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# Tocopherol and Ascorbate Have Contrasting Effects on the Viability of Microencapsulated *Lactobacillus rhamnosus* GG

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**ABSTRACT:** The antioxidants, sodium ascorbate and tocopherol, have contrasting effects on the viability of microencapsulated *Lactobacillus rhamnosus* GG (LGG) spray-dried powders during storage (4 and 25 °C; 32, 57, and 70% relative humidity). The addition of tocopherol improved probiotic viability during storage, while the incorporation of Na-ascorbate alone or in combination with tocopherol had detrimental effects on probiotic survival. The beneficial effect of tocopherol is a consequence of its chemical antioxidative action. The reduced viability in Na-ascorbate containing microcapsule formulations is hypothesized to be due primarily to the production of acetic acids arising from chemical degradation reactions and the catabolism of ascorbate by LGG. This study highlights the importance of considering the detrimental consequences of degradative chemical reactions and the metabolic fate of additives on the viability of probiotics when designing probiotic encapsulant formulations.

**KEYWORDS:** Ascorbic acid, tocopherol, short chain acids, probiotic, microencapsulation, *Lactobacillus rhamnosus* GG, viability

## INTRODUCTION

Probiotics are defined as live microbial organisms that, when administered in adequate amounts, confer a health benefit to the host.[1] The oral intake of probiotics has beneficial effects on intestinal microflora balance. Probiotics are effective in the prevention and treatment of several clinical disorders including diarrhea, lactose intolerance, and certain allergies[2] and in weight management.[3]

Probiotics may be incorporated in various food formats such as yogurt, chocolate, cereal fruit bars, meats, and infant formulas.[4] To maximize benefit to the host, cell viability should be maintained at $\geq 7 \log_{10}$ cfu/g of product.[5] The viability of probiotics is decreased on exposure to low pH, high moisture, high temperature, and oxygen. These stresses can cause changes in DNA, oxidation of membrane lipids, and damage to cell membrane proteins, ultimately resulting in cell death.[6,7]

Maintaining the viability of probiotic preparations during manufacture, storage, and distribution is a major challenge. There is potential for microencapsulation to protect bioactives such as probiotics and also to control their release in the host postconsumption.[8,9] The process of microencapsulation involves entrapping an active core within a secondary material, creating a microenvironment that can protect the active against surrounding unfavorable influences until it is released at a desired time or site.[10] A recent review examines the use of various encapsulant materials and methods for preparation of encapsulated probiotics.[11] However, the effectiveness of encapsulation and various methods for increasing the shelf life of probiotics has to be further examined.[11,12]

Probiotics are typically supplied as freeze-dried cultures. Spray drying is an alternative to freeze drying for the preservation of probiotics and is of commercial interest as it is less expensive than freeze drying. While the process of spray drying and subsequent storage can result in viability loss of probiotics,[13,14] preadaptation of probiotics to heat and the selection of appropriate carriers (e.g., proteins, sugars, and gums) can largely offset this to enhance the survival.[15−17] Encapsulation or the use of carriers during drying has been previously examined for the protection of *Lactobacillus rhamnosus* GG in the dry state. Sugars have been commonly used as carriers for freeze-dried probiotic preparations. Trehalose or trehalose−lactose mixtures were found to be more effective for maintaining viability of LGG during storage as compared to lactose.[18] The possibility of using reconstituted skim milk as a carrier for LGG in spray-dried formulations has been demonstrated. It was found that partial substitution of skim milk with prebiotics improved survival during spray drying but had a detrimental effect on survival during storage.[19] The addition of monosodium glutamate to trehalose containing a LGG formulation has been found to improve the survival of LGG during drying and storage.[20] Spray-dried microencapsulated probiotics prepared from emulsion-based protein−carbohydrate formulations have previously been shown to have enhanced viability during nonrefrigerated storage and gastrointestinal tract transit.[21]

Chemical deteriorative reactions, particularly the oxidation of membrane lipids, have been suggested to be a major cause for death of lactic acid bacteria when exposed to air, high temperature, and high humidity.[22,23] This is evidenced by a decreased ratio of unsaturated to saturated fatty acid following spray drying and during storage.[24] Probiotic bacteria may therefore benefit from the incorporation of antioxidants within probiotic formulations intended for spray drying and extended storage.

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Table 1. Composition of Spray-Dried Blank and LGG Microcapsule Formulations with and without Antioxidants

| ingredient | control (no AO) | | Na-ascorbate (AO1) | | tocopherol (AO2) | | Na-ascorbate + tocopherol (AO1 + AO2) | |
|---|---|---|---|---|---|---|---|---|
| | blank | FDLGG | blank | FDLGG | blank | FDLGG | blank | FDLGG |
| | ingredients (% wt/wt) | | | | | | | |
| hydrolyzed whey protein | 15.0 | 14.7 | 14.25 | 13.95 | 15.0 | 14.7 | 14.25 | 13.95 |
| dextrinized starch | 5.0 | 4.9 | 4.75 | 4.65 | 5.0 | 4.9 | 4.75 | 4.65 |
| hydrolyzed palm stearin | 80.0 | 78.4 | 76.0 | 74.4 | 75.0 | 73.4 | 71.0 | 69.4 |
| FDLGG | | 2.0 | | 2.0 | | 2.0 | | 2.0 |
| Na-ascorbate | | | 5.0 | 5.0 | | | 5.0 | 5.0 |
| tocopherols | | | | | 5.0 | 5.0 | 5.0 | 5.0 |

In this study, LGG was added to an emulsion-based formulation containing hydrolyzed whey protein, dextrinized starch, and palm kernel stearin prior to spray drying. The aim of this study was to investigate the effects of coencapsulation of LGG with antioxidants. The antioxidants chosen were a lipophilic antioxidant (tocopherol) and a hydrophilic antioxidant (sodium ascorbate). These are antioxidants commonly used in food, and in addition, tocopherol and ascorbate are synergistic when added in combination.[25] The viability of microencapsulated LGG powders during storage at refrigerated (4 °C) and nonrefrigerated (25 °C) temperatures under various relative humidity (RH) conditions (32, 57, and 70% RH) were examined. Probiotic viability was examined using standard culturing methods over a period of 6 months. Analysis of headspace volatile components by gas chromatography−mass spectrometry (GC-MS) was an added element in this study to assess whether the development of chemical compounds could be related to loss of viability during storage. The analysis was carried out both for LGG-containing microcapsules and microcapsules without LGG to distinguish between products formed due to degradation of encapsulant components and the metabolism of components in the formulation by the probiotic bacteria.

## MATERIALS AND METHODS

**Materials.** Hydrolyzed whey protein (MyoPure HWP DH17) was from Muscle Brand Pty Ltd. (Greenwich, NSW, Australia), dextrinized tapioca starch (K4484, Batch EJ2045) was from National Starch Pty Ltd. (Bridgewater, NJ), and hydrogenated palm kernel stearin (Ultra Choco) was from Wilmar International Ltd. (56 Neil Road, Singapore). Na-ascorbate was from DSM Nutritional Products AG (Basel, Switzerland). Mixed tocopherol (Covi-ox T-70) was a gift from Cognis Australia Pty Ltd. (Melbourne, Victoria, Australia). It contained 14% $\alpha$-tocopherol, 2% $\beta$-tocopherol, 60% $\gamma$-tocopherol, and 24% $\delta$-tocopherol. Commercial freeze-dried probiotic bacterium (containing 45% sucrose as per product specification), FDLGG, was from Valio Ltd. (Helsinki, Finland).

Magnesium chloride was obtained from BDH Chemicals (AnalaR grade; Kilsyth, Victoria, Australia), Na-bromide was from Fluka Chemicals (Sydney, NSW, Australia), and strontium chloride was from Univar, Ajax Chemicals, P/L (Sydney, NSW, Australia). De Man, Rogosa, and Sharpe (MRS) agar was obtained from Oxoid Ltd. (Hampshire, England).

**Microcapsule Manufacture.** Microencapsulated LGG spray-dried emulsion-based formulations, with or without added antioxidants, were prepared (Table 1). Microcapsules were formulated with Na-ascorbate (AO1), tocopherol (AO2), or a combination of the two (AO1 + AO2); control microcapsules without any antioxidant (no AO) were also prepared.

LGG microcapsules were prepared by spray drying emulsions of hydrogenated palm kernel stearin, hydrolyzed whey protein, and dextrinized tapioca starch containing the LGG. Aqueous mixtures [3:1 protein: dextrinized starch; 6.25% total solids (TS), pH 7.5] were prepared. The hydrolyzed whey protein was dispersed in water (60 °C) and left to hydrate for 30 min. The dextrinized starch was added, and the mixture was stirred for a further 15 min. The mixture was then adjusted to pH 7.5 using 1.0 M NaOH. The palm kernel stearin was added to the aqueous mixture and mixed using a high shear mixer (Type X50/10, Ystral Gmbh, Ballrechten-Dottingen, Germany) operated at 3000 rpm to form a pre-emulsion [25% TS, 4:1 ratio of fat:(hydrolyzed whey protein + dextrinised starch)]. This pre-emulsion was homogenized using a Rannie two-stage homogenizer (APV Homogenizers, Albertslund, Denmark) at pressures of 18/8 MPa. Freeze-dried LGG was mixed into the emulsions at 40 °C using a mixer (Robot Coupe MP450 VV, Robot Coupe USA Inc., Jackson, MS) operated at 5000 rpm for 5 min, and the mixture was fed into a Niro Production Minor spray dryer (Niro A/S, Søborg, Denmark) fitted with a rotary atomizer. The formulation was dried at inlet and outlet temperatures of 170 and 70 °C, respectively. Where Na-ascorbate was included, this was added to the homogenized emulsion prior to addition of LGG. Where tocopherol was included, this was dissolved in the fat phase prior to addition to the aqueous dispersion and homogenization.

Blank microcapsules were prepared in the same way except that the LGG was not added into the formulation prior to drying. The compositions of the blank microcapsules are as given in Table 1.

**Scanning Electron Microscopy.** Spray-dried microcapsules were examined using a Hitachi S4300/N Field Emission, Variable Pressure, and Scanning Electron Microscope. The working distance was 11.3 mm, and the beam energy was 1.2 kV. Before microscopy, the microcapsule samples were mounted on a double-sided carbon tape on a stud and then coated with gold.

**Storage.** Microencapsulated LGG powders were stored in open containers at 25 or 4 °C exposed to various RHs. Saturated salt solutions of magnesium chloride, sodium bromide, and strontium chloride were used to provide atmospheres with RHs of 32, 57, or 70%, respectively.[26] Samples were removed at intervals for analysis during the 6 month storage period.

**Enumeration of Viable LGG.** Microencapsulated LGG samples were dispersed in phosphate-buffered saline (pH 7.0) in a shaking water bath (37 °C, 100 rpm, 1 h). Samples were then diluted (0.1% w/v peptone water) and recovered on MRS agar using a spiral plating method. Plates were incubated under anaerobic conditions at 37 °C for 48 h.[27] Anaerobic conditions were achieved using Oxoid Anaerogen sachets. Duplicate samples were enumerated in triplicate at each sample time. Results are presented as the average ± standard deviation.

**Headspace Volatile Analysis by Solid-Phase Microextraction (SPME)-GC-MS.** The volatile compounds in LGG microcapsules were analyzed with an Agilent gas chromatography mass spectrometry system (GCMS- 6890N model GC and 5975B model MSD) equipped with a CombiPAL robotic autosampler (CTC Analytics AG, Zwingen, Switzerland). The sample (0.1 g) was mixed with internal standard

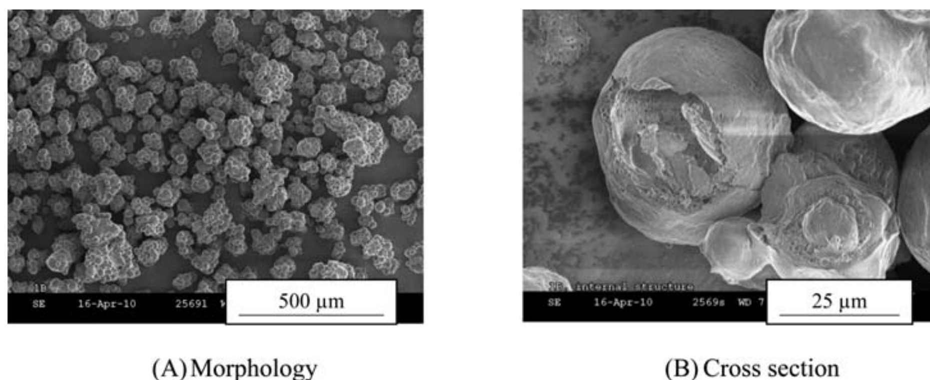


**Figure 1.** Scanning electron micrograph of LGG microcapsules showing (A) the morphology of the microcapsules and (B) a cross-section of the microcapsules.

solution (2-methyl-3-heptanone and 2-ethyl butyric acid in methanol) in a 22 mL screw cap headspace vial. Volatile metabolites in the sample vial was extracted/concentrated on the SPME fiber (carboxen/polydimethylsiloxane, CAR/PDMS; 85 $\mu$m film thickness; 10 mm long; Supelco, Bellefonte, PA) for 30 min at 50 °C. SPME-sampled volatiles were thermally desorbed in the GC inlet (PTV inlet, 260 °C, set in splitless mode, splitless time 1.0 min) and chromatographed on a VF-WAXms column (30 m × 0.32 mm × 1.0 $\mu$m; Varian Inc., Mulgrave, Victoria, Australia) using a temperature gradient of 35−225 at 10 °C/min with initial and final hold times of 3 and 10 min, respectively. Eluted compounds were detected by the MS detector. MS condition were as follows: capillary direct interface temperature, 260 °C; ionization energy, 70 eV; mass range, 30−300 amu; and scan rate, 5 scans/s. Compounds were identified by comparison of collected mass spectra with spectra in NIST08 database (National Institute of Standards Technology mass spectral search program; NIST, United States of America), linear retention indices (determined using a set of saturated alkanes C7−C22), and spectra of an authentic standard. Quantitative analysis was performed using internal standard methodology. Two independent samples were analyzed, and differences between analyses were less than 5%.

## RESULTS AND DISCUSSION

**Visualization of LGG Microcapsules.** Typical morphology and a cross-sectional view of the microcapsules are shown in Figure 1. Spray-dried microcapsules agglomerate into clusters that are typically 50−200 $\mu$m (Figure 1A); individual microcapsules have a diameter of 10−20 $\mu$m and are hollow and porous (Figure 1B). The LGG cells are embedded within the matrix of the powder particle.

A pink color developed in microcapsules containing Na-ascorbate. Pink coloration has previously been observed in freeze-dried LGG preparations containing ascorbate.[28]

**Survival of LGG Cells.** Spray drying caused a loss in viability of LGG. The survival of LGG cells immediately after manufacture was dependent on the formulation used. The loss ($\log_{10}$ CFU/g) was 1.16 ± 0.10 for LGG microcapsules without antioxidants and 1.00 ± 0.02 for microcapsules containing tocopherol. The loss ($\log_{10}$ CFU/g) was greater for microcapsules containing Na-ascorbate (2.15 ± 0.12) and those containing Na-ascorbate and tocopherol (1.90 ± 0.02), as compared to formulations containing tocopherol or formulations without antioxidant. These results suggested that Na-ascorbate had a detrimental effect on the survival of LGG during spray drying.

The viability of LGG decreased with storage time under all conditions (Figures 2 and 3). As expected for the same formulation, the loss of viability increased with increasing storage temperature at a constant water activity and also increased with increased water activity at a constant temperature. Under all storage conditions, the loss in viability was greater for microencapsulated LGG containing Na-ascorbate than that for microencapsulated LGG without the added antioxidants, showing that Na-ascorbate had a detrimental effect on cell viability during storage. On the other hand, the incorporation of tocopherol alone protected the LGG during storage. However, the magnitude of the effects of added tocopherol alone was dependent on both temperature and RH. For instance, at 4 °C, tocopherol improved the survival of LGG at low RH, but the differences at higher RH were relatively minor, whereas at 25 °C, the protection afforded by tocopherol alone was marked at all RH. When tocopherol and Na-ascorbate were used together, the beneficial effect of the tocopherol was overridden by the larger detrimental effect of Na-ascorbate on LGG viability.

**Development of Volatile Compounds in Microcapsules.** GC-MS analysis revealed that a number of volatile products were formed on storage of blank and LGG-containing microcapsules. The major component in the headspace was acetic acid. There were also short chain fatty acids, namely, butanoic, 3-methyl butanoic, and hexanoic acids. Apart from the acids, ethanedioic acid dimethyl ester and methional were also detected (data not shown).

For the same formulation, the concentration of volatiles increased with increasing temperature at constant water activity or with increasing water activity at a constant temperature (Figures 4 and 5). In LGG microcapsules, the compounds in the headspace were generally higher in microcapsules containing Na-ascorbate alone or Na-ascorbate and tocopherol than in microcapsules with added tocopherol only and microcapsules without added antioxidants (Figures 4 and 5). There were also differences between samples containing tocopherol alone and the control (without antioxidants), but these were minor in comparison to the differences between samples containing tocopherol alone and those in samples containing Na-ascorbate alone or Na-ascorbate in combination with tocopherol.

As in the case of LGG microcapsules, the compounds in the headspace were also generally higher in blank microcapsules containing Na-ascorbate alone or Na-ascorbate and tocopherol than in blank microcapsules with added tocopherol only and blank microcapsules without added antioxidants (Figures 4 and 5). A comparison between blank and LGG microcapsules with the same formulation stored under the same conditions showed that the levels of acetic acid were most marked at 25 °C, especially for formulations containing Na-ascorbate (Figures 4 and 5).

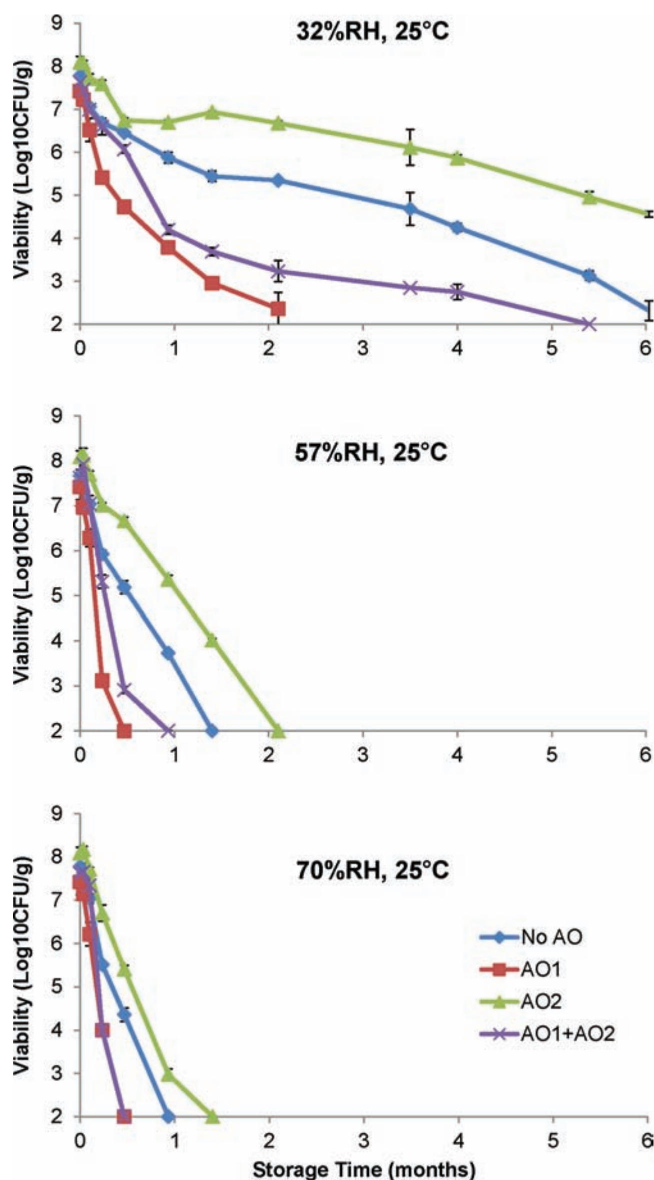


**Figure 2.** LGG viability counts during storage at 25 °C and RH of 32 (top), 57 (middle), and 70% (bottom). Legend: no AO, control; AO1, Na-ascorbate; AO2, tocopherol; and AO1 + AO2, Na-ascorbate + tocopherol.

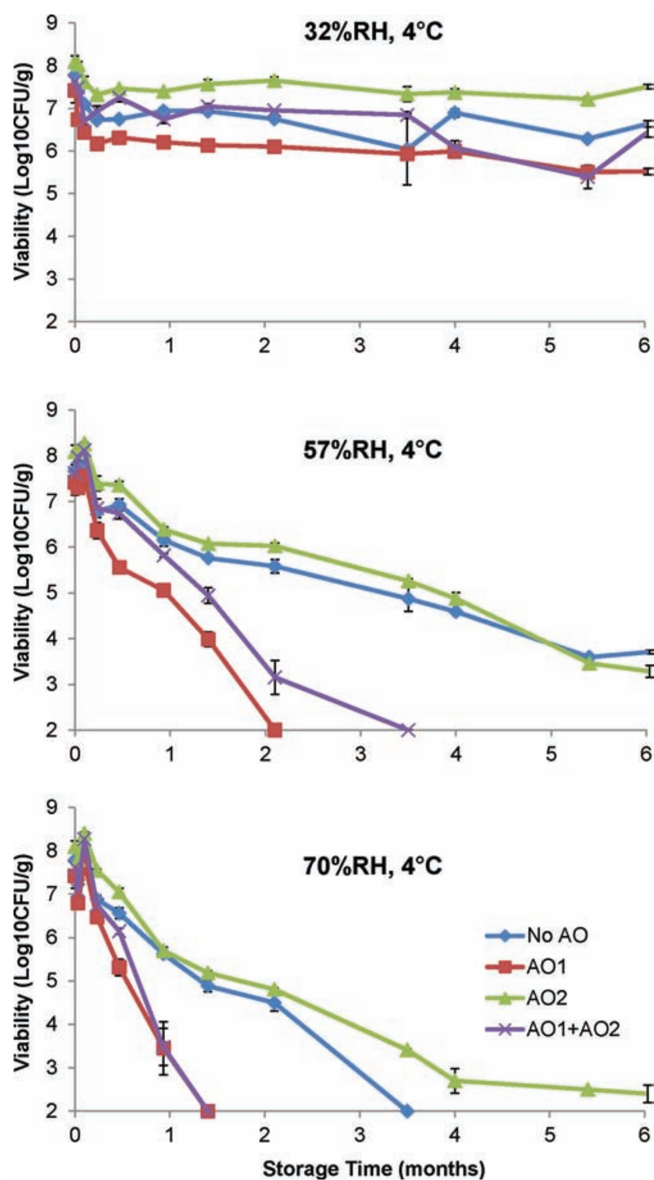


**Figure 3.** LGG viability counts during storage at 4 °C and RH of 32 (top), 57 (middle), and 70% RH (bottom). Legend: no AO, control; AO1, Na-ascorbate; AO2, tocopherol; and AO1 + AO2, Na-ascorbate + tocopherol.

The origin of the detected compounds could be (a) chemical degradation products of individual components, (b) chemical reaction products due to interaction between components in the matrix, and (c) metabolites formed due to the action of the LGG. Both the metabolism of microorganisms and the chemical degradation reactions are increased with increased temperature and humidity. The fact that acetic acid is observed in all formulations suggests that the main matrix components contributed to its development. Acetic acid may be formed as a result of the Maillard reaction between sugars and protein components.[29] All matrices had sugars and proteins that can undergo the Maillard reaction.[30,31] Acetic acid may also be a product of starch/sugar metabolism. The Kyoto Encyclopedia of Genes and Genomes shows that LGG bacteria have the ability to metabolize starch/dextrins into simple sugars with the resultant formation of a range of products including acetic acid through the glycolytic pathway (http://www.genome.jp/kegg). Specifically, LGG is capable of metabolizing glucose.[32]

The higher levels of acetic acid in LGG formulations than in corresponding blank formulations containing Na-ascorbate as compared to those without Na-ascorbate suggest that ascorbate itself had a primary role in acetic acid development. Acetic acid is one of the main volatile products resulting from the chemical degradation of ascorbic acid.[33] Another pathway is via the catabolism of ascorbate by LGG. A recent study has proven that ascorbate is catabolized by LGG to carbon dioxide, acetate, and lactate.[34]

The short chain fatty acids (butanoic acid), 3-methylthiopropanal (methional), and 3-methylbutanoic acid may arise from the Strecker degradation. These are all products that have been found in systems where the Maillard reaction has occurred.[29,35,36]

Interestingly, oxalic acid in dimethyl ester form was also detected in Na-ascorbate containing LGG microcapsules by GC-MS analysis

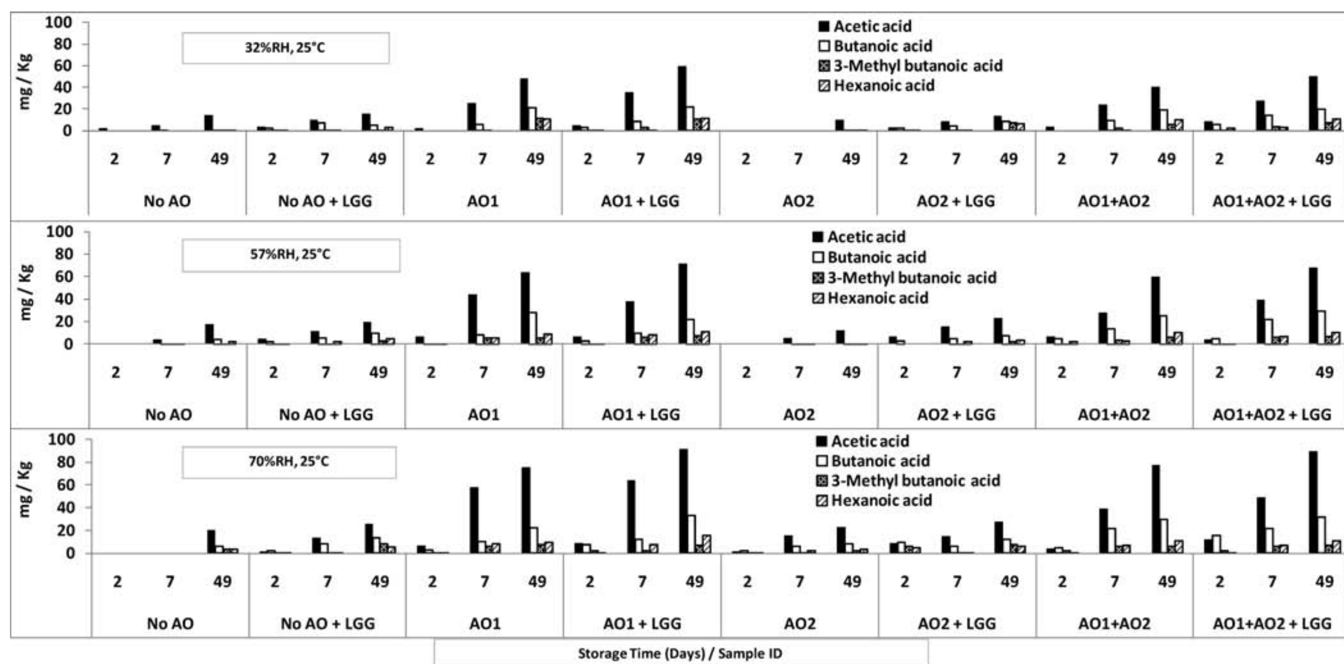


**Figure 4.** Volatile compounds in blank microcapsules and LGG microcapsules during storage at 25 °C and RH of 32 (top), 57 (middle), and 70% RH (bottom) after 2, 7, and 49 days of storage. No AO, control; AO1, Na-ascorbate; AO2, tocopherol; and AO1 + AO2, Na-ascorbate + tocopherol.

(data not shown). The oxidative degradation of ascorbic acid also leads to production of oxalic acid,[37] which is a nonvolatile organic acid commonly found in plant, fungi, and animals.

**Relationship between Headspace Volatiles and LGG Viability.** The trends for the loss in viability (Figures 2 and 3) appeared to be related to the development of headspace volatile compounds (Figures 4 and 5). Short chain fatty acids are well-known microbial inhibitory compounds.[38] The presence of higher concentrations of short chain fatty acids could explain the lower survival of the probiotics in formulations containing Na-ascorbate. There was a significant inverse correlation ($p < 0.01$) between the major volatile compound, acetic acid, and LGG viability across all LGG capsules stored in all environments (Figure 6).

**Contrasting Antioxidant Effects on LGG Viability.** The major observations were that for the same formulation, there was the expected loss in viability with increasing temperature and water activity. The detrimental effects of high temperature and high water activity on dried probiotic survival are well-known.[39]

While the storage environment had the greatest effect on survival, the results also demonstrated that the type of antioxidant used in protein−carbohydrate-based LGG preparations had specific influences on the loss in LGG viability. Although both Na-ascorbate and tocopherol are known to arrest the oxidation of unsaturated lipids and are synergistic when combined in a formulation,[25] it was obvious that the incorporation of Na-ascorbate used alone or in combination with tocopherol had detrimental effects on the viability of microencapsulated LGG (Figures 2 and 3). This finding was not expected, and it demonstrates that factors other than antioxidant effects play an overriding role in influencing the survival of probiotics in Na-ascorbate-containing formulations. Our results also showed that coencapsulation with tocopherol alone increased the long-term storage viability of microencapsulated LGG formulations.

The most likely reason for the beneficial effects of added tocopherol on LGG viability is that it protects the membrane lipids from oxidation during storage. Other authors have suggested that membrane lipid oxidation is a major cause of cell death of lactobacilli bacteria exposed to high temperature and humidity.[22,23] Tocopherol, being a lipophilic antioxidant, can penetrate the plasma membrane and protect membrane lipids from oxidation. Previous work has shown that the addition of a lipophilic antioxidant (butylated hydroxyanisole) to preparations of bacteria (*Weissella paramesenteroides* LC11) containing cryoprotectants inhibited oxidation of lipids and prevented loss of membrane integrity, improving the survival of the freeze-dried bacteria on storage.[40]

In this work, there was a detrimental effect of Na-ascorbate on LGG survival. Both beneficial and detrimental effects of added ascorbate in dried probiotic preparations have been reported. Teixeira et al.[41] showed that the addition of ascorbic acid together with monosodium glutamate protected *Lactobacillus delbrueckii* ssp. *bulgaricus* cells during storage at 4 °C, while at 20 °C, the rate of viability loss was greater than that in samples without added ascorbic acid and monosodium glutamate. Kurtmann et al.[28] demonstrated that incorporation of up to 10% sodium ascorbate in the freeze-drying medium of *Lactobacillus acidophilus* improved storage stability (at 30 °C) after freeze drying.

The observed effects of Na-ascorbate in different systems are confounding. The contrasting effects of Na-ascorbate on probiotic survival during storage with different probiotics strains and formulations suggest that mechanisms other than the chemical antioxidative effects of ascorbate come into play and can override the beneficial antioxidative action of ascorbate on lipids. Kurtmann et al.[28] attributed the beneficial effect of ascorbate in freeze-dried bacteria to the high level of ascorbate (10%) added and its ability to prevent oxidative reactions, which are detrimental to probiotic survival. Other mechanisms obviously dominated the beneficial effects of antioxidant and oxygen scavenging potential of ascorbate in arresting the oxidation of membrane lipids in our

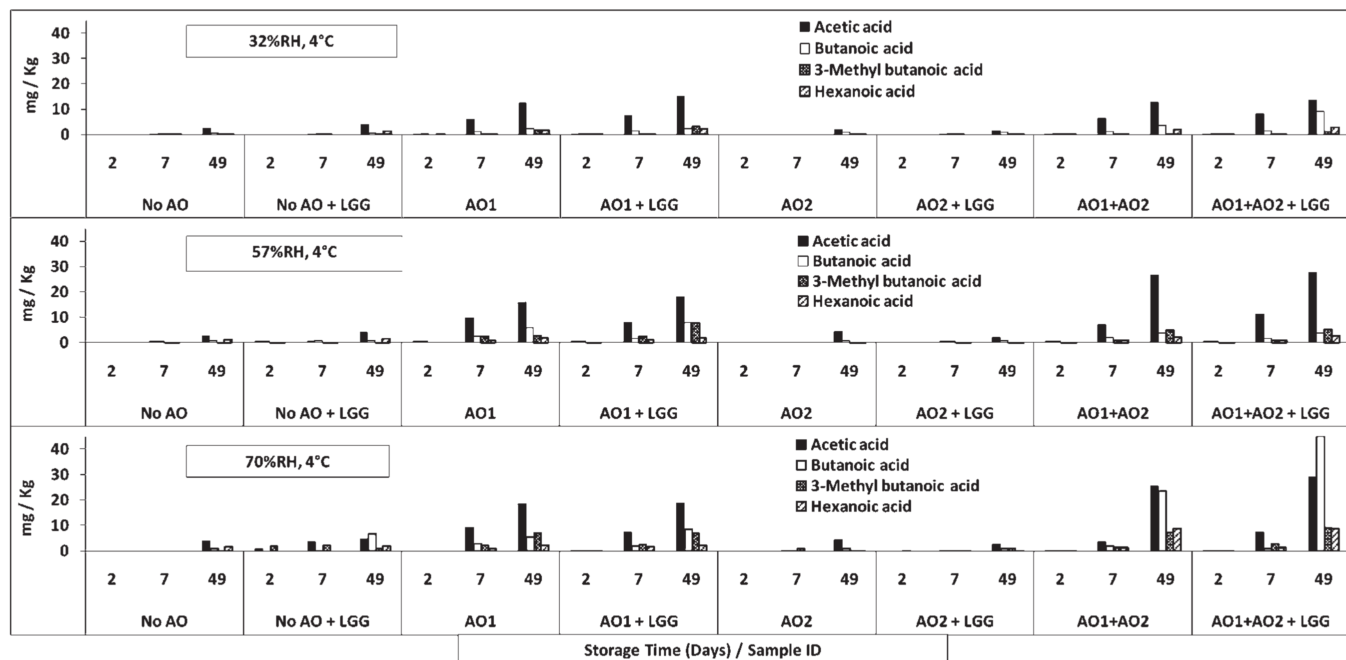


**Figure 5.** Volatile compounds in blank microcapsules and LGG microcapsules during storage at 4 °C and RH of 32 (top), 57 (middle), and 70% RH (bottom) after 2, 7, and 49 days of storage. No AO, control; AO1, Na-ascorbate; AO2, tocopherol; and AO1 + AO2, Na-ascorbate + tocopherol.

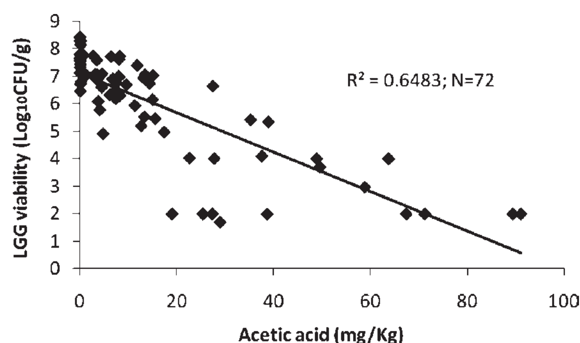


**Figure 6.** Correlation between viability of LGG and acetic acid in headspace of all microcapsules stored under all environments (4 and 25 °C at 32, 57, and 70% RH).

studies where LGG was formulated with protein−carbohydrate encapsulants. This effect was shown by Teixeira et al.[41] where *Lactobacillus delbrueckii* ssp. *bulgaricus* were stored under non-refrigerated conditions. We suggest that the varying effects of added ascorbate are due to (a) the unintended consequences of chemical degradation products of the encapsulants and their interaction with Na-ascorbate and (b) the different abilities of various probiotics to catabolize the ascorbate to form products that are detrimental to cell viability.

In our study, the chemical degradation products may arise from reactions of the encapsulant materials and ascorbate. Others have demonstrated that ascorbic acid interacts with amino acids to form melanoidin products.[42] Also, as previously indicated, LGG, which was used in our study, has the capacity to catabolize ascorbate to acetic acid,[34] which is detrimental to the probiotic. On the other hand, Kurtmann et al.[28] found that ascorbate improved the survival of freeze-dried *L. acidophilus*. By examining the amino acid sequences of genes encoding for enzymes in the catabolism in LGG and carrying out a Blastp search,[43] it was concluded that enzymes involved in L-ascorbate catabolism was not present in *L. acidophilus* strains NCFM and 30SC (Personal communication, Simon Gladman). Thus, the inability of *L. acidophilus* to catabolize ascorbate into acetic acid and other products possibly explains why ascorbate was not detrimental to this probiotic.

In conclusion, the results above showed that stability of probiotics during storage was dependent on the type of antioxidant added in the formulation. Tocopherol was shown to be effective in enhancing the storage stability of LGG microcapsules at refrigerated as well as nonrefrigerated storage. However, Na-ascorbate did not show any protective effect; instead, it had detrimental effects on probiotic survival during storage. The significant correlation between LGG viability and acetic acid suggests that the development of this acid was a major determinant of LGG viability. Although it is not possible to ascribe the relative contributions of the various mechanisms that account for the development of the acetic acid as well as other volatile compounds in the headspace, it appears that the negative effect in the presence of Na-ascorbate was a consequence of a combination of chemical degradative reactions involving Na-ascorbate and the catabolism of this compound to acetic acid by LGG. The main insight from this work is that both the chemical effects of additives and their metabolic fate in the presence of probiotic bacteria have to be taken into account when they are incorporated into probiotic preparations.

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