

## Extending spring roll sheet shelf life against microbial spoilage using preservative combinations

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### Article history:

Received: 18 April 2025

Revised: 16 May 2025

Accepted: 16 July 2025

Published: 16 February 2026

### Keywords:

Spring roll sheet,  
Extending shelf life,  
Preservative combination,  
Microbial spoilage

### DOI:

[https://doi.org/10.26656/fr.2017.10\(1\).099](https://doi.org/10.26656/fr.2017.10(1).099)

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### Abstract

High water activity ( $a_w$ ) and low-acid foods are rapidly spoiled by microorganisms. The preservative was widely used to extend the microbial shelf life of foods. The suitable preservative combinations were potential microbial inhibition/killing because of their synergistic effect, multiple anti-microbial actions, and broad anti-microbial efficacy, enhancing longer shelf life even with a reduced preservative concentration. A spring roll sheet has a short shelf life (2–3 days) because of its high moisture content (~40%) and  $a_w$  (~0.99), which makes it susceptible to microbial spoilage. Extending this short shelf-life is necessary for commercial use. This study extended the shelf life of spring roll sheets stored at room temperature (27–29°C) before microbial spoilage. The process investigated the suitability of using a combination of tartaric acid (T) and potassium sorbate (S)/calcium propionate (P)/mixture of S and P (M) with varied concentrations and in regulated concentrations following the CODEX standards for food additives as preservatives with efficient contents for storage time extension. The advantages of preservative combinations were efficient in broadly inhibiting/killing microorganisms, and the potential for a longer shelf life of the treated product. The experimental preservatives did not affect the moisture content or water activity compared to the control samples (without preservatives); however, the control was adversely affected by rapid microbial spoilage. The higher concentration of T (0.27%) more efficiently enhanced the efficacy of the added S, P, and M to inhibit microbial growth than the lower concentration (0.19%). Only the combination of T (0.27%) with S (0.10%) and the combination of T (0.27%) with a mixture of S (0.05%) and P (0.05%) were efficient as preservatives to extend the shelf life of the spring roll sheet to more than 12 days, which was 4 times the shelf life of the control sample. These suitable preservative combinations inhibited the dominant microbe causing spoilage of the target products, resulting in efficient extension of the shelf life of the product, even though less preservative contents were used. The preservative contents in the spring roll sheet samples were 3.4–7.3 times lower than the maximum permitted levels in CODEX Alimentarius Commission (2023).

## 1. Introduction

The spring roll sheet, composed of wheat flour, is thin and circular (Figure 1A). It has a high moisture content and high-water activity ( $a_w$ ); consequently, it suffers from rapid microbial spoilage that shortens its shelf life to only 2–3 days, as shown in Figure 1B. Extending this short shelf-life is necessary for commercial use. The advantages of a longer shelf life for products containing regulated food additives are reducing the spoilage loss cost, increasing opportunities

for distribution and shelf holding, and increasing profits.

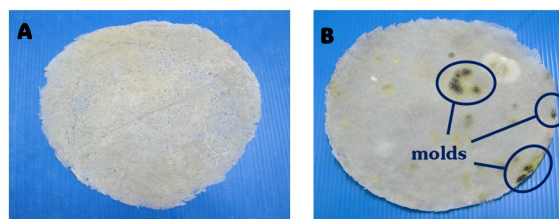


Figure 1. Appearance of spring roll sheet: (A) fresh spring roll sheet and (B) microbially spoiled spring roll sheet, following storage at room temperature for 3 days.

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Adding preservatives is one potential method to retard microbial spoilage; however, the usage content must follow the general standard for food additives of the CODEX Alimentarius Commission (2023). Suitable preservatives should effectively inhibit microbial growth, be stable, not easily degraded, and not destroy the nutritional value of food. Additionally, a preservative should not induce any undesirable color, odor, taste, or off-flavor in the food. Most preservatives are acids and function best as anti-microbials at a low pH with an undissociated form (Rattanapanone, 2014). Furthermore, acids enhance taste, reduce off-flavors, and reduce the pH of food, which could inhibit microbial growth and spore germination. The anti-microbial mechanisms of weak acids and their salts are: (1) inhibition of enzymatic activity (Melnick *et al.*, 1954), causing metabolic processes to be disrupted, resulting in microbial growth inhibition (Davidson and Juneja, 1989); (2) damage to the microbial cell membrane, resulting in the loss of membrane transport (Ronning and Frank, 1987; Booth and Kroll, 1989; Msagati, 2013) and consequently, the microorganism is unable to uptake essential nutrients, leading to the disruption of cellular metabolic processes and eventual cell death (Holoak *et al.*, 1996).

The specificity of each preservative can affect different types of microorganisms; hence, preservative combinations are efficient in extending protection against microbial spoilage because of the resultant broad inhibition or death of microorganisms. Suitable preservative combinations should have synergistic and efficient microbial inhibition of the dominant microbe causing spoilage in the product. A spring roll sheet is a starch-based food with a high  $a_w$  value; hence, it is easily spoiled by yeast, mold, and bacteria. The current study selected the combination of tartaric acid and potassium sorbate and/or calcium propionate. Tartaric acid enhances the efficacy of preservatives, which were the acid form and salt form of acids (Nunheimer and Fabian, 1940; Olson and Macy, 1940; Olson and Macy, 1945; Doores, 1989) since it lowers the pH of the food, preventing undissociated forms, which is an important anti-microbial function (Rattanapanone, 2014). Furthermore, tartaric acid has a mild, sour taste, adds a good flavor to many foods, and produces anti-microbial properties at a low pH value (Nunheimer and Fabian, 1940; Doores, 1989). Potassium sorbate and calcium propionate are salts of organic acids with high water solubility and are widely utilized as food preservatives. Notably, potassium sorbate has been shown to effectively inhibit the growth of yeast, mold, and bacteria, being particularly effective within a pH range of 5.5–6.0 (Gould, 1964; Freese, 1978; Eklund, 1980; Smoot and Pierson, 1981; Blocher *et al.*, 1982; Sofos and Busta, 1983; Davidson and Juneja, 1989; Ochiai *et*

*al.*, 2002; Wang *et al.*, 2006). Calcium propionate is more effective in inhibiting mold growth than yeast and has been reported to inhibit *Bacillus mensentericus* and *B. subtilis*, which are bacteria responsible for ropiness (stringiness) in starch-based foods (Pacher *et al.*, 2022). Calcium propionate produced optimal efficacy in the pH range 4.0–6.0 (O'Leary and Kralovec, 1941; Deane and Downs, 1951; Heseltine, 1952; Woolford, 1975; Davidson and Juneja, 1989). Both sorbate and propionate had high potential anti-microbial ability at higher pH (pH < 6.0) as compared to that of benzoate (pH < 4.5 (Suwannaphan and Klangmuang, 2022), hence sorbate and propionate may be more suitable for low acid foods than benzoate. For this research, the initial pH of the spring roll sheet was about 6.5. Too much acidification enhanced sour taste and the non-acceptance of customers. Therefore, sorbate and propionate were chosen to extend the microbial shelf life of the spring roll sheet as preservatives, and tartaric acid functioned as an acidifying agent and preservative to synergize the antimicrobial potential.

In related studies, Suwannaphan and Klangmuang (2022) demonstrated that spring roll wrappers stored in nylon-linear low-density polyethylene (LLDPE) bags could be preserved for up to 13 days at ambient temperature when the pH was adjusted using vinegar, with the addition of sodium benzoate. Chigozirim and Ahaotu (2023) conducted a 10-day storage study on bread at room temperature. They found that bread treated with sorbate and a combination of sorbate and propionate had similar levels of bacterial inhibition. However, the bread treated with a mixture with a sorbate-to-propionate ratio of 50:50 showed significantly less mold growth compared to all other samples. Patrick *et al.* (2019) found that the combination of sorbate (0.1% of flour) and propionate (0.3%) in bread efficiently retarded fungal growth in bread. However, the addition of ascorbic acid (0.1% w/w of flour) did not enhance fungal growth inhibition of the combination of sorbate and propionate. It may be because bread baking is a high-temperature process (175 to 190°C) and ascorbic acid is severely degraded by heat and is unstable in thermal food processing (Yin *et al.*, 2022); thus, ascorbic acid may lose its functions as an acidifying agent and preservative.

The objective of the current research was to investigate combinations of various preservatives (tartaric acid, potassium sorbate, and/or calcium propionate) at varied concentrations on the microbial spoilage of spring roll sheet.

## 2. Materials and methods

### 2.1 Materials

All-purpose wheat flour, arrowroot starch, salt, coconut milk, and non-dairy creamer were purchased from a local market (Bangkok, Thailand). Tartaric acid and potassium sorbate (Krunghthechemi Co. Ltd., Thailand) and calcium propionate (Caltech Corporation Ltd., Thailand).

### 2.2 Preservative types and concentrations

From our preliminary experiments, using more than 0.27% tartaric acid (T) resulted in an excessively sour taste in the spring roll sheet and the acidification with 0.27% tartaric acid reduced the pH of the spring roll sheet to 5.2 (data not shown), which was in the suitable pH range of 5.0–6.0 for the high anti-microbial potential of sorbate and propionate. Therefore, the maximum concentration of T was set at 0.27% of wheat flour and used at concentrations of 0.19% (T1) and 0.27% (T2) of wheat flour. Potassium sorbate (S) was used at concentrations of 0.05% (S1) and 0.10% (S2) of wheat flour. Calcium propionate (P) was used at concentrations of 0.05% (P1) and 0.10% (P2) of wheat flour. Mixed S and P (labeled as M) with a ratio of 1:1 was used at total concentrations of 0.05% (M1) and 0.10% (M2) of wheat flour.

### 2.3 Preservative combinations

Following CODEX standards for food additives, in pasta and noodle products, calcium propionate was allowed to be used at an appropriate level, while the maximum permitted levels for tartaric acid and potassium sorbate were 5,000 and 2,000 mg/kg, respectively (Codex Alimentarius Commission, 2023). The formulations for preservative combinations used for spring roll sheet preparation are presented in Table 1. The calculated amounts of preservatives in the final

spring roll sheet are shown in Table 2. The preservative contents in all the spring roll sheet samples were 3–7 times lower than the maximum permitted levels regulated for food additives (Codex Alimentarius Commission, 2023).

### 2.4 Spring roll sheet preparation

The spring roll sheet was prepared by mixing 90.74% all-purpose wheat flour, 4.54% arrowroot starch, 0.18% salt, 2.27% coconut milk, and 2.27% non-dairy creamer, with added water. The resultant mixture was kneaded until a dough with a spreadable consistency was obtained. Then, the dough was rested before being spread onto a flat pan and heated to approximately 160°C. A circular sheet with a diameter of approximately 170 mm and a thickness of 1–3 mm was formed and heated for about 30 s. The sheet was removed from the pan, cooled, and packaged.

All sample preparation steps were performed following good food sanitation and in a closed room maintained at 26–27°C to prevent contamination. Work surfaces, utensils, and equipment were sanitized using 70% alcohol. The operators wore sanitized gloves and masks throughout the sample preparation and packing processes.

### 2.5 Storage of spring roll sheet

The six fresh spring roll sheets were packaged in 250 mm × 300 mm nylon/LLDPE plastic bags containing oxygen absorbers (6 g) to reduce the oxygen level within the bags. The investigated storage times of samples at room temperature were 0, 6, and 12 days. The fresh and stored samples were determined for moisture content, water activity, and microbial growth.

### 2.6 Moisture content determination

Aluminum cans were dried in a hot-air oven at

Table 1. Formulations of preservative combinations for spring roll sheet preparation.

Treatment	Preservative combination for spring roll sheet preparation		
	Tartaric acid (T) (% of wheat flour)	Potassium sorbate (S) (% of wheat flour)	Calcium propionate (P) (% of wheat flour)
T1S1	0.190	0.050	-
T1S2	0.190	0.100	-
T2S1	0.270	0.050	-
T2S2	0.270	0.100	-
T1P1	0.190	-	0.050
T1P2	0.190	-	0.100
T2P1	0.270	-	0.050
T2P2	0.270	-	0.100
T1M1	0.190	0.025	0.025
T1M2	0.190	0.050	0.050
T2M1	0.270	0.025	0.025
T2M2	0.270	0.050	0.050

Table 2. Calculated the preservative content in the spring roll sheet.

Treatment	Preservative content in spring roll sheet using calculation		
	Tartaric acid (T) (mg/kg)	Potassium sorbate (S) (mg/kg)	Calcium propionate (P) (mg/kg)
T1S1	1,045.00	275.00	-
T1S2	1,045.00	550.00	-
T2S1	1,485.00	275.00	-
T2S2	1,485.00	550.00	-
T1P1	1,045.00	-	275.00
T1P2	1,045.00	-	550.00
T2P1	1,485.00	-	275.00
T2P2	1,485.00	-	550.00
T1M1	1,045.00	137.50	137.50
T1M2	1,045.00	275.00	275.00
T2M1	1,485.00	137.50	137.50
T2M2	1,485.00	275.00	275.00

\*Calcium propionate was allowed to be used at an appropriate level, while the maximum permitted levels for tartaric acid and potassium sorbate were 5,000 and 2,000 mg/kg, respectively (Codex Alimentarius Commission, 2023).

105°C for 3 h and then cooled in a desiccator and weighed. A sample (3 g) was placed in a weighed aluminum can and then placed in a hot-air oven at 105°C for 3 h, followed by cooling in a desiccator (AOAC Official Method 14.004, 2000). The moisture content was calculated using equation (1):

$$\text{Moisture content (\%)} = \frac{(\text{Weight of sample before drying (g)} - \text{Weight of sample after drying (g)})}{\text{Weight of sample before drying (g)}} \times 100 \quad (1)$$

### 2.7 Water activity ( $a_w$ ) determination

The water activity was determined at 25°C using a water activity meter (LabMASTER-aw; Novasina AG, Switzerland).

### 2.8 Microbial growth determination

The Thai Bureau of Quality and Safety of Food (2017) sets the microbial spoilage of pasta and noodle products based on an aerobic plate count (APC) of  $5 \times 10^5$  colony-forming units (CFU)/g or more, with a yeast and mold count of  $5 \times 10^2$  CFU/g or more. Additionally, visible physical appearance changes indicative of microbial growth, such as yellow spots, fluff or spores, along with mucus formation, were considered indicative of microbial spoilage.

#### 2.8.1 Aerobic plate count

The aerobic plate count (APC) was determined following the method provided in chapter 3 of the Bacteriological Analytical Manual of the U.S. FDA (Maturin and Peeler, 2001). Each sample (50 g) was vigorously mixed with the sterilized diluents (50 mL), and the mixture was diluted using serial dilutions. A 1 mL diluted sample was added to a petri dish, and then agar was poured and swirled to ensure it had mixed well. The poured plate was incubated at 35°C for 48 h. The CFU count was determined, and the APC was calculated

and expressed as colony forming units per gram (CFU/g).

#### 2.8.2 Yeast and mold counts

The yeast and mold counts were determined following the method provided in chapter 18 of the Bacteriological Analytical Manual of the U.S. FDA (Tournas *et al.*, 2001) using dichloran rose-bengal chloramphenicol (DRBC) agar. Sample (50 g) was mixed with 0.1% peptone water (450 mL); the mixture was homogenized using a stomacher for 2 min and then prepared serial decimal dilutions. Diluted sample (0.1 mL) was added and spread onto the agar surface. The inoculated plate samples were incubated at 25°C for 5–7 days. The yeast and mold colonies were counted and expressed as colony forming units per gram (CFU/g).

### 2.9 Statistical analysis

A completely randomized design (CRD) was performed with three factors. The first factor was tartaric acid concentration with two levels (0.19 and 0.27%). The second factor was the type of preservative (S, P, and M). The third factor was preservative concentration (0.05 and 0.10%). Data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test at the 95% confidence level ( $p < 0.05$ ) using the SPSS statistical software (IBM, USA).

## 3. Results and discussion

### 3.1 Moisture content and water activity

The moisture contents of the fresh spring roll sheet in all treatments were within the range 38–43% and  $a_w$  values were approximately 0.99 (Figure 2A), which is typical of foods that are highly susceptible to microbial (bacteria, yeasts, and molds) growth (Rattanapanone,

Table 3. Moisture content (%) of spring roll sheet during storage of 0, 6, and 12 days at room temperature.

Treatment	Storage time (days)		
	0	6	12
Control	42.07±6.03 <sup>abc</sup>	-----Spoiled-----	
T1S1	41.23±0.40 <sup>abcA</sup>	39.68±1.30 <sup>bcdA</sup>	34.02±1.04 <sup>bcB</sup>
T1S2	42.58±0.20 <sup>abA</sup>	41.11±1.30 <sup>bA</sup>	31.66±0.54 <sup>deB</sup>
T2S1	39.77±0.08 <sup>abcA</sup>	38.40±0.77 <sup>deA</sup>	29.96±1.23 <sup>eB</sup>
T2S2	42.78±1.48 <sup>abA</sup>	38.65±1.78 <sup>cdeAB</sup>	33.10±0.98 <sup>bcdB</sup>
T1P1	42.73±0.95 <sup>abA</sup>	36.66±0.85 <sup>efB</sup>	38.59±0.89 <sup>aB</sup>
T1P2	40.98±0.68 <sup>abcA</sup>	39.69±0.84 <sup>bcdA</sup>	32.37±0.66 <sup>cdB</sup>
T2P1	37.88±0.54 <sup>cA</sup>	40.74±0.14 <sup>bcA</sup>	33.06±1.13 <sup>bcdB</sup>
T2P2	37.97±0.83 <sup>cAB</sup>	39.96±0.18 <sup>bcdA</sup>	34.68±1.16 <sup>bB</sup>
T1M1 <sup>NS</sup>	42.41±1.33 <sup>abc</sup>	38.54±1.38 <sup>cde</sup>	37.71±0.43 <sup>a</sup>
T1M2	43.15±0.80 <sup>aA</sup>	40.66±0.29 <sup>bcdA</sup>	34.39±0.80 <sup>bcB</sup>
T2M1	38.40±0.40 <sup>bcA</sup>	35.92±0.21 <sup>fB</sup>	29.98±0.73 <sup>eC</sup>
T2M2	40.22±0.94 <sup>abcB</sup>	45.46±0.13 <sup>aA</sup>	30.11±1.03 <sup>eC</sup>

Values are presented as mean±SD. Values with different lowercase superscripts in the same column are statistically significantly different ( $p < 0.05$ ). Values with different uppercase superscripts in the same row are statistically significantly different ( $p < 0.05$ ).  
<sup>NS</sup> non-significant differences ( $p \geq 0.05$ ) in the same row.

2014). The preservatives had no measurable effects on the moisture content or the  $a_w$  values compared to the control sample (Figure 2A). The preservatives—either acid (T) or salts (S or P)—did not bind water molecules; hence, there was no decrease in  $a_w$  values. After storage times of 6 and 12 days, the ranges in the moisture content were 36–45% and 30–39%, respectively (Figures 2B and C). The moisture content of almost all samples significantly decreased (Table 3) as storage time increased because of water evaporation and water syneresis due to starch retrogradation (Gudmundsson, 1994). However, the  $a_w$  value tended to be constant (~0.97–0.98) throughout storage (Figures 2A, B, and C).

### 3.2 Microbial growth during storage

Table 4 shows the APC and yeast and mold counts in the fresh and stored spring roll sheet. The APC and yeast and mold count in the fresh samples were in the range  $10 - 10^3$  CFU/g and less than 10 CFU/g, respectively. During storage, yellow spots were visually observed in the control sample (without preservative) after storage for 3 days, thus defining the shelf life of the control sample. As storage reached 6 days, the APC and yeast and mold counts of the control sample steeply increased to  $10^8$  CFU/g and  $3.4 \times 10^3$  CFU/g, respectively, indicating rapid spoilage. All treated samples containing the lower T concentration (0.19%; T1) had a shelf life of less than 6 days. T1S1, T1M1, and T1M2 had APC levels higher than  $5 \times 10^5$  CFU/g, which limited microbial spoilage for storage lasting 6 days. In addition, spoilage occurred in the T1P1, T1P2, and T1M1 samples due to yeast and mold because the yeast and mold counts were higher than  $5 \times 10^2$  CFU/g, the maximal allowable before

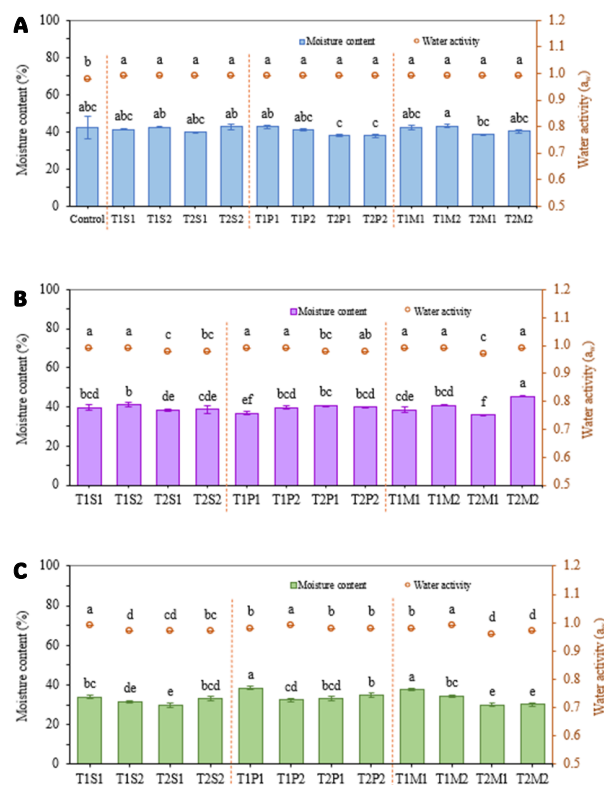


Figure 2. Moisture content (%) and water activity ( $a_w$ ) of spring roll sheet during storage at room temperature: (A) 0 day, (B) 6 days, and (C) 12 days. Data are presented as mean±SD. Different alphabets indicate statistically significantly different ( $p < 0.05$ ).

yeast and mold spoilage. The increase in the T concentration (0.27%; T2) improved the anti-microbial efficacy that was confirmed in all treated samples containing T2, which had a shelf life longer than 6 days. This could have been due to the more acidic environment enhancing the inhibitory effects of the potassium sorbate

Table 4. Microbial growth of spring roll sheet during storage of 0, 6, and 12 days at room temperature.

Sample	0 day		6 days		12 days	
	APC (CFU/g)	Yeast and molds (CFU/g)	APC (CFU/g)	Yeast and molds (CFU/g)	APC (CFU/g)	Yeast and molds (CFU/g)
Control	$1.10 \times 10^3$	<10	$1.20 \times 10^8$	$3.40 \times 10^3$		
			----- Visually observed yellow spots as stored 3 days -----			
T1S1	$6.35 \times 10^2$	<10	$1.50 \times 10^6$	50		
T1S2	$4.60 \times 10^2$	<10	$1.02 \times 10^5$	50	Visually observed mold	
T2S1	$1.55 \times 10^2$	<10	$5.01 \times 10^4$	<10		
T2S2	$1.20 \times 10^3$	<10	$4.50 \times 10^3$	<10	$1.64 \times 10^3$	<10
T1P1	$1.51 \times 10^3$	<10	$6.85 \times 10^4$	$1.50 \times 10^3$		
T1P2	$1.05 \times 10^3$	<10	$2.45 \times 10^5$	$3.80 \times 10^3$	Visually observed mold	
T2P1	$1.55 \times 10^3$	<10	$6.95 \times 10^4$	<10		
T2P2	$2.25 \times 10^3$	<10	$6.80 \times 10^4$	<10		
T1M1	$1.35 \times 10^2$	<10	$6.70 \times 10^5$	$3.45 \times 10^3$		
T1M2	$2.15 \times 10^2$	<10	$5.55 \times 10^5$	<10	Visually observed mold	
T2M1	$2.55 \times 10^2$	<10	$2.67 \times 10^4$	<10		
T2M2	$2.00 \times 10^3$	<10	$1.95 \times 10^3$	<10	$6.01 \times 10^4$	<10

, expressed microbial spoilage.

Thai Bureau of Quality and Safety of Food (2017) sets the microbial spoilage of pasta and noodle products based on an aerobic plate count (APC) of  $5 \times 10^5$  colony-forming units (CFU)/g or more, with a yeast and mold count of  $5 \times 10^2$  CFU/g or more. Additionally, visible physical appearance changes indicative of microbial growth, such as yellow spots, fluff or spores, along with mucus formation, were considered indicative of microbial spoilage.

and calcium propionate (acting as preservatives in salt forms), which have been reported to be most effective in the ranges pH 5.5–6.0 and pH 4.0–6.0, respectively (O'Leary and Kralovec, 1941; Heseltine, 1952; Gould, 1964; Woolford, 1975; Eklund, 1980; Smoot and Pierson, 1981; Blocher *et al.*, 1982; Davidson and Juneja, 1989). In an acidic system (tartaric addition), tartaric acid, potassium sorbate, and calcium propionate were in undissociated form, which were able to penetrate the cell membrane of microorganisms. As they passed into the cytoplasm of microorganisms (higher intracellular pH), tartaric acid was broken down and released protons ( $H^+$ ), resulting in depression of intracellular pH, which disrupted/inhibited enzyme activity and metabolic process (Melnick *et al.*, 1954; Davidson and Juneja, 1989). Moreover, potassium sorbate and calcium propionate induced proton and anion accumulation inside the cell of microorganisms, which disrupted/inhibited metabolic enzymes and destroyed the transport membrane (Suwannaphan and Klangmuang, 2022). These changes caused microbial growth inhibition and eventual cell death. The preservative combination expressed multiple anti-microbial actions, resulting in broader anti-microbial efficacy.

In addition, the higher concentration of tartaric acid (T2) with a lower concentration of potassium sorbate (S1)/calcium propionate (P1)/combination of potassium sorbate and calcium propionate (M1) (as T2S1, T2P1,

and T2M1, respectively) produced higher levels of efficient microbial inhibition than at the lower tartaric acid concentration (T1) but also at the even higher concentrations of potassium sorbate (S2)/calcium propionate (P2)/combination of potassium sorbate and calcium propionate (M2) (as T1S2, T1P2, and T1M2, respectively). These results concluded that a higher acidified system increased the undissociated form of T, S, and P, resulting in higher synergistic anti-microbial potential, even with a smaller amount of usage (sorbate and propionate). These results were consistent with the studies by Olson and Macy (1940) and Olson and Macy (1945) on the efficacy of preservatives (sodium propionate and calcium propionate) against mold in butter, where acidifying butter to pH 5.5 using lactic acid resulted in 5% P being as effective as 10% P in unacidified butter in preventing surface mold growth. Suwannaphan and Klangmuang (2022) studied the shelf life of spring roll wrappers and reported that a combination of vinegar (for pH adjustment) and sodium benzoate preserved the product for up to 13 days at ambient temperature. The acidified system was able to efficiently improve the anti-microbial efficiency of the preservatives in salt form of acids, such as S and P. Furthermore, in the current study, increasing the concentration of S/P/combination of S and P to 0.10% (S2, P2, and M2, respectively) enhanced microbial inhibition compared to the lower concentration of 0.05% (S1, P1, and M1, respectively) at the same T

concentration, due to the higher preservative content in the system.

There were only two treatments (T2S2 and T2M2) that limited the microbial spoilage and increased the shelf life of the spring roll sheet up to 12 days. This may have been due to S providing effective inhibition against a broader range of microorganisms, including yeasts, molds, and many bacteria (Gould, 1964; Freese, 1978; Eklund, 1980; Smoot and Pierson, 1981; Blocher *et al.*, 1982; Sofos and Busta, 1983; Davidson and Juneja, 1989). Notably, none of the combinations of T and P extended the shelf life to 12 days, which may have been due to P, although less effective at yeast and mold inhibition, being able to effectively inhibit the growth of *B. mesentericus* and *B. subtilis*—bacteria known to cause ropiness in starch-based foods such as spring roll sheet (O'Leary and Kralovec, 1941; Deane and Downs, 1951; Davidson and Juneja, 1989). Another study reported that the combination of sorbate and propionate effectively extended the shelf life of bread up to 20 days at room temperature (Patrick *et al.*, 2019), with significantly less mold growth (Chigozirim and Ahaotu, 2023). Hence, T2M2 was the best potential preservative combination because: (1) it could retard microbial spoilage and extend the shelf life of spring roll sheet up to 12 days (4 times longer than the shelf life of the control); (2) it could inhibit a broad range of microorganisms that cause spoilage in starch-based foods; and (3) it contained a lower potassium sorbate content (desirable for consumer health) than T2S2 (Table 2).

#### 4. Conclusion

The combination of preservatives (tartaric acid and potassium sorbate, and/or calcium propionate) improved the anti-microbial effectiveness due to broader inhibition of microorganisms, resulting in a longer shelf life of the spring roll sheet, even with a reduced preservative concentration. Tartaric acid added at the higher concentration (0.27%) for pH adjustment efficiently improved the anti-microbial efficiency of potassium sorbate/calcium propionate more than with a lower concentration (0.19%). The shelf life of the spring roll sheet was extended to 12 days at room temperature, which was 4 times longer than the shelf life of the control sample (without preservative), when the treatment consisted of a combination of tartaric acid (0.27%) and potassium sorbate (0.10%) or a combination of tartaric acid (0.27%) and a mixture of potassium sorbate (0.05%) with calcium propionate (0.05%). However, none of the combinations of tartaric acid and calcium propionate could extend the shelf life of the spring roll sheet up to 12 days. Thus, there was potential for some of the tested preservative combinations to

efficiently inhibit the dominant microbes causing spoilage in the target products.

#### Conflict of interest

The authors declare no known competing financial interests or personal relationships that influenced the results reported in this paper.

#### Acknowledgments

This research was financially supported by the Kasetsart University Research and Development Institute (KURDI), Bangkok, Thailand. The Institute of Food Research and Product Development provides facilities, and Mr. Thongsuk Limcharoen provided the specialized spring roll sheet preparation.

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