Research Article

Microbial Safety of Low Water Activity Foods: Study of Simulated and Durban Household Samples

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Sixty household low water activity foods were examined and a simulative study was conducted in a high sugar, low a_w almond and macadamia butter to determine the survival of *Bacillus cereus* and *Staphylococcus aureus* ATCC 25923. Results obtained from 60 low a_w samples collected at household level had some significant differences ($P \leq 0,05$) within food categories amongst the various tests. Spices had the highest number of aerobic bacteria, aerobic spore-formers, anaerobic spore-formers, and *S. aureus*. Mean aerobic colony counts for nuts and spices were 2.30 log CFU/g and 4.40 log CFU/g, respectively. Pathogens such as *Escherichia coli* and *Cronobacter sakazakii* were present in nuts, whilst *Salmonella* spp. was present in chocolates. This implies that certain low a_w foods may present a public health risk. In the simulative study, temperature and high sucrose concentrations played a significant role in the survival of *B. cereus* and *S. aureus* ATCC 25923. *B. cereus* was found to be more osmotolerant at both reduced and elevated temperatures (18°C and 25°C) in the 12% sucrose sample in both butters, whilst *S. aureus* ATCC 25923 seemed to grow better in sucrose-free samples at both temperatures in both butters. This implies that certain low a_w foods may present a public health risk. Also, *B. cereus*, being a spore-forming bacterium, can be osmotolerant at both reduced and elevated temperatures.

1. Introduction

Food safety, is a global concern with the developing countries being the worst affected. Recently over the years, a number of incidences relating to outbreaks caused by the presence of food-borne pathogens in low water activity (a_w) foods have increased [1]. According to Byrd-Bredbenner et al. [2], households were ranked as the most common setting and primary location in which food-borne illnesses took place in the United States. This has become a serious problem not only for food industries but also for the final consumer consuming these products.

These increased outbreaks have therefore sparked the interest and urgency to investigate and understand the possible causes of the presence of food-borne pathogens in low a_w foods. Low a_w foods that have drawn considerable attention to the food industries, research communities, and the public are those of spices, dry nuts, chocolates, infant and adult cereals, and milk powder, being the most common amongst many other low a_w foods [3].

Low a_w foods are derived from high moisture foods which are often subjected to dehydration or desiccation processes to achieve this, or they could be naturally low in moisture. Furthermore, the water content of such foods could also be achieved by adding solutes (salt or sugar) or freezing to control the quality of the final product. a_w present in foods is a vital element in predicting the stability and safety of a food product with respect to the rate at which deterioration reactions occur [4].

Foods with low a_w contributes to the extended shelf life of the food product which is often desirable to the consumer. However, over the years, this has led to consumer perceptions that such low a_w foods were not of concern from a microbiological or food safety perspective and were safe for human consumption [2].

The main reason for this perception was that microorganisms were not able to thrive and proliferate in dry conditions. However, many studies done by researchers in literature have found that microorganisms have the capability of surviving in low a_w foods [1, 5]. The increased number of food-borne

related illness outbreaks recently, associated with low a_w foods, has portrayed that even though microorganisms cannot proliferate in such foods, there is a likelihood for them to be persistent for extended periods of time [1, 5, 6]. Depending on the type of microorganism, they can cause illness even with their low infectious dose or subsequent temperature abuse that permits the growth of these microorganisms [5]. Furthermore, literature has also demonstrated that Gram-negative bacteria exhibited more sensitive behaviour to low a_w than Gram-positive bacteria. The minimum a_w for all microbial growth is 0.60 and spoilage of foods would not be of a microbiological nature below this value [7].

The main pathogens of concern in literature were those of *Salmonella* spp., *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Cronobacter sakazakii*, *Staphylococcus aureus*, and *Clostridium botulinum* [1, 8]. A crucial step that is often omitted is an additional heating step which has the ability to control or kill potentially contaminated food-borne bacteria. Consumers often consume such foods without additional heating which is the possible cause for food-borne illness outbreaks.

Although food-borne diseases and illnesses have been on the increase in recent times, they mainly have been reported in the developed countries where food safety and quality are a priority. Rarely are such cases reported in Africa or in other developing countries and limited research has been done in Africa to combat this problem. This could be due to a lack of financial resources to conduct such research or a lack of knowledge amongst these developing communities about food safety in general.

The purpose of this study is to determine the prevalence of food-borne pathogens in household samples and to conduct a simulative study in low a_w , high sugar almond and macadamia butters. The samples collected were spices, milk chocolates, nuts, milk powder, and infant and adult cereals. The pathogens of concern in this study are those mentioned above. Since fungal growth has been widely studied in low a_w foods, this study aims to focus on the bacterial growth in such foods. Furthermore, limited research has been done in Africa in this regard and, therefore, the purpose of this research is also to enlighten African communities about food safety of low a_w foods.

2. Materials and Methods

2.1. Collection and Storage of Samples. For the first objective, commercial samples of six different categories were collected from ten Durban homes using sterile Ldpe Ziplock bags and stored at -18°C to prevent the growth of bacteria. At the time of collection, the samples were collected at 25°C . The following samples collected were as follows:

- (1) Adult cereal
- (2) Infant cereal
- (3) Milk powder
- (4) Spices
- (5) Nuts
- (6) Milk chocolate

For the second objective, sugar, almond, and macadamia butters were purchased from a retail food store to conduct the simulative study. These samples were stored at room temperature after the test organisms (*B. cereus* and *S. aureus*) have been confirmed to be absent in them.

2.2. Bacterial Analysis of the Samples. All the sixty samples collected were examined for the presence of *L. monocytogenes*, *S. aureus*, *Salmonella* spp., *E. coli* O157:H7, *C. sakazakii*, and *C. botulinum* using conventional methods. Before microbial analysis was conducted, a_w of each sample was checked using a Novasina AG Labswift a_w meter. Two strains were used for the simulative study on almond and macadamia butters. *S. aureus* ATCC 25923 was purchased from Anatech and a laboratory strain of *B. cereus* was used individually to inoculate the butters to determine its survival over a period of time in a certain sugar concentration.

2.2.1. Conventional Methods

***C. sakazakii*.** 10 ml of the macerated solution was added to 90 ml of buffered peptone water (BPW) and was incubated at 37°C for 24 hours after which 1 ml was introduced into Lauryl Sulphate Tryptose Broth and incubated at 37°C for 24 hours. After incubation, a loopful was streaked onto *Enterobacter sakazakii* Agar plates and incubated at 44°C for 24 hours according to the method by Nokwanda and Ijabadeniyi [9].

***E. coli*.** A modified method by MFHPB [10] was used for this study where one gram of food sample was inoculated into 10 mL double strength of Lauryl Sulphate Tryptose (LST) Broth and incubated for 24 hours. After 24 hours, a 1 ml aliquot was transferred into 10 ml of Brilliant Green Lactose Broth (BGLB) and incubated at 35°C for 24 hours. After 24 hours, 1 ml aliquot was transferred into *Escherichia coli* (EC) Broth tubes in a water bath at 45°C . This was transferred into Eosin Methylene Blue (EMB) Agar, which was incubated in a water bath at 45°C . After 24 hours, EMB plate was incubated at 35°C for 24 hours.

***S. aureus*.** A dilution series was done on all samples. They were prepared using BPW. 0.1 ml of the dilution was thereafter spread-plated onto Baird-Parker Agar with egg-yolk tellurite emulsion and incubated at 35°C for 24 hours according to the method by ISO [11].

***Salmonella*.** The method by SABS [12] was used for this experiment. 25 g of the food sample was preenriched with 225 ml of BPW and was incubated at 35°C for 16 to 20 hours (preenriched sample). 0.1 ml of the preenriched sample was then transferred into a test tube containing 10 ml of Rappaport-Vassiliadis Enrichment Broth (RVB) and another 10 ml of preenriched medium was transferred into a bottle containing 100 ml of sterile Selenite Cystine Medium (SCM). RVB was incubated at 42°C for 24 hours and SCM at 35°C for 48 hours. The culture obtained from RVB after incubation for 24 hours and the culture obtained from SCM after incubation for 48 hours were then inoculated onto the surface of *Salmonella* HiCrome Agar and XLD Agar to obtain

isolated colonies. All of these plates were incubated at 35°C for 24 hours and thereafter examined for growth.

L. monocytogenes. The method by Ijabadeniyi and Naidoo [13] was used for this experiment. Half-strength and full-strength Fraser Listeria Selective Enrichment (FLSE) Broth were prepared as stipulated by the manufacturer. 1 ml of the test sample was inoculated into 9 ml of half-strength FLSE and incubated at 35°C for 24 hours. 0,1 ml of the culture obtained in the previous step was then inoculated into a test tube containing 10 ml of full-strength FLSE and incubated at 35°C for 48 hours. The culture obtained from the half-strength FLSE incubated for 24 hours was streaked onto Oxford Listeria Selective Agar to observe growth of colonies and incubated microaerobically at 35°C for 24 hours. The plates were reincubated if growth was slight or if no colonies were observed for a further 24 hours. The same procedure was followed for the culture obtained from the full-strength FLSE.

Aerobic and Anaerobic Spore-Formers Count. The test sample was prepared by adding 25 g of sample to 225 ml of BPW. 20 ml of this 10^{-1} dilution was then pipetted into two test tubes, whereby one test tube served as a control. These two test tubes were then placed into a water bath with a thermometer placed in the control test tube. These test tubes were held at 75°C for 20 minutes. A dilution series was done and thereafter 0.1 ml of the sample will be pipetted into Petri dishes and pour-plated with Trypticase Soy Agar. Both aerobic and anaerobic plates will be incubated at 35°C for 48 hours. The anaerobic plates will be placed into anaerobic jars with Anaerocult according to the method of MFLP [14].

Aerobic Colony Count. The test sample was prepared in the same way that they were prepared for aerobic and anaerobic spore-formers count. A tenfold dilution series was done in duplicate. 0,1 ml of the dilutions was spread-plated onto Nutrient Agar and incubated for 37°C for 24 hours according to the method of ISO [15].

2.2.2. Simulative Study

Preparation of Inoculum. A modified method by Nummer et al. [16] was used. Two strains were used for this simulative study: a laboratory strain of *B. cereus* and *S. aureus* ATCC 25923. *B. cereus* cells were received as a lawn of cells on TSA and *S. aureus* ATCC 25923, which was received in the form of a swab, was swabbed directly onto Baird-Parker Agar. Baird-Parker Agar plates were incubated at 37°C for 24 hours. After incubation, the cells from the respective plates were washed with 5 ml nutrient broth and were transferred into 15 ml centrifuge tubes. A pellet was obtained by centrifugation at 2200g for 10 minutes. The pellets were then resuspended into 1 ml nutrient broth and this was used to inoculate the butters.

Inoculation of the Butters. Almond and macadamia butters were separated into 25 g samples. Sucrose was added to added in 3 g and 5 g quantities into the 25 g samples of almond and macadamia butter and mixed thoroughly. a_w of the samples were taken before the two strains were

TABLE 1: Average water activities for the low a_w food samples collected from households.

Food category	Average a_w
Infant cereal	0,352
Adult cereal	0,381
Milk powder	0,284
Nuts	0,648
Spices	0,437
Chocolates	0,427

individually inoculated. 1 ml of the two strains, *S. aureus* ATCC 25923 and *B. cereus*, were individually inoculated into each butter sample aseptically. The concentration of the cells of both strains was approximately $1,3 \times 10^7$ CFU/ml, which is approximately 7 log CFU/ml which was compared to a 0,5 McFarland standard for approximate turbidity using the method by [13].

Recovery and Enumeration of S. aureus ATCC 25923 and B. cereus. The inoculated almond and macadamia butter samples (25 g) were assayed for *S. aureus* ATCC 25923 and *B. cereus* in duplicate at 1, 7, 14, 21, and 28 days, respectively. These samples were stored at 18°C to 20°C and at 25°C. Serial dilutions were done whereby 1 g of all samples was transferred into 9 ml of nutrient broth and the method by ISO [11] was used to recover and enumerate *S. aureus* ATCC 25923 and the method by MFLP [14] was used to recover and enumerate *B. cereus*.

2.3. Statistical Analysis. One-Way Analysis of Variables (ANOVA) and Tukey's comparison test were done to determine if there were significant differences ($P \leq 0,05$) between the household samples as well as to compare the mean values for the simulative study.

3. Results and Discussion

The average a_w per food category was calculated and is depicted in Table 1. Table 2 shows the results achieved from the first objective which was to assess the quality of low a_w foods collected from Durban households.

Based on these average a_w readings (refer to Table 1), all of the samples were found to have had a_w below 0,7. a_w for samples such as nuts (0,648) and spices (0,437) corresponds with the values from literature in which such samples had a_w of <0,6 [3]. Infant cereal (0,352), adult cereal (0,381), and milk powder (0,284) a_w values also corresponded with values in the literature by [1, 17].

The samples were stored in clean air-tight containers prior to collection. Furthermore, upon collection, the food was of good quality, whereby no visible fungi or mould was present. Furthermore, all samples were still within their shelf life. As per the results obtained from household samples collected, as seen in Table 2, most of the samples had low counts. Spices had the highest counts of aerobic bacteria, aerobic spore-formers, anaerobic spore-formers, and *S. aureus* followed by nuts. This could be due to poor hygiene practices in industry

TABLE 2: Microbiological quality of low a_w activity food presented in means of ACC, ASF, AnSF, and *S. aureus* (Log CFU/g) collected at household level.

Category	ACC (Log CFU/g)	ASF (Log CFU/g)	AnSF (Log CFU/g)	<i>S. aureus</i> (Log CFU/g)
Infant cereal	2,022 ^a ± 0,83	1,90 ^{ab} ± 0,76	0,19 ^c ± 0,31	0,30 ^{cd} ± 0,34
Adult cereal	1,00 ^a ± 0,77	ND ^b	ND ^{bc}	0,19 ^{bcd} ± 0,25
Milk powder	1,30 ^a ± 0,72	0,092 ^b ± 0,29	0,096 ^{bc} ± 0,30	0,15 ^{bcd} ± 0,29
Nuts	2,30 ^a ± 2,10	0,66 ^{abcd} ± 1,40	0,97 ^{abcd} ± 2,10	0,37 ^d ± 1,20
Spices	4,40 ^a ± 0,33	3,30 ^b ± 0,40	2,70 ^{bc} ± 1,00	1,30 ^d ± 1,10
Chocolates	0,74 ^a ± 0,33	0,07 ^b ± 0,23	0,065 ^{bc} ± 0,21	0,024 ^{bcd} ± 0,075

(1) Results represented as Means ± Standard Deviation. (2) Means ($n = 60$). (3) Means with same superscript letters in rows are not significantly different ($P \geq 0,05$). (4) ACC: aerobic colony count; ASF: aerobic spore-formers count; AnSF: anaerobic spore-formers count; ND: not detected.

and prolonged exposure to air. Furthermore, spices and nuts originate from natural sources and could also have been contaminated from the natural environment as mentioned in the literature [1, 3].

There was no growth detected for aerobic and anaerobic spore-formers counts in adult cereal, indicating that these samples were well kept at household level and were not exposed to the air for prolonged periods of time. Infant cereal and milk powder exhibited a similar trend with regard to their colony counts indicating that there were no significant differences ($P > 0,05$) between these two samples. Most of the food categories apart from spices and nuts had relatively low colony counts amongst ACC, ASF, AnSF, and *S. aureus*. Chocolate samples compared to the other five food categories had the least colony counts. Whilst significant differences ($P < 0,05$) were noted amongst the different colony counts within a food category, spices and nuts were the only two categories that differed significantly from the rest of the categories in this regard.

Foods such as infant cereal, adult cereal, and milk powder have been desiccated to achieve to their desired organoleptic properties. According to [17], desiccation in turn increases the concentration of solutes in the remaining water available. The osmotic potential of water is increased by the addition of solutes. Whilst cereals contain added sugar (usually in the form of sucrose), the patterns of effect on microbial responses have exhibited similar qualities when KCl, NaCl, sucrose, and glucose were used. Glycerol as a humectant on the other hand permitted growth at lower a_w , although there were exceptions: *S. aureus*, for example, was more inhibited by glycerol than NaCl.

Presence and absence tests for pathogens such as *C. sakazakii*, *E. coli*, *L. monocytogenes*, and *Salmonella* spp. were also conducted on the collected household samples.

Whilst *C. sakazakii*, *E. coli*, *L. monocytogenes*, and *Salmonella* spp. were not recovered from infant cereals, adult cereals, milk powder, and spices. Nut samples had *C. sakazakii* and *E. coli* which were found in 60% and 100% of the samples, respectively. Chocolates, on the other hand, had *Salmonella* present in all (100%) of the samples [1, 8].

According to [7, 17], Gram-negative bacteria are more susceptible to low a_w foods than Gram-positive bacteria. Whilst Gram-negative bacteria can only survive in foods

with $a_w \geq 0,95$, Gram-positive bacteria, on the other hand, can withstand a_w in foods as low as $<0,8$. However, in the present study, *C. sakazakii*, *E. coli*, and *Salmonella* which are Gram-negative pathogens survived in low a_w foods being nuts and chocolates which had an average a_w of 0,648 and 0,427, respectively, which is well below the limit as stipulated in the literature. Gram-positive bacteria like *L. monocytogenes* was absent in these samples. Aerobic spore-formers like *B. cereus* and anaerobic spore-formers like *C. botulinum* are Gram-positive bacteria that also survived in low a_w foods in the present study. A study done by Doyle and Glass [18] showed the prevalence of *C. botulinum* in dried dairy products. This could be due to the presence of spores that these bacteria possess since they are spore-forming bacteria and, by having the presence of spores, would enhance their survival in stressed environments. According to Beuchat et al. [1], such bacteria survive at a_w ranging between 0,93 and 0,92. a_w of the foods in the present study were significantly lower than the values stipulated in the literature implying that Gram-positive aerobic and anaerobic spore-formers like *B. cereus* and *C. botulinum* have the ability to survive in foods that possess a_w lower than this range as seen in Table 1.

It would be highly acceptable to have an absence of pathogens in food samples such as infant and adult cereals and milk powder, due to the desiccation process that they undergo as well as spices being a naturally occurring low a_w food that is high in antioxidants, therefore possessing high antimicrobial activity [9]. Although these pathogens of importance were absent in these samples in the present study, according to review articles and studies conducted by [1, 3, 9], pathogens such as *C. sakazakii* were found to be present in foods such as spices and powdered infant formula and *E. coli* was found to be present in infant rice cereal.

Tables 3–6 represent the results obtained from the second objective which was a simulative study that was conducted on two different butters, almond and macadamia butter with an adjusted sugar content, stored at two different temperatures (18°C and at 25°C) for a period of 28 days.

The almond butter used for this simulative study contained no added sugar or salt. Although there were significant differences amongst the groups within pathogen for both *S. aureus* ATCC 25923 and *B. cereus* (refer to Table 3), it was noted that the growth of *S. aureus* ATCC 25923 at 18°C to

TABLE 3: Populations of *S. aureus* ATCC 25923 and *B. cereus* in almond butter at varying concentrations of sugar stored at 18°C.

Concentration of sucrose at 18°C to 20°C	Populations (Log CFU/g) over storage time (days)				
	1	7	14	21	28
<i>S. aureus</i> ATCC 25923					
0%	5,34 ^a	5,25 ^{ab}	5,23 ^{abc}	4,73 ^d	3,59 ^e
12%	5,033 ^{ns}	4,32 ^{ns}	2,72 ^{ns}	ND	ND
20%	4,72 ^a	ND	ND	ND	ND
<i>B. cereus</i>					
0%	5,033 ^a	4,74 ^b	4,53 ^c	ND	ND
12%	4,56 ^{ns}	4,14 ^{ns}	3,45 ^{ns}	3,30 ^{ns}	3,00 ^{ns}
20%	3,17 ^a	2,88 ^b	ND	ND	ND

(1) Means with same superscript letters in rows within pathogen are not significantly different ($P \geq 0.05$). (2) Means ($n = 2$). (3) ND: not detected; ns: no significance.

TABLE 4: Populations of *S. aureus* ATCC 25923 and *B. cereus* in macadamia butter at varying concentrations of sugar stored at 18°C.

Concentration of sucrose at 18°C	Populations (Log CFU/g) over storage time (days)				
	1	7	14	21	28
<i>S. aureus</i> ATCC 25923					
0%	5,29 ^a	4,94 ^{ab}	4,51 ^{bc}	3,92 ^d	3,45 ^e
12%	4,74 ^a	4,31 ^b	4,029 ^c	ND	ND
20%	4,61 ^a	ND	ND	ND	ND
<i>B. cereus</i>					
0%	4,29 ^a	3,94 ^{ab}	2,24 ^c	ND	ND
12%	5,053 ^a	5,021 ^{ab}	4,86 ^c	4,40 ^d	4,30
20%	3,64 ^a	ND	ND	ND	ND

(1) Means with same superscript letters in rows within pathogen are not significantly different ($P \geq 0.05$). (2) Means ($n = 2$). (3) ND: not detected; ns: no significance.

TABLE 5: Populations of *S. aureus* ATCC 25923 and *B. cereus* in almond butter at varying concentrations of sucrose stored at 25°C.

Concentration of sucrose at 25°C	Populations (Log CFU/g) over storage time (days)				
	1	7	14	21	28
<i>S. aureus</i> ATCC 25923					
0%	5,40 ^a	5,39 ^{ab}	5,22 ^c	4,87 ^d	4,53 ^e
12%	5,074 ^a	4,93 ^b	4,63 ^c	4,56 ^{cd}	4,00 ^e
20%	4,89 ^a	4,79 ^{ab}	4,65 ^{abc}	4,49 ^{bd}	3,15 ^{de}
<i>B. cereus</i>					
0%	4,88 ^a	4,77 ^b	4,51 ^c	ND	ND
12%	5,25 ^{ns}	5,22 ^{ns}	5,041 ^{ns}	3,77 ^{ns}	3,53 ^{ns}
20%	4,80 ^{ns}	4,76 ^{ns}	4,72 ^{ns}	3,69 ^{ns}	3,22 ^{ns}

(1) Means with same superscript letters in rows within pathogen are not significantly different ($P \geq 0.05$). (2) Means ($n = 2$). (3) ND: not detected; ns: no significance.

20°C in almond butter did not survive as well as *B. cereus* in the 12% sucrose sample. Furthermore, both strains did not survive very well in the 20% sucrose sample, as the growth of *S. aureus* ATCC 25923 had ceased by the 7th day and *B. cereus* by the 14th day. However, *S. aureus* ATCC 25923

survived very well in a sucrose-free medium, whilst *B. cereus* survived moderately well in a low sucrose content medium (12% sucrose). The growth of *B. cereus* was inhibited after the 21st day. This indicates that a high sucrose content inhibits the growth of both these bacteria at slightly lower temperatures.

TABLE 6: Populations of *S. aureus* ATCC 25923 and *B. cereus* in macadamia butter at varying concentrations of sucrose stored at 25°C.

Concentration of sucrose at 25°C	Populations (Log CFU/g) over storage time (days)				
	1	7	14	21	28
<i>S. aureus</i> ATCC 25923					
0%	5,34 ^a	5,32 ^{ab}	4,59 ^c	4,47 ^d	4,38 ^{de}
12%	5,00 ^a	4,99 ^{ab}	4,85 ^{abc}	4,67 ^{abcd}	3,15 ^e
20%	3,97 ^a	3,67 ^b	3,00 ^c	ND	ND
<i>B. cereus</i>					
0%	5,39 ^a	5,066 ^b	ND	ND	ND
12%	5,18 ^a	4,67 ^b	4,66 ^{bc}	4,19 ^d	3,45 ^e
20%	4,20 ^a	3,88 ^b	3,72 ^c	3,60 ^d	3,49 ^e

(1) Means with same superscript letters in rows within pathogen are not significantly different ($P \geq 0.05$). (2) Means ($n = 2$). (3) ND: not detected; ns: no significance.

Whilst limited studies implicate the presence of *B. cereus* and *S. aureus*, several have implicated the presence of *E. coli* and *Salmonella* [1, 8, 16, 19, 20]

The macadamia butter used in this simulative study had a carbohydrate content of 5 g of which of total sugar amounted to 3,3 g per 100 g (3,3%). A similar trend was noticed in macadamia butter stored at 18°C despite the butter having the contained added sugar. *S. aureus* ATCC 25923 grew well in the sucrose-free sample (0%) but, because of the butter already containing 3,3% sucrose, it can be deduced that *S. aureus* did not survive after 14 days at very low sucrose contents. *B. cereus*, on the other hand, survived after 14 days but ceased to grow after the 21st day in a very low sucrose medium indicating that *B. cereus* is more osmotolerant than *S. aureus* ATCC 25923. Furthermore, like almond butter, *B. cereus* grew fairly well in the 12% sugar sample. This is, in addition to the 3,3% sucrose that butter had already contained, implying that *B. cereus* grows moderately well in 12–18% sucrose medium. Both strains of bacteria did not survive very well at 20% sucrose content, implying that a high sucrose content would inhibit their growth.

Temperature seemed to have significantly influenced the growth of both bacteria. It was observed that the growth of *S. aureus* ATCC 25923 had grown fairly well in both the 12% and 20% sucrose samples. The growth of *B. cereus* had ceased after the 21st day in the sucrose-free (0%) sample but was now able to thrive in the 20% sucrose sample, implying that *B. cereus* is more osmotolerant compared to *S. aureus* ATCC 25923. However, at 25°C, *S. aureus* ATCC 25923 was able to metabolise sucrose as a carbon source than it did at a reduced temperature.

A similar trend was noticed in macadamia butter stored at 25°C. It was observed that the growth of *S. aureus* ATCC 25923 was inhibited after the 21st day in the 20% sucrose sample, whilst the growth of *B. cereus* ceased after the 7th day in the 0% sucrose sample. However, *B. cereus* grew fairly well throughout the 28 days in both the 12% and 20% sucrose samples.

From this simulative study, it can be deduced that the concentration of sugars (sucrose) and the effect of temperature affected the growth of both strains of bacteria.

Whilst *S. aureus* ATCC 25923 was able to thrive in a sample that was sucrose-free (0%), *B. cereus* proved to be more osmotolerant surviving in the 12% sucrose concentration at reduced temperatures (18°C to 20°C). Both strains did not survive very well at 20% sucrose concentration at reduced temperatures. At an elevated temperature (25°C), both *B. cereus* and *S. aureus* ATCC 25923 were able to grow at all three concentrations of sucrose in almond butter. There were no significant differences between the growth of both bacteria in both types of butter, except for 20% sucrose concentration in almond butter.

According to Finn et al. [5], bacteria have osmoprotectants which aid in bacterial survival in low a_w environments. The function of osmoprotectants is to balance the osmolarity within the bacterial cell to that of the external environment to avoid any loss of water.

In this present study, temperature and humectants (sucrose) induced a stressed environment for the bacteria to grow. According to Carlin et al. [21], despite the optimum growth limits for bacteria to thrive, bacteria have to adjust to changes in temperature. Mechanisms of modification to their lower or higher temperature growth limits show significant differences.

There could be a high possibility that, due the spore-forming ability of *B. cereus* in an unfavourable environment, the vegetative cell itself is protected from the stressed environment as opposed to *S. aureus* which is not a spore-forming bacterium and is more susceptible to stressed environments. Due to the fact that a_w of the nut butter has increased after sucrose was added, it would be possible for both strains of bacteria to survive. Carbon as a source of nutrition also plays a vital role, when present in low concentrations.

4. Conclusion

From this study, it can be concluded that low a_w food samples possessed low colony counts except, for spices and nuts which had the highest colony counts as well as aerobic spore-formers, anaerobic spore-formers, and *S. aureus* compared to the other food categories. Overall, good manufacturing practices should be followed in industry and in the home

to avoid outbreaks of food-borne illness. Future work can be conducted on fungi producing mycotoxins, which have been in the spotlight in recent years; furthermore since fungal growth has been highly associated in low a_w foods, it would be apt to conduct such work.

Disclosure

This work has been accepted for presentation at IAFP 2017 Annual Meeting in Florida, USA.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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