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Suitability of a Freeze Dried Product as a Vehicle for Vitamin Fortification of Military Ration Packs: A Preliminary Study

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Human Protection and Performance Division
Defence Science and Technology Organisation

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ABSTRACT

The intake of adequate energy and nutrients plays a fundamental role in ensuring that Australian Defence Force (ADF) personnel are operationally ready. Combat ration packs (CRP) may be provided to soldiers when it is not practical to feed them with fresh food. CRP may be fortified with vitamins to offset losses during storage and to minimise the impact of other factors that could otherwise lead to inadequate vitamin intakes by consumers. This study investigates the suitability of a freeze dried meal as a carrier for vitamins C, A and E. A fortified freeze dried meal was prepared and was subjected to a storage trial. Vitamin losses during fortification and storage were determined. Losses during storage were low for all vitamins, although losses during fortification were high for vitamin E. There was evidence of protective effects when vitamins were added in combination.

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Suitability of a Freeze Dried Product as a Vehicle for Vitamin Fortification of Military Ration Packs: A Preliminary Study

Executive Summary

Australian Defence Force (ADF) soldiers are provided with food in the form of combat ration packs (CRP) when access to fresh food is not practical. The CRP storage and distribution system provides for CRP to remain in the system for up to three years and six months before issued to the consumer. During this period vitamin levels decline—in some cases dramatically—due to a range of factors that affect their stability.

Fortification of CRP components can make a significant contribution to resolving the problems of loss of vitamins during processing and storage, selective consumption by troops, uneven distribution of nutrients across the range of components and naturally low levels of certain vitamins. Fortification can reduce the risk that soldiers may fail to meet the military recommended dietary intake (MRDI) for particular vitamins. Fortification can also be used to boost the levels of certain vitamins, such as those with antioxidant properties that may confer special benefits to the soldier.

Vitamins C, A and E were selected as fortificants in this study as they are at risk of being underconsumed, due to low initial levels, poor distribution across CRP components and high losses during processing and storage.

The aims of this study were to:

1. Investigate the suitability of a freeze dried meal as a vehicle for vitamin fortification.
2. Determine the losses of fortificants during processing.
3. Determine the losses of fortificants during storage.
4. Determine the protective effects if any when fortificants are added in combination.
5. Make recommendations based on the experimental results.

The product selected for fortification was Chicken Tetrazzini. The freshly cooked meal was fortified with each vitamin and with combinations of the vitamins. The product was freeze dried and samples were placed on storage for up to 24 months at temperatures of 20, 30, 37 and 48 °C. At designated time points samples were withdrawn from storage and analysed to determine vitamin levels.

Conclusions

1. This study has demonstrated that a freeze dried meal matrix provides a suitable vehicle for fortification with vitamins C, A and E when added as coated ascorbic acid, calcium ascorbate, beta-carotene and vitamin E acetate.
2. Losses of vitamin C—added as coated ascorbic acid and calcium ascorbate—from the point of fortification to the end of the storage trial were low (up to 16%) for samples stored at 20–37 °C for 12 and 24 months. Losses during the freeze drying and storage steps are negligible with most losses occurring during fortification. Reduced processing losses of ascorbic acid were observed when added as coated ascorbic acid together with beta-carotene and vitamin E acetate. During storage a clear loss trend was evident for all treatments but only at 48 °C.
3. Losses of vitamin A—added as beta-carotone alone—were moderate (49–50%) for samples stored at 20–37 °C for 12 and 24 months. Approximately half the overall loss occurred during storage. A protective effect was observed with no storage loss trend observed for vitamin A added as beta-carotene in combination with ascorbate and vitamin E acetate.
4. Losses of vitamin E—added as DL-alpha-tocopherol acetate—were very high during fortification whether added alone (88%) or in combination (76–79%). A protective effect was observed when vitamin E was added in combination with vitamins C and A. No loss trends were observed during storage at any temperature.
5. The data obtained during this study, particularly during the storage trial, was characterised by large variations suggestive of analytical errors or methodological difficulties. This complicated and to some extent limited our interpretation of the data.

Recommendations

It is recommended that further research be conducted to confirm and extend the findings of this study. Future work should:

1. Exercise greater control over vitamin analyses to maximise confidence in the data.
2. Determine changes in organoleptic characteristics during storage.
3. Investigate the performance of alternative fortificants, vehicles and processes.
4. Further investigate the vitamin losses.

Recommendations 3 and 4 are expanded in the report.

Authors

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Lan has been employed at DSTO-Scottsdale since 2004. Her R&D work experience has been in the fields of food science and technology and food chemistry, with particular interests in food fortification, microencapsulation, product development and food analysis. She graduated from Victoria University, Melbourne (VUT) firstly with BSc (Hons) and then a PhD in Food Science and Technology in 2004. She is a member of the Australian Institute of Food Science and Technology (MAIFST).

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Ross commenced work at DSTO-Scottsdale in 1988 and has gained broad experience in the fields of chemistry, food technology, microbiology and nutrition. He holds a MBA - Technology Management (Latrobe University), B.App.Sc. in Chemistry and Geography (University of Tasmania) and an Assoc.Dip. in Laboratory Technology (Riverina-Murray Institute of Higher Education). He is a Member of the Royal Australian Chemical Institute and a Member of the Nutrition Society of Australia. Ross is currently Site Manager and a Task Leader of work programs focussed on nutrition and food science and technology.

Contents

1. Introduction

1.1 Combat ration packs and nutrition

Australian Defence Force (ADF) soldiers are provided with food in the form of combat ration packs (CRP) when access to fresh food is not practical. Australian CRP comprise a range of individual food items assembled into packages of sufficient quantity and variety to sustain one person for one day (Combat Ration One Man and Patrol Ration One Man) or five persons for one day (Combat Ration Five Man). CRP may be the sole food source for several weeks, therefore, it is important that they provide sufficient nutrients to not only sustain the consumer but to ensure that they are as well nourished as possible.

Although CRP may contain adequate levels of nutrients immediately after manufacture, the components are not consumed at that time. The CRP storage and distribution system provides for CRP to remain in the system for up to three years and six months before issue to the consumer (DMO, 2009). During this period vitamin levels decline, in some cases dramatically, due to a range of factors that affect their stability (Figure 1). Depending on the storage temperature and duration, the vitamin C loss from some CRP food items (e.g. Chocolate Ration, Fruit Grains and Fruit Spreads) may exceed 90%, while thiamine losses from main meal items may exceed 60% of initial levels[1] (Table 1). Significant losses have also been reported for commercially available products (Table 2).

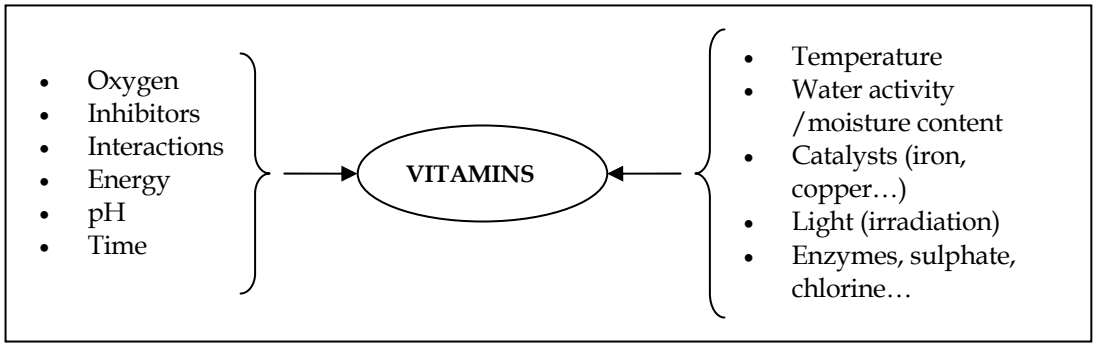

Figure 1: Factors affecting the stability of vitamins (Ottaway, 2008).

The potential for vitamin deficiencies in CRP menus has been investigated (Bui *et al.*, 2005). The initial levels of vitamins may not be high enough to sustain high losses during storage and still retain adequate amounts at the time of consumption. In fact, due to the processed nature of CRP food components, initial levels may be quite low unless raised through fortification.

One of the main concerns is the level, high loss rate and poor distribution of vitamin C across the range of CRP components. Although vitamin C is the vitamin most at risk post-storage,

[1] DSTO-Scottsdale unpublished data, 2004. These values are based on the measured losses, compared to initial levels, from samples of these foods stored at 37 °C for 12 months. This is comparable to the losses that would be expected due to storage at a lower temperature for a longer period; 24 months at 25–30 °C is expected to be approximately equivalent to 12 months at 37 °C, based on the assumption that the reaction rates approximately double with a temperature increase of 10 °C.

thiamin and riboflavin are also of some concern. Vitamin A levels have been found to be marginal post-storage and poorly distributed across CRP components (Coad *et al.*, 1996). Similarly, Vitamin E levels in CRP have been assessed as inadequate and poorly distributed with up to 70% contained in two components (Stephenson and Walker, 1997).

Table 1: Vitamin C, A and E losses during storage (DSTO - unpublished data - Ration built in 2002/2003, stored and analysed in 2004–2006)

Vitamin	**Losses during storage (%)**	
DSTO data	**% loss**	**Fortified food items, stored at 37 °C/12month**
Ascorbic acid (Vitamin C)	100%	Fruit grains, Fruit spread
	≥ 90%	Baked beans, Chocolate ration
	≥ 50%	Retort pouched meals
Vitamin E	> 75%	Retort pouched meals, Noodles, instant
	> 50%	Chocolate, ration
Vitamin A	> 30%	Cheese, processed, cheddar
	> 50%	Scotch-finger biscuit
	> 80%	Chocolate ration
Thiamin	Up to 60%	Baked beans
	Up to 80%	Chocolate ration, Beef kai si ming, Chicken, pasta & vegetable
Total folate	> 50%	Potato & onion powder, Tomato soup powder
	Up to 75%	Vegemite

The problems of vitamin losses and naturally low levels in some components are compounded by the fact that CRP are generally under-consumed (Booth *et al.*, 2001; Carins, 2002; Carins and Tennant, 2010). This, coupled with the poor distribution of vitamins across the range of components, results in the adequacy of intake being subject to the choices of the consumer. Consequently, for a variety of reasons, soldiers may fail to meet the military recommended dietary intake[2] (MRDI) (Forbes-Ewan, 2009) for particular vitamins.

[2] MRDI have been developed for a range of activity levels, age groups and for male and female ADF members (Forbes-Ewan, 2009).

Table 2: Vitamin C, A and E losses during storage (literature review)

Vitamin	**Losses during storage**	
	% loss	**Fortified food items, stored at various short time and low temperature**
Vitamin C	41 to 52%	Fortified whole milk, chocolate milk and low fat milk, 4 °C/36 days (Head and Hansen, 1979)
	63%	Fortified instant noodles (Sanyoto *et al.*, 2008)
	29 to 41%	Fruit juices (closed containers), room temperature/4 mth (Kabasakalis *et al.*, 2000)
	23%	Multivitamin tablet after 6 mth in plastic containers at 25 °C and 75% relative humidity (RH) (Ottaway, 2008)
	60 to 67%	Orange juice, 4 °C/31 days, open containers (Kabasakalis *et al.*, 2000)
Vitamin A	44%	Multivitamin tablet 25 °C/6 mth, 75% RH, in plastic containers (Ottaway, 2008)
	50%	Flour (37 °C/6 mth or 45 °C/3 mth) (Anderson and Pfeifer, 1970)
	>90%	Vitamin A palmitate in corn flakes, room temperature/ 6–8 weeks (Kim *et al.*, 2000)
	50%	Flour (40 °C/6 mth) (Parrish *et al.*, 1980b)
Vitamin E	80%	Wheat flours, (alpha-tocopherol and alpha-tocotrienol) stored at room temperature/12mth (Piironen *et al.*, 1988)
	60%	Wheat flour (beta-tocopherol and beta-tocotrienol) stored at room temperature/12 mth (Piironen *et al.*, 1988)
	33%	Vitamin E added to bread (Ranhotra *et al.*, 2000)
	26%	Vitamin E-fortified fresh-cut pears stored at 2 °C and 88% RH (Lin *et al.*, 2006)

1.2 Fortification

Over the last 70 years, food fortification has played a major role in the health of populations around the world by providing a means to eliminate many nutritional deficiencies. It has the advantage of being able to improve the quality of nutritional intake to a target population without requiring radical changes in food consumption (Mannar and Wesley, 2008). The potential benefits of fortification are so significant that some vitamin additions are mandated (Mannar and Wesley, 2008), such as the fortification of flour with thiamine and folic acid in Australia (FSANZ, 2010).

In a military feeding context, fortification of CRP components can make a significant contribution to resolving the problems of loss of vitamins during processing and storage, selective consumption by troops, uneven distribution of nutrients across the range of components and naturally low levels of certain vitamins. This has been recognised by Australia, the USA (Baker-Fulco *et al.*, 2001) and other nations who currently fortify CRP components.

Fortification can also be used to boost the levels of certain vitamins, such as those with antioxidant properties that may confer special benefits to the soldier. Military personnel have unique needs that require them to perform at peak physical and/or psychological levels under difficult conditions, including in hot environments. Improving the nutritional fitness of the military has been recommended (Scott *et al.*, 2010) and a need to increase antioxidants during exercise in hot environments has been suggested (Burke, 2001; AIS, 2009). In a review of nutritional concerns and countermeasures related to space flight, Cena, Sculati and Roggi (2003)

observed that supplementary dietary antioxidants such as vitamin C, vitamin E and beta-carotene may be of benefit to people engaged in physical exercise and training.

The current report provides details of a study conducted by DSTO to investigate the fortification of CRP with vitamins that are at risk of being present at low levels at the time of consumption.

1.3 Selection of fortificants

There were two key decisions to be made regarding the selection of fortificants for this study. Firstly, the vitamins that were to be added needed to be identified. Secondly, the form in which those vitamins should be added needed to be determined.

For the reasons already given, vitamins C, A and E were selected as fortificants for this study. The forms in which the vitamins were to be added are stated in the Materials and Methods section. There is evidence of a protective effect when antioxidant vitamins are present together in a matrix (Morais *et al.*, 2002; Sanchez-Moreno *et al.*, 2003). Therefore, in this study the vitamins were added alone and in combination to observe any protective effects that may result.

1.4 A vehicle for fortification

The Australian Defence Force Food Specifications (ADFFS) has long required that certain CRP components, such as main meals, confectionery and beverages, be fortified with vitamins (A, C, E, thiamine, niacin and riboflavin) (DoD, 2008). However, there is a particular concern about the loss of vitamins from retort pouched main meal items—50–80% loss of vitamins C and E in some main meals during storage—as these are generally well consumed compared to other components (Probert *et al.*, 2010). Whilst the main meal items offer the advantage of being well-consumed, there is the disadvantage of being a food matrix that results in fairly rapid loss of vitamins.

DSTO-Scottsdale has a long history of producing freeze dried meals for military ration packs. Freeze drying reduces the moisture to very low levels—less than 2% w/w—thereby reducing the water activity and significantly slowing many chemical reactions. Vitamin degradation is reduced at low water activities (Lee and Labuza, 1975). The freeze dried products are nitrogen flushed and vacuum packed in high barrier foil laminate providing further protection from reactions involving oxygen and water. Freeze drying results in foods that can be stored and distributed under non-refrigerated conditions while minimising changes in nutritional value and acceptability (Ratti, 2001).

Freeze dried meals were selected as the vehicle for fortification in this study. The advantages include: the main meal items are highly acceptable, the fortificants are in a highly protective environment, the products are shelf-stable and we had ready access to the food production and fortification process.

1.5 Shelf life of CRP

A warranty period of two years from the date of acceptance is applicable to CRP, however it may be up to three and a half years before they are consumed (DMO, 2009). The ADFFS requires that individual CRP food components have a shelf life of two years when stored at

30 °C (DoD, 2008). Therefore, CRP should continue to meet the MRDIs for at least two years when stored at 30 °C, and preferably for three and a half years. The stability of fortificants over this period is of interest in this study.

1.6 Aims of the study

The aims of this study were to:

1. Investigate the suitability of a freeze dried meal as a vehicle for vitamin fortification.
2. Determine the losses of fortificants during processing.
3. Determine the losses of fortificants during storage.
4. Determine the protective effects if any when fortificants are added in combination.
5. Make recommendations based on the experimental results.

2. Materials and Methods

2.1 Fortificants

Commercially available forms of vitamins have been reviewed for their suitability as fortificants for CRP components (Bui, 2005). The forms that were selected for this study are all approved food additives[3] (FSANZ, 2010) and are described in the following paragraphs.

2.1.1 Vitamin C (ascorbic acid)

Vitamin C was added as CVC™ Type A Coated Ascorbic Acid and C·CAL-97™ Calcium Ascorbate[4]. Both are white or almost white in colour and virtually odourless. Calcium ascorbate provides the additional benefit of enhancing calcium levels.

2.1.2 Vitamin A

Beta-carotene is nutritionally and commercially the most important form of pro-vitamin A and is of similar stability to vitamin A, being sensitive to oxygen, light and acids (Ottaway, 2008). Vitamin A was added as Lucarotin® 10 CWD S/Y Beta-carotene[5]. It is a free-flowing, red-orange powder that is cold water dispersible (CWD). The beta-carotene in this product is embedded in a matrix of soya protein and glucose syrup and has been stabilised with ascorbyl palmitate and DL-alpha-tocopherol. It has a claimed stability of at least 24 months at room temperature when stored in the unopened original containers (BASF, 2003).

[3] Ascorbic acid, approved food additive number 300; calcium ascorbate, 302; beta-carotene (vitamin A), 106a; DL-alpha-tocopherol (vitamin E), 307.
[4] CVC™ Type A Coated Ascorbic Acid is manufactured by Weisheng Pharmaceutical (Shijiazhuang) Co. Ltd and contains a minimum of 97.5% ascorbic acid (product specification). C·CAL-97™ Calcium Ascorbate is manufactured by Takeda Chemical Industries Ltd and contains 97% calcium ascorbate (product specification). Both products were purchased from Bronson & Jacobs, Sydney who sourced them from BASF Australia Ltd.
[5] Lucarotin® 10 CWD S/Y Beta-carotene was purchased from Bronson & Jacobs, Sydney who sourced it from BASF Australia Ltd. The manufacturer was not declared on the accompanying documentation.

2.1.3 Vitamin E

DL-alpha-tocopherol acetate, the predominant form of vitamin E used in foods and vitamin supplements, was used in this study. It was added as Vitamin E Acetate 500[6]. It is a white to off-white powder. It can be added to even mildly flavoured foods such as bread at relatively high levels without adversely affecting sensory quality (Ranhotra *et al.*, 2000). It is very stable in the presence of air and elevated temperatures (BASF, 2002) and is recommended for fortification of foods (USAID, undated). It is virtually unaffected by the oxidising influences of air and light, including UV light (Merck and Co., 1996).

2.1.4 Level of fortification

When fortifying foods it is normal practice to add a greater amount of fortificant than would be necessary to attain the required concentration. To compensate for future losses an additional amount, termed an 'overage', is added. However, there may be considerations aside from loss compensation that influence the level of overage. For example, beta carotene imparts a strong orange colour—it is often used to colour food—therefore it is important to consider the impact on the organoleptic characteristics of the food when deciding on the level of overage.

The MRDI for vitamin C is 45 mg/day for men and women over the age of 19 years, but there is no upper limit of intake specified (Forbes-Ewan, 2009). The Australian Institute of Sport (AIS) has suggested 500 mg vitamin C per day for 1–2 weeks when commencing a period of increased training stress (AIS, 2009).

Due to the expected poor stability during processing and storage a high level of overage was used for vitamin C. Coated ascorbic acid was added at the rate of approximately 8 x MRDI/100 g of freshly cooked product. In the case of calcium ascorbate, the overage was reduced to approximately 7 x MRDI/100 g of product due to a chalky taste being experienced at higher levels. Based on the level of fortification with calcium ascorbate each freeze dried meal (a single serve of 110 g) would contribute approximately 1410 mg of ascorbate and 160 mg of calcium[7]. The coated ascorbic acid would contribute 1720 mg of vitamin C per serve. The calcium content is a useful contribution towards the MRDI of 1000 mg/day (Forbes-Ewan, 2009). This may be important in a military environment where an increased requirement for calcium has been suggested (Forbes-Ewan, 2009).

Vitamin A is highly sensitive to light. Therefore a high level of overage was used (approximately 8 x MRDI/100 g of freshly cooked product). Beta-carotene is not considered to be toxic because absorption becomes inefficient at high intakes, possibly because conversion of beta-carotene and other provitamin A carotenoids is regulated by the vitamin A status of the individual (Northrop-Clewes and Thurnham, 2002).

[6] Vitamin E Acetate 500 was purchased from Bronson & Jacobs, Sydney who sourced it from BASF Australia Ltd. The manufacturer was not declared on the accompanying documentation.
[7] The calculations assume moisture content of 75% in the fresh product and 2.5% in the freeze dried product.

As Vitamin E acetate is relatively stable, a low overage was used (3 x MRDI/100 g of freshly cooked product). If there were no processing or storage losses this would equate to approximately 125 mg of vitamin E per single serve of the final product. The AIS has stated that 500 IU (~335 mg) of vitamin E per day for 1–2 weeks may be suitable for certain training situations (AIS, 2009).

Six treatments (Table 3) are used in this study. Each vitamin is added alone and in combination with the other vitamins. The vitamin C treatments have been termed C_1 and C_2 and the combination treatments Mix 1 and Mix 2. The target levels of fortification for each treatment are also presented in Table 3.

Table 3: Treatments and target fortification levels

Treatment	Target fortification level in freeze dried meal		
	C (mg/100 g)	A (µg/100 g)	E (mg/100 g)
C_1	1560		
C_2	1280		
A		29250	
E			114
Mix 1	1560	29250	114
Mix 2	1280	29250	114

Notes
1. C_1 = CVC™ Type A Coated Ascorbic Acid
2. C_2 = C·CAL-97™ Calcium Ascorbate
3. Mix 1 = C_1 + A + E
4. Mix 2 = C_2 + A + E

2.1.5 Fortification vehicle

Chicken Tetrazzini was selected for this study. In addition to the previously stated benefits of using freeze dried meals as a vehicle, this particular meal is the most popular of the range. The pH of the freshly cooked meal—6.0—is also favourable (Figure 2), although at the lower end of the range for vitamin A.

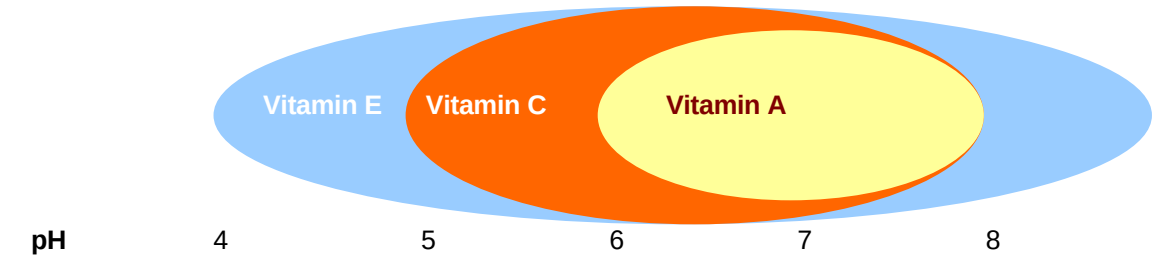

Figure 2: pH optima for vitamins E, C and A in aqueous formulations (data sourced from Bronson & Jacobs, 2004).

2.2 Production of fortified freeze dried product

The Chicken Tetrazzini was prepared from diced chicken, pasta, cream, butter, cheese, mushrooms and other ingredients cooked in a large steam-jacketed kettle and spread on trays prior to freezing and freeze drying.

Each fortificant treatment was added to the food on a tray-by-tray basis. In order to minimise thermal degradation, the additions were made at the end of the cooking stage when the temperature of the cooked food was reduced to ~ 70 °C. A 4.2 kg amount of the cooked meal was removed from the kettle and placed in a bucket. A pre-measured dose of the fortificant was mixed into a small quantity (approximately 250 mL) of the sauce from the cooked meal and then stirred into the bucket of food and mixed thoroughly. The fortified cooked food was spread out on trays and cooled for an hour before transferring to the blast freezer to freeze over night (~ 20 hrs/-20 °C).

The trays of frozen food were loaded into the freeze dryer and dried over an eight hour period. The freeze dried product was removed from the freeze dryer and transferred to plastic bags (one bag per treatment). While in the bag the pieces of freeze dried product were manually broken and tumbled to mix. The bags were placed in drums, evacuated and backflushed with food-grade nitrogen, stored overnight then vacuum packed in 110 g amounts in a heavy duty laminate which included a 25 µm foil layer[8].

Samples were taken during the above process for storage trial as detailed below. A flowchart of the whole process is presented in Figure 3.

[8] This is the final product. Prior to consumption the 110 g single serve package of freeze dried product is reconstituted with water to make 460 g of ready-to-eat Chicken Tetrazzini.

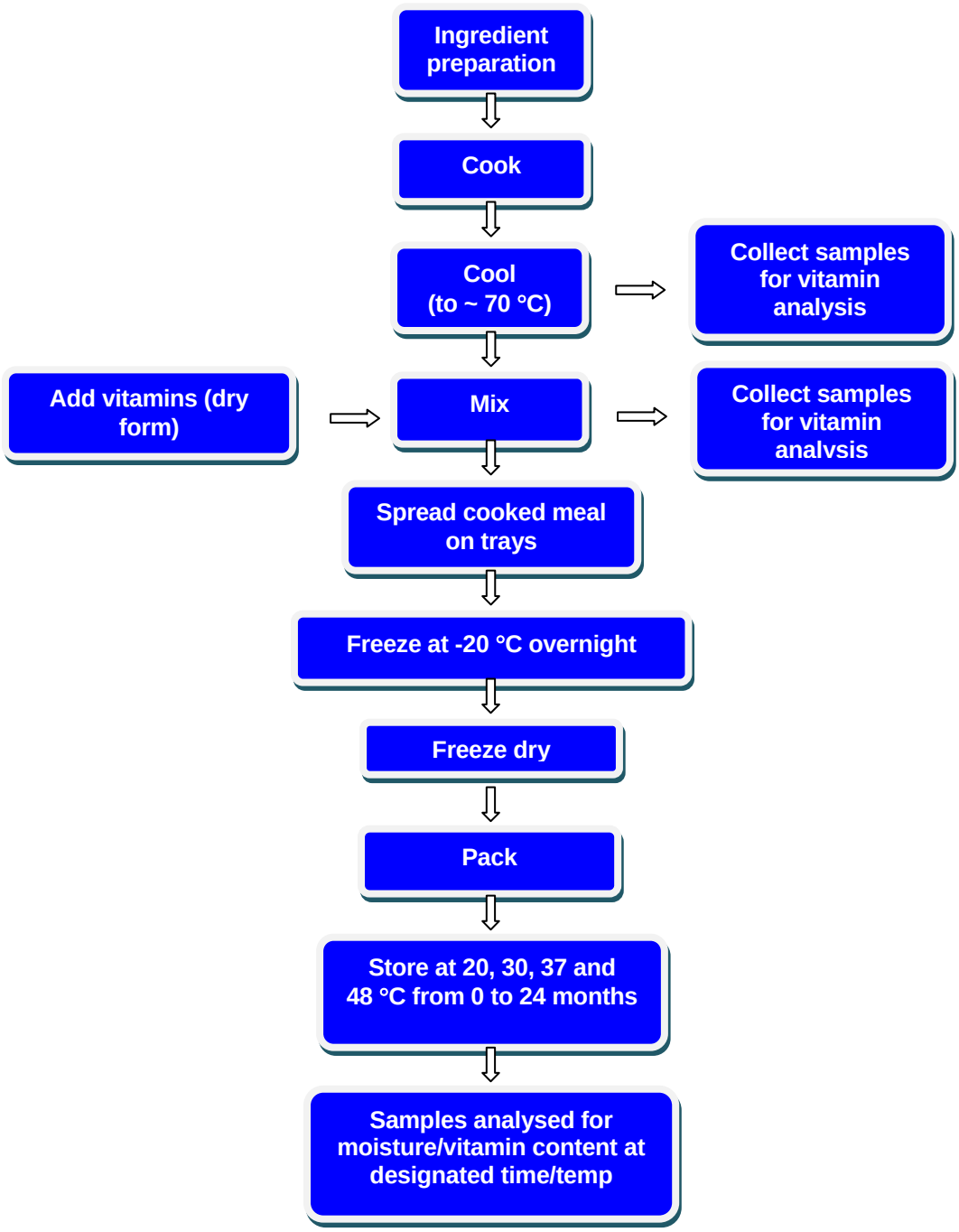

Figure 3: Flowchart for preparation and sampling of fortified Chicken Tetrazzini

2.3 Storage trial

Packages of the freeze dried, fortified product were placed on storage using the profile in Table 4. This is the standard storage profile used by DSTO-Scottsdale to conduct shelf-life evaluation of ration pack components. Each storage trial sample was an unopened 110 g package of freeze dried product. Sufficient samples were allocated to each point in the profile to meet the sampling protocol requirements detailed below.

Table 4: Storage profile

Temperature (°C)	**Storage time (months)**						
	0	1	2	3	6	12	24
	✓						
20		✓		✓	✓	✓	✓
30		✓		✓	✓	✓	✓
37		✓		✓	✓	✓	✓
48		✓	✓	✓	✓		

2.4 Sampling protocol

The following types of samples were collected:

1. Background (samples of the freshly cooked product prior to fortification).
2. Fortified (samples of the freshly cooked product after fortification).
3. Treatment controls (samples of the fortified freeze dried product at zero time).
4. Treatment test samples (samples of the fortified freeze dried product at all other time/temperature points in the storage profile).

Background samples were taken directly from the kettle at the completion of cooking and prior to the meal being spread on the trays. Samples (n=3) were placed in plastic bags (approximately 200 g/bag), cooled to room temperature over a period of one hour, stored in a freezer at -20 °C for 2 days and then express freighted to the laboratory for moisture and vitamin analysis.

Fortified samples (n=9) were collected immediately after fortification using the same technique as for background samples and were submitted for analysis.

Freeze dried treatment control samples (n=9) were collected after packaging was completed. These were the zero time point samples. These samples were submitted to the laboratory for analysis without any need for freezer storage or express freight. Samples of the unfortified freeze dried product were stored at 1 °C and were collected (n=3) at the same time points as the treatment test samples and submitted for analysis.

Treatment test samples (n=3/treatment/storage time point/temperature point) were collected at each point in the storage trial and were held at 1 °C until they were submitted for analysis (Table 5).

Table 5: Storage trial sampling plan

Treatment	Month(s)					
	0	**1**	**3**	**6**	**12**	**24**
C_1	9	3	3	3	3	3
C_2	9	3	3	3	3	3
A	9	3	3	3	3	3
E	9	3	3	3	3	3
Mix 1	9	3	3	3	3	3
Mix 2	9	3	3	3	3	3

Note The numbers in the table represent the numbers of samples taken at each time/temperature point in the storage profile.

2.4.1 Methods for moisture and vitamin analysis

The methods of analysis were: high performance liquid chromatography for the vitamin determinations, sand moistures for wet samples and convection oven moistures for the freeze dried samples. These analyses were outsourced to a commercial NATA Accredited laboratory[9].

2.4.2 Statistical model and calculation methods

The purpose of the calculations and statistical analysis is to determine vitamin losses during processing and storage and to determine the presence of protective effects.

The loss of vitamins during addition to the meals was calculated as the difference between the amount added (weight of fortificants) plus that naturally present and the amount measured in the samples of fortified food. The moisture content was determined in the samples of freshly cooked food pre-fortification, post-fortification and in the freeze dried product. This enabled a calculation of the theoretical amount of vitamins in the freeze dried product, which in turn enabled a calculation of the loss during freeze drying.

The theoretical level of vitamins in the freeze dried product was calculated as:

$$Fortified\ level_{post\text{-}FD} = Fortified\ level_{pre\text{-}FD} * (100 - moisture_{post\text{-}FD}) / (100 - moisture_{pre\text{-}FD})$$

Therefore these were calculated levels based on the amount of vitamin added to the product plus the background levels. No adjustment was made for losses occurring after addition and before freeze drying.

The reduction in vitamin levels following freeze drying was calculated as:

$$Percentage\ Loss_{post\text{-}FD} = 100 * (Fortified\ level_{post\text{-}FD} - Measured\ level_{post\text{-}FD}) / Fortified\ level_{post\text{-}FD}$$

The Percentage Loss $_{post\text{-}FD}$ is a cumulative loss from the moment of addition until completion of the freeze drying process.

[9] NATA Accredited = Accredited by the National Association of Testing Authorities to the requirements of ISO 17025.

Vitamin losses during storage have been calculated as the difference between the initial levels and the levels post storage (the level of the vitamin in the product after storage for the time period under consideration). The initial levels were generally considered to be the measured levels of vitamins in the product after freeze drying, however in some instances—detailed in the results section—the target levels of fortification (Table 2.1) were used as the values for the initial levels.

One of the factors of interest is whether there are any differences in loss characteristics among fortificants, in particular for each vitamin or in combination, and whether losses are greater during fortification or storage. Differences between treatments were evaluated using Student's t-test.

Evidence of loss trends during storage was also determined by plotting vitamin levels against time. Vitamin levels were plotted as the measured value and, where considered useful, using the log scale. Linear regression curves were fitted to the data and their coefficients of determination (R^2) calculated. Bar graphs included error bars representing the standard deviation at each data point. In some cases the standard deviation was zero as all three values at that data point were equal, therefore error bars do not appear for all plotted data points.

Where relevant, calculations on the storage trial data included estimates of the half life of vitamin treatments. The estimate was based on the integrated rate law for a first order reaction,

$$\ln[\mathrm{A}] = -kt + \ln[\mathrm{A}]_0$$

where, [A] is the concentration at time t, k is the rate constant and $[\mathrm{A}]_0$ is the initial concentration. The rate constant was estimated from a plot of $\ln([\mathrm{A}]/[\mathrm{A}]_0)$ vs t, which has a slope of $-k$. The half life, $t_{1/2}$, was calculated using the relationship,

$$t_{1/2} = \ln(2) / k$$

The rate of change of reaction rates with changes in temperature is often quantified using the term Q_{10}, which is the change due to increasing the temperature by 10 °C. It is calculated as:

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{10/(T_2 - T_1)}$$

Where, R_1 is the reaction rate at T_1 and R_2 is the reaction rate at T_2. Q_{10} is typically in the range of 2 to 4 for degradation reactions taking place in food matrices (Sewald and DeVries, undated). This relationship is used in this report to assist with the interpretation of the results obtained during storage trials.

3. Results and Discussion

3.1 Vitamins in freshly cooked product

The initial levels of vitamins C, A and E in the freshly cooked product, are summarised in Table 6 and are also presented in a graph form (Figure 4) in order to compare the MRDIs and serving size (460 g) levels. The moisture content of the freshly cooked product is 75%.

Table 6: Vitamin content in freshly cooked product versus the MRDIs (Forbes-Ewan, 2009)

MRDIs and vitamin levels in fresh product	C (mg)	A (ug)	E (mg)
MRDI (for men aged 19-30)	45	900	10
MRDI (for women aged 19-30)	45	700	8
Vitamins/100 g	1.9	14	0.37
Vitamins/serving size (460 g)	8.7	64	1.7
Percentage MRDI (men) /serving size (460 g)	19%	7%	17%

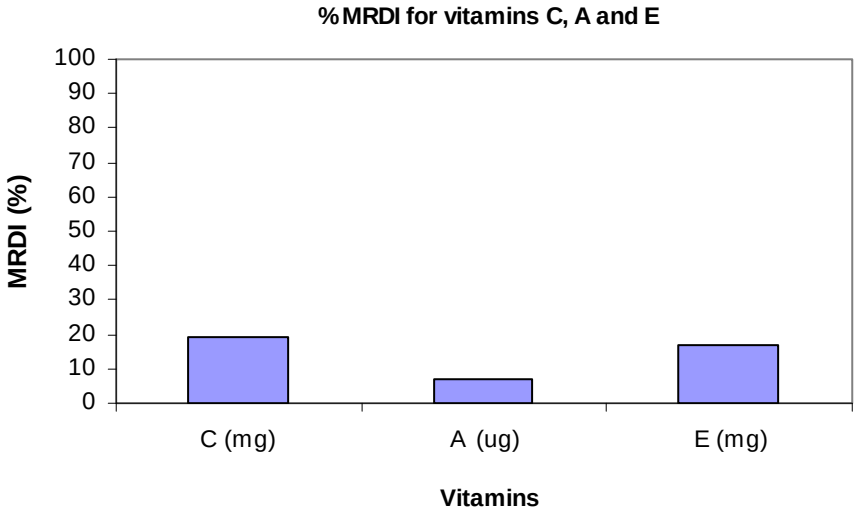

Figure 4: Vitamins per serving size (460 g of fresh cooked meal) versus MRDIs

This meal, despite being of a large serve size, makes a poor contribution to dietary intake of vitamins C, A and E providing only 19, 7 and 17% of the MRDIs respectively. The deficit must be obtained from the remaining CRP food items among which the vitamins are poorly distributed. Due to discards and vitamin storage losses, there is a risk of deficiency in vitamin intake.

3.2 Vitamin levels post fortification, prior to freeze drying

The effect of treatment on vitamin levels is presented below (Table 7). Significant differences between treatments are indicated.

Table 7: Vitamin levels in the meal post fortification (prior to freeze drying)

Treatment	Vitamin levels in meal - Mean (SD)		
	C (mg/100 g)	A (µg/100 g)	E (mg/100 g)
C_1	338 (16) [a, b, c]		
C_2	306 (13) [a, d]		
A		3630 (1060)	
E			3.5 (0.9) [f, g]
Mix 1	369 (21) [b, d, e]	3519 (1248)	7.2 (1.3) [f]
Mix 2	313 (40) [c, e]	4148 (598)	6.2 (0.6) [g]

Notes
1. For each value, n = 9.
2. Moisture content = 75%
3. Within a column, values followed by the same letter are statistically different from each other (P< 0.05).

Consistent with the differing levels of fortification, the vitamin C levels are different for C_1 and C_2 and for Mix 1 and Mix 2. In addition, there appears to be a protective effect when C_1 is added together with vitamins A and E in Mix 1, as the vitamin C level is significantly higher when the fortificant is Mix 1 compared to C_1.

There was no significant difference between treatments for vitamin A. However, there was evidence of a protective effect for vitamin E when added together with vitamins C and A for both Mix 1 and Mix 2, this was highly significant (P<0.001). The difference in vitamin E level between Mix 1 and Mix 2 is not statistically significant (P=0.06).

3.3 Vitamins losses prior to freeze drying process

The losses of vitamins from the fortified meals prior to freeze drying was determined (Table 8). The losses were highest for vitamin E and lowest for vitamin C, with vitamin A being approximately midway between the other two.

3.3.1 Vitamin C

Vitamin C losses during fortification ranged from 5–16%. The higher figure may be in part due to issues with the analysis—this is discussed in a later section. These losses are relatively low and may have been aided by the high rate of fortification (Bui and Small, 2007; Butt *et al.*, 2007).

3.3.2 Vitamin A

Losses of vitamin A ranged from 45–53% of the added amount. These losses are not unexpected as the addition was made at moderate temperatures, in the presence of air and light. Furthermore, the pH of the meal is at the lower end of the preferred pH range. Further work in

this area should anticipate approximately 50% loss of vitamin A when added in this form (stabilised beta-carotene).

Table 8: Vitamins losses during fortification

Vitamin	**Losses (%)**
C_1	16
Vitamin C (Mix 1)	8
C_2	7
Vitamin C (Mix 2)	5
A	52
A (Mix 1)	53
A (Mix 2)	45
E	88
E (Mix 1)	76
E (Mix 2)	79

Note For each value, n = 9.

3.3.3 Vitamin E

Losses of vitamin E acetate during fortification were higher than expected considering its claimed resistance to degradation due to air, temperature and light. The losses may be due to the effects of freezing the samples prior to transporting them to the laboratory and thawing prior to analysis. Therefore it is not certain that the fortification process leads directly to the degradation.

The next step in the production process is to freeze the meals prior to freeze drying, therefore, if there were losses due to freezing the samples this may simply replicate what would happen during production. Another consideration is that the potency of the added vitamin E acetate was not measured—the claimed purity was assumed to be correct.

Future fortification of vitamin E, at least as vitamin E acetate, whether alone or in combination with vitamins A and C, should make allowance for approximately 90% loss when added in this manner.

3.4 Vitamin levels after freeze drying

The effect of treatment on vitamin levels after freeze drying is presented below (Table 9). Significant differences between treatments are also indicated. The moisture content of the freeze dried product is 2.5%.

Table 9: Vitamin levels in the meal post fortification (after freeze drying)

Treatment	Levels in meal (Mean and SD)		
	C (mg/100 g)	A (µg/100 g)	E (mg/100 g)
C_1	1600 (71) [a, b, c]		
C_2	1333 (132) [a]		
A		18778 (1986)	
E			18 (1.6) [d, e]
Mix 1	1311 (298) [b]	18556 (2404)	29 (3.1) [d, f]
Mix 2	1178 (199) [c]	18667 (1936)	22 (1.9) [e, f]

Note
1. For each value, n = 9.
2. Moisture content = 2.5%
3. Within a column, values followed by the same letter are statistically different from each other (P< 0.05)

There are significant differences between C_1 and C_2, as expected, but not between Mix 1 and Mix 2. The data is relatively scattered and the ability to detect protective effects, if present, has been compromised. In contrast to the results prior to freeze drying, it now appears that C_1 is better added alone rather than together with vitamins A and E.

There are no significant differences between treatments for vitamin A.

The previously observed protective effect experienced with vitamin E has remained. The effect is larger with Mix 1. A significant difference between the two treatments, Mix 1 and Mix 2 is also evident. The differences for all pairs of treatments are highly significant (P<0.001).

3.5 Effect of freeze drying process on the stability of vitamins

The total vitamin losses from fortification through to completion of freeze drying are presented in Table 10. Comparison of these results with those presented in Table 8 reveals that there is no evidence of loss of vitamins E and C during the blast freezing and freeze drying process. The main losses of these vitamins occur during the addition and mixing stages. It has been reported that no significant losses of vitamin C were found when freeze drying carrot slices (Lin *et al.*, 1998).

Losses of vitamins C (added as C_1) and A are lower post-freeze drying than pre-freeze drying. This anomaly may be due to factors affecting the samples taken prior to freeze drying. Possible problems include analytical errors and losses during freezer storage, transport and sample preparation at the laboratory.

Table 10: Vitamin losses following freeze drying

Vitamin	Losses (%)
C_1	0
Vitamin C (Mix 1)	0
C_2	16
Vitamin C (Mix 2)	8
A	36
A (Mix 1)	37
A (Mix 2)	36
E	84
E (Mix 1)	74
E (Mix 2)	81

Note For each value, n = 9

No literature reports of other work investigating the effect of freeze drying on added vitamins in the types of product studied here could be located.

3.6 Storage trial results

The aim of the storage trial is to enable an assessment of the stability of the added vitamins in the freeze dried meal matrix under conditions that may be experienced by components in the CRP supply chain.

3.6.1 Vitamin C

The analytical data for some points in the storage profile were inconsistent with overall trends and reasonable expectations[10]. Due to these concerns, and to allow for a consistent approach, all calculated losses for the vitamin C storage trial are based on the target level of fortification as the initial level.

Charts of the storage trial results for 20, 30 and 37 °C (Figure 5 to Figure 8) are shown below for each vitamin C treatment. The charts also show the target level of fortification and the upper and lower 95% confidence limits (UCL, LCL) for the initial level[11] (time zero). The charts provide visual representations of the data trends, inconsistencies and wide ranging standard deviations.

[10] For example, the vitamin C levels after storage for 1 month at 20 °C were approximately double the initial levels. This was queried with the laboratory which corrected a calculation error and re-reported results at approximately the expected levels. In other instances blocks of data appear to be higher or lower than could be reasonably expected; no further calculation errors were reported. The levels at the beginning of the storage trial were found, in some cases, to be relatively low and of higher standard deviation than subsequent points.

[11] The charts show the initial level as measured, whereas losses have been calculated based on the target level of fortification.

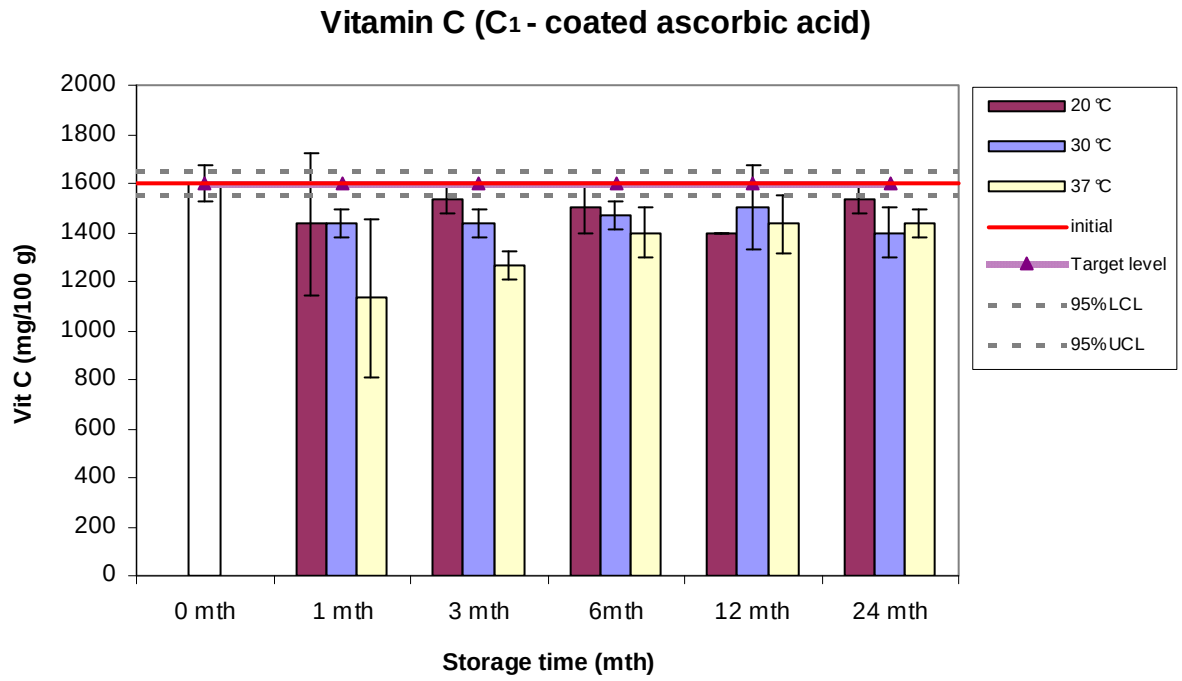

Figure 5: Vitamin C levels following fortification with coated ascorbic acid and storage at 20, 30 and 37 °C for up to 24 months

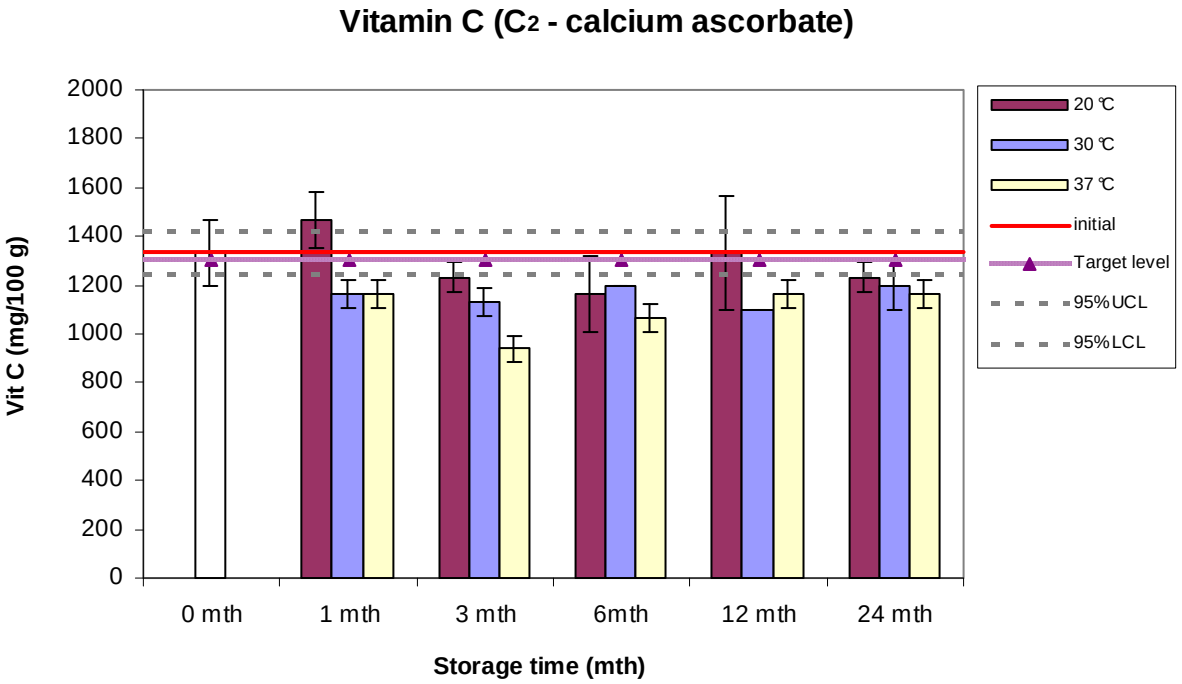

Figure 6: Vitamin C levels following fortification with calcium ascorbate and storage at 20, 30 and 37 °C for up to 24 months

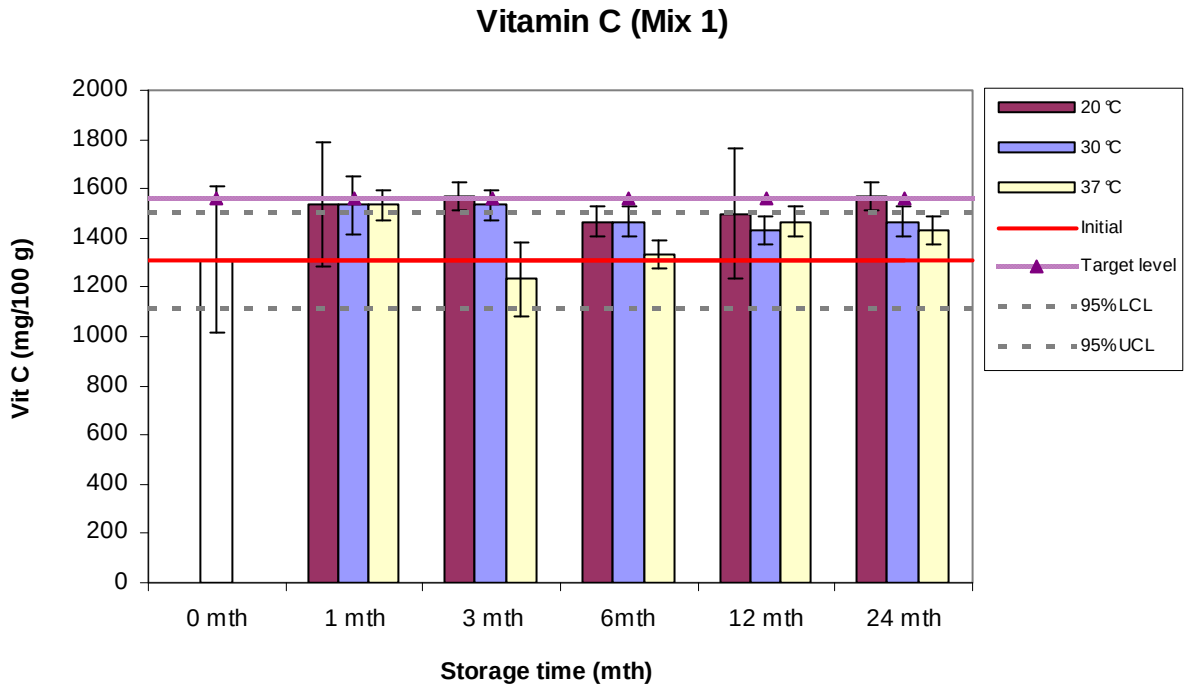

Figure 7: Vitamin C levels following fortification with Mix 1 and storage at 20, 30 and 37 °C for up to 24 months

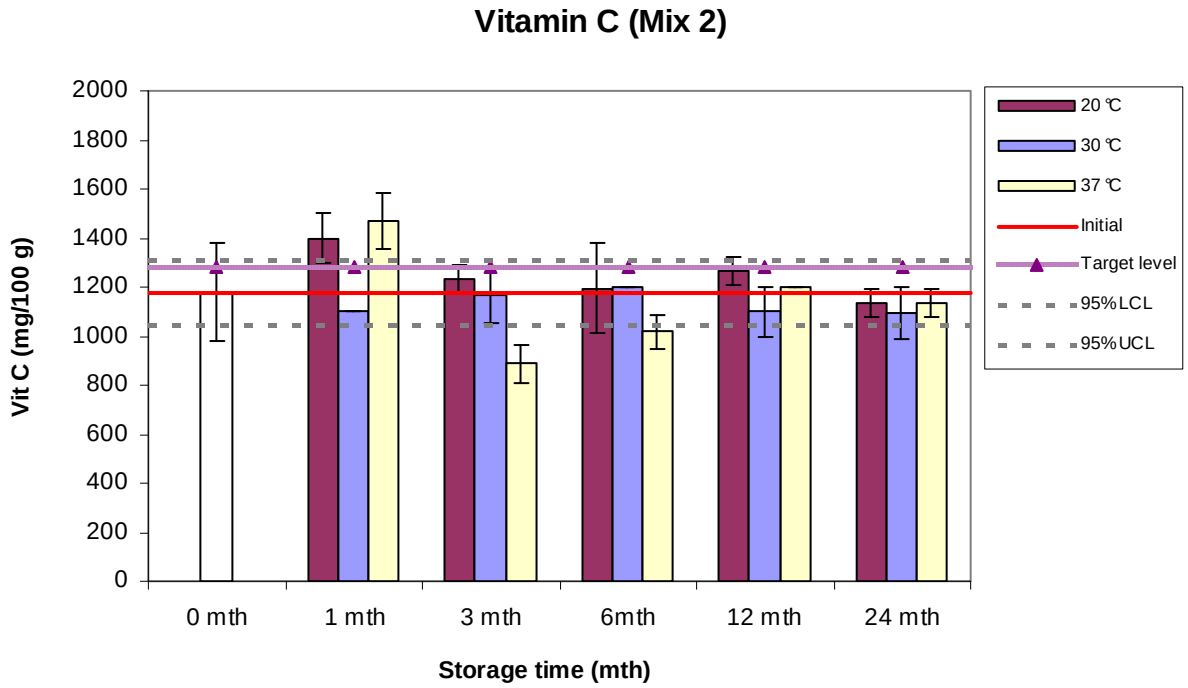

Figure 8: Vitamin C levels following fortification with Mix 2 and storage at 20, 30 and 37 °C for up to 24 months

The charts indicate that the vitamin C levels do not decrease over the storage period when the product is stored at 20–37 °C. If there are any loss trends at 20, 30 and 37 °C these are lost in the data scatter. There is, however, a clear decrease in vitamin C values over time when stored at 48 °C for three months (Figure 9). The differences between C_1 and C_2 and between Mix 1 and Mix 2 are due to the different levels of fortification. The plot of $\log_{10}$ of the vitamin C concentration versus time (Figure 10) clearly shows the decline with good R^2 values (0.73–0.93). Similar plots were also prepared for 20, 30 and 37 °C but have not been presented; no trends or correlations were found.

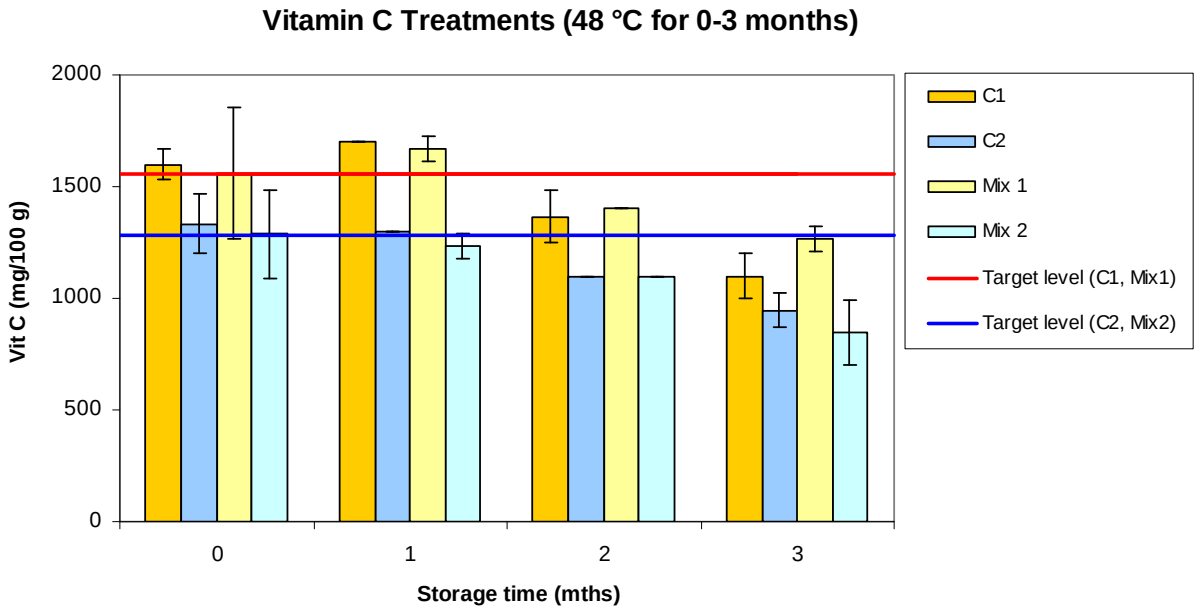

Figure 9: Vitamin C levels during storage for up to 3 months at 48 °C

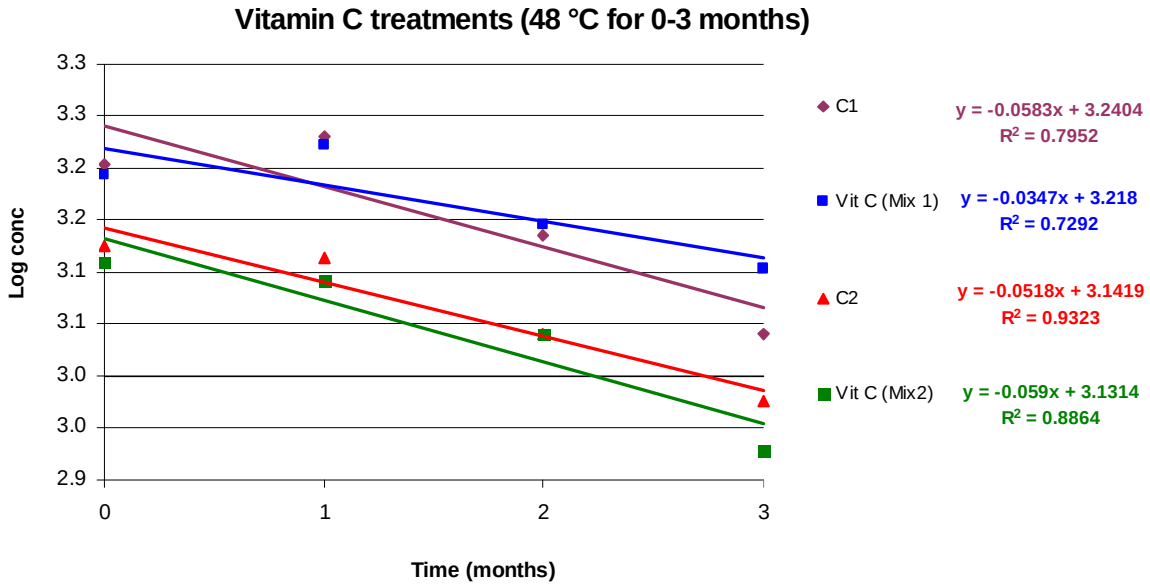

Figure 10: Log_{10} of vitamin C levels during storage for up to 3 months at 48 °C

Although there are no loss trends at 20, 30 and 37 °C there are measurable losses, with one exception, from the initial levels. These are presented in Table 11 and provide an indication of the level of losses that may be occurring without any trend being present. The figures should be viewed with caution because of the large variation in the results. Losses are higher at 48 °C, in line with the information in Figure 9 and Figure 10.

Table 11: Losses of vitamin C during storage (%)

Vitamin C	**Loss of vitamins during storage (%)**						
	20 °C		30 °C		37 °C		48 °C
	12 mth	24 mth	12 mth	24 mth	12 mth	24 mth	3 mth
C_1	10	2	4	10	8	8	30
C_2	-4	4	14	16	9	9	26
Mix 1	4	0	8	6	6	8	19
Mix 2	1	11	14	14	6	11	34

Notes: For each value, n = 3.

Based on the data collected over the three month storage period at 48 °C, the loss rate and half life of vitamin C has been calculated for each treatment (Table 12).

Table 12: Rate of loss and half life ($t_{½}$) of vitamin C during storage at 48 °C for 3 months

Treatment	**Rate of loss** (%/month)	*$t_{½}$ (months)*
C_1	11.8	5.2
C_2	10.6	5.8
Mix 1	7.4	8.6
Mix 2	11.3	5.1

The half life at 48 °C has been used to estimate the half life at 30 °C assuming a Q_{10} of 4 for the temperature range 30–48 °C. On that basis, the half life at 30 °C ranges from 63–105 months for the four treatments, equating to losses of 15–23% over 24 months. An assumed Q_{10} of 2 equates to losses of 42–60% over 24 months storage at 30 °C.

Compared to the losses experienced with other food matrices (Table 1 and Table 2) the losses reported here are relatively low. This provides good evidence of the suitability of freeze dried meals as a vehicle for fortification with vitamin C. There is no clear evidence of a protective effect for vitamin C when added in combination with vitamins A and E.

3.6.2 Vitamin A

Concerns about some of the data are similar to those expressed in relation to vitamin C. Some data was considered to be unrealistic and repeat analyses were requested. It was not, however, practical or economical to investigate all suspect data points, therefore, more critical data points were targeted.

Consequently, the focus was on samples toward the end of the storage trial—12 and 24 months—at 20, 30 and 37 °C. The vitamin A levels in the samples that had been stored for 12 months at 20, 30 and 37 °C were approximately 40% higher than the initial levels. When a second set of samples were analysed the vitamin A levels were found to be equal to or slightly lower than the initial levels, and more in line with expectations. The data set for the samples stored for 3 months at 20, 30 and 37 °C was excluded from the data analysis—without repeat analysis being requested—due to all results being approximately 30% lower than subsequent points in the storage trial.

The charts of the storage trial results for 20, 30 and 37 °C are shown below for each vitamin A treatment (Figure 11 to Figure 13). The charts also show the target level of fortification and the initial level (time zero) with upper and lower 95% confidence limits (UCL, LCL).

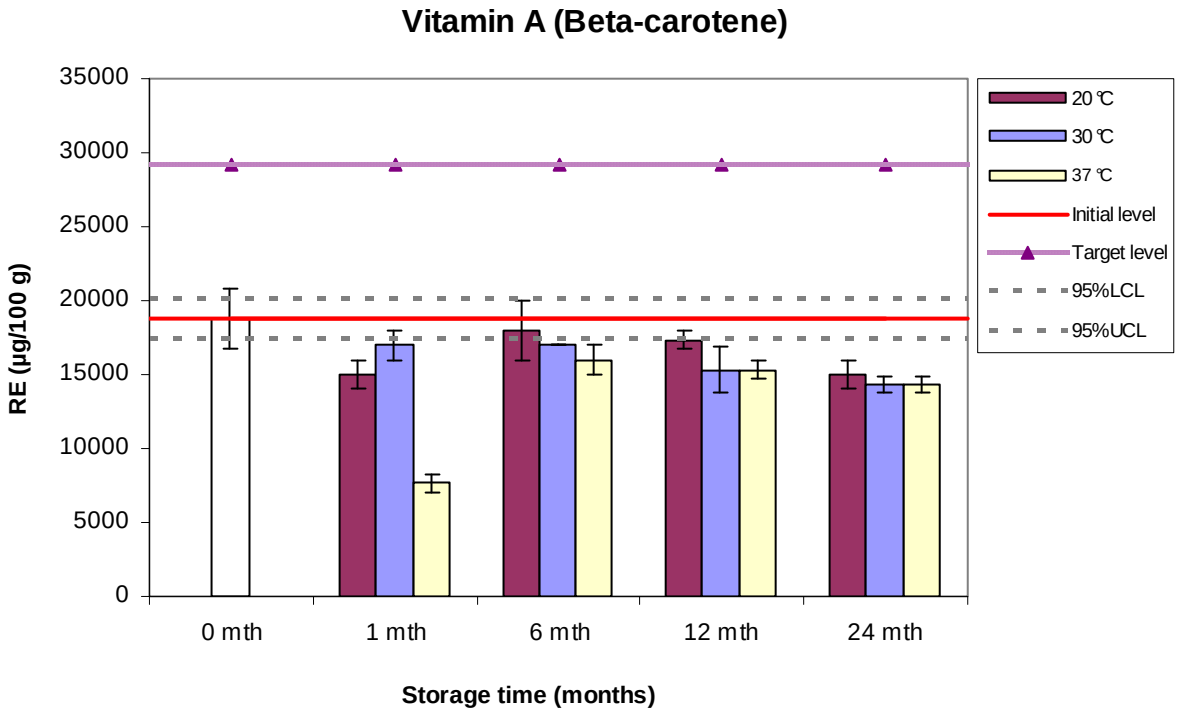

Figure 11: Vitamin A levels, in units of retinol equivalents (RE), following fortification with Beta-Carotene and storage at 20, 30 and 37 °C for up to 24 months

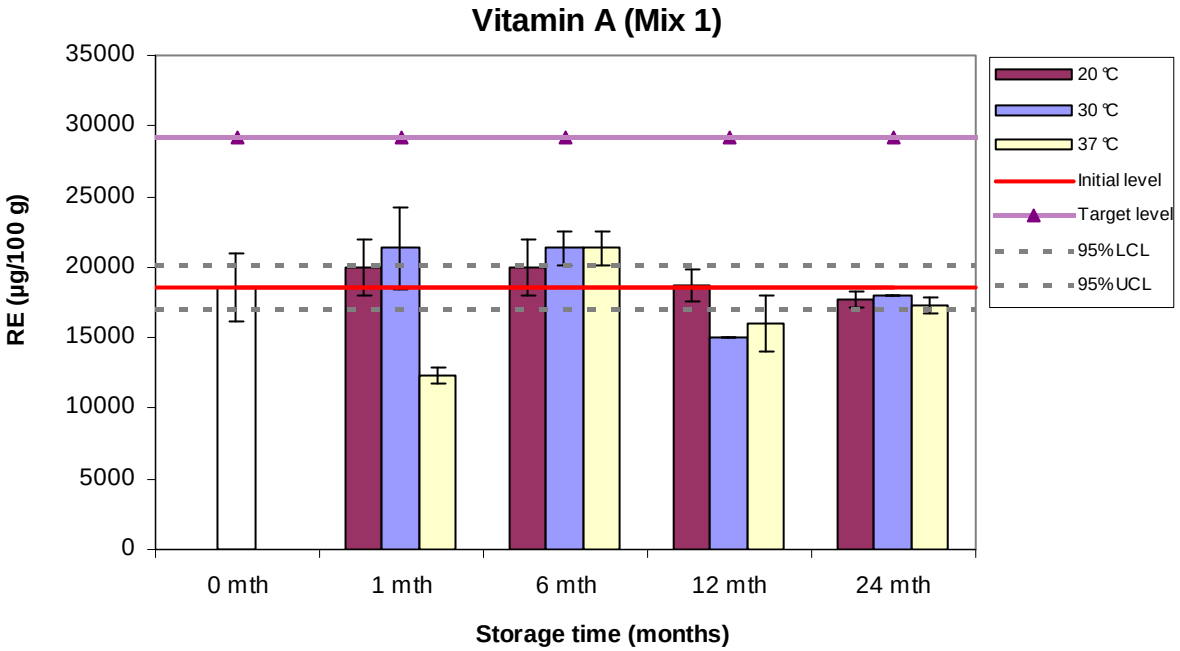

Figure 12: Vitamin A levels, in units of retinol equivalents (RE), following fortification with Mix 1 and storage at 20, 30 and 37 °C for up to 24 months

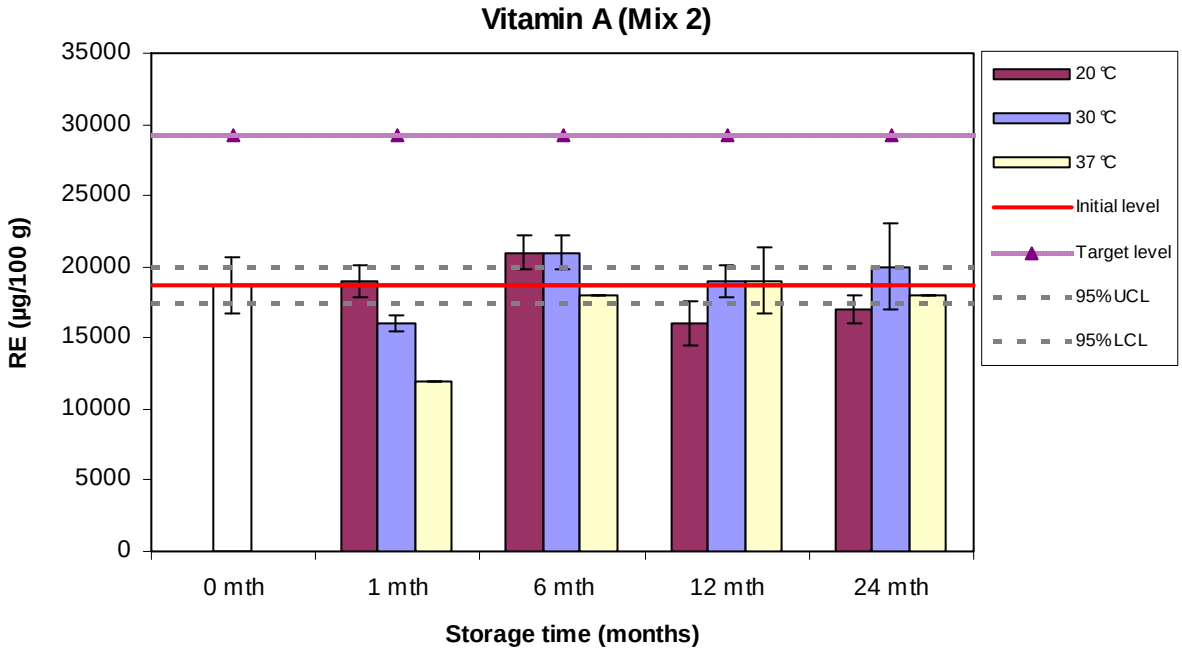

Figure 13: Vitamin A levels, in units of retinol equivalents (RE), following fortification with Mix 2 and storage at 20, 30 and 37 °C for up to 24 months

Visually, there appears to be a downward trend for the first treatment (vitamin A added as Beta-carotene alone) but little or no trend when added in Mix 1 and Mix 2. This has been

explored by plotting the $\log_{10}$ of the concentrations against time (0, 6, 12 and 24 months) and applying a linear fit to each data set (Figure 14 to Figure 16).

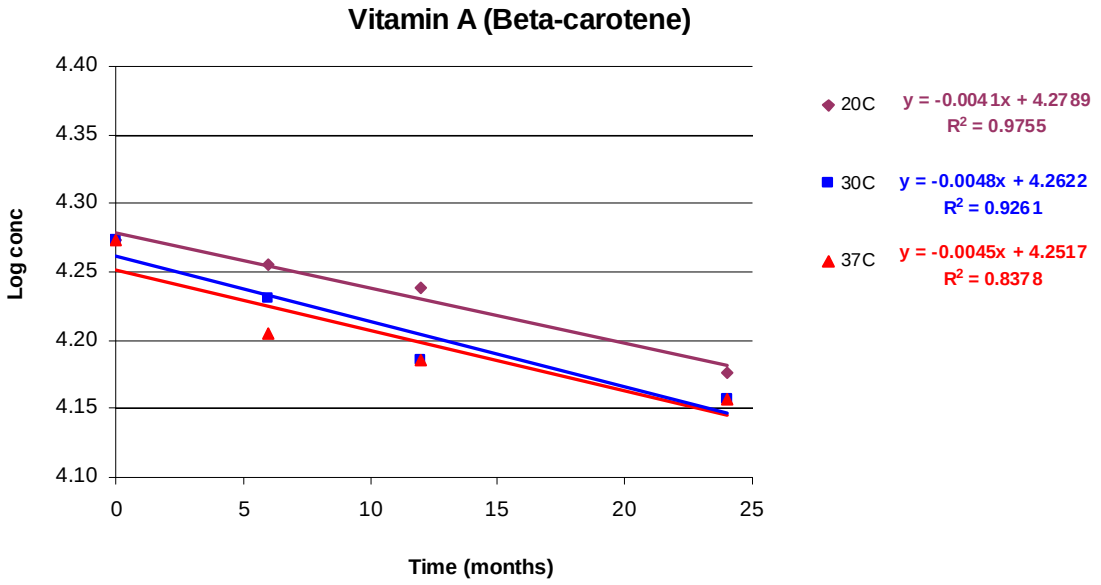

Figure 14: $\log_{10}$ of vitamin A levels following fortification with Beta-Carotene and storage at 20, 30 and 37 °C for up to 24 months

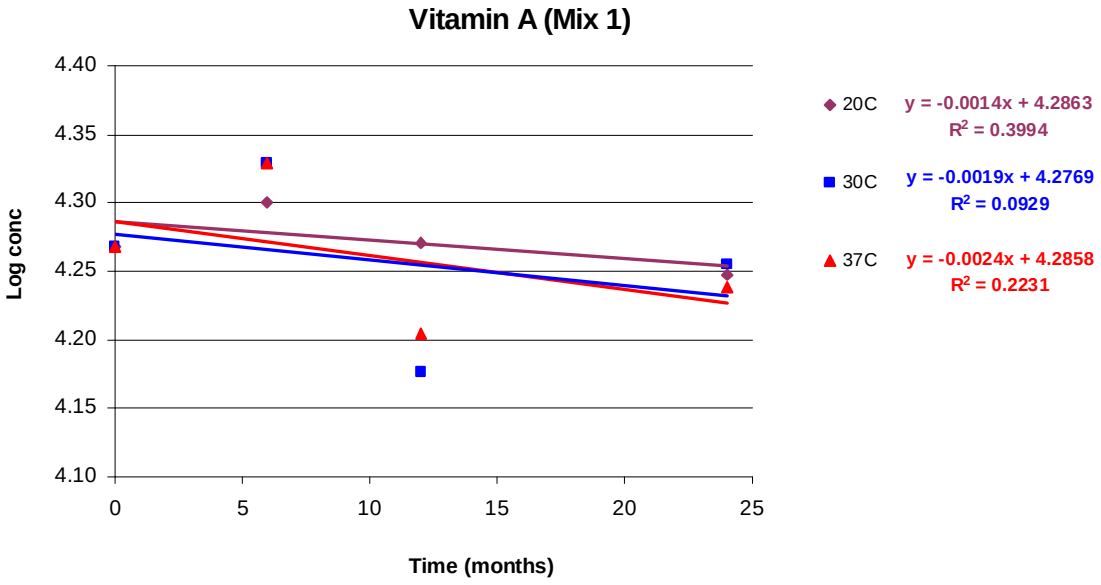

Figure 15: $\log_{10}$ of vitamin A levels following fortification with Mix 1 and storage at 20, 30 and 37 °C for up to 24 months

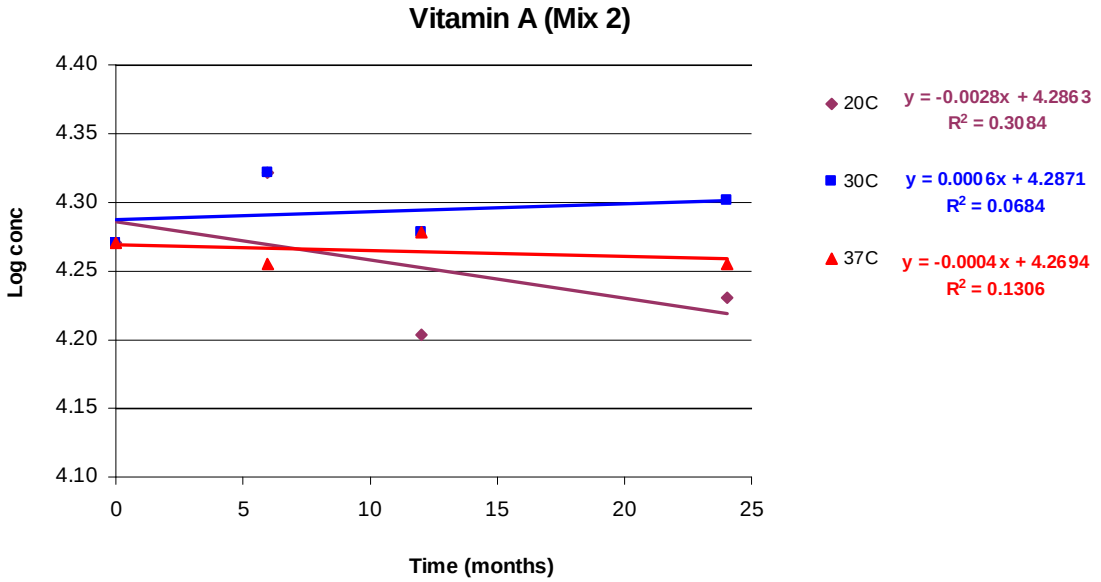

Figure 16: Log_{10} of vitamin A levels following fortification with Mix 1 and storage at 20, 30 and 37 °C for up to 24 months

As previously stated, there were concerns about the analytical data for some points in the storage trial, consequently data for the 1 month time point was excluded from the plots presented in Figure 14 to Figure 16.

The data sets in Figure 8 demonstrate good linear correlations (R^2 0.84–0.98) and very similar slopes (0.0041, 0.0045 and 0.0048). Therefore, the loss rate for vitamin A added as beta-carotene—without vitamins C and E—is well described by the data and is relatively independent of storage temperature. The half lives at 20, 30 and 37 °C are 73, 63 and 67 months respectively, that is, in excess of five years at each of those temperatures.

The plots for Mix 1 and Mix 2 (Figure 15 and Figure 16) do not indicate any loss trend, suggesting that the beta-carotene may be protected when added with vitamin E and either coated ascorbic acid or calcium ascorbate. The previously stated concerns, however, about some of the analytical data and the lack of clear trends suggest that a cautious approach should be adopted and the observed effect verified by further trials.

Ascorbic acid has previously been observed to improve vitamin A (palmitate) stability, for example when added to ready-to-eat cereal (Anderson *et al.*, 1976). Vitamin A palmitate was also found to benefit from the presence of a vitamin mixture (B_1, B_6, B_{12}, C and D) added to corn flakes (Kim *et al.*, 2000). The addition of ascorbic acid to paprika significantly improved the stability of beta-carotene during storage for up to 54 days at 22 °C (Morais *et al.*, 2002). Ascorbyl palmitate and DL-alpha-tocopherol have been utilised to protect the beta-carotene preparation —Lucarotin® 10 CWD S/Y Beta-carotene—used in our work (see section 2.1 above). The vitamin E acetate used in our study is a preferred form for fortification but is not considered to provide any protective effect, unlike the free alcohol form (Anderson *et al.*, 1976).

Changes observed during storage at higher temperatures for shorter periods of time may provide early warning of changes that would occur at lower temperatures over longer storage periods. Figure 17 shows the results of storage at 48 °C for up to 3 months for all three treatments. The initial levels are very similar for all three treatments, therefore, they appear as a single line on the chart. There is no clear trend towards lower levels over time for Mix 1 and Mix 2; this is supported by the plots in Figure 15 and Figure 16 where the R^2 values are poor for each data set.

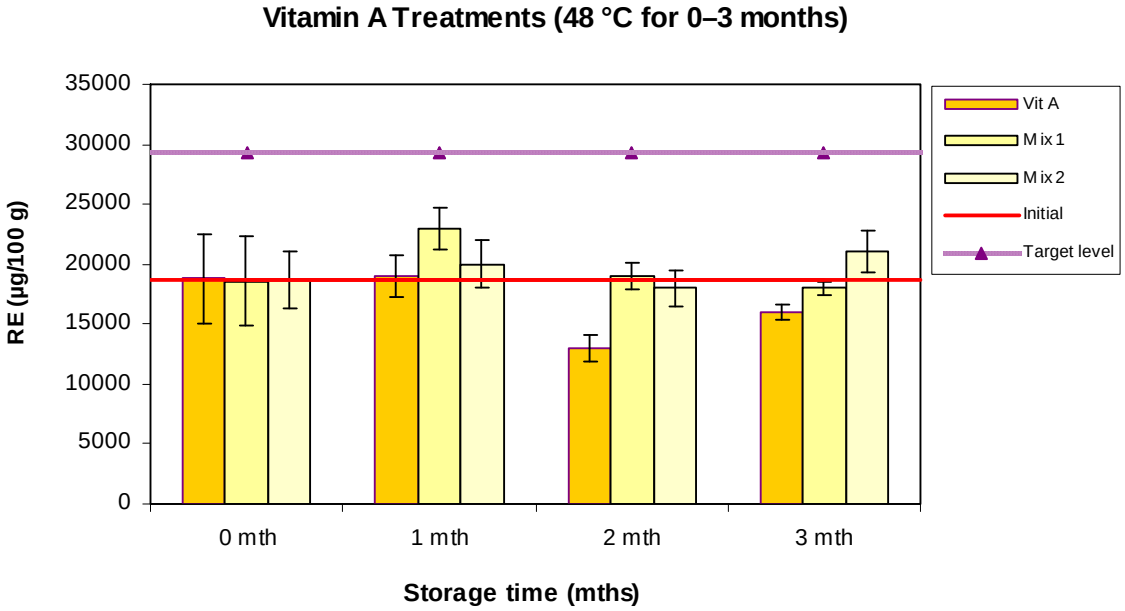

Figure 17: Vitamin A levels, in units of retinol equivalents (RE), for all three treatments following storage for up to 3 months at 48 °C

Losses of Vitamin A—when added as beta-carotene—have been summarised below in Table 13. These have been calculated as the difference between the measured levels at time zero and at the specific time points. Due to the good data fit (Figure 14) similar values are obtained through application of the half life values to obtain predicted losses of 20, 23 and 22% following 24 months of storage at 20, 30 and 37 °C respectively.

Table 13: Losses of vitamin A during storage (%)

	Losses during storage (%)						
	20 °C		30 °C		37 °C		48 °C
	12 mth	24 mth	12 mth	24 mth	12 mth	24 mth	3 mth
Vitamin A	8	20	18	24	18	24	15

Note: For each value, n = 3

In summary, cumulative losses of vitamin A—whether added alone or in combination with vitamins C and E—amounted to 36–37% at the start of the storage trial. Estimated additional losses following storage at 20–37 °C for 24 months were 20–23% of the residual vitamin A content added as beta-carotene alone, totalling losses of vitamin A over the entire process of 49–50% of the added amount. These values compare favourably with the values cited in Table 1

and Table 2 for losses during storage alone and indicates that freeze dried meals are suitable candidates for fortification with vitamin A. There is evidence of a protective effect for vitamin A when added in combination with vitamins C and E.

3.6.3 Vitamin E

Charts of the storage trial results for 20, 30 and 37 °C are shown below for each vitamin E treatment (Figure 18 to Figure 20). The 48 °C storage trial results for the three treatments are also presented (Figure 21). Unlike the charts for vitamins C and A, the target level of fortification has been omitted to avoid compression of the main area of interest.

Visually, there is no clear loss trend for any treatment over the 24 month period, or 3 month period in the case of storage at 48 °C. There are rises and falls in the data which may mask minor trends. This variation may be due to analytical problems[12], uneven fortification across the production run or causes unknown. A tighter set of data and more accurate estimate of loss trends may be possible with a different fortification regime and a reduction in initial losses.

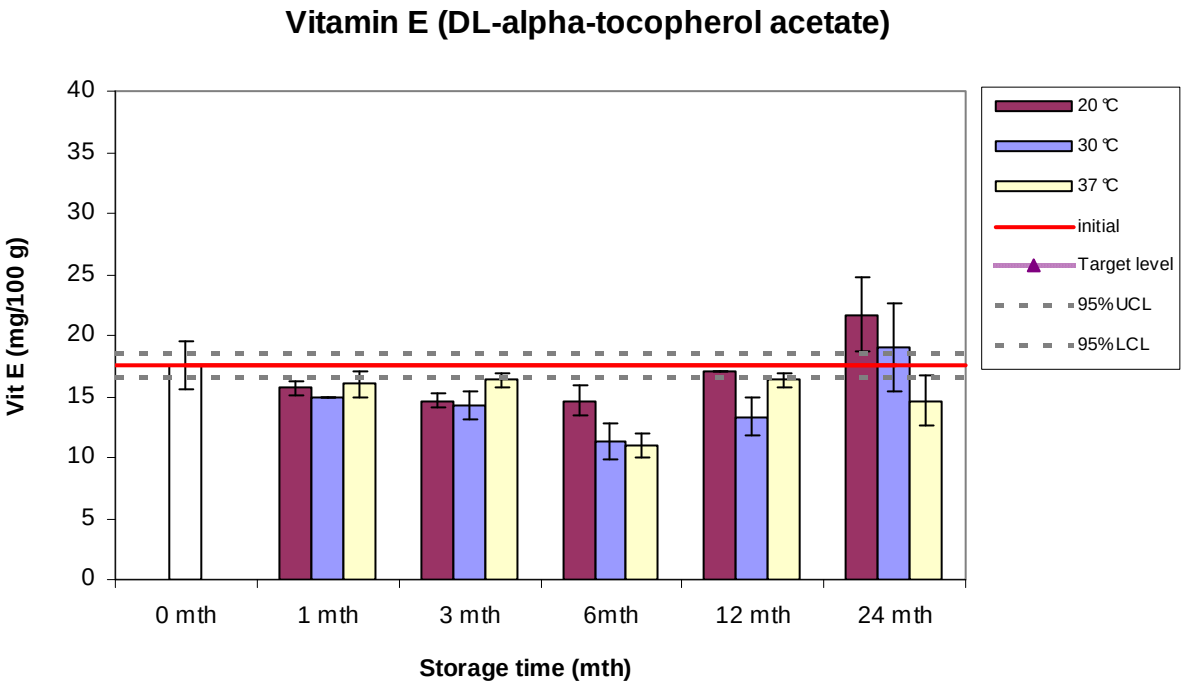

Figure 18: Vitamin E levels, following fortification with Vitamin E acetate and storage at 20, 30 and 37 °C for up to 24 months

[12] The data was queried with the laboratory. Some anomalies were due to a calculation error. A second set of samples were submitted for 24 months at 20, 30 and 37 °C as the first set of results was suspect. The second set of results was used in the charts and calculations.

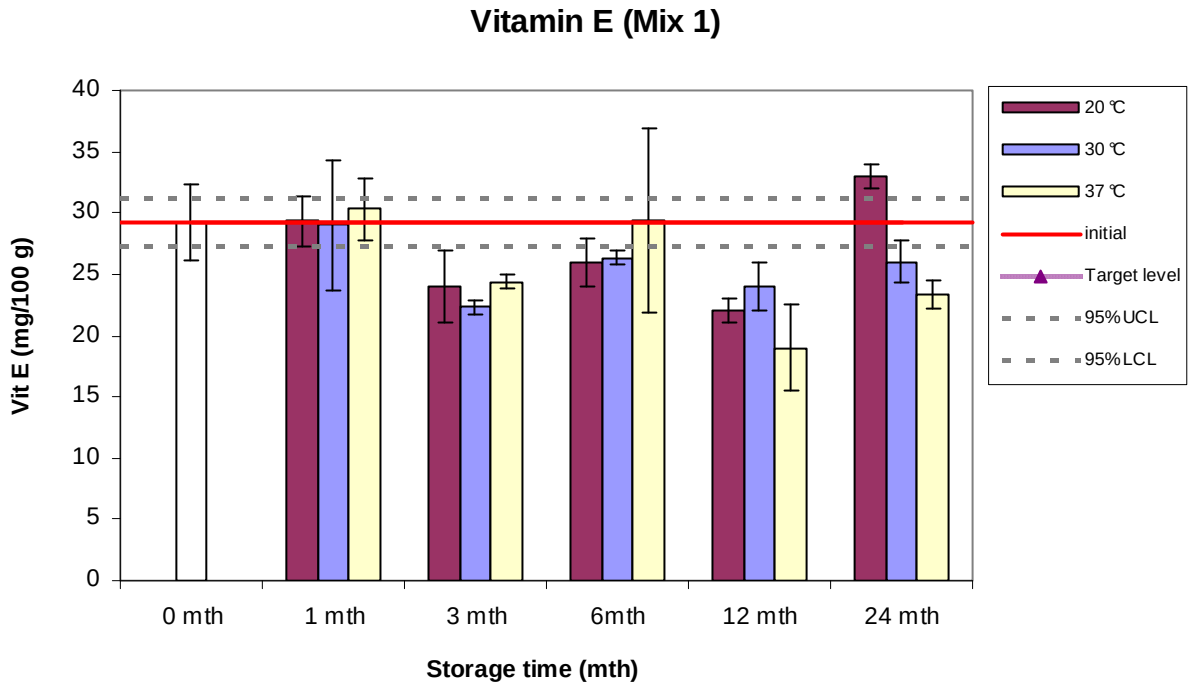

Figure 19: Vitamin E levels, following fortification with Mix 1 and storage at 20, 30 and 37 °C for up to 24 months

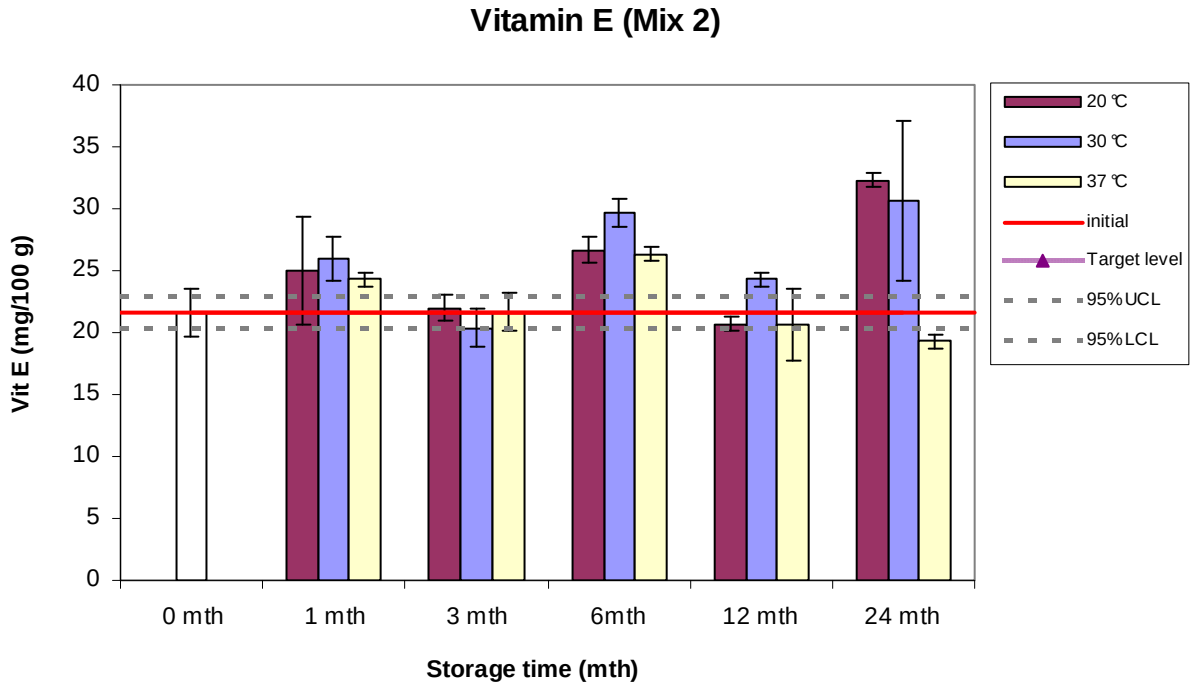

Figure 20: Vitamin E levels, following fortification with Mix 2 and storage at 20, 30 and 37 °C for up to 24 months

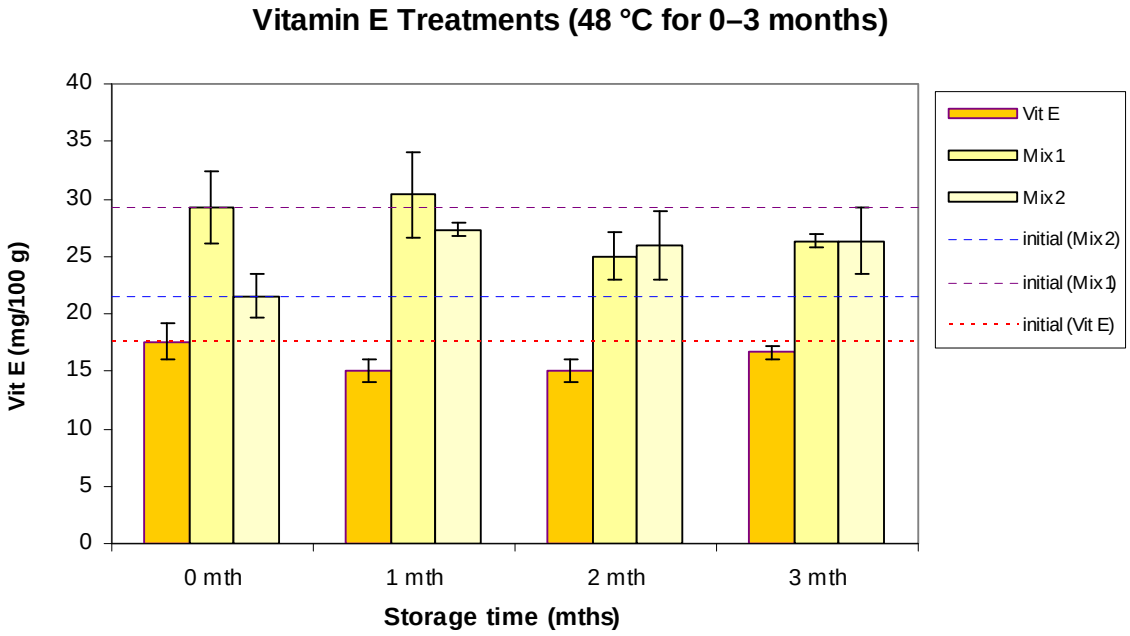

Figure 21: Vitamin E levels, for all three treatments following storage for up to 3 months at 48 °C

3.7 Protective effects

The protective effects that have been observed in this study are summarised in Table 14. The data indicates some evidence of protective effects during fortification (vitamins C and E) and during storage (vitamin A).

Table 14: Summary of observed protective effects and where they occurred during the study

Treatments	Fortification			Freeze drying			Storage		
	C	A	E	C	A	E	C	A	E
Mix 1	✓	–	✓	–	–	–	–	✓	–
Mix 2	–	–	✓	–	–	–	–	✓	–

4. Conclusions

1. One of the aims of this study was to investigate the suitability of a freeze dried meal as a vehicle for vitamin fortification. This study has demonstrated that a freeze dried meal matrix provides a suitable vehicle for fortification with vitamins C, A and E when added as coated ascorbic acid, calcium ascorbate, beta-carotene and vitamin E acetate.
2. Losses of vitamin C—added as coated ascorbic acid and calcium ascorbate—from the point of fortification to the end of the storage trial were low (up to 16%) for samples stored at 20–37 °C for 12 and 24 months. Losses during the freeze drying and storage steps are negligible with most losses occurring during fortification. Reduced processing losses of ascorbic acid were observed when added as coated ascorbic acid together with beta-carotene and vitamin E acetate. During storage a clear loss trend was evident for all treatments but only at 48 °C.
3. Losses of vitamin A—added as beta-carotone alone—were moderate (49–50%) for samples stored at 20–37 °C for 12 and 24 months. Approximately half the overall loss occurred during storage. A protective effect was observed with no storage loss trend observed for vitamin A added as beta-carotene in combination with ascorbate and vitamin E acetate.
4. Losses of vitamin E—added as DL-alpha-tocopherol acetate—were very high during fortification whether added alone (88%) or in combination (76–79%). A protective effect was observed when vitamin E was added in combination with vitamins C and A. No loss trends were observed during storage at any temperature.
5. The data obtained during this study, particularly during the storage trial, was characterised by large variations suggestive of analytical errors or methodological difficulties. This complicated and to some extent limited our interpretation of the data.

5. Recommendations

It is recommended that further research be conducted to confirm and extend the findings of this study. Future work should:

1. Exercise greater control over vitamin analyses to maximise confidence in the data.
2. Determine changes in organoleptic characteristics during storage.
3. Investigate the performance of alternative fortificants, vehicles and processes.
   a. Investigate other freeze dried meal recipes for suitability as fortification vehicles.
   b. Investigate fortification options that are more suitable to routine production, for example the use of a powder dispenser capable of delivering milligram amounts during the packing step.
   c. Investigate other target levels of fortification using the current data as a guide to study design.
   d. Review the range of commercially available fortificants to identify alternative stabilised forms.

4. Further investigate the vitamin losses.
   a. Measure vitamin losses upon reconstitution of the freeze dried product.
   b. Determine whether there is an absolute loss plus a proportional loss.
   c. Exploit protective effects to minimise losses during processing and storage.

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19. ABSTRACT

The intake of adequate energy and nutrients plays a fundamental role in ensuring that Australian Defence Force (ADF) personnel are operationally ready. Combat ration packs (CRP) may be provided to soldiers when it is not practical to feed them with fresh food. CRP may be fortified with vitamins to offset losses during storage and to minimise the impact of other factors that could otherwise lead to inadequate vitamin intakes by consumers. This study investigates the suitability of a freeze dried meal as a carrier for vitamins C, A and E. A fortified freeze dried meal was prepared and was subjected to a storage trial. Vitamin losses during fortification and storage were determined. Losses during storage were low for all vitamins, although losses during fortification were high for vitamin E. There was evidence of protective effects when vitamins were added in combination.