.25±0.16^{bc}$	$3.89±1.17^{b}$	$3.58±0.17^{c}$	$26.58±1.54^{ab}$
28	$2.48±0.11^{b}$	$47.27±0.81^{a}$	$14.66±0.39^{cd}$	$4.40±0.10^{ab}$	$4.99±0.47^{b}$	$26.21±0.88^{ab}$

Lowercase letters within the column with different superscripts are significantly different ($p \leq 0.05$).

4.3.4.7 Effect of yogurt storage on viable cell count of LAB and probiotic stains of yogurts

The effect of storage on the viable cell count of LAB and probiotic strains in cow milk yogurt and soy milk yogurt over a 28-day period (Table 30). The results showed that the number of LAB and probiotic bacteria in both types of yogurt increased as storage time progressed. In cow milk yogurt, LAB counts were initially high and continued to increase until the final day of storage. Similarly, soy milk yogurt exhibited a steady rise in LAB throughout the storage period, although the initial counts were lower than those in cow milk yogurt. By the end of storage, both types of yogurt demonstrated a significant increase in viable LAB and probiotic cell counts compared to the initial day ($p \leq 0.05$).

The increase in the viable cell count of LAB and probiotics during storage is consistent with the known behavior of these microorganisms in fermented products. The continuous growth of LAB throughout the storage period can be attributed to the favorable environment created by the yogurt matrix, which provides nutrients like lactose in cow milk yogurt and sugars in soy milk yogurt that support bacterial metabolism (Agil et al. 2013). The slight differences in growth patterns between cow milk yogurt and soy milk yogurt could be due to variations in the composition of substrates available for the LAB, such as the type of sugars (lactose vs. sucrose) and proteins present in the two types of yogurt. In cow milk yogurt, lactose is the primary sugar, which serves as a substrate for LAB. LAB strains such as *L. delbrueckii subsp. bulgaricus* and *S. thermophilus* metabolize lactose into lactic acid during fermentation. These LAB strains possess specific enzymes, such as β-galactosidase, that break down lactose efficiently during fermentation (Jan et al. 2022). In contrast, soy milk yogurt contains sucrose as its main sugar, which is metabolized differently by LAB. LAB strains vary in their ability to utilize sucrose, leading to differences in fermentation efficiency and acid production. LAB metabolism of sucrose can be less efficient due to variability in their enzymatic capacities, as not all LAB strains are equally adept at utilizing sucrose. This can lead to slower acid production and differences in the sensory and textural properties of soy-based yogurts. Moreover, the protein and carbohydrate matrices in soy milk differ significantly from those in cow milk, impacting the growth dynamics of LAB and their metabolic pathways (Dan et al. 2023).

Moreover, the increase in LAB during storage may also be linked to the gradual acidification of the yogurt, as LAB are known to produce lactic acid during fermentation, which lowers the pH of the yogurt. This acidic environment favors the growth of acid-tolerant strains, allowing for continued proliferation during cold storage (Nguyen et al. 2014). However, this increase in LAB numbers could eventually reach a plateau or even decline if the pH drops too low, which can inhibit further bacterial growth (Granato et al. 2010). Furthermore, (Cui et al. 2021) reported that the yogurts made from soy and cow milk were affected by the addition of probiotics during storage at 4 °C for 28 days with a 7-day interval. Cow milk yogurt had slightly higher *S. thermophilus* (ST), *L. delbrueckii* subsp. *bulgaricus* (LB) and *L. acidophilus* La-5 (LA) counts than soy milk yogurt, but *B. animalis* subsp. *lactis* BB-12 (BB) counts were significantly higher in soy milk yogurt than in cow's milk yogurt. However, the findings suggest that both cow milk and soy milk yogurts can maintain and even increase the viability of probiotic strains during storage, which is crucial for their functional benefits. Maintaining high viable cell counts of probiotics is important for the health-promoting effects of yogurt, as these bacteria contribute to gut health, immune function, and digestion (Granato et al. 2010; Ranadheera et al. 2010; Vinderola et al. 2019). The ability of LAB to remain viable over extended storage periods also enhances the shelf life and probiotic efficacy of yogurt products.

Table 25 Lactic acid bacteria counts and probiotics of cow milk and soy milk yogurts during storage time at 4°C for 28 days.

Storage time (day)	**LAB (cfu/g)**		**Probiotic (cfu/g)**	
	cow milk yogurt	**soy milk yogurt**	**cow milk yogurt**	**soy milk yogurt**
1	$2.0^{d} \times 10^{4}$	$3.3^{e} \times 10^{4}$	(-)	(-)
7	$2.2^{cd} \times 10^{4}$	$4.5^{d} \times 10^{4}$	(-)	(-)
14	$2.4^{bc} \times 10^{4}$	$7.1^{c} \times 10^{4}$	(-)	(-)
21	$2.7^{ab} \times 10^{4}$	$7.6^{b} \times 10^{4}$	(-)	(-)
28	$2.9^{a} \times 10^{4}$	$8.0^{a} \times 10^{4}$	1.1×10^{5}	8.5×10^{7}

Lowercase letters within the column with different superscripts are significantly different ($p \leq 0.05$) and (-) represents an amount that was not determined.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study comprehensively evaluated the effects of co-fermentation of yogurts with yogurt bacteria and probiotic strains on key bioactive components, particularly melatonin, tryptophan, and antioxidant activity of cow and plant-based milk. The results demonstrated that yogurt produced through co-culturing traditional yogurt starters with probiotics exhibited significant improvements in melatonin, tryptophan, and antioxidant activity compared to conventional yogurt made without probiotics. The probiotic strains used, the combination of *Lactobacillus acidophilus* and *Bifidobacterium lactis*, produced the highest levels of melatonin and demonstrated the most potent antioxidant activity, as measured by DPPH, ABTS, and FRAP assays. These assays revealed that the probiotic-enhanced yogurts had a significantly stronger ability to neutralize free radicals and oxidative stress compared to traditional yogurt. This suggests that the inclusion of specific probiotic strains boosts the nutritional value of yogurt and enhances its functional properties, particularly its antioxidant potential. Furthermore, the combination of multi-strain YC and probiotics had comparable TA and pH values, color values (L*, a*, b*) and TPA (consistency and cohesiveness) to regular yogurt whereas the addition of probiotics altered some physicochemical and textural properties.

The study also explored the impact of adding tryptophan to yogurt formulations. The addition of tryptophan to cow milk and soy milk yogurts (MPBY+tryp 1.5% and SBBY+tryp 1.5%) led to an increase in melatonin content and its derivatives compared to unfermented milk, underscoring the role of tryptophan as a precursor in melatonin biosynthesis during fermentation. The concentrations of tryptophan enhanced the antioxidant activity of the yogurt, indicating that tryptophan fortification also benefits melatonin production and improves the overall health-promoting properties of yogurt by boosting its capacity to counteract oxidative stress. Furthermore, the study investigated the effect of storage on various properties of the yogurt, including melatonin and its derivatives content, antioxidant activities, ACE inhibition, and physicochemical characteristics such as pH, titratable acidity, syneresis, and proximate composition. For storage, significant changes ($p \leq 0.05$) were observed in melatonin and its derivatives content, antioxidant activities, ACE inhibition, and physicochemical characteristics for both cow milk and soy milk yogurts fermented with YC and probiotics. These findings suggest that while yogurt maintains its beneficial properties during storage, careful consideration must be given to the storage conditions and duration, as these can influence its functional qualities.

In summary, this research underscores the potential of yogurt as a functional food, rich in bioactive compounds like melatonin and tryptophan, contributing to various health benefits, including improved antioxidant defense and potential ACE inhibition. The successful co-fermentation of traditional yogurt cultures with probiotic strains offers a promising approach for the dairy industry to create innovative products that cater to the growing consumer demand for health-promoting foods. By selecting optimal combinations of probiotic strains and incorporating additional precursors like tryptophan, yogurt manufacturers can enhance their products' nutritional and functional qualities, offering consumers a food that supports gut health and promotes overall well-being through enhanced antioxidant activity and bioactive compound content. The insights gained from this study will be instrumental for the dairy industry in the development of next-generation probiotic yogurt products, particularly those enriched with bioactive metabolites like melatonin and tryptophan. These findings provide a scientific rationale for selecting specific probiotic strains and optimizing yogurt formulations to maximize health benefits, thereby contributing to the advancement of functional foods in the marketplace.

5.2 Recommendations

To increase the biological activity of high-melatonin yogurt, further research is suggested as follows

5.2.1 Developing storage protocols to maintain content of melatonin, tryptophan, and antioxidant activity throughout the shelf life of the product

5.2.2 In-depth scientific investigations, including human impact studies or simulate the digestive system and regulatory approvals for health claims related to melatonin, tryptophan, and antioxidants

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APPENDICES

Appendix A

LC-MS/MS chromatograms of standard of melatonin, free tryptophan, and serotonin

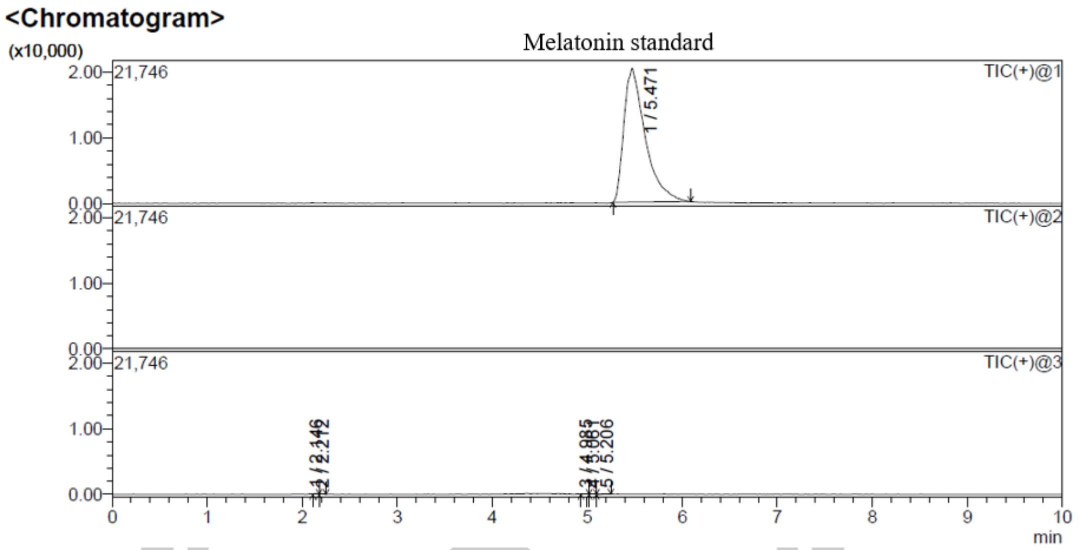

Figure 38 LC-MS/MS chromatogram of melatonin standard (1.0 µg/mL).

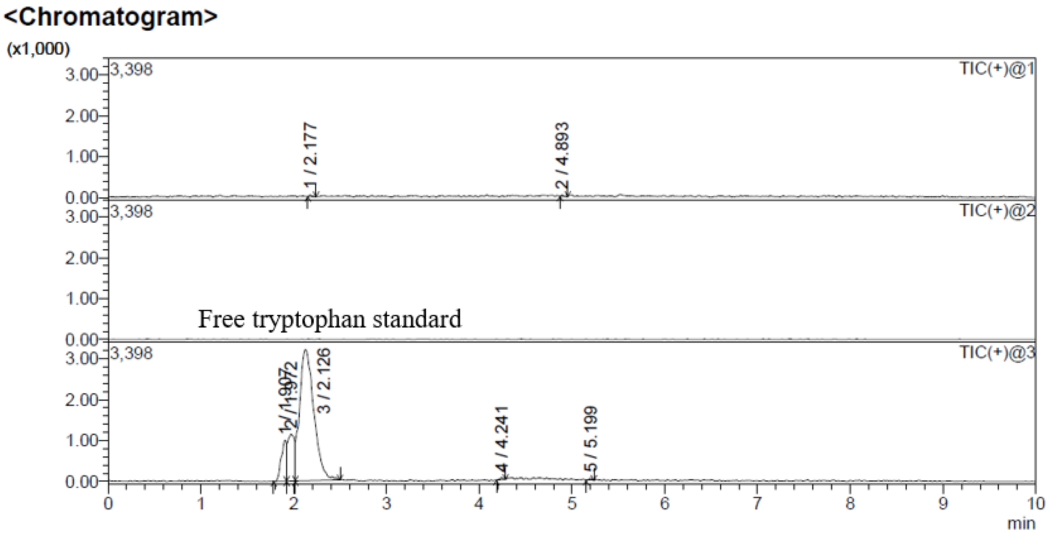

Figure 39 LC-MS/MS chromatogram of free tryptophan standard (1.0 µg/mL).

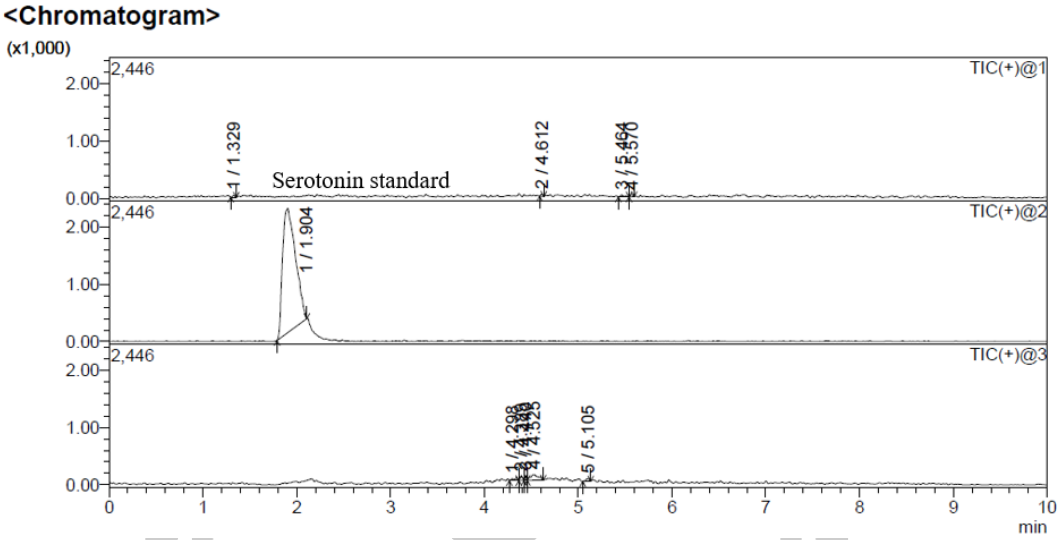

Figure 40 LC-MS/MS chromatogram of serotonin standard (0.5 µg/mL).

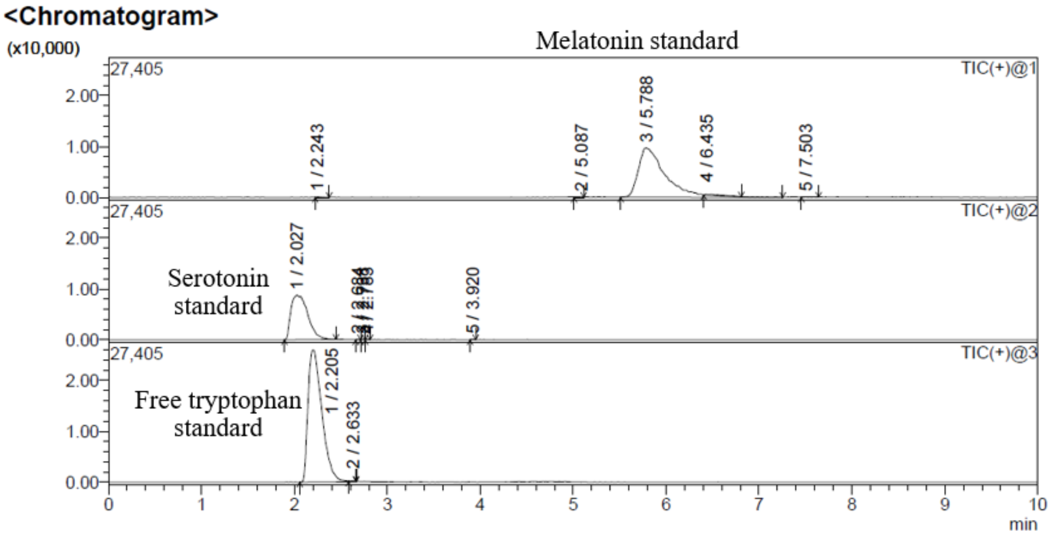

Figure 41 LC-MS/MS chromatogram of mixed standard.
Melatonin standard (1.0 µg/mL), free tryptophan standard (5.0 µg/mL), and serotonin standard (5.0 µg/mL)

Appendix B

Calibration curves of standard of melatonin, free tryptophan, serotonin, and antioxidant activity

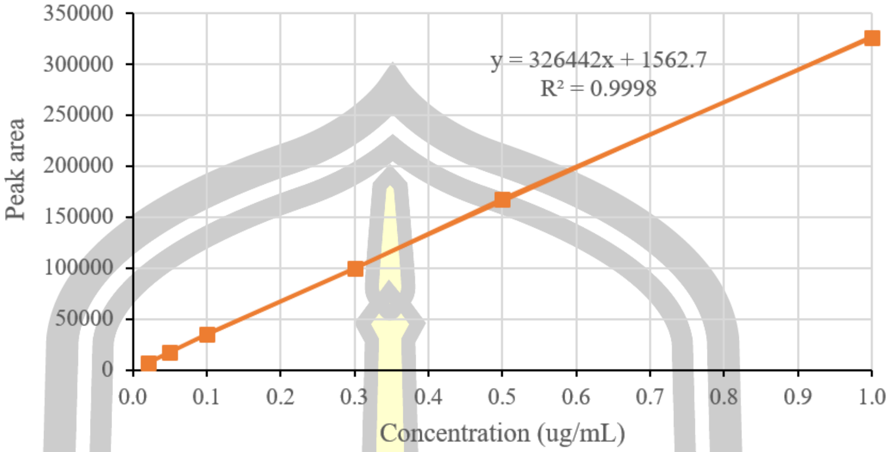

Figure 42 Calibration curve of melatonin standard.

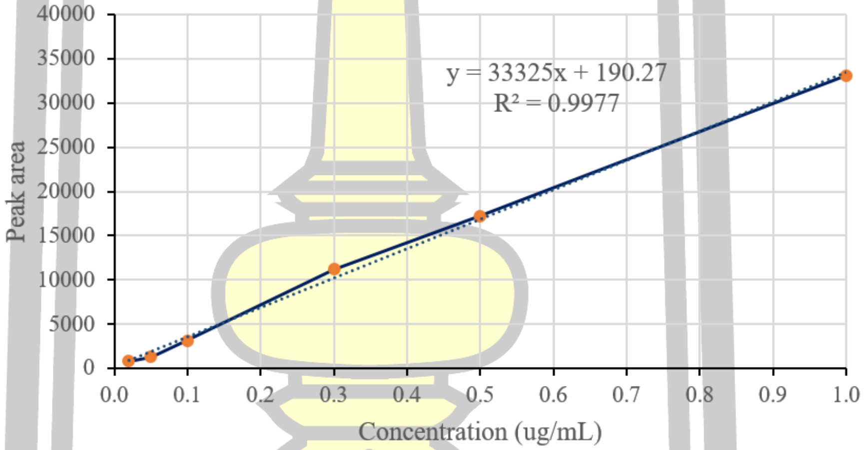

Figure 43 Calibration curve of free tryptophan standard.

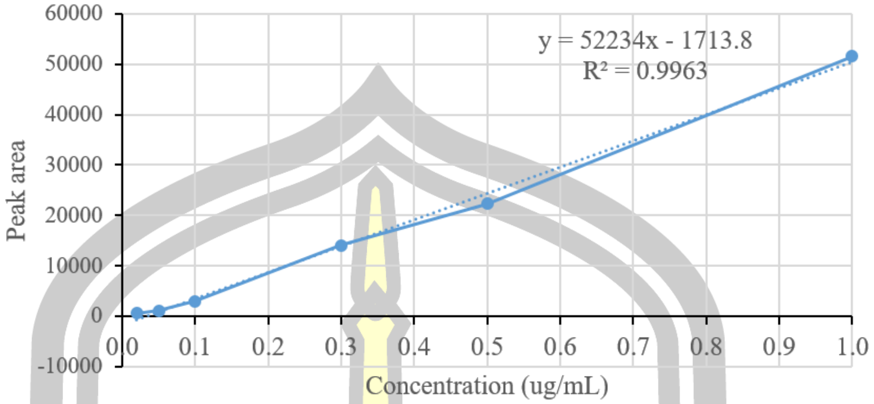

Figure 44 Calibration curve of serotonin standard.

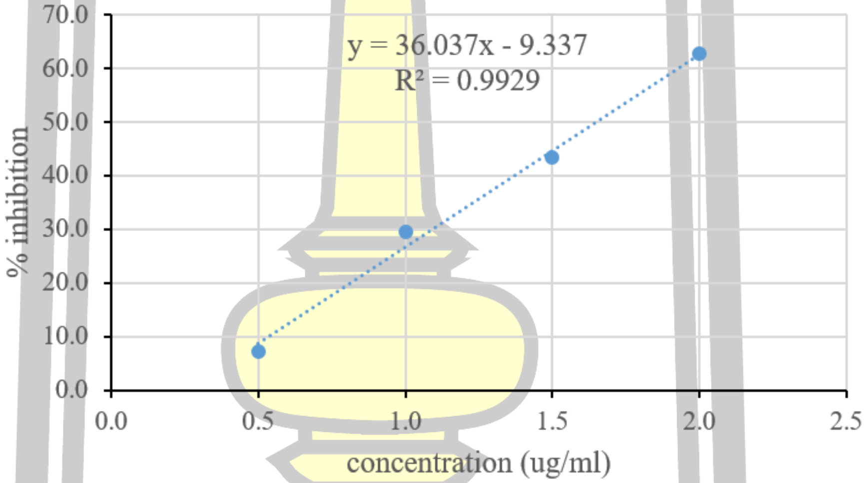

Figure 45 Calibration curve of L-ascorbic acid standard using DPPH assay.

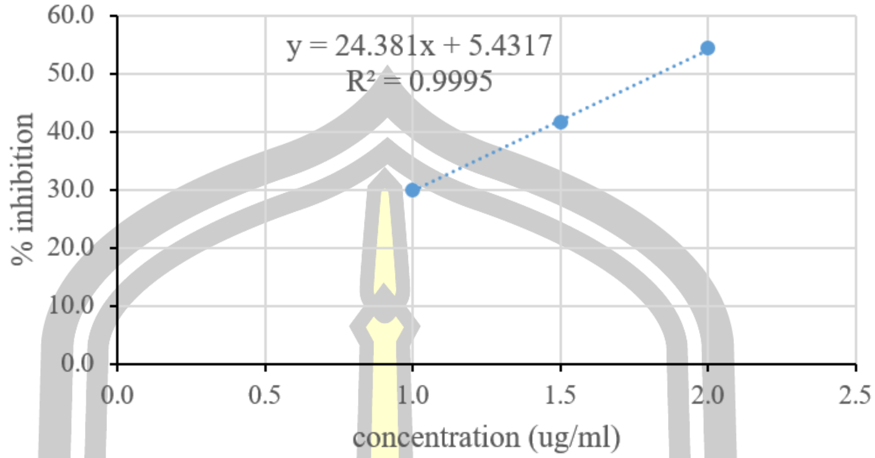

Figure 46 Calibration curve of L-ascorbic acid standard using ABTS assay.

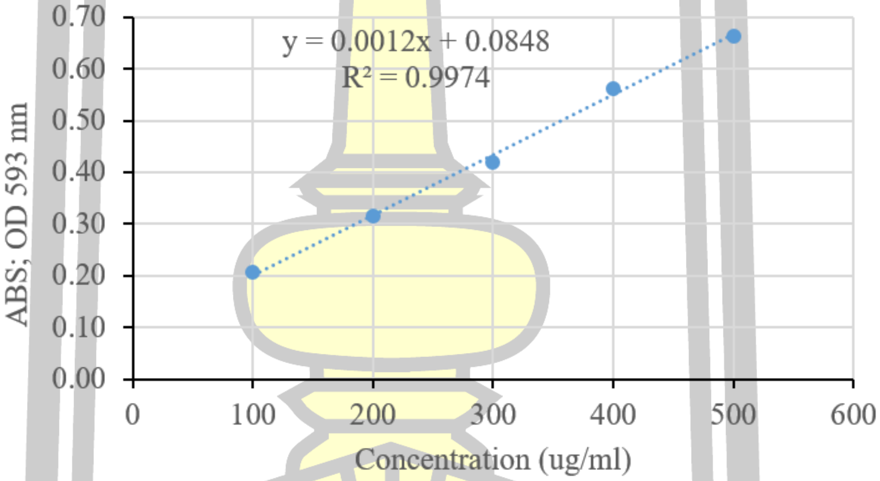

Figure 47 Calibration curve of L-ascorbic acid standard using FRAP assay.

Appendix C

Table of results

Table 26 pH value and titratable acidity during yogurt fermentation.

Fermentation time (h)	pH value				Titratable acidity (%)			
	MP-YC	MP-BY	MP-BT	MP-LA	MP-YC	MP-BY	MP-BT	MP-LA
0	6.08±0.04	6.06±0.00	6.09±0.03	6.10±0.00	0.21±0.01	0.29±0.01	0.26±0.01	0.23±0.01
1	5.82±0.02	5.51±0.01	5.30±0.08	5.26±0.13	0.31±0.01	0.57±0.03	0.66±0.04	0.49±0.02
2	5.21±0.09	4.85±0.01	4.87±0.01	4.92±0.02	0.56±0.02	0.75±0.01	0.80±0.02	0.56±0.01
3	4.47±0.05	4.55±0.01	4.58±0.02	4.62±0.02	0.88±0.02	0.76±0.02	0.86±0.01	0.57±0.02
4	4.27±0.04	4.43±0.00	4.51±0.06	4.48±0.01	0.99±0.02	0.86±0.02	0.88±0.01	0.56±0.02
5	4.14±0.01	4.37±0.01	4.46±0.01	4.43±0.01	1.07±0.01	0.86±0.02	0.88±0.03	0.56±0.01
6	4.03±0.01	4.32±0.00	4.36±0.02	4.37±0.01	1.11±0.04	0.81±0.03	0.89±0.02	0.58±0.03
7	3.99±0.01	4.30±0.00	4.32±0.01	4.33±0.03	1.16±0.06	0.78±0.04	0.74±0.03	0.57±0.00
8	3.97±0.02	4.32±0.01	4.31±0.00	4.32±0.01	1.23±0.02	0.82±0.03	0.73±0.02	0.59±0.02

Mean values are expressed as mean ± standard deviation of replicate experiments (n = 3)

Table 27 Antioxidant activity by DPPH, ABTS, and FRAP of yogurt fermented by different mixed cultures.

Fermentation time (h)	DPPH IC_{50} (mg/mL)				ABTS IC_{50} (mg/mL)				FRAP (µmol$FeSO_4$/g)			
	MP-YC	MP-BY	MP-BT	MP-LA	MP-YC	MP-BY	MP-BT	MP-LA	MP-YC	MP-BY	MP-BT	MP-LA
0	46.58	55.03	43.89	57.65	14.74	22.24	18.37	19.28	1154.48	1116.90	1158.13	971.69
2	50.56	64.31	40.36	62.72	12.88	21.03	13.96	21.93	1175.96	1094.06	1080.52	909.14
4	56.00	42.66	42.11	70.15	12.79	20.44	18.81	17.67	1035.85	1126.28	1069.88	868.43
6	41.51	42.92	43.04	63.68	17.85	21.10	16.25	16.57	1124.41	1178.08	1037.02	877.70
8	44.615	39.720	47.907	57.111	15.13	19.90	16.37	14.11	1027.24	1035.38	983.20	849.15

Mean values are expressed as mean of replicate experiments (n = 3)

Table 28 pH value and titratable acidity during fermentation of soy milk yogurt.

Fermentation time (h)	**pH value**				**Titratable acidity (%)**			
	SB-YC	**SB-BY**	**SB-BT**	**SB-LA**	**SB-YC**	**SB-BY**	**SB-BT**	**SB-LA**
0	6.24±0.03	6.07±0.02	6.26±0.01	6.12±0.00	0.23±0.01	0.21±0.00	0.24±0.01	0.20±0.04
2	5.40±0.00	5.27±0..04	5.15±0.01	5.65±0.00	0.38±0.00	0.37±0.01	0.42±0.03	0.29±0.03
4	4.72±0.01	4.55±0.00	4.60±0.01	4.70±0.01	0.60±0.02	0.61±0.01	0.31±0.06	0.39±0.03
6	4.59±0.01	4.20±0.01	4.40±0.00	4.31±0.00	0.68±0.01	0.84±0.00	0.41±0.05	0.49±0.02
8	4.53±0.01	4.09±0.01	4.26±0.00	4.07±0.00	0.75±0.01	0.99±0.00	0.74±0.06	0.93±0.05

Mean values are expressed as mean ± standard deviation of replicate experiments (n = 3)

Table 29 pH value and titratable acidity during fermentation of chickpea yogurt.

Fermentation time (h)	**pH value**				**Titratable acidity (%)**			
	CP-YC	**CP-BY**	**CP-BT**	**CP-LA**	**CP-YC**	**CP-BY**	**CP-BT**	**CP-LA**
0	6.19±0.01	6.22±0.02	6.17±0.01	5.96±0.01	0.16±0.01	0.19±0.00	0.16±0.01	0.18±0.00
2	5.46±0.01	4.98±0.01	4.95±0.02	5.86±0.01	0.21±0.02	0.35±0.00	0.33±0.00	0.19±0.00
4	4.58±0.01	4.39±0.01	4.61±0.01	5.39±0.02	0.42±0.02	0.48±0.02	0.38±0.01	0.26±0.00
6	4.35±0.00	4.31±0.01	4.42±0.01	4.66±0.01	0.49±0.02	0.51±0.00	0.43±0.01	0.45±0.06
8	4.29±0.01	4.27±0.00	4.34±0.01	4.44±0.02	0.55±0.01	0.54±0.02	0.47±0.01	0.45±0.02

Mean values are expressed as mean ± standard deviation of replicate experiments (n = 3)

Table 30 pH value and titratable acidity during fermentation of white sesame yogurt.

Fermentation time (h)	pH value				Titratable acidity (%)			
	SS-YC	SS-BY	SS-BT	SS-LA	SS-YC	SS-BY	SS-BT	SS-LA
0	6.44±0.02	6.15±0.00	5.97±0.01	5.81±0.04	0.05±0.00	0.08±0.01	0.10±0.01	0.15±0.01
2	5.22±0.06	5.56±0.02	5.22±0.01	5.20±0.03	0.20±0.01	0.13±0.00	0.22±0.03	0.28±0.05
4	4.58±0.00	4.82±0.01	4.76±0.01	4.13±0.01	0.29±0.01	0.32±0.04	0.35±0.00	0.60±0.03
6	4.41±0.01	4.40±0.01	4.50±0.00	3.92±0.00	0.34±0.00	0.45±0.01	0.49±0.00	0.72±0.01
8	4.33±0.01	4.18±0.00	4.36±0.01	3.80±0.00	0.35±0.00	0.54±0.01	0.57±0.00	0.85±0.01

Mean values are expressed as mean ± standard deviation of replicate experiments (n = 3)

Table 31 Antioxidant activity by DPPH, ABTS, and FRAP assays of soy milk yogurt.

Fermentation time (h)	DPPH IC_{50} (mg/mL)				ABTS IC_{50} (mg/mL)				FRAP (µmol$FeSO_4$/g)			
	SB-YC	SB-BY	SB-BT	SB-LA	SB-YC	SB-BY	SB-BT	SB-LA	SB-YC	SB-BY	SB-BT	SB-LA
0	61.80	20.58	26.27	11.27	2.49	2.82	2.59	2.32	2185.69	1793.20	2481.63	2597.88
2	65.60	56.16	37.17	20.95	6.30	2.72	2.75	4.86	1918.32	1660.92	2204.52	2409.40
4	107.92	9.03	13.59	10.69	2.86	4.54	4.28	0.51	1577.20	1734.03	2362.03	2488.68
6	109.61	18.25	19.01	11.66	4.72	4.19	3.99	4.21	1850.61	1735.97	2306.11	2317.07
8	38.71	24.23	70.63	32.29	2.48	6.20	5.20	3.31	2331.04	1928.01	2468.92	2577.86

Mean values are expressed as mean of replicate experiments (n = 3)

Table 32 Antioxidant activity by DPPH, ABTS, and FRAP assays of chickpea yogurt.

Fermentation time (h)	DPPH IC$_{50}$ (mg/mL)				ABTS IC$_{50}$ (mg/mL)				FRAP (µmolFeSO$_4$/g)			
	CP-YC	CP-BY	CP-BT	CP-LA	CP-YC	CP-BY	CP-BT	CP-LA	CP-YC	CP-BY	CP-BT	CP-LA
0	27.51	26.87	42.14	39.47	10.49	15.66	19.83	25.94	1806.97	2477.69	1590.89	1914.38
2	26.11	25.86	31.39	59.57	11.45	14.82	19.72	21.19	1818.95	1729.02	1602.48	2218.55
4	24.05	25.29	29.65	64.72	11.07	12.23	16.53	20.23	1841.55	2011.65	1856.07	2026.62
6	23.19	24.12	31.47	28.17	10.57	3.04	17.93	26.09	1827.16	1976.71	1885.37	2288.92
8	23.22	28.52	29.75	30.74	7.90	12.84	12.31	22.32	1968.76	2007.72	1901.51	1888.90

Mean values are expressed as mean of replicate experiments (n = 3)

Table 33 Antioxidant activity by DPPH, ABTS, and FRAP assays of white sesame yogurt.

Fermentation time (h)	DPPH IC$_{50}$ (mg/mL)				ABTS IC$_{50}$ (mg/mL)				FRAP (µmolFeSO$_4$/g)			
	SS-YC	SS-BY	SS-BT	SS-LA	SS-YC	SS-BY	SS-BT	SS-LA	SS-YC	SS-BY	SS-BT	SS-LA
0	84.65	53.17	66.00	41.03	23.16	25.46	27.04	26.99	1486.44	1175.83	1351.65	1332.18
2	43.06	50.14	45.31	38.78	25.20	25.34	28.85	29.27	1011.13	1125.37	1122.23	1126.83
4	46.69	45.19	44.58	41.88	27.69	26.22	28.21	31.08	919.64	972.79	981.19	1006.77
6	48.77	41.87	48.58	39.40	27.83	29.87	30.49	34.71	859.07	1208.18	1136.05	1103.98
8	50.76	39.88	48.81	41.01	25.56	30.68	27.82	32.23	1112.27	1044.84	1099.75	1166.28

Mean values are expressed as mean of replicate experiments (n = 3)

Table 34 pH value and titrated acidity during fermentation of cow milk yogurts adding tryptophan.

Treatment	pH value			Titratable acidity (%)		
	0	4	8	0	4	8
MPBY+tryp 0	6.06±0.01	4.56±0.02	4.05±0.00	0.28±0.00	0.74±0.01	1.11±0.03
MPBY+tryp 0.5	6.06±0.00	4.55±0.07	4.14±0.01	0.29±0.01	0.75±0.01	1.05±0.03
MPBY+tryp 1.0	6.03±0.01	4.58±0.01	4.14±0.00	0.26±0.01	0.74±0.00	1.07±0.01
MPBY+tryp 1.5	6.09±0.00	4.76±0.01	4.22±0.01	0.27±0.00	0.76±0.01	1.02±0.00
MPBY+tryp 2.0	6.29±0.01	5.98±0.01	4.97±0.01	0.18±0.00	0.32±0.00	0.57±0.01

Mean values are expressed as mean ± standard deviation of replicate experiments (n = 3)

Table 35 Antioxidant activity of extracted from cow milk yogurts adding tryptophan measured using DPPH IC_{50}, ABTS and FRAP assays.

Treatment	DPPH IC_{50} (mg/mL)			ABTS (%)			FRAP (μmolFeSO$_4$/g)		
	0	4	8	0	4	8	0	4	8
MPBY+tryp 0	55.03	42.66	39.72	51.71	58.93	57.25	2098.80	1902.33	1785.48
MPBY+tryp 0.5	223.99	257.37	102.71	93.67	89.87	91.54	66.92	55.78	145.42
MPBY+tryp 1.0	125.97	144.26	150.95	93.45	92.74	92.50	241.60	158.85	289.20
MPBY+tryp 1.5	100.93	94.54	96.50	93.50	93.69	93.08	116.54	486.82	389.76
MPBY+tryp 2.0	159.41	99.31	77.61	91.80	94.48	93.94	206.07	376.95	334.64

Mean values are expressed as mean of replicate experiments (n = 3)

Table 36 pH value and titrated acidity during fermentation of soy milk yogurts adding tryptophan.

Treatment	**pH value**			**Titratable acidity (%)**		
	0	**4**	**8**	**0**	**4**	**8**
SBBY+tryp 0	5.88±0.01	4.64±0.00	4.22±0.00	0.23±0.02	0.58±0.01	0.68±0.01
SBBY+tryp 0.5	5.86±0.00	4.69±0.00	4.28±0.00	0.28±0.00	0.60±0.01	0.68±0.01
SBBY+tryp 1.0	5.88±0.00	4.80±0.00	4.36±0.00	0.27±0.00	0.61±0.00	0.65±0.00
SBBY+tryp 1.5	5.88±0.00	4.95±0.00	4.44±0.00	0.27±0.01	0.64±0.00	0.44±0.00
SBBY+tryp 2.0	5.89±0.00	4.96±0.00	4.46±0.00	0.30±0.01	0.64±0.00	0.45±0.01

Mean values are expressed as mean ± standard deviation of replicate experiments (n = 3)

Table 37 Antioxidant activity of extracted from soy milk yogurts adding tryptophan measured using DPPH IC_{50}, ABTS and FRAP assays.

Treatment	**DPPH IC_{50} (mg/mL)**			**ABTS (%)**			**FRAP (µmolFeSO$_4$/g)**		
	0	**4**	**8**	**0**	**4**	**8**	**0**	**4**	**8**
SBBY+tryp 0	134.48	201.55	170.98	67.36	60.14	60.34	482.14	371.54	475.07
SBBY+tryp 0.5	97.81	23.368	96.54	96.41	95.95	95.25	360.24	319.33	529.64
SBBY+tryp 1.0	142.83	109.49	107.51	92.77	90.36	85.89	285.81	332.68	385.21
SBBY+tryp 1.5	118.65	98.14	94.90	93.34	94.41	90.91	340.55	500.66	489.33
SBBY+tryp 2.0	100.27	102.88	91.97	96.84	96.30	85.96	496.77	488.76	567.07

Mean values are expressed as mean of replicate experiments (n = 3)

Table 38 Sensory evaluation of cow milk yogurts fermented with different LAB and probiotics.

Treatment	Attributes					
	Appearance	**Color**	**Odor**	**Texture**	**Flavor**	**Overall**
Ref.MPY	8.12±0.46	8.26±0.76	8.00±0.34	8.40±0.45	8.20±1.10	8.30±1.24
MP-YC	6.56±1.04	7.05±1.11	6.88±1.01	5.62±1.00	5.20±0.97	5.17±1.21
MP-BY	6.40±1.27	7.18±1.13	7.00±1.25	5.69±0.99	4.21±1.01	5.38±0.89
MP-BT	6.23±1.06	7.11±1.02	6.78±1.09	5.22±1.13	4.09±1.14	5.26±1.19
MP-LA	6.38±1.42	7.02±1.18	6.22±1.26	5.16±1.11	4.11±1.06	5.11±1.32

Ref.MPY refers to reference yogurt (commercial yogurt)

Mean values are expressed as mean ± standard deviation of replicate experiments (n = 50)

Table 39 Sensory evaluation of soy milk yogurts fermented with different LAB and probiotics.

Treatment	Attributes					
	Appearance	**Color**	**Odor**	**Texture**	**Flavor**	**Overall**
Ref.SMY	6.30±1.23	6.10±1.30	6.85±1.11	6.42±1.32	5.66±0.51	6.12±1.12
SB-YC	6.64±1.41	6.18±1.28	3.64±0.98	5.10±1.22	3.54±0.73	4.30±0.99
SB-BY	5.78±1.03	5.40±1.47	3.90±0.77	4.96±1.65	2.88±1.19	4.00±1.62
SB-BT	5.79±1.53	5.97±1.36	3.58±1.15	4.88±1.20	3.27±1.70	4.15±1.39
SB-LA	3.21±1.68	4.24±1.71	4.12±1.34	2.63±1.49	2.54±1.06	2.73±1.16

Ref.SMY refers to reference soy milk yogurt (commercial soy milk yogurt)

Mean values are expressed as mean ± standard deviation of replicate experiments (n = 50)

Table 40 Sensory evaluation of cow milk and soy milk yogurts.

Treatment	Attributes						
	Appearance	**Color**	**Odor**	**Texture 1**	**Texture 2**	**Flavor**	**Overall**
Ref.MPY	8.24±0.74	8.36±0.87	8.20±0.83	8.36±0.90	8.46±1.22	8.12±1.30	8.36±1.04
MP-BY	7.14±1.25	7.48±1.03	7.38±1.21	6.22±1.46	6.16±1.73	6.04±0.99	6.10±1.30
Ref.SMY	6.64±1.72	6.36±1.80	6.80±1.83	7.10±1.62	6.86±1.91	5.66±1.81	6.12±1.66
SB-BY	6.02±1.73	5.70±1.53	4.38±1.31	5.24±1.98	4.04±1.54	4.08±1.35	4.12±1.33

Ref.MPY refers to reference yogurt (commercial yogurt)

Ref.SMY refers to reference soy milk yogurt (commercial soy milk yogurt)

Texture 1 refers to visual texture: the smoothness of the yogurt

Texture 2 refers to taste-tested texture: the smoothness of the yogurt

Mean values are expressed as mean ± standard deviation of replicate experiments (n = 50)

BIOGRAPHY

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Research grants & awards	1) Mahasarakham University (graduate students; Ph.D.) 2) The Agricultural Research Development Agency (Public Organization)
Research output	1) Utilization of yogurt bacteria and probiotic combination enhanced the melatonin content and antioxidant activity of yogurt (International Food Research Journal) 2) Co-culturing yogurt bacteria with probiotics increased melatonin content and enhanced the antioxidant activity of soy milk yogurt (Food Technology and Biotechnology Journal)