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Research Article

Application of Trehalose Dihydrate for the Improvement of Cooked Rice Quality and Extension of Shelf Life

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Cooking methods, such as steaming, may cause quality issues in cooked rice during storage due to the retrogradation of gelatinised starch and other related structural changes. This study is aimed at enhancing the shelf life of cooked rice blocks (nigiri) by applying trehalose dihydrate, thereby improving the quality and palatability of the samples over their shelf life without compromising food safety. Two samples (4TH15, containing 15% trehalose and 5% sucrose, and a control [C4]) were prepared after the initial sensory (preference) testing, and they were analysed for physicochemical (pH, water activity and textural properties) and nutritional properties. Microbial analysis involved testing for yeast and moulds, total viable count (TVC) and challenge testing for *Bacillus cereus* and *Listeria monocytogenes*. Sensory analysis to determine shelf life extension included triangle testing. The application of trehalose dihydrate at 15% and sucrose at 5% improved the perceived quality of cooked rice, with a notably higher preference over a 7-day testing period. No ascertainable difference was found when comparing 2-day older trehalose-containing samples to control samples over shelf life, and this suggested that quality degradation was inhibited. This was further demonstrated through texture analysis of relevant quality parameters, with slower increases in hardness and firmness and improved retention of stickiness during shelf life. No yeast and mould growth was detected during shelf life testing, with TVC levels remaining insufficient to yield perceivable spoilage. The growth of *B. cereus* and *L. monocytogenes* was not supported. Therefore, trehalose was suitable for improving the shelf life and quality of cooked-acidified rice without posing a detriment to food safety.

Keywords: cooked rice; quality; sensory; shelf life; trehalose dihydrate

1. Introduction

During chilled storage and over shelf life, cooked rice undergoes quality degradation due to retrogradation, moisture redistribution and loss by evaporation, yielding firm and hard rice, with reduced adhesiveness, eating quality and acceptability [1–4]. This is inevitable in starchy foods with moisture contents equal to or greater than 35% or water activity (a_w) values greater than 0.90, including cooked rice [3]. Retrogradation is the recrystallisation of amylose and amylopectin. It predominantly involves amylose–amylose interactions and those between amylose and long internal chains of amylopectin [5, 6]. Rice starch begins to retrograde at temperatures ranging from 80°C to 95°C during cooling [7]. Rapidly diminishing rice quality is the limiting factor for shelf life, with rice typically becoming unpalatable after 4 days from preparation. This is a significant problem for ready-to-eat cooked, chilled rice products, such as sushi and rice salads, commonly referred to as 'food-to-go'.

Rice-cooking methods, such as steaming, will not prevent quality issues or degradation due to the gelatinisation and subsequent starch retrogradation during storage [3, 8]. Hence, the application of rice quality improvers is required. The novel rice-improver trehalose dihydrate, or

simply trehalose, is used in similar and starch-based products [9].

Trehalose is a nonreducing disaccharide, first isolated from the ergot of rye. It is ubiquitous in nature, present in plants, prokaryotes, lower eukaryotes, fungi, insects and invertebrates. It possesses multiple functions such as preservative, antioxidant, flavour enhancer, desiccant/humectant, sweetener, cryopreservative/cryoprotectant and stabiliser [10–16]. Therefore, trehalose finds application in various foodstuffs, though additional functions continue to be discovered. Over 8000 applications of trehalose in foodstuffs are reported in the literature, with usage at concentrations ranging from 0.5% to 5% w/w [17, 18]. Such applications include pastries, bakery goods, breakfast cereals, frozen and dried products, chilled goods, meat, meat products and substitutes [13].

Trehalose was first approved in the UK in 1991 as a cryoprotectant at a maximum concentration of 5%. In 1995, trehalose was approved as a food additive in Japan. The FDA granted it 'generally regarded as safe' (GRAS) status in 2000. Hence, trehalose is safe for consumption [9]. Subsequently, Commission Decision 2001/721/EC authorised its sale as a novel food or ingredient in the EU. However, when using trehalose in foods, it must be declared in the ingredients list, followed by a prominently displayed statement, 'trehalose is a source of glucose', in a typeface at least as large as the ingredient list [19].

Currently, there are no maximum usage concentrations in food. Following the withdrawal of the UK from the EU, application of novel foods and ingredients is now controlled by the Novel Food Regulations, whereby only novel foods contained in Regulation (EC) 2017/2470, as amended by Regulation (EU) 2018/1023, are permitted by Article 6(1) of Regulation (EU) 2015/2283, in which trehalose is contained [20–23].

Trehalose may reduce the extent of desiccation in foods due to its ability to inhibit the hardening of gelatinised starch and retrogradation in starch systems more effectively than sucrose or glucose [18, 24]. The inhibition of retrogradation is proposed to involve interactions between trehalose and water molecules or between trehalose and molecular chains of starch polysaccharides in gelatinised starch, whereby it interferes with reassociation during storage [25]. Others propose that the higher number of equatorial hydroxyl groups of trehalose compared to other sugars results in increased sugar–starch interactions, thereby increasing the stability of amorphous starch regions and mitigating recrystallisation, particularly during chilled storage [26–28]. However, the extent of inhibition is dependent on the starch type, storage period and temperature, with these parameters also influencing the retrogradation rate [6].

Low-molecular-weight sugars, including glucose, fructose, sucrose and trehalose, penetrate starch granules and complex with amylose and amylopectin. Furthermore, they may increase the gelatinisation temperature (T_{gel}), thus inhibiting the initiation of retrogradation, possibly due to incomplete or partial starch gelatinisation. Amongst low-molecular-weight sugars, the ones that tend to have higher molecular weights, such as trehalose, are more effective [2, 29–31]. Although retrogradation is more prominent at higher T_{gel}, partial gelatinisation inhibits retrogradation [32].

A study reported enhanced moisture retention in cooked rice at concentrations of 3% by dry weight, alongside improved palatability and shelf life, without noticeably enhancing sweetness [10]. Similar work with identical concentrations demonstrated texture retention in cooked rice when stored at 20°C, and following freezing [9]. Trehalose has also been shown to inhibit retrogradation and enhance the softness of cooked rice when applied at concentrations of 5%–15% [2]. Another study reported that applying trehalose to cooked-refrigerated rice at 1%–3% w/w decreased retrogradation, albeit to a lesser extent than gellan gum applied at 0.1%–0.3% w/w. Interestingly, both additives were less effective in reducing retrogradation than ohmic heating, although this is not widely utilised within the food industry [33].

Previous research reported that applying trehalose at 2%–5% of the dry rice weight improved sensory characteristics compared to nontreated, sucrose- or glucose-containing rice [9, 34]. One study reported that 85% of participants preferred the flavour of trehalose over sucrose [35]. Hence, trehalose may be an effective alternative to sucrose in sugar-containing rice products. However, the removal of sucrose and its replacement with alternative sugars may alter the flavour profile of foods, although the impact of trehalose on flavour is similar to that of sucrose. The sweetness intensity of trehalose is lower than that of other sugars. Trehalose has a relative sweetness of 45% compared to sucrose at a concentration of 10% w/w, with sweetness intensity increasing as the concentration increases. However, it may produce more persistent and long-lasting sweetness than other sugars [16]. Contrarily, one study reported that large amounts of added trehalose yielded an 'unnatural sweetness' compared to conventionally used saccharides (including glucose, maltose and dextrin), which perceptibly altered the flavour of cooked plain rice [36].

Although there has been considerable interest in and various applications and functions of trehalose in foods in published research, few papers have involved the application of trehalose to cooked rice using industrial manufacturing equipment, nor have they investigated the application of trehalose as an acidified rice quality and shelf life improver. Therefore, this study is aimed at enhancing the shelf life of cooked rice by inhibiting quality degradation, thereby improving quality and palatability during later stages of shelf life, whilst applying trehalose dihydrate without compromising food safety.

2. Materials and Methods

2.1. Preparation of Rice Samples. Trehalose dihydrate (CAS6138-23-4, TREHA, Hayashibara) was provided by the Cornelius Group. Round grain Selenio variety white sushi rice (*Oryza sativa* L. subsp. *japonica*) was purchased from S&B Herba. Ellsey's Vinegar provided sushi seasoning. Rapeseed oil (Rapeseed Oil 1080, KTC Edibles) and sucrose (Caster Sugar, Silver Spoon) were also used. The total sugar content of the sushi seasoning was 36.7 g/100 g, equating to

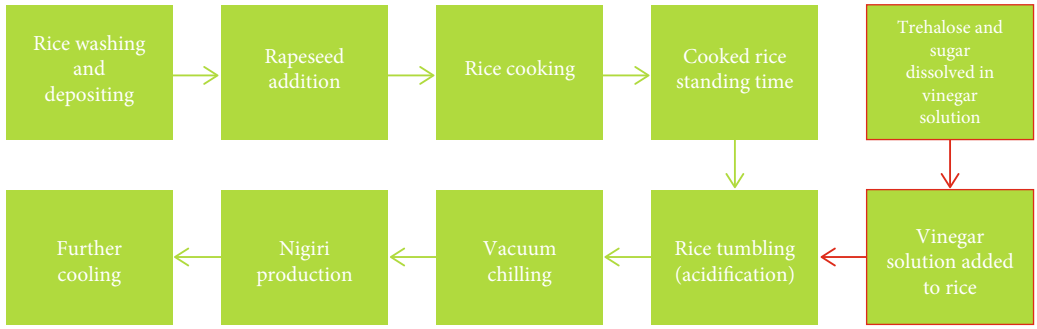


FIGURE 1: Sample preparation (steps outlined in red apply only to trehalose samples).

10.7% of dry rice weight. Most of this value is derived from the sucrose solution, with rice and spirit vinegar containing around 0.13 and 3.20 g of sugar per 100 g, respectively.

A commercial sushi manufacturer prepared the rice samples. Figure 1 outlines the general process, with steps outlined in red applying only to trehalose samples. Rice was washed using a batch rice washer (KP90KN, Kubota) on the wash setting '+1', corresponding to a 4-min wash with a P+06 water addition rate. After washing, the rice washers deposited 5.1 kg of washed rice and cooking water into net-lined steel rice-cooking pots. Fifty grammes of rapeseed oil was deposited into the pots, and the solution was briefly hand-agitated. The contained rice was covered with nets, and the pots were sealed with bespoke pot lids. Rice pots were transferred into automatic electric rice ovens (FRC162FA, Fujimak) and cooked for approximately 38 min to a core temperature greater than 90°C. Cooking times and temperatures were controlled automatically by the rice cooker. Cooked rice was allowed to stand for 20 min whilst covered. Subsequently, the rice and the nets were weighed before the rice was emptied into a food industry mixer (75GS-VSM Gentle Mixer, Glass).

Trehalose and sucrose were dissolved by hand agitation within a vinegar solution comprised of no-added-sugar sushi seasoning (a blend of spirit vinegar, rice vinegar, water and salt) with an acidity of 3.87% and potable water at 945 and 710 g, respectively (based on 5.1 kg of rice). This was evenly poured over cooked rice and then mixed at 6 RPM for 30 s in the vertical position, followed by 90 s in the horizontal position to achieve acidification. Rice was always tumbled within 45 min of the end of cook time. Following mixing, rice was deposited into trays, and the pH was checked to be between 3.40 and 4.20.

Acidified rice was cooled to 45°C–49°C using a vacuum chiller (R5-0255D, Busch). The cooled rice was reweighed in tared containers and formed into $23 \pm 1\ g$ blocks, known as 'nigiri', using a high-speed nigiri press (SMPM-155, Fujiseiki). Nigiri were trayed and cooled to a temperature lower than 5°C within 1 h of machining. Finished samples were stored at $4°C \pm 1°C$ for up to 8 days before further testing.

Initially, the following samples were prepared for the preference testing: 4TH5 (5% trehalose and 7.5% sucrose), 4TH7.5S5 (7.5% trehalose and 5% sucrose), 4TH7.5S7.5 (7.5% trehalose and 7.5% sugar), 4TH15 (15% trehalose and 5% sucrose) (all percentages are provided by dry rice weight) and two control samples (4CSF and C4). Control 4CSF contained no-added-sugar sushi seasoning, and control C4 comprised rice vinegar, spirit vinegar and salt. Both control samples contained sugar at a concentration of 10.7% of the dry rice weight.

Following numerous preliminary trials at varying trehalose concentrations, a recipe of 15% trehalose dihydrate blended with 5% sucrose (4TH15) by dry rice weight yielded the greatest favourability in the initial sensory tests implemented (flavour, grain softness, grain definition, stickiness and moistness) compared to other samples trialled, and therefore, this sample was chosen for further investigation. Figure 2 shows the mean scores obtained from the organoleptic testing.

2.2. pH Measurement. pH testing was conducted on rice blocks using a handheld pH metre (pHScan30, Oakton, Cole-Parmer Instrument Co., United Kingdom) with an accuracy and resolution of 0.01. Checks were completed by pushing the pH metre into hand-formed rice balls, thereby giving a reading of the rice surface pH.

2.3. a_w Measurement. Testing was conducted using an AquaLab water activity metre (Decagon Devices Inc., Pullman, Washington) for up to 7 days (pack plus [P+] 0, P+3, P+5 and P+7), as specified in BS ISO 21807:2004 [37]. Samples were stored at $4°C \pm 1°C$ throughout the testing period ($n = 3$).

2.4. Nutritional Analysis. Nutritional testing was conducted by a UKAS-accredited laboratory using a 150 g composite sample taken from the start, middle and end of the batch (Table 1). Total fat, saturated fatty acids, available carbohydrates, total sugar, total dietary fibre, protein, sodium, salt, ash and moisture contents were measured in triplicate. The energy content of the samples was calculated.

2.5. Texture Analysis. Texture analysis was conducted using a texture analyser (TA.XTplus, Stable Micro Systems, Godalming, United Kingdom) with a 500 N load cell. All analyses were conducted on formed oblong rice blocks weighing $23 \pm 1\ g$. Stored samples were maintained at $4°C \pm 1°C$ until the end of P+3, then at $8°C \pm 1°C$ to simulate customer storage conditions. Samples were tested on Days P+0, P+3, P+4, P+5, P+6 and P+7 ($n = 3$). The parameters measured and test conditions were as follows.

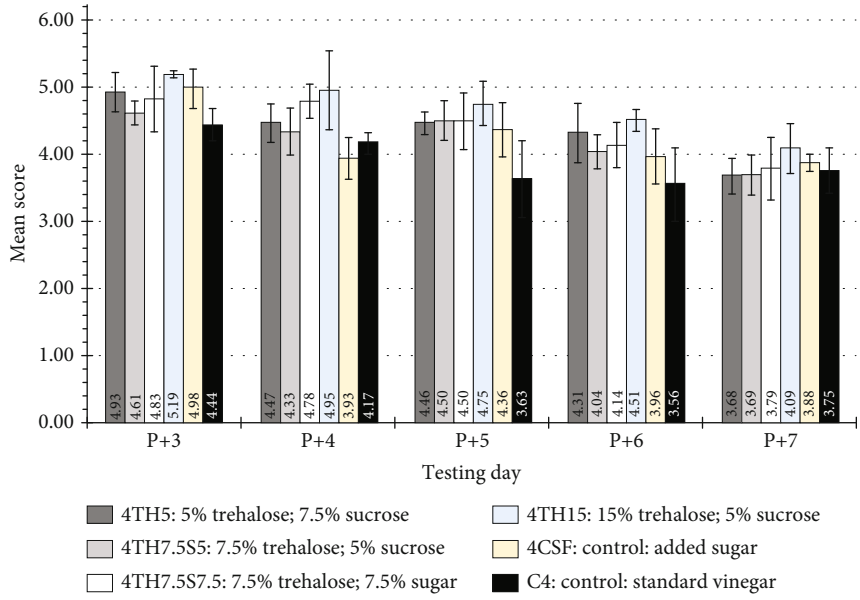


FIGURE 2: Mean scores from organoleptic assessment of rice formulated with varying concentrations of trehalose. The scores were derived from the degree of liking of flavour, grain softness, grain definition, stickiness and moistness. P stands for packaging, and the numbers following denote the days of storage after packaging.

TABLE 1: Nutritional analysis methods.

Component	Method
Energy (kcal/kJ)	Obtained by calculation
Total fat	Oven drying and pulsed nuclear magnetic resonance (NMR) (AM/C/1015)
Saturated fatty acids	Gas chromatography based on BS EN ISO 12966-2:2017 [38] (AM/C/107)
Available carbohydrates	Obtained by calculation
Total sugar	Ion-exchange chromatography (AM/C/1014)
Total dietary fibre	AOAC method No. 985.29
Protein	Dumas method (AM/C/224)
Sodium	Inductively coupled plasma optical emission spectrometry (ICP-OES) (AM/C/1002)
Ash	BS 4401: Part 1:1998 [39]
Moisture (loss on drying)	Oven drying and pulsed NMR

Hardness and stickiness were measured as the peak force and negative peak force, respectively. A 35-mm diameter aluminium cylinder (P/35) was used with the following test conditions applied: Test mode: compression, pretest speed: 0.50 mm/s, test speed: 0.50 mm/s, posttest speed: 10.00 mm/s, strain value: 90% and trigger force: 3.0 g.

Firmness was measured as the maximum force using Warner-Bratzler Compression Blade (HDP/BSW) with the following test conditions: Test mode: compression, pretest speed: 1.50 mm/s, test speed: 1.50 mm/s, posttest speed: 10.00 mm/s, distance: 40.00 mm and trigger force: 40 g.

The maximum shear force was measured using a Kramer Shear Cell 5-Blade Attachment (HDP/KS5) under the following test conditions: compression mode, a test speed of 3.00 mm/s and a distance of 20.00 mm.

2.6. Microbial Analysis

2.6.1. Spoilage Organisms. The samples were tested for yeast and moulds (ISO 4833-1:2013) [40] and total viable count (TVC) (ISO 21527-1:2008) [41] by a UKAS-accredited laboratory. Testing days were P+0, P+2, P+6 and P+8 ($n = 3$). Samples were stored at $4^{\circ}C \pm 1^{\circ}C$ until the end of P+3 and then at $8^{\circ}C \pm 1^{\circ}C$ until the end of testing, simulating consumer storage conditions.

2.6.2. Challenge Testing. The testing is aimed at determining whether trehalose application facilitates the growth and survival of pathogens of concern (*Bacillus cereus* and *Listeria monocytogenes*), thereby compromising food safety in acidified rice. Samples were stored at $4^{\circ}C \pm 1^{\circ}C$ until the end of P +3, followed by $8^{\circ}C \pm 1^{\circ}C$ until the end of testing.

Growth potential (δ) is the difference between $\log_{10}$ cfu/g values at the end of the test and $\log_{10}$ cfu/g values at the beginning of the test following inoculation [42, 43]. These values were obtained by taking the median of the $\log_{10}$ cfu/g concentration amongst the test units at the beginning and end of the study, respectively. The growth potential of the microorganisms in the samples was calculated using the equation given below [44]:

$$\text{Growth potential } (\delta) = \log_{10}\text{cfu/g}_{(\text{Day7})} - \log_{10}\text{cfu/g}_{(\text{Day0})}. \quad (1)$$

2.7. Sensory Analysis. During sensory testing, samples were stored in sealed containers at $4^{\circ}C \pm 1^{\circ}C$ from P+0 and then moved to $8^{\circ}C \pm 1^{\circ}C$ at the end of P+3 to simulate consumer

storage conditions. Samples were removed from refrigeration 30 min before consumption.

Panellists involved in sensory analysis were selected based on their experience and familiarity with rice quality and associated defects. They were aged between 21 and 65, with an even distribution of males and females. Before testing, the panellists were informed of the risk of intolerance to trehalose. Individuals with allergies, intolerances or aversions to trehalose or foods containing trehalose were excluded.

2.7.1. Preference Testing. Preference testing was conducted by ISO 4121:2003 [45] and ISO 6658:2017 [46], involving eight panellists. In line with ISO 4121:2003, a numerical hedonic scale was utilised based on the degree of 'liking' of a quality parameter (flavour, grain softness, grain definition, stickiness and moistness), as discussed in ISO 6658:2017. The hedonic scale was as follows: 1, dislike very much; 2, dislike moderately; 3, dislike slightly; 4, neither like nor dislike; 5, like slightly; 6, like moderately and 7, like very much. Any score below 4 was considered unacceptable. The same panellists were recruited for each stage of preference testing. Although this may be perceived as a limitation, it ensures that the obtained data is consistent and reliable [47, 48]. Samples scoring the highest in a defined parameter were considered the most preferable.

2.7.2. Triangle Testing. Forty-eight panellists were recruited, with the same panellists present throughout the testing period. Samples were assessed based on testing for differences, and the data were analysed according to ISO 4120:2021 [49]. Initially, testing was conducted on trehalose samples produced 1 day prior to the control samples, followed by a subsequent trial using trehalose samples produced 2 days before the control samples. Assessment was conducted on an individual basis over 5 days from P+3 to P+7.

Data analysis utilised an α-risk of 0.001; a difference at this level provides strong evidence of a perceptible difference. Confidence intervals were calculated using a critical value (z_α) of 2.33 to give a 99% confidence interval for the proportion of the population capable of distinguishing the samples, as described in the now superseded ISO 4120:2007 [50]. The presence of a difference during triangle testing was used to determine the potential for shelf life improvement and efficacy of trehalose dihydrate as a rice quality improver, with no difference inferring equivalent quality in the older samples.

2.8. Ethics Statement. All work contained herein was approved by the University of Lincoln's Ethics Board, under Review Reference 2021_6430.

2.9. Statistical Analysis. The data were analysed using Microsoft Excel 2019. All values were reported as mean ± standard deviation. The significance testing used the T.TEST function with a two-tailed distribution, assuming unequal variance. ANOVA tests have been used to determine the effect of sample type and storage time on the sensory and textural parameters. A p value < 0.05 was considered significant.

3. Results and Discussion

3.1. Initial Sensory Measurements (Preference Testing). Samples were individually assessed from P+3 to P+7 for flavour, grain softness and definition, stickiness and moistness. This is the shelf life at which the product would enter retailer stores (P+1: collected from the facility, P+2: picked up at the depot and P+3: delivery, chilled storage and sale from this point on). As such, only the retailable life is of interest to be studied.

Figure 2 outlines the preference testing results, where mean scores were derived from the degree of liking of flavour, grain softness, grain definition, stickiness and moistness. Individual scores of the initial sensory test are provided in Figure S1. During sample preparation, no processability issues were encountered, such as product sticking to the spindles of the high-speed nigiri press. Hence, it can be concluded that trehalose can be utilised as a rice quality-improving ingredient within the food industry up to concentrations of at least 15%.

Overall, the trehalose-containing samples scored more favourably than the control samples. The perceived crumbliness of the control samples increased more rapidly than that of the trehalose samples. This is likely linked to a reduction in stickiness and increased hardness associated with retrogradation and moisture migration, therefore indicating inhibition of retrogradation [51, 52]. However, organoleptically, the samples were not significantly different ($p > 0.05$), indicating that trehalose yielded a slight improvement in quality. Conversely, another study with a larger panel size reported a significant difference in sensory scoring after applying trehalose at 15% of dry rice weight, although this work utilised untrained panellists [2]. Nonetheless, this trial should be replicated with an increased number of experienced panellists to ascertain if trehalose would result in a quality improvement.

Sample 4TH15 scored most favourably and remained organoleptically acceptable until P+7; hence, it can be concluded that quality and shelf life improvements were achieved. However, on P+7, scores were not significantly different ($p > 0.05$). Therefore, it was advisable that P+6 would be the maximum achievable life. Also, 4TH15 generally scored highest on flavour, indicating an improved flavour profile. However, this was difficult to conclude from a small sensory panel ($n = 8$). Furthermore, no significant difference was present between the 4TH15 and the control samples.

Sample 4TH15 received the highest score for perceived moistness throughout testing (data not shown), and this difference was significantly different from both control samples on P+4 and P+5 ($p < 0.05$). This was a desirable attribute, and it could relate to the relationship between sweetness and perceived wetness, whereby perceived sweetness correlates with strong sugar–water hydrogen bonding [53], given that this sample contained the highest total sugar concentration.

4TH15 possessed preferential perceived softness throughout testing, significantly greater ($p = 0.039$) than control (C4) on P+6, which inferred an improvement of grain softness. However, a significant difference ($p < 0.05$) was generally absent. On P+7, all samples scored similarly on grain softness, thus affirming that P+6 was the maximum

TABLE 2: pH and a_w values of vinegar solutions and cooked-vinegared rice samples. P stands for packaging, and the numbers following denote the days of storage after packaging.

		15% trehalose + 5% sucrose (4TH15)	Control (C4)	*p* value
pH values				
	Vinegar solution	2.66 ± 0.16	2.42 ± 0.09	0.16
	Rice posttumbling	3.83 ± 0.27	3.88 ± 0.11	0.87
	Rice postvacuum chilling	4.06 ± 0.13	4.03 ± 0.11	0.89
Testing days	P+0	4.25 ± 0.03	4.26 ± 0.08	0.87
	P+3	4.31 ± 0.01	4.18 ± 0.05	0.06
	P+5	4.29 ± 0.01	4.19 ± 0.08	0.23
	P+7	4.32 ± 0.05	4.19 ± 0.05	0.06
a_w values				
Testing days	P+0	0.974 ± 0.010	0.972 ± 0.009	0.87
	P+3	0.959 ± 0.002	0.960 ± 0.001	0.55
	P+5	0.978 ± 0.000	0.975 ± 0.009	0.62
	P+7	0.973 ± 0.009	0.974 ± 0.009	0.95

attainable life. Nevertheless, all samples showed a decreasing trend for grain softness, flavour and stickiness. This may relate to an increased rate of retrogradation, as reported by others [2].

The remainder of this study will present the results obtained for sample 4TH15 (containing 15% trehalose and 5% sugar) and the control (C4).

3.2. pH and a_w Values. The pH values are presented in Table 2. The pH of the control sample decreased over shelf life, although not significantly ($p > 0.05$). Xue et al. [54] reported a reduced pH due to increased microbial counts and the fermentation of carbohydrates into acids. However, microbial growth in the current study was generally insignificant, as outlined in Section 3.5. Hence, the decrease in pH may result from the decomposition of starch into its constituent monosaccharides [55]. Conversely, the pH of the trehalose sample increased during storage, although this change was not statistically significant. Betoret et al. [56] reported a similarly increased pH of probiotic juices by adding 10% trehalose and *Lactobacillus salivarius* spp. *salivarius*. The higher growth reported is likely related to the acid stress adaptation of *L. salivarius*, which may utilise trehalose. Other organisms, including *Propionibacterium freudenreichii*, accumulate trehalose as part of their acid tolerance response, thereby increasing cell viability, acid resistance and subsequent outgrowth [57–59]. Bacteria can adapt to acidic environments by producing acidophilic proteins or by secreting compounds such as ammonia through the action of urease. Consequently, pH increases, thus enhancing microbial survival and proliferation [60].

The a_w of both samples decreased on P+3, with a subsequent increase on P+5 (Table 2). The a_w was comparable between the samples ($p \geq 0.05$). However, both samples had a_w values sufficient for the growth of pathogens of concern and their spores [61]. Similar work applying trehalose to bread at concentrations of 2%, 4% and 6% also reported a decreasing a_w in all samples, including the control, between P+0 and P+2, with no significant difference between the trehalose samples and the control [62]. Conversely, another study, which applied trehalose to bread at similar concentrations, reported increased moisture content and a_w values at higher concentrations [63]. Li et al. [64] reported a decrease in a_w of starch without trehalose as a function of storage time during refrigerated and ambient storage, similar to what was observed on P+3. Such a reduction may result from moisture loss during cooking, cooling and subsequent chilled storage. Increased starch and solid concentrations in cooked rice, resulting from moisture loss during storage, were found to decrease a_w [65]. Additionally, free, unbound water may bind with starch and other substances during storage, thus reducing the a_w [66].

3.3. Nutritional Profile. Reformulation with 15% trehalose and 5% sucrose resulted in significant changes to energy, sodium and moisture content ($p < 0.05$) when compared to the control (Table 3). Adding trehalose significantly increased the calculated total energy content (161 ± 2 kcal against 154 ± 0 kcal/100 g). This was somewhat expected, given the higher total sugar content of sample 4TH15. However, the nutritional analysis inaccurately suggested reduced sugar content in the trehalose-containing sample. This could relate to the absence of extreme hydrolysis conditions and an enzyme (trehalase) capable of hydrolysing trehalose into its glucose moieties [67, 68]. Additionally, other carbohydrate-hydrolytic enzymes present are incapable of hydrolysing trehalose [9]. Thus, trehalose-derived sugar was not determined. This may hinder the broader adoption of trehalose dihydrate as a food additive.

Ion-exchange chromatography (IEC) can be used to determine the total sugar content, including trehalose-derived sugars. One such example is the use of IEC with pulsed amperometric detection, which has a limit of quantification of 0.05 mg/L for trehalose [69]. Similarly, another

TABLE 3: Nutritional values per 100 g of sample.

	15% trehalose + 5% sucrose (4TH15)	Control (C4)	*p* value
Energy (kJ/kcal)	685 ± 8/161 ± 2	654 ± 1/154 ± 0	0.03*
Fat (g)	0.37 ± 0.09	0.47 ± 0.05	0.27
Of which saturates (g)	0.09 ± 0.02	0.10 ± 0.01	0.62
Available carbohydrates (g)	37.33 ± 0.17	34.50 ± 1.28	0.09
Total sugar (g)	2.00 ± 0.08	4.50 ± 0.22	<0.05*
Fibre (g)	< 0.50	< 0.50	> 0.05
Protein (g)	2.10 ± 0.04	2.97 ± 0.04	0.41
Salt (g)	0.29 ± 0.00	0.36 ± 0.00	0.02*
Sodium (g)	0.12 ± 0.00	0.14 ± 0.00	0.02*
Moisture (g)	59.83 ± 0.45	61.73 ± 0.45	0.02*
Ash (g)	0.23 ± 0.05	0.94 ± 0.05	0.35

*Statistical significance ($p \leq 0.05$).

study utilised high-performance anion-exchange chromatography coupled with pulsed amperometric detection for a rapid carbohydrate assay [70]. However, neither study involved foodstuffs; they analysed pharmaceuticals and rice fungus, respectively. Another technique is the anthrone or anthrone/sulphuric acid spectrophotometric method at 620 nm. However, this technique is prone to overestimating sugar as anthrone reacts with other contained sugars; although for total sugar assays, this should not be a significant issue [71–73]. Gas chromatography–mass spectrometry can also be used to quantify trehalose. However, this requires additional time-consuming processes that may introduce inadvertent errors. However, a highly sensitive liquid chromatography–tandem mass spectrometry capable of detecting and quantifying trehalose and trehalose-derived total sugar is proposed in the literature [73].

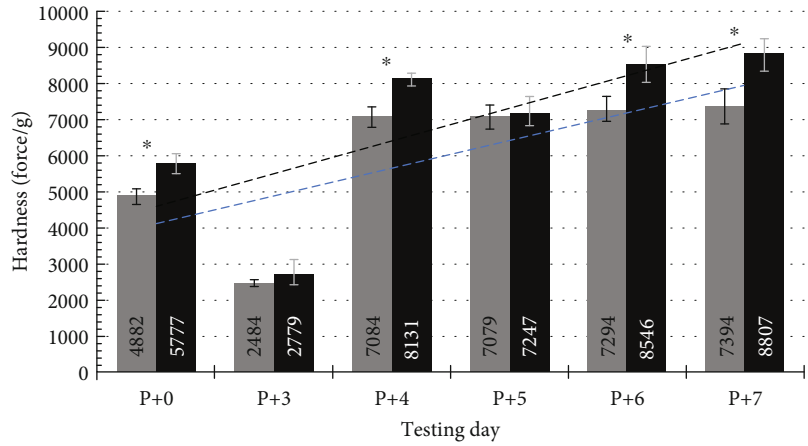
The trehalose-containing sample had significantly lower moisture and sodium (salt) content than the control. All samples were prepared from the same batch of rice. Hence, the apparent reductions were attributed to the addition of trehalose and sucrose, which exceeded the sucrose content of the control recipe.

3.4. Texture Measurements

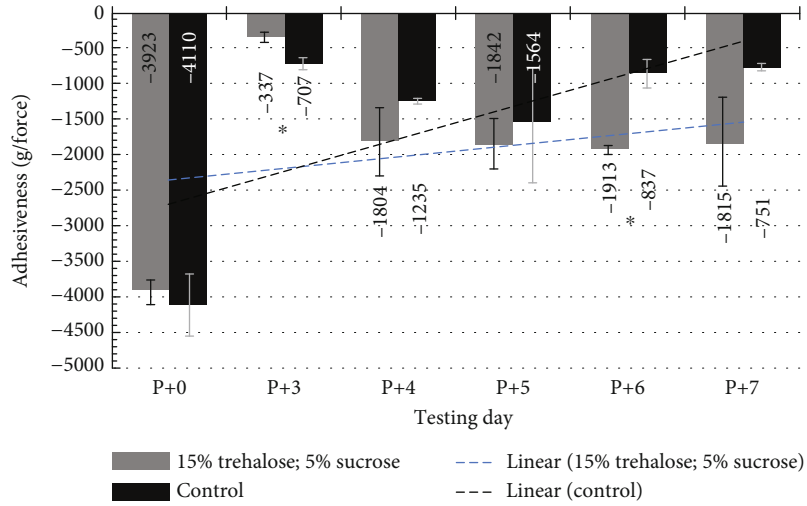
3.4.1. Hardness. Hardness generally increased in both samples during storage (Figure 3a). The trehalose sample had lower hardness values ($p \leq 0.05$). This was similar to previous work using 15% trehalose, which reported inhibited hardness during storage [2]. However, the study reported a decrease in initial hardness with increasing trehalose concentrations but a lower increase in hardness at lower trehalose concentrations. This was explained by the decreased inhibition of retrogradation at increasing sugar concentrations due to interactions and reactions of trehalose with other food matrix components. Other studies have reported a similar effect, with the 'softening effect' of trehalose being greater than that of different sugars, including glucose and sucrose [26, 27]. These studies documented that increasing concentrations of trehalose up to 5% resulted in a greater reduction of hardness.

The lower hardness of the trehalose sample may indicate the inhibition of retrogradation, with a known correlation between increased hardness and the extent of retrogradation [4]. However, hardness values appeared to stabilise after P+4. This may relate to the association of starch molecules in cooked rice grains. Although water is increasingly expelled, some water remains within grains, resulting in a gradual increase in hardness and eventually a plateau during storage [4, 74]. The apparent plateau in the trehalose sample after P+4 may indicate the inhibition of long-term retrogradation associated with amylopectin [75]. However, it may similarly relate to the increased storage temperature (8°C). Although 8°C is within the maximum retrogradation temperature, starch retrogrades more slowly than when stored at 4°C [28, 76]. Hence, the significant increase in hardness between P+0 and P+4 may relate to amylose's rapid, short-term retrogradation at refrigeration temperatures ($4^{\circ}C \pm 1^{\circ}C$). Similarly, the lesser increase in hardness from P+4 may relate to the slower recrystallisation of amylopectin and minor contribution to short-term starch retrogradation, with the apparent stability in the trehalose sample inferring the inhibition of amylopectin retrogradation compared to the control sample [6, 7, 75].

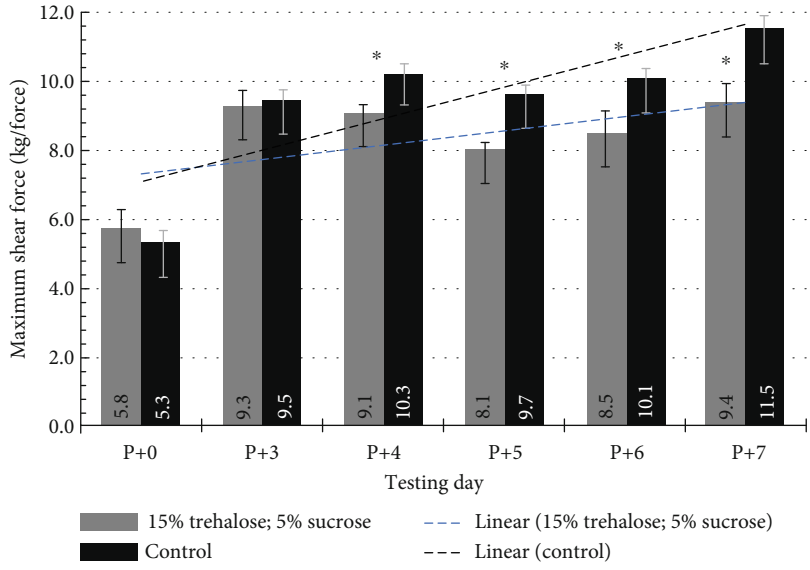
The lower hardness of the trehalose sample may indicate that trehalose competes with starch for water. Sugar–starch and sugar–water interactions exhibit an antiplasticising effect, thereby diluting the starch components required for retrogradation. Nonetheless, trehalose-containing foodstuffs are thought to absorb and retain water due to the high glass transition temperature (T_g) of trehalose, and the presence of trehalose is believed to regulate moisture content, thus delaying hardening [11, 27]. T_g is the temperature at which polymer substrates change from rigid glassy materials to soft, nonmelted materials. Molecular movement begins at T_g. Molecules below this temperature are immobile, producing glass-like, hard materials. Above these temperatures, materials become elastic and soft, allowing for molecular



(a)

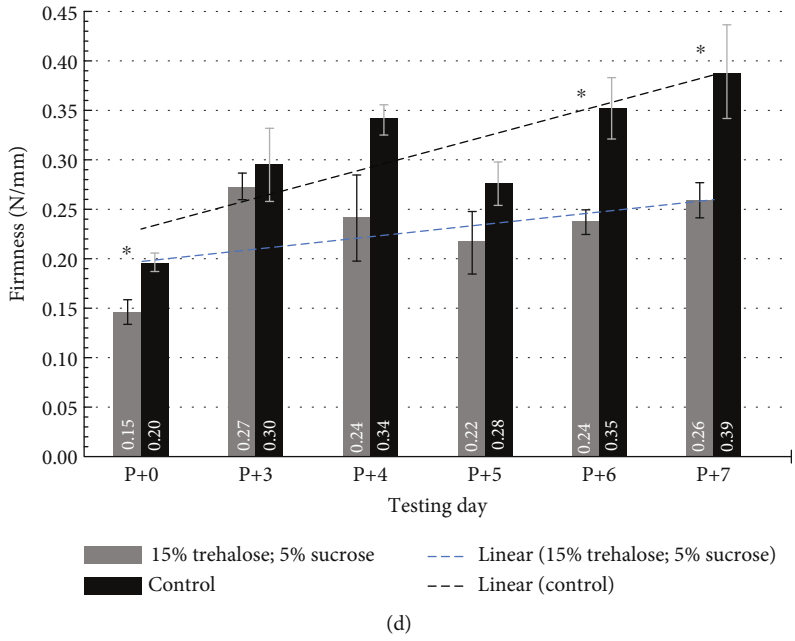


(b)



(c)

Figure 3: Continued.



(d)

FIGURE 3: Texture measurements (a) hardness, (b) adhesiveness, (c) maximum shear force and (d) firmness. P stands for packaging, and the numbers following denote the days of storage after packaging. Asterisk (∗) denotes significant differences between the trehalose and control samples within the same testing day ($p \leq 0.05$).

movement [77]. This strongly suggested that a shelf life improvement was possible.

3.4.2. Adhesiveness. The high initial adhesiveness is typical of freshly cooked, low-amylose rice used for sushi [78]. In the current study, a lower initial adhesiveness was observed in trehalose-containing samples compared to the control samples, although this difference was not statistically significant (Figure 3b). Another study [34] reported a similar reduction in adhesiveness when trehalose was applied at a concentration of 5%. Adhesiveness decreased rapidly during storage at 5°C, with the control sample retaining a higher adhesiveness during the 180-min test. However, this short testing time was deemed insufficient to demonstrate changes in adhesiveness during refrigerated storage.

A decrease in adhesiveness over shelf life was similarly reported in the literature, attributed to an increased rate of retrogradation [4]. A reduction in adhesiveness was observed in the trehalose sample after P+0, with adhesiveness remaining somewhat constant after P+4. This correlation was similarly observed in hardness. Compared to the control sample, a significant decrease in adhesiveness was observed over time, despite the initial higher adhesiveness. Akin to the hardness changes, a considerable reduction in adhesiveness was seen on P+3 in both samples. Others have similarly reported this due to moisture loss, although a subsequent increase in adhesiveness was not observed [79].

Increasing water content may reduce the interactions between gelatinised starch granules, particularly amylopectin, thus lowering adhesiveness [80]. Interactions may be further reduced by trehalose–starch and trehalose–water interactions, thus alluding to the initially lower adhesiveness of the trehalose sample [2, 27]. However, evidence of trehalose–water interactions is contradictory [11, 81]. The addition of sugars, including sucrose and maltose, maintains the adhesiveness of cooked rice stored at 4°C [80]. Although the influence of trehalose was not reported previously, it may be concluded from Figure 3b that trehalose possessed a greater ability to maintain adhesiveness than the sucrose present in the vinegar. The fact that the adhesiveness values remained unchanged throughout storage implied that quality attributes were preserved, suggesting that a shelf life extension was possible [82, 83]. The apparent stabilisation of adhesiveness after P+4 in the trehalose sample may indicate stabilisation of amylopectin interactions responsible for stickiness [84]. This may have resulted from the notably higher hydrogen bond strength provided by trehalose compared to that of sucrose in the control sample, as well as the proposed inhibition of amylopectin retrogradation, which explains the lower adhesiveness in the control sample following P+4 [75, 85].

3.4.3. Maximum Shear Force. Initially, the trehalose sample registered a higher maximum shear force (Figure 3c). However, neither sample differed on P+0 and P+3 ($p \geq 0.05$). Maximum shear force increased faster in the control compared to the trehalose sample, with samples significantly different to each other from P+4 until P+7 ($p \leq 0.05$). Another study reported a similarly increasing shear force in nigiri stored at 4°C for 8 days [86]. The study reported a gradual increase in shear force from P+1 to P+4, followed by significant increases after P+4. The authors proposed a positive correlation between shear force and hardness, similar to what was observed in the current study. However, increased shear force necessitates increased force for mastication, thus negatively affecting desirability. The association of increased

TABLE 4: Total viable count colony-forming units per gramme of the samples and challenge testing during shelf life.

		Testing day	15% trehalose + 5% sucrose (4TH15)	Control (C4)	*p* value
Total viable count (cfu/g) ($n = 3$)		P+0[1]	< 10	< 10	N/A
		P+2[1]	220 ± 33	110 ± 54	0.08
		P+4[2]	70 ± 28	40 ± 27	0.30
		P+6[2]	<10 ± 0	90 ± 42	0.12
		P+8[2]	<10 ± 0	70 ± 14	0.03*
Challenge testing during shelf life (log_{10} cfu/g) ($n = 3$)	*Bacillus cereus*	P+0[1]	2.70 ± 0.03	2.41 ± 0.09	<0.05*
		P+3[1,3]	2.60 ± 0.08	2.57 ± 0.29	0.49
		P+5[2]	2.38 ± 0.14	2.08 ± 0.28	0.46
		P+7[2]	2.08 ± 0.13	2.08 ± 0.16	0.93
		Growth potential (δ $\log_{10}$ cfu/g)[4]	−0.62	−0.34	
	Listeria monocytogenes	P+0[1]	7.20 ± 0.06	7.45 ± 0.03	0.15
		P+3[1,3]	7.20 ± 0.06	7.32 ± 0.15	0.48
		P+5[2]	6.65 ± 0.03	7.38 ± 0.72	0.10
		P+7[2]	6.71 ± 0.19	6.95 ± 0.11	0.16
		Growth potential (δ $\log_{10}$ cfu/g)[4]	−0.49	−0.50	

[1]Stored at 4°C ± 1°C.
[2]Moved to 8°C ± 1°C at the end of the day.
[3]Stored at 8°C ± 1°C.
[4]Calculated value.
*Statistical significance ($p \leq 0.05$).

shear force with hardness may suggest a relationship between increased shear force and a higher rate of retrogradation. Unfortunately, this has not been cited in current literature. The significantly lower shear force observed after P+4 may suggest an improved quality of trehalose-containing nigiri and could infer an extension of shelf life.

3.4.4. Firmness. The firmness of both samples generally increased during storage (Figure 3d). However, the trehalose sample registered a lesser increase and lower overall firmness. Additionally, firmness remained somewhat stable in the trehalose sample between P+4 and P+7 ($p \geq 0.05$). The latter may relate to the possible interactions and subsequent stabilisation of starch and water matrices by trehalose, thus inhibiting retrogradation-associated firmness [24, 25]. Akin to hardness, decreased firmness is observed with P+5.

Similar work [27] reported significant inhibition of firming following trehalose application due to the influence of trehalose on nucleation and increased T_g. Hence, trehalose was proposed as an effective antistaling agent. A similar influence could be possible, given the lower firmness values of the trehalose sample and how these values remained relatively stable after P+4. Another study reported a lower, albeit insignificant, firmness in rice cakes following trehalose application. However, this negatively affected the quality and acceptability [87].

Rice firmness influences consumer preference, with overall quality and favourability negatively correlated with increasing firmness [88, 89]. Given the higher overall preference scores of the trehalose sample, this is likely reflected in Figure 2. Hence, the lower firmness may correlate with improved quality, implying that a shelf life extension is plausible.

3.5. Microbial Growth. Table 4 outlines the enumerated growth during shelf life. No yeast or mould growth was determined during testing; therefore, only the TVC data is shown. Both samples demonstrated negligible growth on P+0. However, the growth in the trehalose sample was twice that of the control on P+2. Then, the TVC rapidly declined in the trehalose sample after P+2 to negligible levels on P+6 and P+8. Although TVC was lower in the control until P+4, growth was subsequently higher on P +6 and P+8. Despite residing in the temperature danger zone, storage at 8°C did not encourage growth in the trehalose sample. However, increased growth in the control was observed ($p > 0.05$), which may be related to the higher storage temperature [90].

Although others reported a shelf life of 5–7 days for cooked, refrigerated rice before spoilage became evident; this was not observed in the current study, as the enumerated growth was insufficient to yield perceptible microbial spoilage. Typically, this occurs at TVC levels greater than 10^7 cfu/g [91]. The absence of spoilage was likely related to low pH (Table 1), with a pH < 4.6 purportedly sufficient to achieve a good microbial status in cooked rice [92]. Due to its low pH, cooked and acidified rice is a poor substrate for microbial growth. However, it contains fermentable carbohydrates that may stimulate the growth of lactic acid bacteria

(LAB), potentially causing spoilage, although the levels in acidified rice have remained low in the literature [93].

LAB species, including *Lactococcus lactis*, are present in rice vinegars used in sushi seasonings. Hence, the growth observed in Table 4 may be related to LAB growth, as reported in similar work, albeit at insignificant levels [93, 94]. Trehalose significantly enhances the growth of bacteriocin-producing LAB compared to other sugars, which may account for the higher TVC levels observed in the trehalose sample on P+2. Similarly, the rapid decline in the TVC of the trehalose sample could indicate the presence of bactericidal activity [59]. However, it may equally indicate the endogenous (death) phase of the contained microorganisms due to the presence of metabolic waste, toxic materials and depleted nutrients [95]. This may similarly relate to the increased bactericidal activity associated with trehalose, given the increased growth observed and association with increased bacteriocin production during the exponential phase. Nevertheless, this declines during the death phase. Others reported a death phase of bacteriocin-producing LAB at 36–72 h [59, 96, 97], which may coincide with the reduced growth observed after P+2 in the trehalose sample. The application of trehalose as a rice quality improver at an increased total sugar concentration did not compromise microbial stability. Hence, microbial spoilage was unlikely to occur. Thus, shelf life could be increased without negatively impacting quality.

Table 4 shows the results of the challenge testing with samples inoculated with *B. cereus* and *L. monocytogenes*. Although initially significantly different between the samples, *the B. cereus* populations in both samples continued to decrease, with no significant difference later present. The trehalose sample demonstrated a lower growth potential (δ), despite possessing a higher pH and high a_w.

B. cereus causes emetic and diarrhoeal illness depending on the context in which it grows and is ingested. Emetic illness occurs due to the growth of pathogens within a foodstuff where conditions (pH, a_w, and temperature) are favourable, or by attaching itself to the target cells of the host. Outbreaks are associated with the ingestion of implicated foods containing concentrations of toxins ranging from 10^5 to 10^8 cfu/g, which contain significant amounts of heat-stable emetic toxins responsible for emesis [98]. Conversely, diarrhoeal illness is caused by a large number of ingested vegetative cells or spores that grow in the small intestine (so-called enterotoxins). Infective doses are associated with 10^5–9.5^8 cfu/g. However, emetic and diarrheal outbreaks have been reported with levels of less than 10^3 cfu/g and as low as 400 cfu/g [99].

Despite the role of trehalose as a microbial stress metabolite and the ability of *B. cereus* to utilise trehalose as a substrate, the presence of trehalose does not appear to have yielded a protective effect to *B. cereus* nor contributed to its acid tolerance [100, 101]. Given the optimal pH growth range of 4.5–9.5 of *B. cereus*, the absence of increasing populations in both samples was likely related to the low pH of the samples [98, 102]. Both samples had a pH less than 4.3 until P+7; the trehalose sample reached a pH of 4.32 (Table 2). Vegetative *B. cereus* cells are destroyed at a pH < 4.3. Hence, it was likely that vegetative cells were killed in both samples. However, spores, which may germinate and cause diarrheal poisoning, remain viable within a pH range of 1–9 [98].

L. monocytogenes is commonly associated with deaths from foodborne pathogens, particularly amongst vulnerable populations and those with compromised immune systems. It is capable of surviving at low temperatures (1°C–45°C), making it a pathogen of concern for ready-to-eat chilled foods, such as chilled cooked sushi rice [103]. According to Regulation (EC) No. 2073/2005, the concentration of *L. monocytogenes* in ready-to-eat foods shall not exceed 100 cfu/g during the product's shelf life [104]. However, a single viable cell can multiply to infectious levels within stored foods [105]. As such, any growth of the organism is considered problematic, with a calculated growth potential (δ) greater than 0.5 $\log_{10}$ cfu/g, inferring a foodstuff that supports pathogenic growth [43].

In general, both samples demonstrated reduced populations of *L. monocytogenes*. Although not significantly different, the control showed a lower growth potential than the trehalose sample. The trehalose and control samples demonstrated increased *L. monocytogenes* populations on P+7 and P+5, respectively. Although the increases were insignificant ($p \geq 0.05$), they may demonstrate acid adaptation in *L. monocytogenes*. The increases may be associated with the increased storage temperature on P+3, given the known efficacy reduction of pH as a product hurdle at increasing temperatures [106]. The δ of 0.06 in the trehalose and control samples between P+5 and P+7 and P+3 and P+5, respectively, suggested that growth was not supported. Both samples demonstrated an overall δ that was less than 0.5 $\log_{10}$ cfu/g. Therefore, it could be concluded that neither sample supported the growth of *L. monocytogenes* [43]. It can be suggested that applying trehalose as a rice improver would not pose a risk to food safety or legality concerning *L. monocytogenes*.

3.6. Sensory Tests to Determine Shelf Life Extension (Triangle Testing). Triangle testing was used to assess the possibility of extending shelf life by 1 and 2 days. The proportion of correct responses (pc) demonstrated an upward trend in the 1-day shelf life extension test (Figure 4). This suggested that the difference between the samples became more pronounced as the shelf life progressed. However, a perceptible difference was only present on P+5/6. Given the proportion of distinguishers (pd), it was believed that 32.1% of the panellists could perceive a difference on this day. However, a perceptible difference was not typically present. Thus, the 1-day older trehalose sample likely possessed a similar quality to the fresher control sample. It can be concluded that 1 day of additional life was attainable, suggesting that trehalose has the potential to be applied as a shelf life improver for rice.

In contrast to the 1-day triangle test, a declining pc trend was observed when testing for a 2-day life extension (Figure 5). This suggested that no perceivable difference existed between the two samples, which could indicate that the quality was very similar, despite the trehalose sample being 2 days older. This was underpinned by the very low pd values and the upper confidence limit present. On P+4/6, the 3.6% pd suggests a minimal difference between the samples. Hence, it

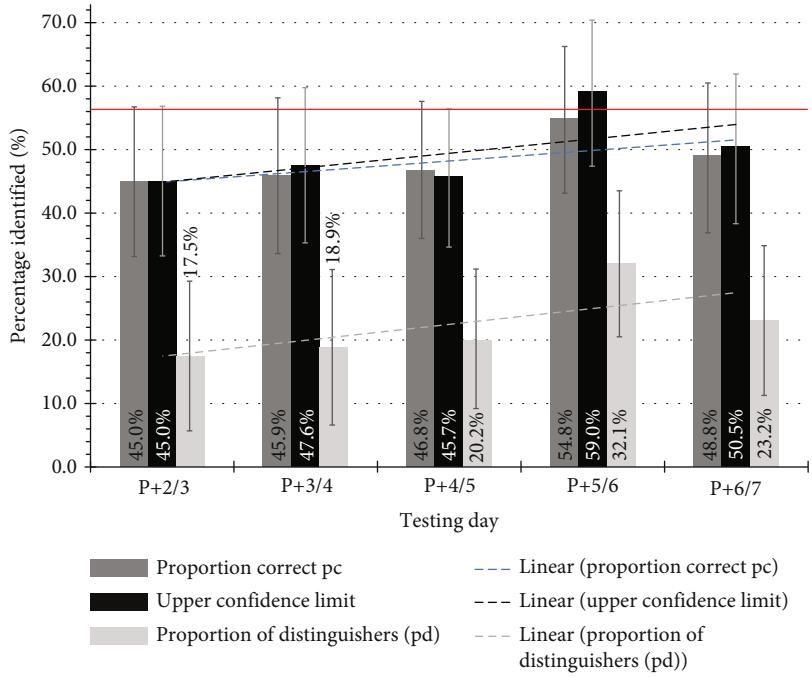


Figure 4: Results from triangle testing to ascertain the potential for a 1-day shelf life extension. Trehalose samples were compared to a control sample that was 1 day fresher than the trehalose sample. The correct identification of 56.3% of the odd samples was the perceptible difference threshold, represented by the red horizontal line. P stands for packaging, and the numbers following denote the days of storage after packaging.

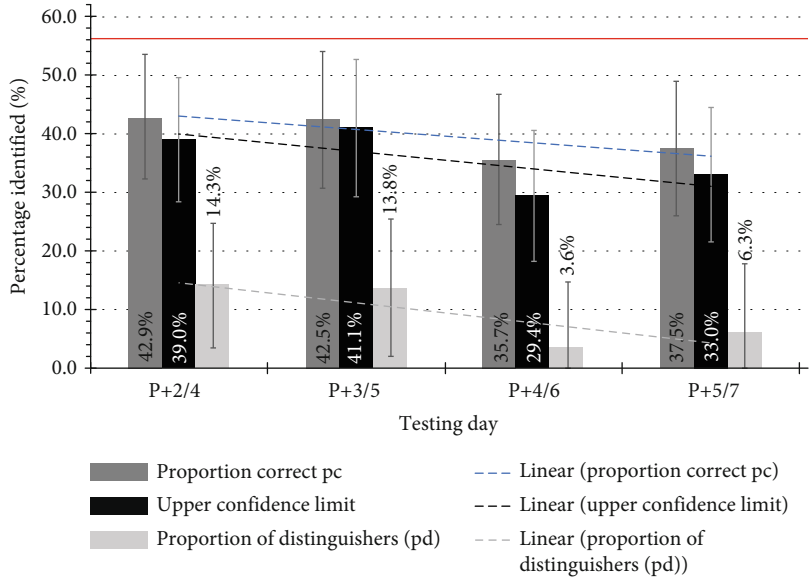


Figure 5: Results from triangle testing to ascertain the potential for a 2-day shelf life extension. Trehalose samples were compared to a control sample, 2 days fresher than the trehalose sample. The correct identification of 56.3% of the odd samples was the perceptible difference threshold, represented by the red horizontal line. P stands for packaging, and the numbers following denote the days of storage after packaging.

could be proposed that 2 days of additional life were possible. However, based on the preference test results, an extension beyond 2 days was impossible due to the poor preference scores in all samples. Additionally, the organoleptic quality of the nonrice sushi components may have become the limiting factor for shelf life extension, with microbial stability maintained for up to 8 days after packing. However, this is mainly dependent on the exact product formulation.

4. Conclusion

The application of trehalose dihydrate and sucrose at 15% and 5%, respectively, improved the perceived quality of cooked sushi rice compared to conventionally prepared samples, with a notably higher preference over a 7-day testing period. No ascertainable difference was found when comparing 2-day older trehalose-containing samples to control samples over shelf life, and this suggested that quality degradation was inhibited. Therefore, trehalose proved suitable for enhancing the shelf life and quality of cooked, acidified rice. This was further demonstrated through texture analysis of relevant quality parameters, with slower increases in hardness and firmness and improved retention of stickiness (adhesiveness) during storage compared to the control sample. The shear force, hardness and firmness of the trehalose sample remained lower than those of the control, indicating minimisation of quality degradation due to the presence of trehalose. However, the attainment of improvements came at the cost of significantly increased total sugar content. No yeast and mould growth was detected during shelf life testing, with TVC levels remaining insufficient to yield perceivable spoilage. Hence, applying trehalose and increased total sugar content did not negate microbial stability during storage, nor did it produce a significant difference in microbial counts compared to the control sample. The growth of *B. cereus* and *L. monocytogenes* was not supported. Hence, applying trehalose as a rice improver does not pose a detriment to food safety.

This work should be replicated with a larger organoleptic panel to increase the accuracy of the findings, thereby ascertaining with greater precision the presence and significance of a perceivable quality improvement. It would also be worthwhile expanding this work to other rice cultivars, notably different varieties of *Oryza sativa* L. subsp. *japonica*, given their current application as sushi rice, but also to long-grain *indica* and broad-grain *javanica*. The behaviour of trehalose–starch interactions and retrogradation enthalpy analysis (by differential scanning calorimetry) should also be investigated further, particularly since recent literature has not extensively explored these topics. Furthermore, there is an absence of studies focusing on such interactions where vinegars and other acidic conditions are present.

Data Availability Statement

Research data are not shared.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualisation: D.B.; formal analysis: D.B.; investigation: D.B.; methodology: D.B. and A.A.T.; supervision: A.A.T.; validation: A.A.T.; writing—original draft: D.B. and A.A.T.; writing—review and editing: A.A.T. and D.B.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. *(Supporting Information)* Figure S1: The individual scores obtained for flavour, grain softness, grain definition, stickiness and moistness in the initial sensory test.

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