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Microencapsulation of Ascorbic Acid for Enhanced Long-term Retention during Storage

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ABSTRACT

A trial to evaluate a range of microcapsules prepared using various encapsulating agents individually and in combination has been completed. The aim was to provide a means of protecting ascorbic acid (AA) from harsh conditions of elevated temperature over extended storage periods, and to obtain a basis for the selection of encapsulating agents for the AA fortification of specialised food products. Microcapsules were prepared using a pilot scale spray dryer; good yields and high recoveries of AA were obtained. The resultant materials were fine powders of relatively uniform particle size, spherical with varying degrees of indentation but no evidence of mechanical damage. Samples of the microcapsules were stored at temperatures of 20, 30, 37 and 48 °C for periods of up to 15 months. There were significant variations in AA retention between treatments and encapsulating agents. It is recommended that six encapsulating agent combinations and loading levels be further evaluated to determine their potential for the fortification of selected food products.

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Microencapsulation of Ascorbic Acid for Enhanced Long-term Retention during Storage

Executive Summary

Australian soldiers rely on combat ration packs (CRP) as their source of energy and nutrients when access to fresh foods is not practicable. CRP components may be fortified to improve the levels of vitamins. Ascorbic acid (AA)—vitamin C—is one of the vitamins added to some CRP components. However, AA is susceptible to degradation during processing and storage of foods, therefore, it is important to protect the added AA to ensure that adequate levels are present at the time of consumption.

The purpose of the research presented in this report was to evaluate the protective properties of microcapsules prepared by spray drying, against harsh conditions of elevated temperature over extended storage periods, reflecting conditions encountered by military personnel and supplies in the field.

Nine hydrocolloids, including various commercial starch-based ingredients, in 13 combinations were selected for evaluation as encapsulating agents. Microcapsules were prepared under controlled conditions and samples were collected for analysis to determine AA content, particle size distribution and examination of the structure by environmental scanning electron microscopy. Samples were also reserved for a storage trial.

Samples were stored at 4 temperatures (20, 30, 37 and 48 °C) for periods of up to 15 months. The storage times and temperatures were based on the standard storage profile used by DSTO-Scottsdale to conduct shelf-life evaluation of ration pack components.

All microcapsule combinations lost AA during storage. The rates of loss at 48 °C ranged from 1.4% to 41% per month. The six best performing combinations exhibited losses of less than 3% per month at 48 °C and less than 1.2% per month at 30 °C.

Conclusions

1. Yields of microcapsules obtained on a pilot scale spray dryer were satisfactory (75–95%).
2. Recoveries of AA during microencapsulation by spray drying were excellent (98–100%), showing virtually no losses under the conditions used.
3. Microcapsules prepared by spray drying consisted predominantly of particles in the range of 10–50 μm diameter. Little variation in size was observed for the various combinations of encapsulating agents used in this investigation.

4. Microscopy confirmed the particle size data obtained by laser diffraction. The images obtained by ESEM also demonstrate that the microcapsules are basically spherical in shape. The surfaces appear to be continuous and show indentations to varying extents.
5. X-ray diffraction analysis showed that for the wall materials displaying a degree of crystallinity, the resultant capsules also have crystalline patterns. However these microcapsules do not demonstrate enhanced retention of AA.
6. The storage trials demonstrated that AA retention levels were dependent upon the particular combinations of encapsulating agents.
7. The results indicate that six combinations of encapsulating agents—F30 + Instant 449, F30 + Instant MAPS, Hi Cap 100, F30 + GA + Hi Maize, F17 + Instant 449 and F30 + GA (combinations 5, 8, 11, 6, 2 and 4 respectively)—warrant further evaluation.
8. Hi Maize appears to enhance the protective effect in the combinations studied.
9. The limited investigation of the effect of loading level of AA within the microcapsules indicated that higher levels are associated with significantly enhanced retention.

Recommendations

1. Further evaluate the most promising of the combinations of encapsulating agents by incorporating the microcapsules into a selection of food products so that the effectiveness of protection can be established within food matrices.
2. Conduct a series of trials to more fully investigate the impact of loading of AA within capsules.
3. Investigate extension of the results of this work to other water soluble bio-active components and micronutrients.

Authors

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Marissa graduated from RMIT University with a BAppSc (Hons) in Food Science in 2005. She then received a post-graduate scholarship through DSTO-Scottsdale early in 2006 and has been conducting her research as a PhD candidate at RMIT University, Melbourne. Her thesis is entitled: "Microencapsulation of ascorbic acid for enhanced long term retention during storage".

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Darryl is Associate Professor of Food Chemistry in the School of Applied Sciences, College of Science, Engineering and Health at RMIT University where he is responsible for lecturing on the chemistry and applications of food molecules at both undergraduate and postgraduate levels. His areas of research include cereal grain foods, procedures for food analysis, the potential of enzymes in food processing as well as enhancement of stability and availabilty of micronutrients. He originally completed his undergraduate and PhD studies at the University of Sydney and has gained broad experience during his career encompassing his work in a variety of industrial, regulatory and research roles.

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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).

Contents

1. Introduction

The intake of adequate energy and nutrients plays a fundamental role in ensuring that ADF personnel are operationally ready (Malberg and Forbes-Ewan, 2010). Australian soldiers use combat ration packs (CRP) as their source of food when access to fresh foods is not practicable. As the period of continual use may be for several weeks, it is essential that CRP provide adequate energy and nutrients during that period. Certain CRP components are fortified to enhance the levels of vitamins that are normally readily obtained from fresh foods, but are typically present at low levels in the processed foods used in CRP. Ascorbic acid (AA), also known as vitamin C, is one of the vitamins added to some CRP components.

Vitamins are chemically reactive and their stability has been studied in a range of foods (Gregory, 2008). Many of the vitamins are relatively unstable in the conditions found during food processing and storage, although the stability characteristics of individual vitamins vary widely (Gregory, 2008). Some recent studies have focussed upon the retention of the water soluble vitamins including riboflavin (vitamin B2) (Bui and Small, 2009), vitamin B6 (Bui and Small, 2008a), thiamin (vitamin B1) (Bui and Small, 2007a, 2007b, 2008b), and the folate group that includes folic acid used as a fortificant (Bui and Small, 2007c, 2007d, 2007e; Hau, 2008; Hau *et al.*, 2007, 2008, 2009). In all cases, considerable losses ranging between 40 and 97% occur during processing and preparation of selected foods, with product pH being a significant factor in some cases. On the other hand, niacin is much more stable in foods (Gregory, 2008; Kawila *et al.*, 2008). Data on ascorbic acid (AA) also indicates significant losses during processing and storage of various foods (Gregory, 2008; Hoare *et al.*, 1993; Ma *et al.*, 2007; Sanyoto *et al.*, 2008).

AA is essential for normal functioning of the body and maintenance of metabolic integrity. It is an antioxidant, a cofactor in collagen formation and other reactions. AA also plays a role in reducing physical stress and maintenance of the immune system (Wintergerst *et al.*, 2007). A number of chemical and physical factors affect the stability of AA in foods during processing as well as storage. These include pH, the presence of oxygen, temperature and light. AA is highly susceptible to oxidation, especially in the presence of metal ions, and the rate of oxidation is increased by exposure to heat and light (Gregory, 2008).

Two broad approaches are available to ensure that intakes of nutrients are optimal, not only for general health and wellbeing, but so that physical and mental performance can be sustained. The consumption of a “wide variety of nutritious foods” is one of the dietary guidelines for Australian adults (NHMRC, 2003). However fortification can also be useful where the diversity of foods is restricted or in cases of increased requirements for nutrients. AA has been used for fortification of food aid commodities (Food and Nutrition Board, 1997) and selected ration pack components. Commercially, AA is used widely to fortify foods, particularly in conjunction with other nutrients in order to enhance or maintain their availability or function (Johnson and William, 1991).

Significant barriers remain to the effective fortification of foods with water soluble vitamins, particularly AA. Significantly, AA is highly soluble in water with more than 300 g dissolving in a litre of water at room temperature (Budavari, 2001). This can result in losses especially when foods are boiled or steamed, but of greater concern is the fact that the direct addition of powdered AA to a food can result in rapid losses simply due to the presence of water. In one

Australian study it was demonstrated that even during refrigerated storage of orange juices at 4 °C, the losses of added AA were significant, reducing the AA content below the legal lower limit prior to these products reaching their use-by dates (Hoare *et al.*, 1993).

Microencapsulation technology provides a strategy for enhancing the retention of sensitive and expensive food components, including AA, through protection from adverse conditions and allowing delivery to the target site at the required time (Augustin and Hemar, 2009). Although this approach has been widely used in pharmaceutical and other industrial applications, it is relatively recently that its potential has been recognised for foods (Vilstrup, 2001).

Many techniques have the potential for microencapsulation of food materials (Augustin and Hemar, 2009; Vilstrup, 2001) including spray drying, spray cooling, spray chilling, fluidised bed coating, extrusion, coacervation lyophilisation, co-crystallisation, liposome entrapment and inclusion complexation. Of these, however, a number have only been evaluated on a laboratory scale. Spray drying (Gharsallaoui *et al.*, 2007) currently has immediate scope for adaptation to a commercial scale and has advantages due to the low thermal effect on materials during drying, a large throughput capacity, while producing a fine, free-flowing powder. Commercially, spray drying has been used for many components, including flavouring agents, fats and oils, vitamins and minerals, microorganisms, enzymes, sweeteners and colorants (Gharsallaoui *et al.*, 2007). The selection of spray drying for this project reflects both its suitability as well as its potential for rapid commercial adoption.

Much of the previous research on microencapsulation has been focussed upon fat soluble components, particularly flavourings for which losses of unprotected materials can be rapid. It has been recommended previously that it may be necessary to develop a “tailor made” solution for each application and for individual components requiring protection (Augustin *et al.*, 2001). Additionally it is important for a strategy to be designed to suit a particular food. In a recent study the release of water soluble acids was achieved by entrapping them within a lipid matrix that could be incorporated into a bakery dough (Al-Widyan and Small, 2005).

Relatively few other publications have reported on strategies that might be suitable for the protection of water soluble components of foods. Furthermore there have been only two previous reports specifically on the microencapsulation of AA (Trindade and Grosso, 2000; Uddin *et al.*, 2001). These provided a range of approaches that might be considered for the research reported here, but some of the encapsulating agents previously applied to AA are not safe for use in food products, particularly the agents selected for cross linking of proteins. Careful consideration of the wide variety of food ingredients that can be used as microencapsulating agents is an important first step in the development of microcapsules. The selection depends upon the characteristics of the components to be protected as well as the nature of the processing, storage and the conditions that may be encountered during these.

The purpose of the research presented in this report was to evaluate the protective properties of microcapsules prepared by spray drying, using various encapsulating agents, against harsh conditions of elevated temperature over extended storage periods, reflecting conditions encountered by military personnel and supplies in the field. The broad aim was to provide a means of protecting and enhancing the stability of AA with the specific objective of screening a wider range of encapsulation agents, so that a limited number of suitable combinations can be identified. These will provide a basis for further steps to find which of these are best suited for fortification of particular food formulations in ration packs.

2. Methods and Materials

2.1 Ingredients for microcapsule preparation

Food grade ingredients were used in the preparation of microcapsules. The details of these are presented in Table 1.

Table 1: Food grade ingredients used in the formulation of microcapsules

Ingredient	Description	Manufacturer
AA food grade (99.0–100.5%)	Product code: 158000	Bronson & Jacobs Pty. Ltd, Melbourne, Australia
Gum Arabic (GA)	Luxara 3A Product 4417	Arthur Branwell & Co Ltd, United Kingdom
Maltodextrins, 2 types: a. Fieldose 17 (F17), DE[1] = 17 b. Fieldose 30 (F30), DE = 30	 F17, and F30	Penford Australia Ltd, Melbourne, Australia
Pregelatinised, modified waxy maize starch	Instant MAPS	
Pregelatinised, modified waxy maize starch	Instant 449	
Unmodified high amylose maize starch	Hi Maize™1043	National Starch, Sydney, Australia
Modified waxy maize starch	CAPSUL®	
Tapioca dextrin	K4484	
Modified waxy maize starch	Hi CAP™100	

2.2 Preliminary considerations for microencapsulation

For this study, spray drying was selected as a suitable technology for encapsulating AA, partly due to the high solubility of AA in water. Spray drying has previously been applied to the encapsulation of various food components and has a number of advantages including cost effectiveness, versatility, potential for scale-up and ready availability of commercial facilities (Gharsallaoui *et al.*, 2007; Gohel, *et al.*, 2009; Thies, 1996, 2004).

The second aspect that was considered was the selection of encapsulating agents. The basis of selection was that these encapsulating agents should be readily available as food grade, safe ingredients with the potential to provide protection for the active component, AA. One other factor was the requirement for the ultimate release within the digestive tract (Desai and Park, 2005; Patty *et al.*, 2010).

Based upon these requirements, previous research (King, 1995; Krishnan *et al.*, 2005; Loksuwan 2007; Gharsallaoui *et al.*, 2007) and information provided by food ingredient suppliers, a number of hydrocolloids, including various commercial starch-based ingredients, were chosen.

A series of preliminary trials of different combinations and ratios of coating materials, as well as spray-dryer operating conditions, were conducted to confirm the suitability of the selected

[1] DE = Dextrose Equivalent of the polysaccharide.

range of capsule wall materials and the optimal spray dryer operating conditions for application to ascorbic acid (Bui *et al.*, 2008). These included partially hydrolysed starches in the form of maltodextrins (F17 and F30) and others promoted as potential microencapsulating agents (Loksuwan, 2007; Trindade and Grosso, 2000). Another starch product (Hi Maize™ 1043) was also trialled as this is high in resistant starch, a component now known to provide significant health benefits, an ability to act as a prebiotic in the digestive system and possibly providing desirable delayed release properties when used as a microencapsulating agent (Patty *et al.*, 2010).

2.3 Preparation of microcapsules incorporating AA

Extensive preliminary studies were used to assess a wide range of options. The results of these trials then allowed the establishment of a more restricted range of potential wall materials and particular combinations which warranted further investigation. Thirteen specific combinations were established (Table 2) and in all cases, the combination could be readily prepared by hydration, suspension and mixing, utilising the procedures described (Section 2.4). The pH was carefully controlled as elevated values (pH 6.0 and above) result in rapid losses of AA.

2.4 Preparation of solutions for spray drying

For each of the combinations selected for this series of trials (Table 2), the wall materials (encapsulating agents) were either dissolved or suspended in a suitable amount of distilled water such that the total volume of water used was 3 L. Initial mixing was by stirring rod for 5 minutes prior to covering with aluminium foil and overnight storage in an incubator set at 50 °C.

The solutions were mixed as required, to give a level of solid material corresponding to approx. 30% (w / v) using a high speed overhead stirrer (Model 0-10 LFA 0-318; Laboratory Supply P / L, Melbourne, Australia) at a setting of 3 that ensured that a vortex was maintained. Mixing was continued for a period of 30 minutes or longer (up to 60 minutes) if this was necessary to achieve homogeneity. Once homogeneity was achieved, AA was added slowly into the vortex taking approximately 15 minutes. Finally, the pH was adjusted to 4.0 using a dilute solution of hydrochloric acid.

Table 2: Spray drying trials combinations of encapsulating agents and their proportions

No.	Core loading (% AA)	Combination of encapsulating agents (wall materials)			Ratio of wall materials
1	6	F17	GA		1 : 1
2	6	F17	Instant 449		8.3 : 1
3	6	F17	GA	Hi Maize™ 1043	2.5 : 2.5 : 1
4	6	F30	GA		1 : 1
5	6	F30	Instant 449		8.3 : 1
6	6	F30	GA	Hi Maize™ 1043	2.5 : 2.5 : 1
7	6	F17	Instant MAPS		8.3 : 1
8	6	F30	Instant MAPS		8.3 : 1
9	6	CAPSUL®			1
10	6	F17			1
11	6	Hi CAP™100			1
12	6	K4484			1
13	6	K4484	Hi Maize™ 1043		5 : 1
14	18	F17	GA		1 : 1
15	36	F17	GA		1 : 1

2.5 Preparation of microcapsules by spray drying

A pilot scale spray drier (Niro Rotary Atomiser Minor, Copenhagen, Denmark) unit was used in accordance with the instructions issued by the manufacturer (Anon, undated). Prior to introducing the prepared feed material containing AA and hydrocolloids, the spray drier chamber was warmed to 120 °C for approximately 15–20 minutes during which no liquid was pumped into the chamber (the settings were: main controller I and fine adjustment setting of 6.0–6.8). Next, warm water (approx. 35 °C) was introduced to the chamber using the peristaltic pump (Masterflex® peristaltic pump, Model: 7512-35, Cole-Palmer Instrument, Chicago, United States). The air pressure controller was then turned on and the setting increased to 5.0 kg / m². Care was taken to increase the pressure smoothly and gradually over a period of 2 minutes. The pumping of warm water was typically continued for a period of approximately 20 min and at the same time the stability of the measured temperatures for both the inlet and outlet air flow was monitored. The temperature achieved at the outlet during this warm-up period was within the range of 72–82 °C. Other parameters were: feed flow rate 10–12 mL / min, final outlet temperatures 80–87 °C.

Microcapsules were collected in a receiver jar and the yield was calculated using the following equation, in which total dry weight is calculated as the sum of all of the solid ingredients added to the food solution.

$$\text{Yield (\%)} = \frac{\text{weight of microcapsules}}{\text{total dry weight}}$$

Subsamples of the microcapsules were reserved for the storage trial and the remaining portion was kept in a closed sealed jar until it was analysed for AA content and particle size distribution. Structural characteristics were also examined by environmental scanning electron microscopy (ESEM).

2.6 Design of the storage trial for microcapsule preparations

The purpose of the storage trial was to compare the performance of the microcapsules prepared with different combinations of encapsulating agents (Table 2). Samples were to be stored at a range of temperatures, based on the standard storage profile used by DSTO-Scottsdale to conduct shelf-life evaluation of ration pack components. Accordingly, the samples were stored at four temperatures (20, 30, 37 and 48 °C) for periods of up to 15 months.

For each combination of microencapsulation agents (hydrocolloids), three spray drying runs were conducted and sub-samples (approx. 5 g in each case) were individually sealed in Cryovac vacuum packaging bags. This gave 225 sub-samples (3 runs x 75 sub-samples) for each combination (Table 2) in the storage trial. For Hi Cap™100 (trial combination 11 in Table 2) only one spray drying run could be carried out so only one set of sub-samples was available for this combination.

The matrix of treatments (involving temperature and period of storage) selected for investigation is presented in Table 3. Treatments are identified using a tick (✓). The sub-samples were assembled into groups corresponding to each of the time-temperature combinations (Table 3). The sub-samples were removed from storage at the selected time points for analysis of AA content and ESEM examination.

Table 3: Storage trial design

Temperature (°C)	**Storage period (months)**								
	Initial	**1**	**2**	**3**	**4**	**5**	**6**	**12**	**15**
	✓								
20				✓	✓	✓	✓	✓	✓
30				✓	✓	✓	✓	✓	✓
37				✓	✓	✓	✓	✓	✓
48		✓	✓	✓	✓	✓	✓		

2.7 AA analysis using capillary electrophoresis

AA was analysed by capillary electrophoresis using a procedure based upon those of Trenerry and coworkers (Marshall *et al.*, 1995; Thompson *et al.*, 1995a, 1995b). An Applied Biosystems instrument (model 270 A-HT) was used with a fused-silica capillary (undeactivated, 75 µm internal diameter, Agilent Technologies). The buffer was sodium orthophosphate-sodium tetraborate (0.02 M, pH 8.6) containing sodium deoxycholate. D-erythorbic acid (D-*iso*-AA) was used as internal standard and the operating conditions were: +15 kV applied voltage, 28 °C temperature condition and 254 nm for UV detection.

For analysis, a small amount of microcapsule (approx 0.10 g) was mixed with aqueous 0.2% D,L-dithiothreitol (10 mL) using a magnetic stirrer. For each solution, 1 mL was transferred to a 10 mL volumetric flask along with sufficient D-*iso*-AA stock solution to give 50 µg / mL of the internal standard when the flask was made up to 10 mL with Milli-Q water. All extracts and other solutions were filtered through a 0.45 µm nylon filter before use. In order to enhance the performance of the system and prevent blockage of the capillary routine procedures were followed. These included rinsing of the capillary between each run using sodium hydroxide (0.1 M) for 2 minutes followed by Milli-Q water for 2 minutes. Data analysis was performed using Shimadzu Class LC-10 Software and the results obtained for spray dried microcapsules were used in calculation of the apparent recovery of AA. This reflects the retention of AA within the microcapsules following spray drying.

Samples on storage were analysed at the specific time points (Table 3) using the same method.

2.8 Characterisation of microcapsules

The moisture content of the microcapsules was determined to verify the effectiveness of the spray drying process. Moisture analysis, using oven drying, was based upon the official method of the Association of Official Analytical Chemists (procedure 925.45 - 44.1.03) (AOAC, 2000). A sample of microcapsules (2 g) was placed in a moisture dish and dried to constant weight in an air oven at 102 °C for 12 hours. The result was expressed as percentage moisture (Appendix A, Table A1).

For particle size analysis, Laser beam scattering (Malvern Mastersizer X, Model MSX025A) was used following the procedure described by Cornell *et al.*, (1994). A 300 mm range lens (suitable for a particle size range of 1.2–600 µm) was used. Small amounts of microcapsules were dispersed in *iso*-butanol using a cell designed for powdered materials. This allowed continuous stirring to maintain the dispersion using a magnetic stirrer arrangement. Malvern software was used for data analysis.

ESEM was used to observe the outer structure of microcapsules with an FEI Quanta 200 instrument. The settings were: accelerating voltage of 30 kV, pressure of 0.5 Torr, spot size of 5.0 nm and working diameter of 10 mm. A small quantity of each microcapsule preparation was mounted on a metal stub using double sided adhesive tape. The features were viewed under various magnifications in ‘low vacuum’ mode.

X-ray diffraction was used to evaluate the chemical structure of materials used in the microencapsulation as well as for the resultant capsules containing AA. The instrument used was a Bruker D8 Advance X-ray. The samples were scanned at a step time of 2.0 seconds, a scatter slit of 1 mm and scanning speed 2.000° 2θ / min, within a range of 2θ values from 2.000° to 52.010°, where θ is the Bragg angle. The resultant patterns were assessed for the presence of prominent peaks corresponding to those previously reported for starches. These peaks would indicate the presence of a degree of uniform packing or arrangement of the molecules which is commonly referred to as crystallinity. A lack of such peaks would demonstrate that the arrangement is less ordered; this is described as an amorphous structure. Where peaks were observed, values for relative crystallinity were calculated using the approach recently described by Frost and co-workers (Frost *et al.*, 2009).

2.9 Calculation of rate of loss from storage trial data

The mean values of AA contents for each microcapsule combination at each time / temperature of storage were used in the discussion and graphs. Data for each time / temperature combination were plotted using Microsoft Excel software. Trendlines were fitted to the plots using the scatter option. The resultant regression equations and corresponding correlation co-efficients were reviewed to see how well the data fitted the linear model. The slopes were used wherever the correlation coefficient exceeded 0.95 or the intercept was within the range 98–102. In the relatively few cases for which neither criteria were met, the initial slope of the curve was used. This was estimated as the slope obtained when only the first two points of the graph were used for regression.

2.10 Statistical analysis

Statistical analysis was conducted with the assistance of a professional statistician (Glen McPherson Consultancy). SPSS (Statistical Package for the Social Sciences, version 12.0, 2003, SPSS Inc., Chicago, IL, USA) was used and was applied to data formed by averaging the readings for two extracts obtained from each sample, except in the one instance where there was a missing extract. In the latter case, the single value was used.

A linear, additive model was fitted with a series of variables. The first was a ‘product’ variable that identified the 15 different combinations as one treatment variable and the second was a ‘temperature’ variable with values 20, 30, 37, 48. In addition, ‘storage time’ (months) was included as the third variable with values of 0, 3, 4, 5, 6, 12, 15 for temperatures 20, 30 and 37 °C, and values of 0, 1, 2, 3, 4, 5, 6 for 48 °C. The design variables were treated as the fourth treatment variable with ‘Batch’ having three levels, and ‘Reps within batch’ also at three levels where these were included as design variables. A ‘temperature’ × ‘storage time’ interaction was also included in the model. The product ‘Hi Cap 100+6%AA’ is lacking data for two of the three batches, hence comparisons with this product have a lower power which implies that larger differences are required to obtain statistical significance.

An analysis of variance was applied with checks on the model and data that indicated the model was appropriate and there were no gross data errors. The primary test was for evidence that patterns over time differed between products at the different temperatures. Further analysis was undertaken at individual temperatures. Two forms of comparison were employed using t-tests for pairwise comparisons, with the residual mean squares from the analysis of variance tables providing the variance estimate. The first test, applied separately to each product, sought evidence of a change in AA level from the initial reading at each of the storage times. The second test compared changes in AA level from the initial reading to a reading obtained at a specific storage time between a pair of products. The tests were applied to all pairs.

Given that statistical evidence of change does not necessarily imply a change that is of biological importance, the statistical test results were overlain with a check that any significant difference obtained from the statistical test was of practical importance. To this end, a difference of 3 units was taken as the minimum of practical importance, and differences exceeding this value were identified.

3. Results and Discussion

3.1 Yields of microcapsules prepared by spray drying

In all the spray drying trials the yields of capsules were high. These were typically in the range of 75–95% where these are expressed as the amount of capsules recovered compared to that of the total solids fed to the spray drying unit in the form of the liquid feed material. Relatively little material adhered to the inner surfaces of the spray drying chamber and no recovery of residual material from within the spray drier was attempted, in order to minimise contamination of the capsular materials. Based upon extensive experience with spray drying of food materials, it is expected that significantly higher yields of capsular material would be routinely obtained if these operations were scaled up from the very small scale used in this work to a commercial level (Gharsallaoui *et al*., 2007).

During the spray drying trials, the recovery of AA was also measured. This was done by directly comparing the amount of AA measured in the capsules by the capillary electrophoresis procedure described in Section 2.7, with that which was incorporated into the feed liquid prior to spray drying. In all cases the recoveries were high (97–100%) indicating that the pH adjustment described in Section 2.4 was effective and that the heating occurring as part of the spray drying process was sufficiently short to have little or no impact on the AA content.

3.2 Characterisation of microencapsulated AA by ESEM

All capsules prepared by spray drying were evaluated by ESEM to assess the overall shape and the surface appearance as well as the integrity of the capsules and any evidence of damage or breakage. Micrographs showing the typical appearance of the capsules are presented in Figure 1.

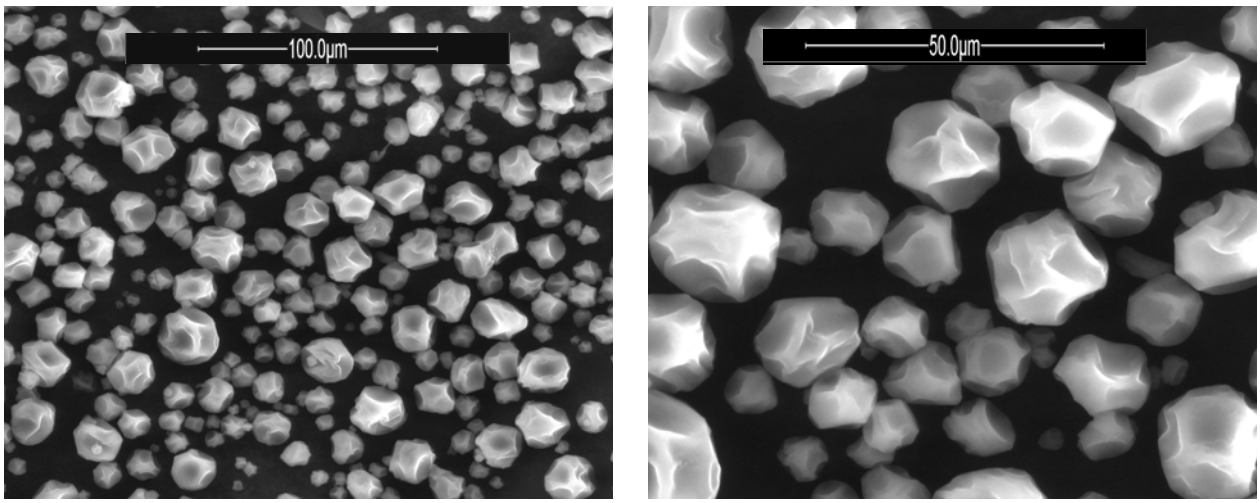

Figure 1: The morphology of microcapsules using K4484+6% AA (combination 12, Table 2) under magnifications of 1000× (left) and 2500× (right).

These demonstrate that the preparations consist of very fine particles which are basically spherical. In addition, the particles are of relatively consistent size. The surface appearance is uniform with some indentations having formed during the drying process within the spray drier. The example shown is consistent with the general observations that there appeared to be no contaminant materials present, no visual evidence of crack formation in the outer surface, mechanical damage or breakage on any of the freshly prepared capsules in this study.

Micrographs for capsules prepared with each of the remaining combinations of encapsulating agents are presented in Appendix B. These demonstrate minor variations in the degree of indentation observed for different microcapsules.

3.3 Characterisation of microencapsulated AA – particle size distributions

In order to effectively measure the diameter of the microcapsules, the technique of laser diffraction was employed (Section 2.8). A typical set of results is seen in Figure 2, demonstrating a relatively uniform size distribution as virtually all particles have diameters within the range of 10–50 µm. The results for the fifteen combinations of encapsulating agents are summarised in Figure 3.

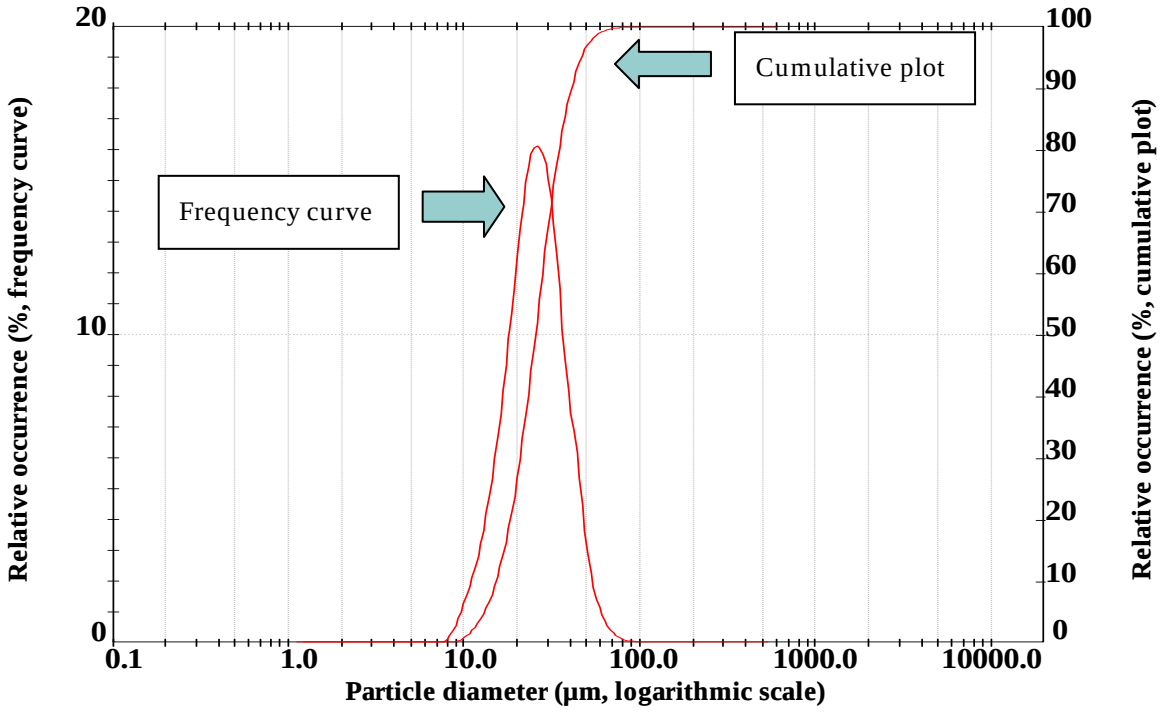

Figure 2: An example of the particle size distribution curves for spray dried microcapsules. Data is for combination 6 (F30+GA+ Hi Maize™ 1043+6% AA)

The graph shows that the results are similar regardless of which of the common measures of mean particle size are used. In addition, all of the combinations have a similar mean diameter. The diameters reflect those that are typically experienced when the technique of spray drying is used (Gharsallaoui *et al.*, 2007) and are comparable to those of milled wheat flour used in breadmaking and other food processing applications. This size provides significant advantages for the use of the resultant microcapsules in the formulation of foods. The fineness, shape and lack of angularity ensures that there will be no adverse impact on textural attributes of the food.

The fine particles cannot be discerned as “gritty or sandy”. A further aspect of the size is that this will facilitate the uniform distribution of the active agent within the food product. Large particle sizes will tend to give less uniform distribution.

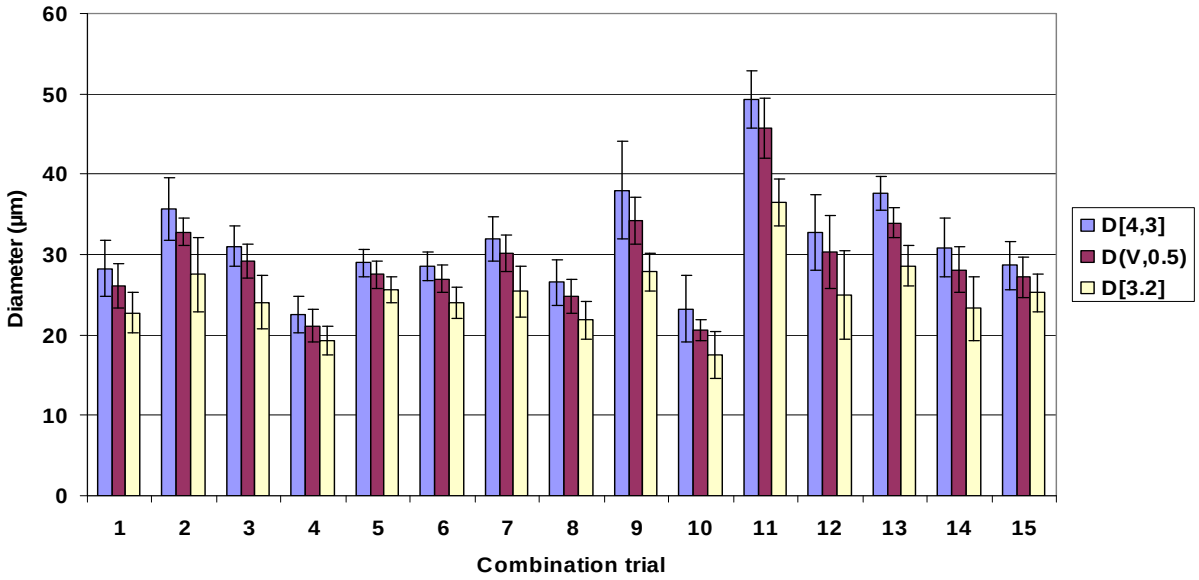

Figure 3: A comparison of particle sizes for the 15 combinations

Notes:

1. *The trial numbers are those presented in Table 2.*
2. *D [4.3] refers to the volume mean diameter.*
3. *D (V, 0.5) is the median diameter at which 50% of the mass of the sample is smaller and 50% is larger than this size.*
4. *D [3.2] is the surface area mean diameter.*
5. *Error bars represent the standard deviation values obtained from replicate determinations; each mean was calculated from nine values which represented triplicate values obtained on each of three replicates.*

3.4 The characterisation of capsular materials using X-ray diffraction

Samples of AA, all encapsulating agents and each of the microcapsule preparations containing AA were analysed by wide angle X-ray diffraction; the results are summarised in Table 4. Each of the individual scans is presented in Appendix C.

Table 4: Results of X-ray diffraction analysis of the materials used for microencapsulation and microcapsules containing AA

	Ingredient/microcapsule preparation	**Observations on crystallinity**
	Capsule material	
	AA	Highly crystalline
	GA	Amorphous
	F17	Amorphous
	F30	Amorphous
	Instant MAPS	Amorphous
	Instant 449	Amorphous
	Hi Maize™1043	Clear crystalline pattern (42%)
	CAPSUL®	Strong crystalline pattern (47%)
	K4484	Strong crystalline pattern (46%)
	Hi CAP™100	Amorphous
No.	**Microcapsules containing AA**	
1	F17+GA	Amorphous
2	F17+Instant 449	Amorphous
3	F17+GA+ Hi Maize™ 1043	Some crystallinity evident (37%)
4	F30+GA	Amorphous
5	F30+Instant 449	Amorphous
6	F30+GA+ Hi Maize™ 1043	Some crystallinity observed (36%)
7	F17+Instant MAPS	Amorphous
8	F30+Instant MAPS	Amorphous
9	CAPSUL	Clear crystalline pattern (38%)
10	F17	Amorphous
11	Hi Cap 100	Amorphous
12	K4484	Some crystallinity observed (54%)
13	K4484+ Hi Maize™ 1043	Some crystallinity observed (58%)
14	F17+GA	Amorphous
15	F17+GA	Amorphous

Notes:

1. *Where the pattern indicated the presence of uniform packing of molecules, a relative crystallinity value has been calculated. These are expressed as percentage values in parentheses.*
2. *The patterns for microcapsules with F17 and GA as wall materials were similar regardless of loading levels of AA (Appendix C, Figures C5 and C9).*
3. *Microcapsules containing 6% AA: samples no. 1 to 13; 18% AA sample no. 14 and 36% AA sample no 15.*

The data indicate that some degree of crystallinity was observed for three of the encapsulating agents. All the others showed X-ray patterns consistent with a totally amorphous structure. In contrast, the AA used in the formulations for the capsules was highly crystalline, as expected. The data for the microcapsules demonstrated that where the original wall materials showed a degree of crystallinity, this was at least partially maintained during spray drying and was evident in the resulting microcapsules. Furthermore, the calculated values of relative crystallinity, before and after spray drying, were very similar. This is consistent with the retention of the granular integrity of starch granules in these microcapsules during the spray drying process. On the other hand, in all instances where the original analyses showed amorphous structure in each of the wall materials, no crystallinity developed when the capsules were made.

3.5 The retention of AA in microcapsules during storage at 48 °C

Storage trial results for samples stored at 48 °C are presented in Figure 4. The general trend is the same as that observed for the lower storage temperatures: as anticipated, losses were highest at 48 °C.

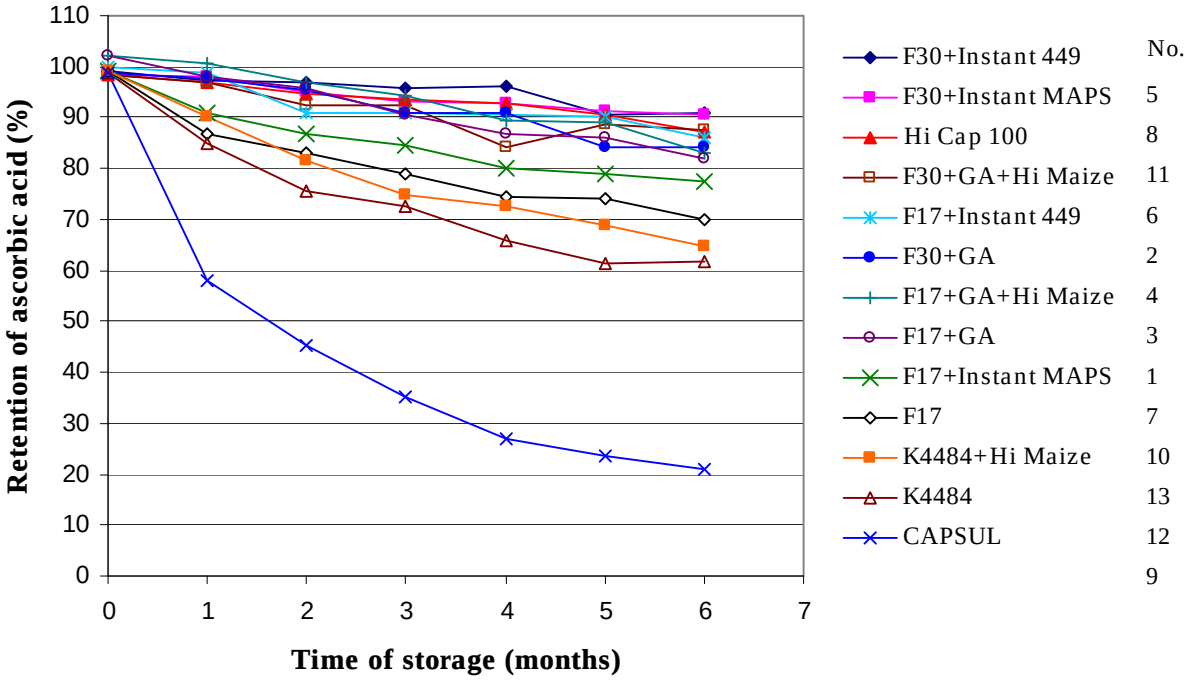

Figure 4: Mean of AA contents of microcapsules (6% AA loading), stored at 48 °C

Note: These microcapsules are ranked according to their decreasing effectiveness in retaining AA. This ranking was based upon the rates of loss calculated using the approach described in Section 2.9.

The results for all combinations (Figure 4) show that there was some decline in AA contents of capsules over the 6 month period. However, the extent of the decline varied very widely among the combinations of encapsulating agents used, with the highest level of retention at 90% for F30 in combination with Instant 449 and Instant MAPS, over the period of the trial. This contrasts with retention as low as 20% for microcapsules prepared using CAPSUL.

In order to compare the data, the rate of loss has been expressed as the percentage loss per month (Table 5). The values obtained at 48 °C ranged between 1.4 and 40.8% per month.

Table 5: Rate of loss of AA for microcapsules stored at 48 °C/ 6 month

Combination of encapsulating agents		Differences observed	Rate of loss of AA (%/mth)
5	F30+Instant 449	a	-1.40
8	F30+Instant MAPS	a	-1.43
11	Hi Cap 100	a	-1.71
6	F30+GA+Hi Maize™ 1043	a,b	-2.06
2	F17+Instant 449	b,c	-2.10
4	F30+GA	c	-2.67
3	F17+GA+Hi Maize™ 1043	d	-3.15
1	F17+GA	d	-3.37
7	F17+Instant MAPS	d	-3.42
10	F17	e	-9.09
13	K4484+Hi Maize™ 1043	f	-12.5
12	K4484	f	-14.0
9	CAPSUL	g	-40.8

Notes:

1. *Combinations are presented in order based upon the retention of AA.*
2. *Those followed by the same letter within the column designated 'Differences observed' are not statistically different from each other at $P < 0.01$.*
3. *The rate values are expressed in units of percentage of initial levels per month.*
4. *Values are negative indicating losses over time.*
5. *The combinations highlighted with a yellow background are those recommended for further evaluation (see Section 5) whereas those shown with light blue have a lower potential to enhance AA retention.*

The work reported here did not investigate the stability of microencapsulated AA in food matrices, however it is of interest to compare the results in Table 5 with previously published losses of AA in food products. In liquid milks approximately 50% of AA was lost per month during storage at 4.4 °C (Head and Hansen, 1979). In a recent study of losses during processing of noodles involving steaming, frying and boiling, losses of AA used as a fortificant were 10–20% per minute (Sanyoto *et al.*, 2008).

In the only previous report of loss of AA from microcapsules made using starch and combinations with β-cyclodextrin (Uddin *et al.*, 2001), the apparent losses were 2–7% during storage at 38 °C for 4 days. The encapsulation using starch in that report demonstrated a strong advantage in that microencapsulation prevented the discoloration that was seen for unprotected AA in the crystalline form. While direct comparisons with the published data are difficult, the low rates obtained in the current study confirm that the protection provided by the microcapsules has potential.

Based on the rates of loss during storage at 48 °C, six combinations of encapsulating agents are recommended for further evaluation. They are F30 + Instant 449, F30 + Instant MAPS, Hi Cap 100, F30 + GA + Hi Maize™ 1043, F17 + Instant 449 and F30 + GA (combinations 5, 8, 11, 6, 2 and 4 respectively).

3.6 The retention of AA at four different storage temperatures

The overall pattern of retention of AA at each of the four temperatures was similar to that shown in Figure 4 and Table 5. As expected the losses were lower as the temperature of storage was reduced. As a specific example, the pattern of results obtained for the combination of F30+GA is shown in Figure 5. This is typical of that observed for all the other combinations. The losses increased as higher temperatures were used for storage.

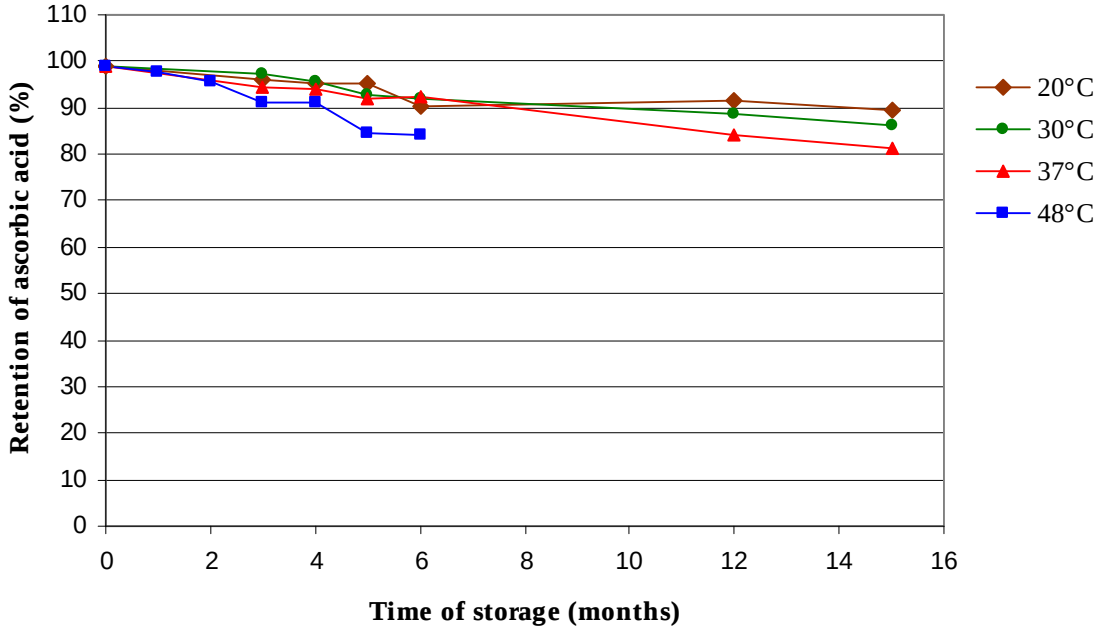

Figure 5: Effect of temperature of storage upon retention of AA for microcapsules prepared from F30+GA+6% AA (combination 4)

Note: The pattern was similar for each of the other combinations prepared in this study.

3.7 Comparison of the combinations at different storage temperatures

To facilitate the direct comparison of the results obtained at each temperature, the rates of loss of AA for each combination / temperature / time were calculated using the approach detailed in Section 3.5. Based upon the resultant rate values, the capsule combinations were ranked in order from highest to lowest retention of AA for each temperature. The results are summarised in Table 6.

The tabulation firstly shows that similar rankings were obtained at each of the temperatures of storage. For many of the preparations the performance was consistent across the trial. For example, CAPSUL (combination 9) afforded the lowest retention at all of the temperatures, whereas those prepared with F30 in combination with Instant 449 and Instant MAPS both provided very high levels of retention. Interestingly some combinations showed less consistent patterns and the most obvious of these was F17+GA+ Hi Maize™ 1043 (Table 6, combination 3).

The data from Table 6 and that obtained at 48 °C (Figure 4) lead to the conclusion that the combinations that gave less than 80% AA retention after 6 months of storage at 48 °C, also showed relatively low retention of AA at the lower temperatures of storage.

A further observation is that for encapsulating agents for which crystallinity was observed in the study of X-ray diffraction patterns (Section 3.5) the retention of AA tended to be lower at all temperatures (Table 6). This indicates that amorphous structure, possibly associated with the presence of lower molecular weight carbohydrate fragments in the walls of the capsules, provides enhanced protection of the AA that has been encapsulated. Accordingly, the presence of crystallinity as determined by X-ray diffraction does not appear to be useful in enhancing stability. This, however, does not preclude a potential advantage of crystallinity in terms of release characteristics of the active material. Further studies may be warranted to evaluate release properties along with the potential role of crystalline structure in this context.

Table 6: AA stability during the storage trial for all microcapsule preparations (6% AA loading)

Rate of loss (% /mth)												
20 °C			30 °C			37 °C			48 °C			
3	F17+GA+Hi Maize	-0.47	5	F30+Instant 449	-0.38	8	F30+Instant MAPS	-0.55	5	F30+Instant 449	-1.40
5	F30+Instant 449	-0.48	8	F30+Instant MAPS	-0.53	5	F30+Instant 449	-0.63	8	F30+Instant MAPS	-1.43
4	F30+GA	-0.56	11	Hi Cap 100	-0.57	11	Hi Cap 100	-0.68	11	Hi Cap 100	-1.71
8	F30+Instant MAPS	-0.58	4	F30+GA	-0.84	3	F17+GA+Hi Maize	-0.88	6	F30+GA+Hi Maize	-2.06
6	F30+GA+Hi Maize	-0.63	6	F30+GA+Hi Maize	-0.87	6	F30+GA+Hi Maize	-1.06	2	F17+Instant 449	-2.10
1	F17+GA	-0.86	7	F17+Instant MAPS	-1.12	4	F30+GA	-1.15	4	F30+GA	-2.67
2	F17+Instant 449	-0.96	2	F17+Instant 449	-1.17	2	F17+Instant 449	-1.24	3	F17+GA+Hi Maize	-3.15
10	F17	-0.96	1	F17+GA	-1.23	1	F17+GA	-1.47	1	F17+GA	-3.37
11	Hi Cap 100	-1.11	3	F17+GA+Hi Maize	-1.26	10	F17	-3.44	7	F17+Instant MAPS	-3.42
7	F17+Instant MAPS	-2.47	10	F17	-2.55	7	F17+Instant MAPS	-4.05	13	K4484+Hi Maize	-9.09
12	K4484	-4.11	12	K4484	-4.32	13	K4484+Hi Maize	-4.52	10	F17	-12.5
13	K4484+Hi Maize	-4.22	13	K4484+Hi Maize	-4.52	12	K4484	-4.65	12	K4484	-14.0
9	CAPSUL	-13.7	9	CAPSUL	-15.0	9	CAPSUL	-17.0	9	CAPSUL	-40.8

Notes:

1. *Data for each temperature has been presented as combination number (refer Table 2), followed by the details of the encapsulating agents and then the rate value obtained for loss of AA, expressed in units of %/ month.*
2. *The rate values are negative indicating losses over time.*
3. *For each temperature, the combinations of encapsulating agents are ranked in order from highest retention of AA during storage (uppermost) to least (lowest in the columns for each temperature).*
4. *The background colours designate the particular combination of encapsulating agents. The colour coding has been used to facilitate comparisons between the rankings of retention for the four temperatures.*
5. *The approach used in the calculation of the values for rate of loss of AA is described in Section 2.9.*
6. *Hi Maize™1043 has been abbreviated to Hi Maize.*

3.8 The impact of selected encapsulating agents upon retention of AA

The combinations of encapsulating agents used for the microcapsules provide data that is novel and may prove useful as a basis for choosing ingredients for other spray drying applications. Firstly, these allow the direct comparison of maltodextrins with differing dextrose equivalent values, reflecting differences in the extent of hydrolysis during manufacture. The relevant data has been summarised in Table 7. This shows that the rate of loss of AA is higher for samples where F17 was used compared to those with F30. On this basis, in the selection of maltodextrins

for encapsulation of water soluble active ingredients, F30 appears to confer better retention properties to the resultant capsules.

Table 7: Effect of the dextrose equivalent of the maltodextrin used in microencapsulation on retention of AA (6% loading)

No.	Combination	Rate of loss (%/mth)			
		20 °C	30 °C	37 °C	48 °C
1	F17+GA	-0.86	-1.23	-1.47	-3.37
4	F30+GA	-0.56	-0.84	-1.15	-2.67
3	F17+GA+Hi Maize™ 1043	-0.47	-1.26	-0.88	-3.15
6	F30+GA+Hi Maize™ 1043	-0.63	-0.87	-1.06	-2.06
7	F17+Instant MAPS	-2.47	-1.12	-4.05	-3.42
8	F30+Instant MAPS	-0.58	-0.53	-0.55	-1.43
2	F17+Instant 449	-0.96	-1.17	-1.24	-2.10
5	F30+Instant 449	-0.48	-0.38	-0.63	-1.40

Notes:

1. *Rate values are expressed as AA lost per month*
2. *The values are negative indicating losses over time*
3. *The background colours are provided to facilitate comparisons and are for capsules with F17 (light blue) and F30 (yellow)*

A further interesting aspect of the data is the association between the use of Hi Maize™ 1043 and AA retention. This ingredient was selected for the trials as it is high in resistant starch and might be expected to retard release during digestion following consumption. The data (Table 8) suggests some effect of enhanced retention at the highest temperatures studied (37 and 48 °C). This observation should be further investigated.

Table 8: Effect of incorporation of Hi-Maize™ 1043 as an encapsulating agent upon retention of AA during storage at 4 temperatures

No.	Combination	Rate of loss (% AA/mth)			
		20 °C	30 °C	37 °C	48 °C
1	F17+GA	-0.86	-1.23	-1.47	-3.37
3	F17+GA+Hi Maize™ 1043	-0.47	-1.26	-0.88	-3.15
4	F30+GA	-0.56	-0.84	-1.15	-2.67
6	F30+GA+Hi Maize™ 1043	-0.63	-0.87	-1.06	-2.06
12	K4484	-4.11	-4.32	-4.65	-14.0
13	K4484+Hi Maize™ 1043	-4.22	-4.52	-4.52	-9.09

Notes:

1. *Rate values are expressed as %AA lost per month.*
2. *The values are negative indicating losses over time.*

3.9 The impact of varying loading levels on retention of AA

A further aspect of microencapsulation which was briefly investigated has been the impact of varying levels of AA incorporation. This is often referred to as the loading of the active component. Microcapsules were prepared using F17+GA corresponding to combinations 1, 14 and 15 in Table 2. The only difference between these was the loading of AA. The relative amounts added were 6, 18 and 36% and in all three cases the capsules were quite similar in particle size (Figure 3). The morphology was also similar although the extent to which the capsules appeared indented was reduced for those capsules with 36% AA loading. (Appendix B, Figures B1 and B8).

The results from AA analyses following the storage trial show that there is an effect on stability that may be of practical significance (Figure 6). The retention of AA obtained for the 18 and 36% AA loading was very high, and substantially enhanced protection of AA was observed for these compared to that observed for the 6% AA loading.

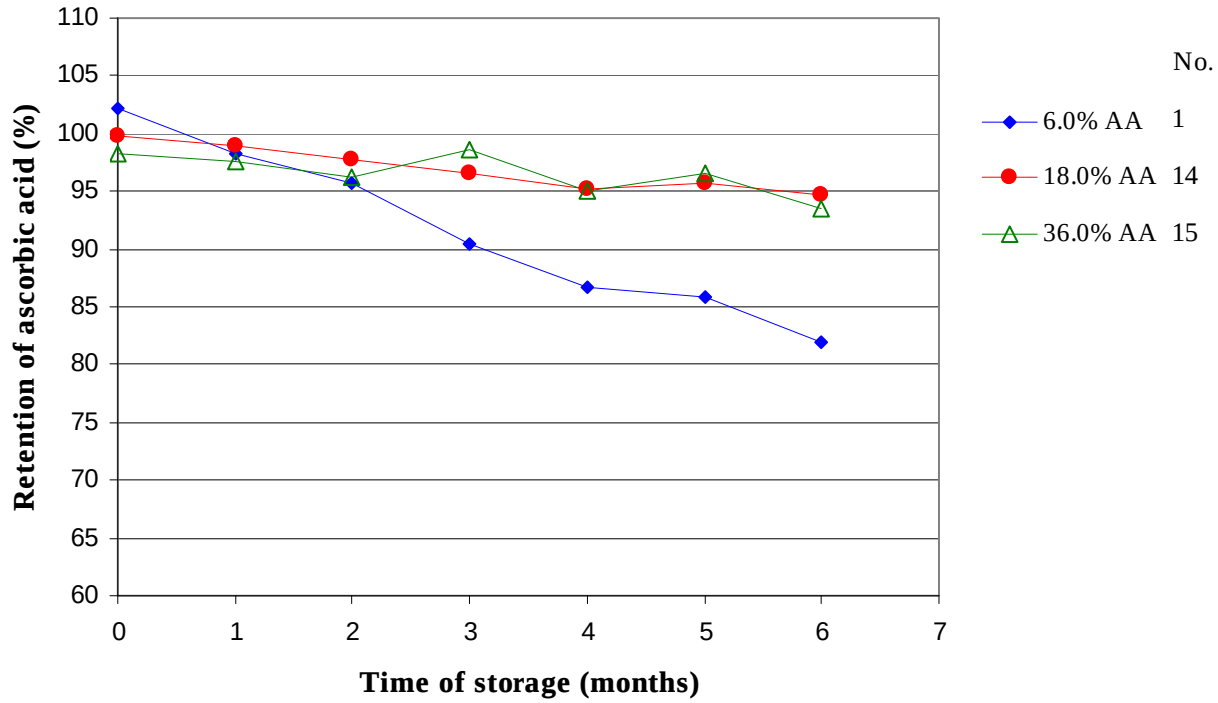

Figure 6: The impact of varying loading levels of AA into the formulation for preparation of microcapsules using the combination of F17+GA (samples stored at 48 °C)

It is therefore likely that even greater stabilities would be achieved if the combinations of encapsulating agents giving the best overall stabilities during storage were used in conjunction with higher rates of addition of AA to the capsule formulations. The potential enhancement of retention at higher levels of incorporation indicated by the current results warrants further investigation. It is recommended that this include additional levels of AA as only three levels of incorporation were trialled. These might be used in conjunction with those combinations of encapsulating agents identified from the storage trial data (Table 5 and Table 6) as having the highest recoveries under adverse storage conditions.

4. Future Research

The results of the current study establish a basis for further research. This could include a project to incorporate microcapsules into food matrices representative of those used in combat ration packs. The specific questions now remaining to be addressed are:

1. What is an optimal level of incorporation of AA into microcapsules to provide the highest level of protection? Additional experiments need to be undertaken to extend the preliminary data indicating that higher loading levels resulted in increased retention of AA.
2. What level of retention is achieved when microencapsulated AA is added to foods and these are stored under harsh conditions?
3. Do different combinations of encapsulating agents have application for particular food products? In order to evaluate this, up to five food products might be selected so that a range of food properties are encompassed. Among those that might be useful in the context of combat ration packs are muesli bars, beverage powder, biscuits, chocolate or other confectionery product, as well as a selection of freeze dried meals.

5. Summary and Conclusions

This research project has sought to develop strategies for enhancing the stability of AA added to foods as a fortificant. A series of microencapsulation agents were used to prepare capsules by spray drying. The resultant preparations were characterised and subjected to storage at a range of temperatures up to 48 °C. The results demonstrate that:

1. Yields of microcapsules obtained on a pilot scale spray dryer were satisfactory (75–95%).
2. Recoveries of AA during microencapsulation by spray drying were excellent (98–100%), showing virtually no losses under the conditions used.
3. Microcapsules prepared by spray drying consisted predominantly of particles in the range of 10–50 μm diameter. Little variation in size was observed for the various combinations of encapsulating agents used in this investigation.
4. Microscopy confirmed the particle size data obtained by the laser diffraction technique. The images obtained by ESEM also demonstrate that the microcapsules are basically spherical in shape. The surfaces appear to be continuous and show indentations to varying extents.
5. X-ray diffraction analysis established that for the wall materials displaying a degree of crystallinity, the resultant capsules also have crystalline patterns. However these microcapsules do not show enhanced retention of AA.
6. The storage trials demonstrated that AA retention levels were dependent upon the particular combinations of encapsulating agents.

7. The results indicate that six combinations of encapsulating agents—F30 + Instant 449, F30 + Instant MAPS, Hi Cap 100, F30 + GA + Hi Maize™ 1043, F17 + Instant 449 and F30 + GA (combinations 5, 8, 11, 6, 2 and 4 respectively)—warrant further evaluation.
8. Hi Maize™ 1043 appears to enhance the protective effect in the combinations studied.
9. The limited investigation of the effect of loading level of AA within the microcapsules indicated that higher levels are associated with significantly enhanced retention.

6. Recommendations

The recommendations from this work are:

1. Further evaluate the most promising of the combinations of encapsulating agents by incorporating the microcapsules into a selection of food products so that the effectiveness of protection can be established within food matrices.
2. Conduct a series of trials to more fully investigate the impact of loading of AA within capsules.
3. Investigate extension of the results of this work to other water soluble bio-active components and micronutrients.

7. Acknowledgements

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Appendix A: Moisture Content of Microcapsules

Table A1: Moisture content of microcapsules prepared with different hydrocolloid agents

Combination of encapsulating agents		**Moisture content (%)**
1	F17+GA	4.67 ± 0.42
2	F17+Instant 449	4.04 ± 0.70
3	F17+GA+Hi Maize	5.72 ± 0.70
4	F30+GA	5.86 ± 0.67
5	F30+Instant 449	4.31 ± 0.44
6	F30+GA+Hi Maize	5.55 ± 0.62
7	F17+Instant MAPS	4.82 ± 0.82
8	F30+Instant MAPS	6.06 ± 0.28
9	CAPSUL	7.49 ± 0.45
10	F17	6.02 ± 0.52
11	Hi Cap 100	5.20 ± 0.82
12	K4484	6.12 ± 0.63
13	K4484+Hi Maize	4.15 ± 0.47
14	F17+GA+18% AA	4.46 ± 1.12
15	F17+GA+36% AA	4.10 ± 0.48

Notes:

1. *The numbering of combinations is that presented in Table 2, Section 2.4.*
2. *Data are expressed in the form of mean value ± the 95% confidence interval values.*
3. *Combinations 1 to 13 were prepared with 6% AA.*

Appendix B: Electron Micrograph Images of Microcapsules for Each Combination of Encapsulating Agent Used in the Investigation

The following figures (B1 to B8) show the typical external features for the various preparations of microcapsules using ESEM. All images are at the same magnification (2500×) to facilitate direct comparisons. The microcapsules shown in each figure contain 6% AA loading, with the exception of Figure B8 which shows microcapsules with 18 and 36% AA loading. These can be directly compared with Figure B1 as these all used the same combination of encapsulating agents: F17+GA.

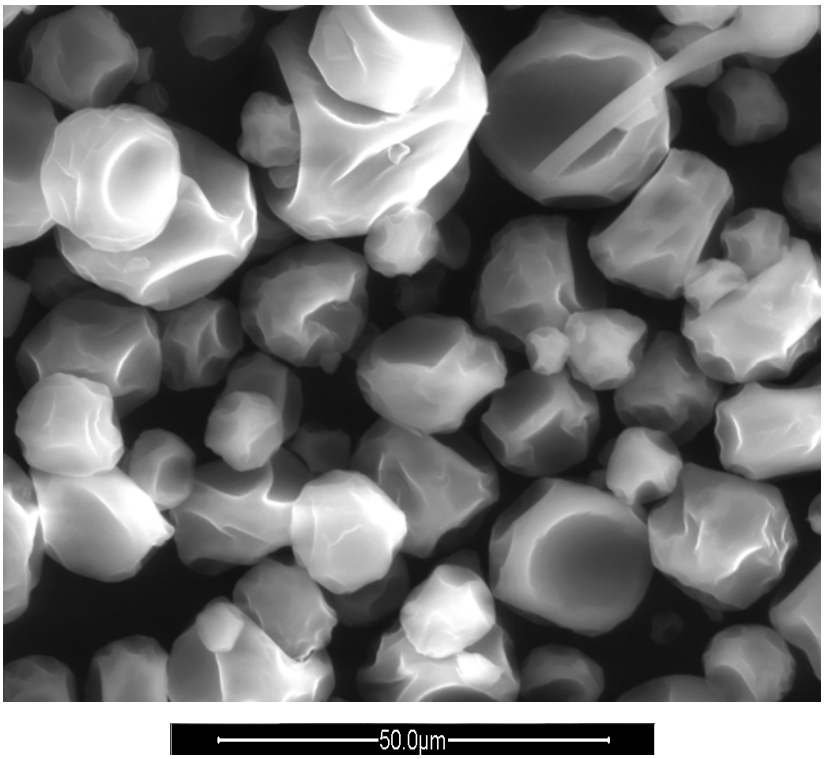

Figure B1: SEM image of microcapsules prepared with F17+GA (combination 1, Table 2).

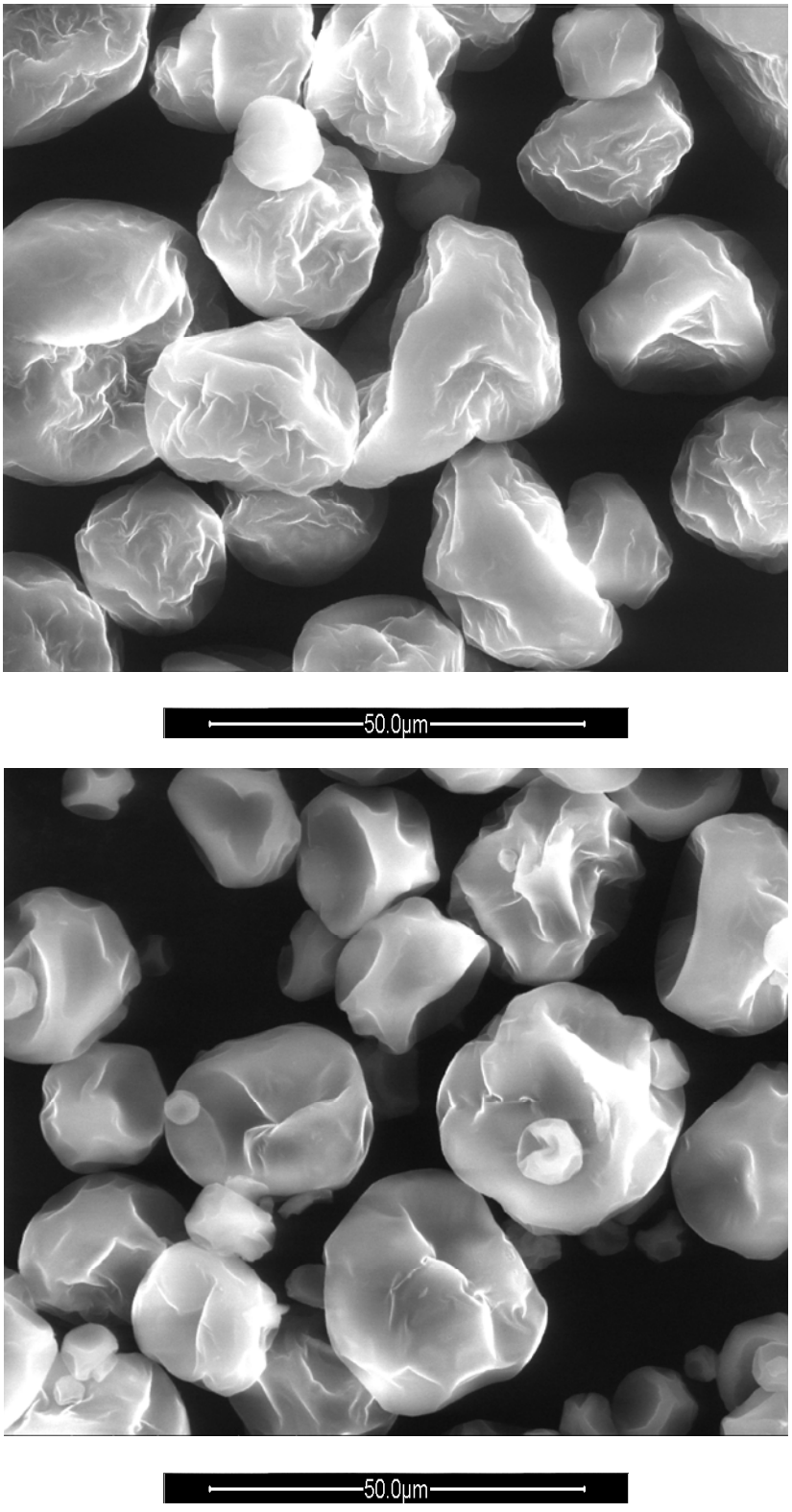

Figure B2: SEM images of microcapsules prepared with F17+Instant 449 (upper) and F17+GA+ Hi Maize™ (lower) (combinations 2 and 3 respectively, Table 2).

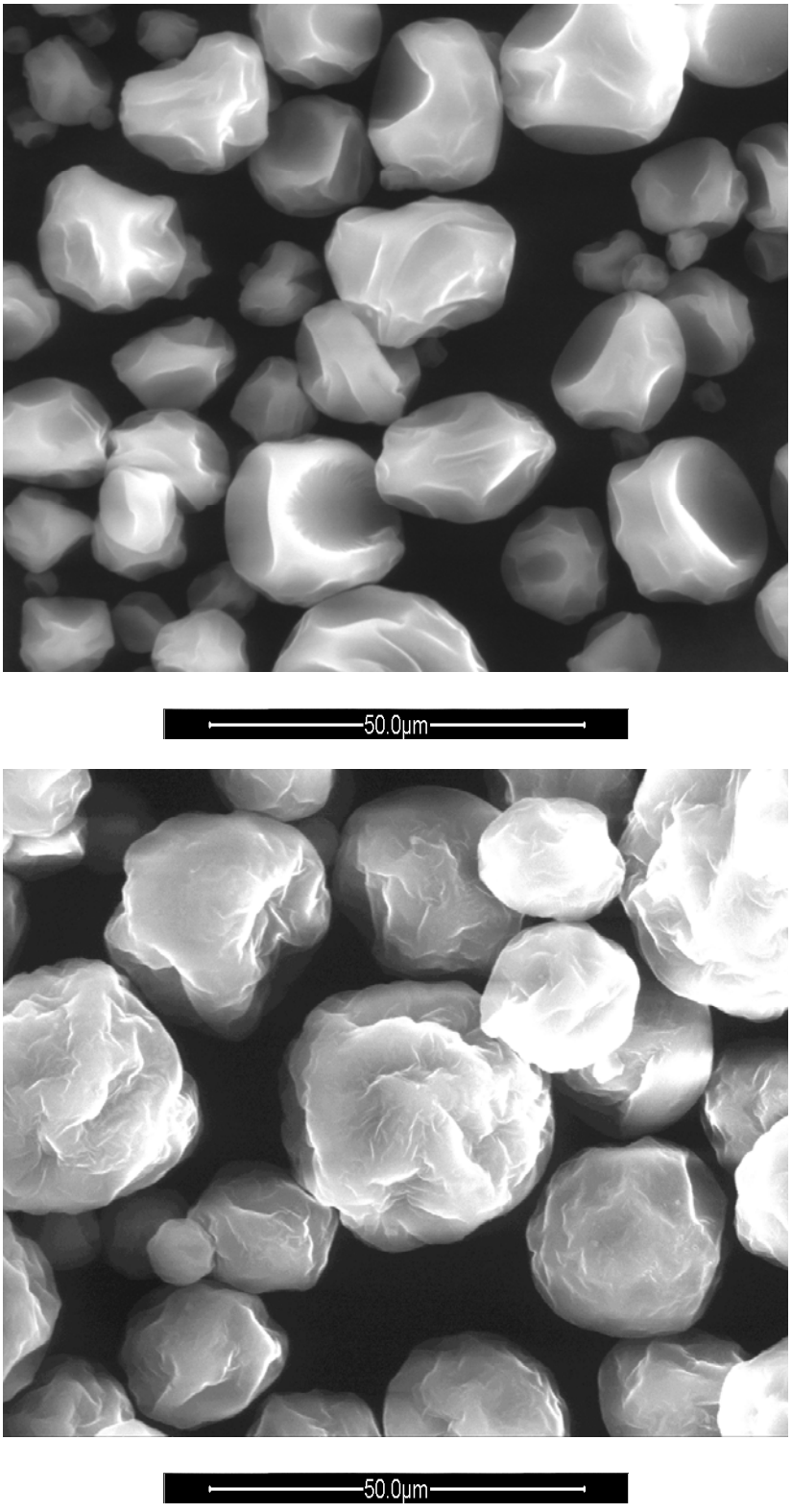

Figure B3: SEM images of microcapsules prepared with F30+GA (upper) and F30+Instant 449 (lower) (combinations 4 and 5 respectively, Table 2).

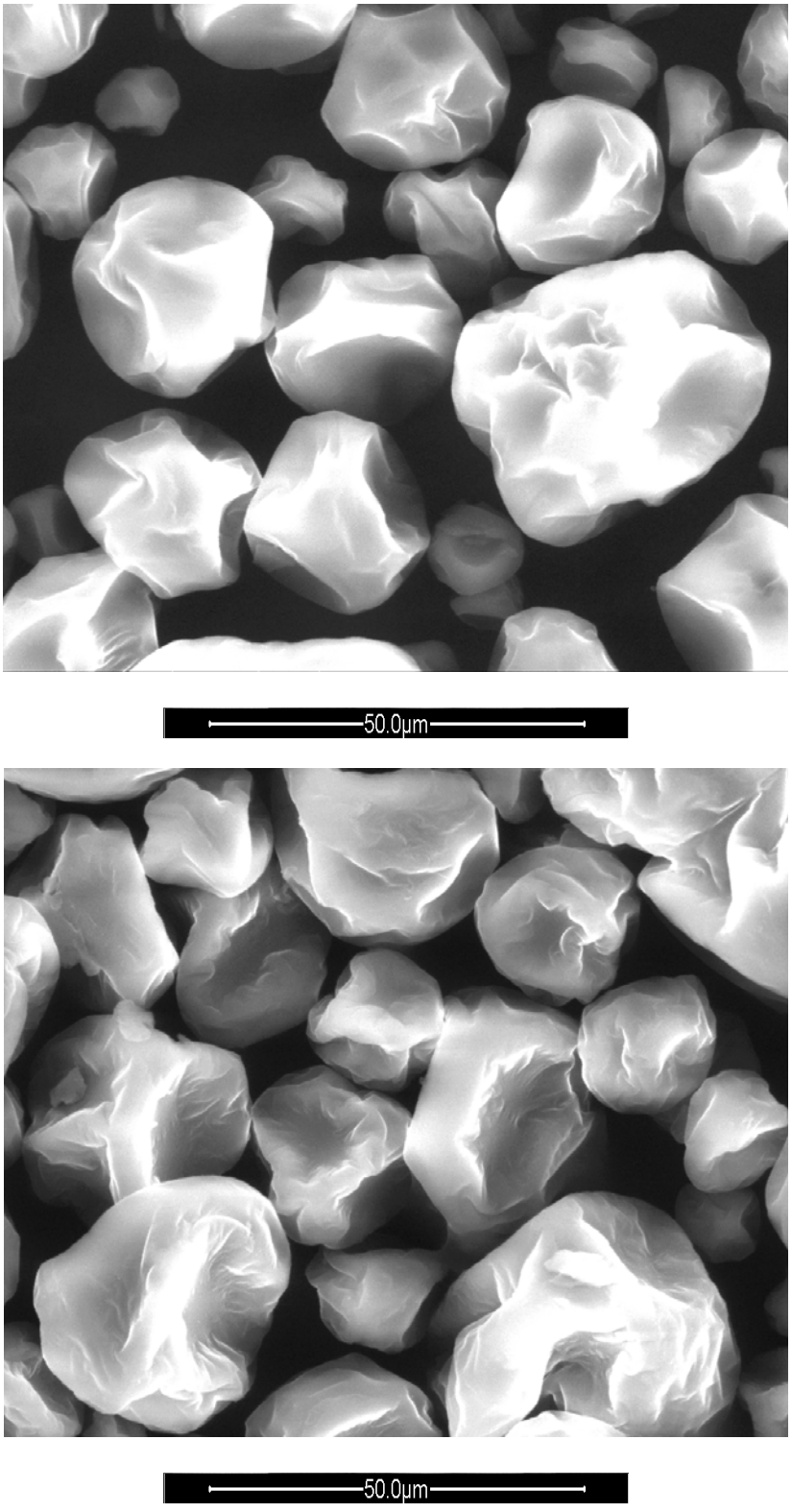

Figure B4: SEM images of microcapsules prepared with F30+GA+Hi Maize™ 1043 (upper) and F17+Instant MAPS (lower) (combinations 6 and 7 respectively, Table 2).

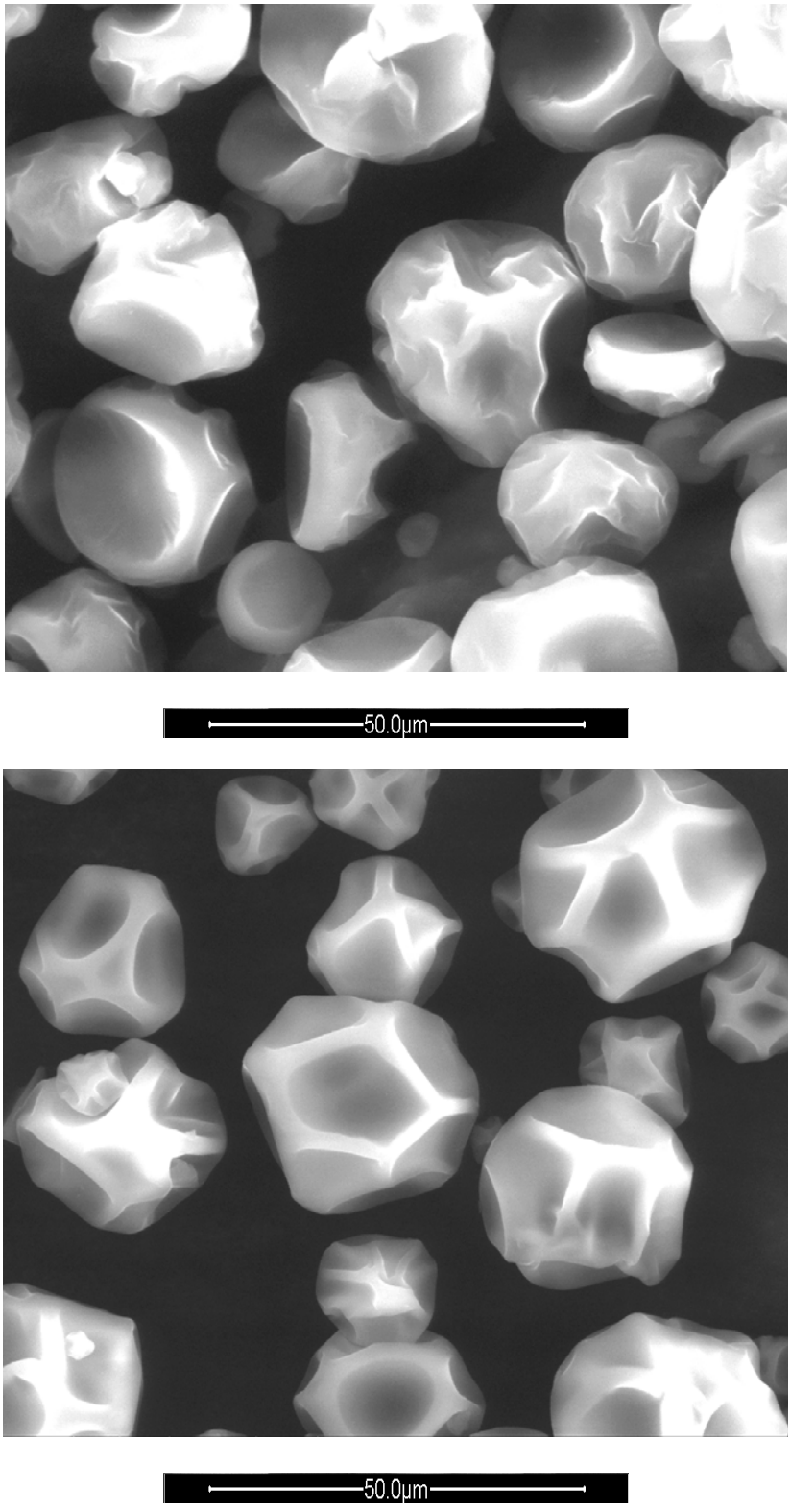

Figure B5: SEM images of microcapsules prepared with F30+Instant MAPS (upper) and CAPSUL® (lower) (combinations 8 and 9 respectively, Table 2).

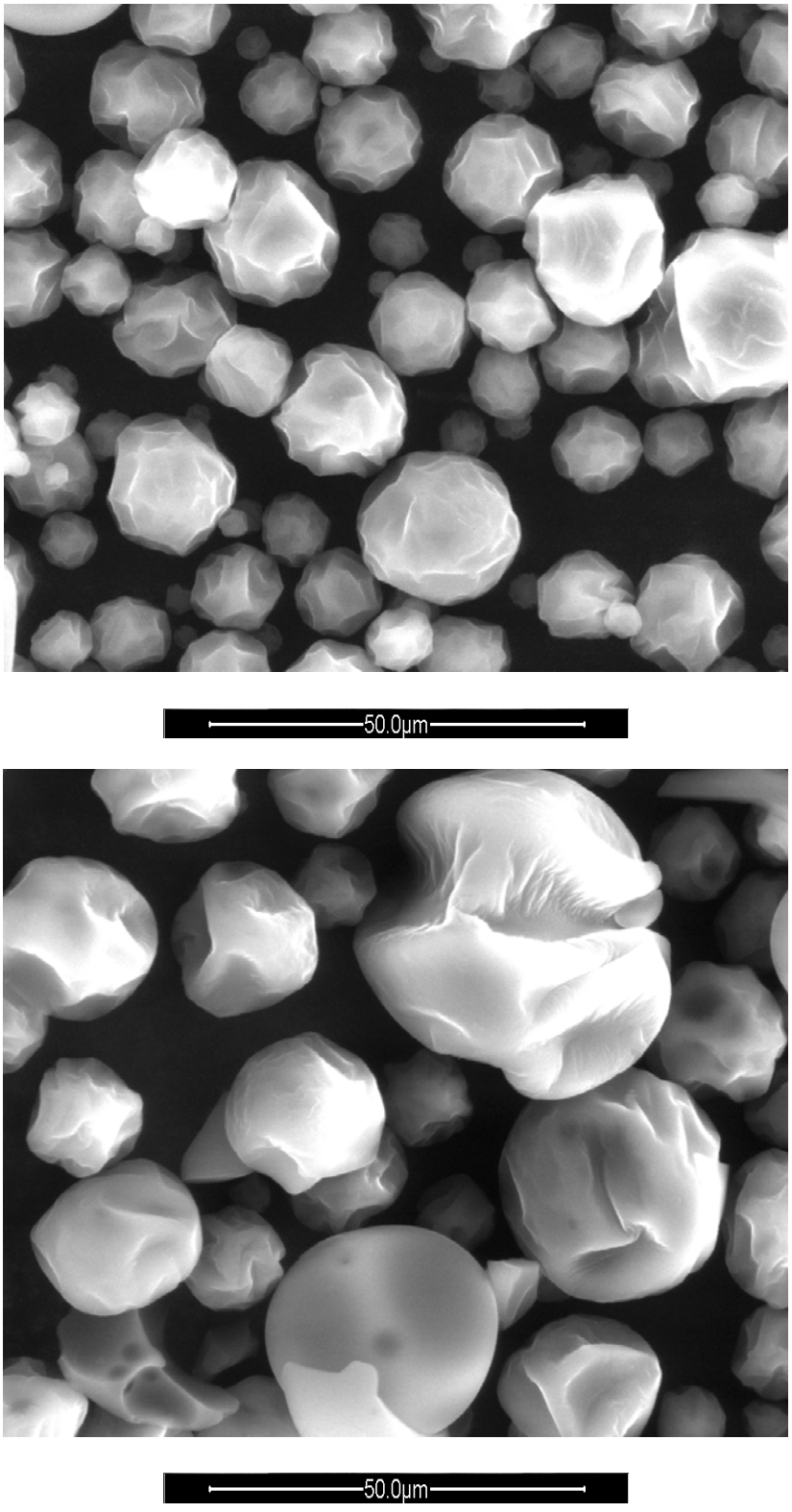

Figure B6: SEM images of microcapsules prepared with F17 (upper) and Hi CAP™ 100 (lower) (combinations 10 and 11 respectively, Table 2).

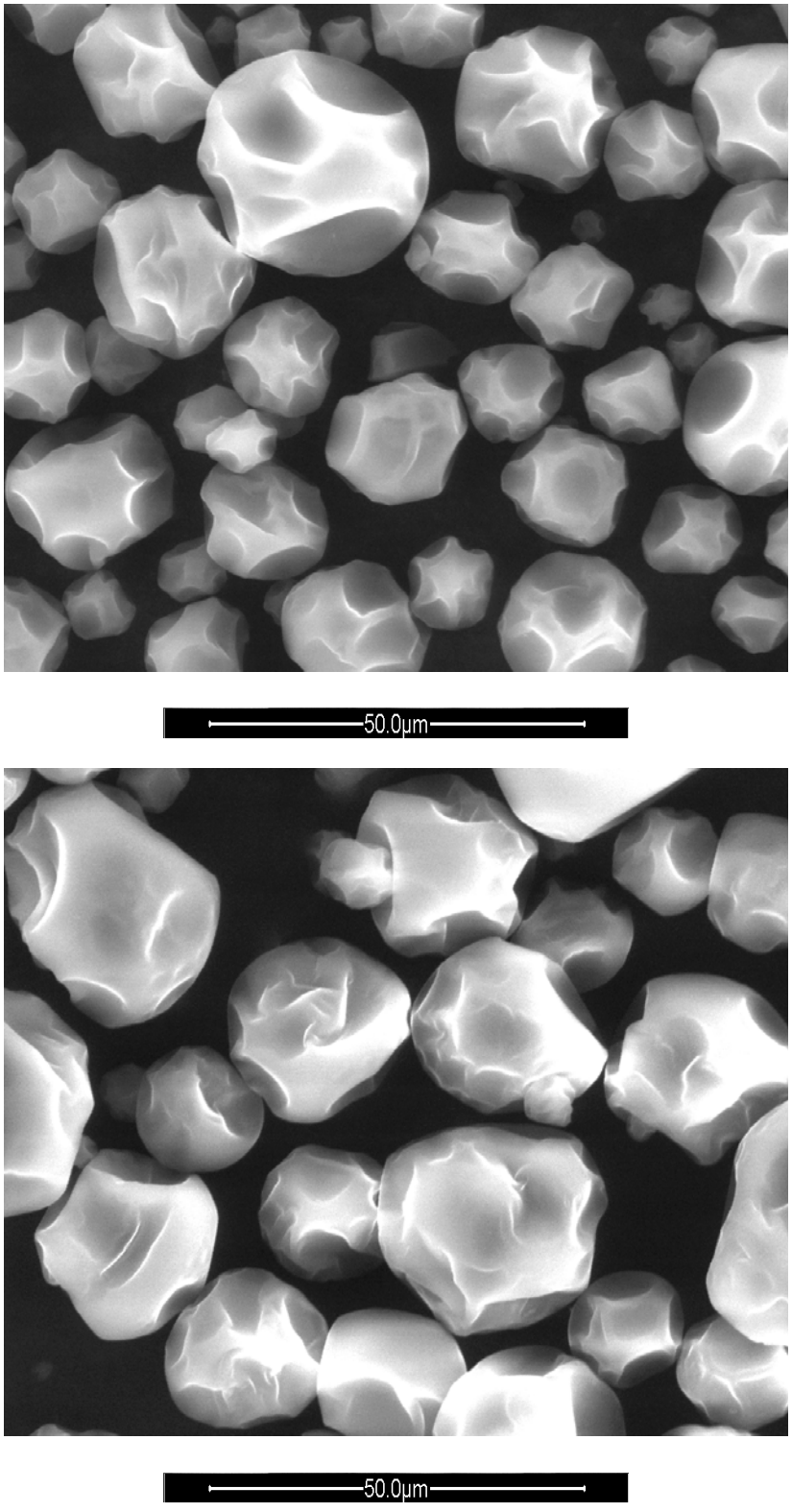

Figure B7: SEM images of microcapsules prepared with K4484 (upper) and K4484+Hi Maize™ 1043 (lower) (combinations 12 and 13 respectively, Table 2).

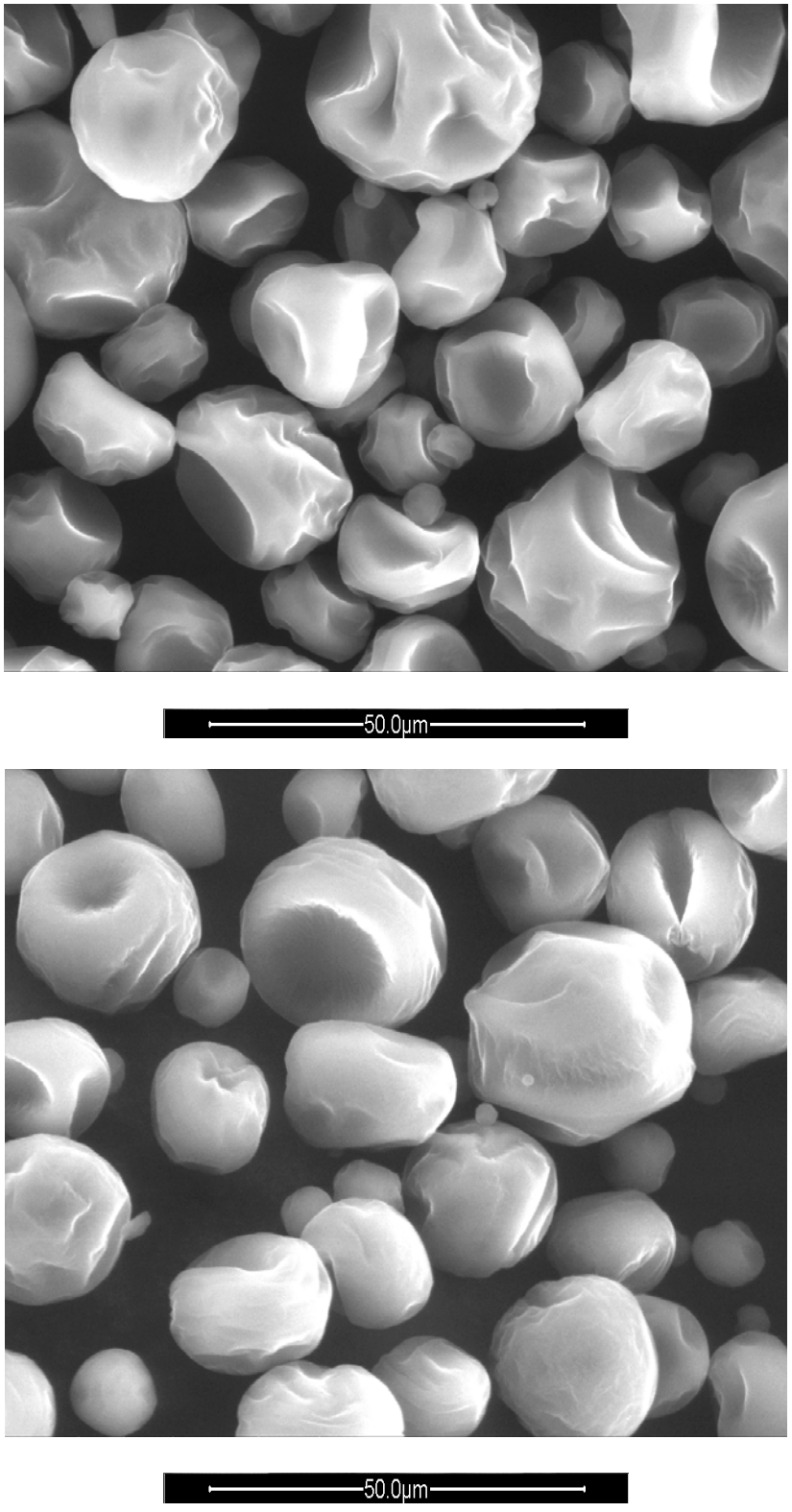

Figure B8: SEM images of microcapsules prepared with F17+GA+18% AA (upper) and 36% AA (lower) (combinations 14 and 15 respectively, Table 2).

Appendix C: X-ray Scans of Encapsulating Agents, Ascorbic Acid and Microcapsule Preparations

The following figures (C1 to C9) show diffraction patterns obtained by wide angle X-ray analysis. The first figure shows the scan for the pure AA used as an ingredient for encapsulation. Then a set of scans are presented for the encapsulating agents (wall materials) used in the preparation of the feed solution used for spray drying. These are followed by those for the various microcapsule preparations (in the same order as they are listed in Table 2, Section 2.4). For the microcapsule samples, each of these contained 6% AA loading other than those in Figure C9 where higher levels are specifically indicated.

It is noted that all figures are shown with the same horizontal and vertical axes in order to facilitate direct comparisons. It is emphasised, however, that for AA (Figure C1), the vertical axis is different. This reflects the highly crystalline nature of the pure AA used for microencapsulation, in comparison with all of other samples.

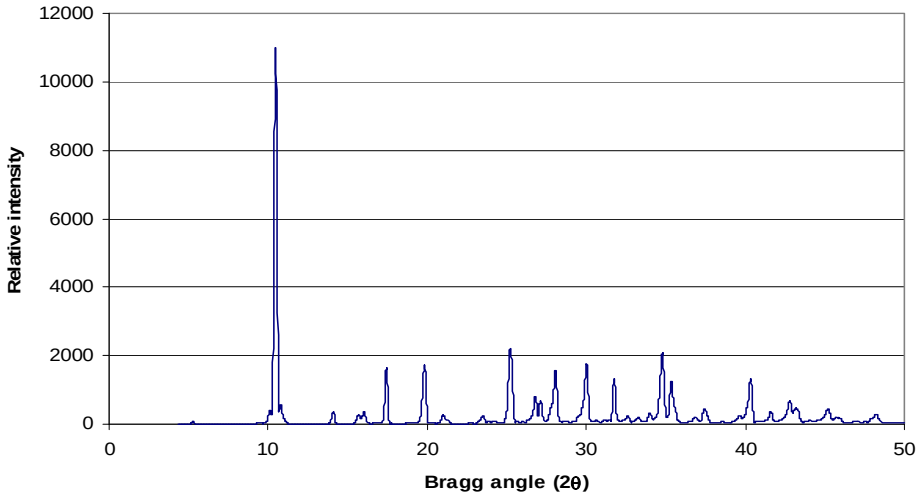

Figure C1: X-ray diffraction pattern for pure crystalline AA

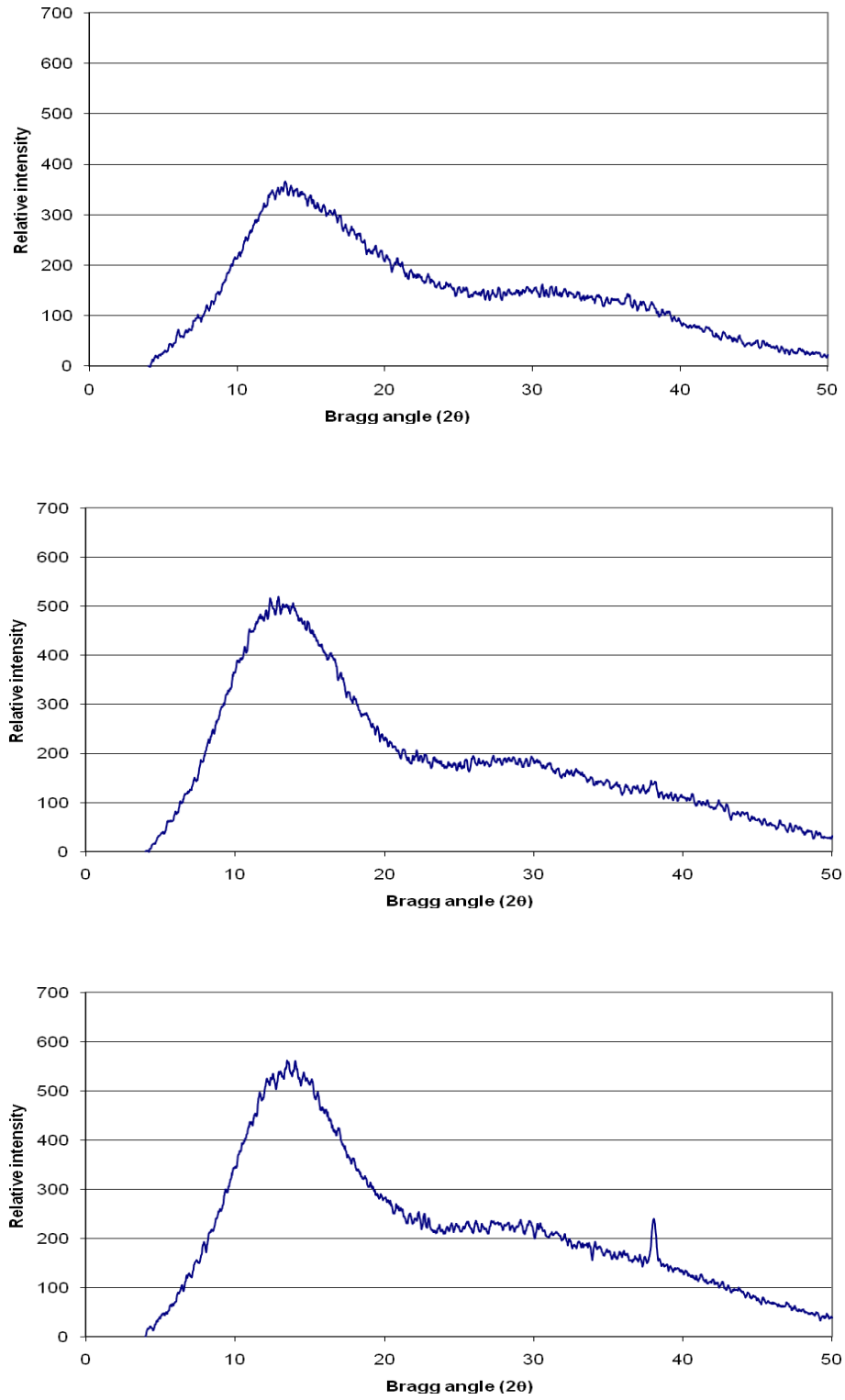

Figure C2: X-ray diffraction patterns for wall materials used in encapsulation of AA: GA (upper), F17 (centre) and F 30 (lower).

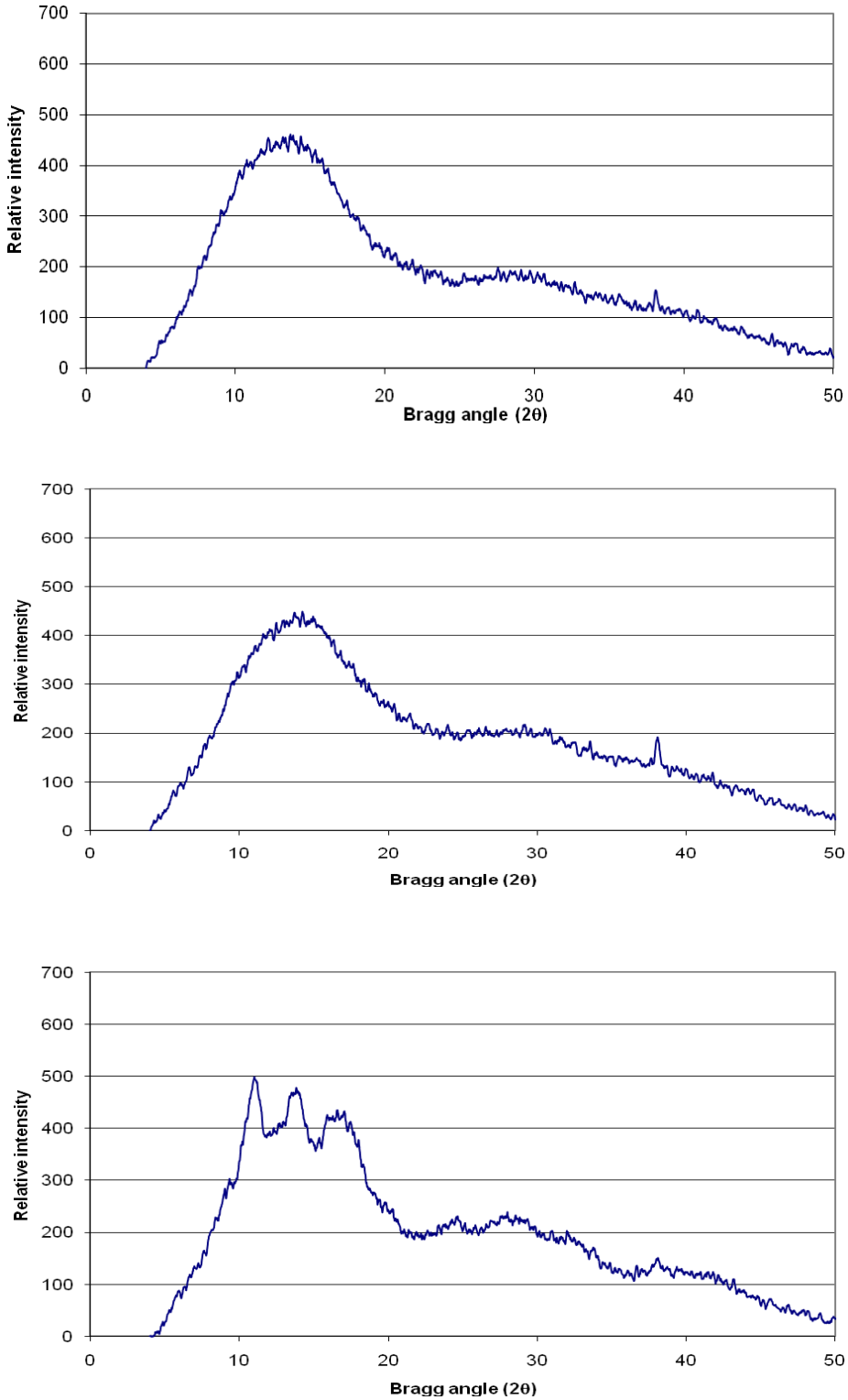

Figure C3: X-ray diffraction patterns for wall materials used in encapsulation of AA: Instant MAPS (upper), Instant 449 (centre) and Hi Maize™ 1043 (lower).

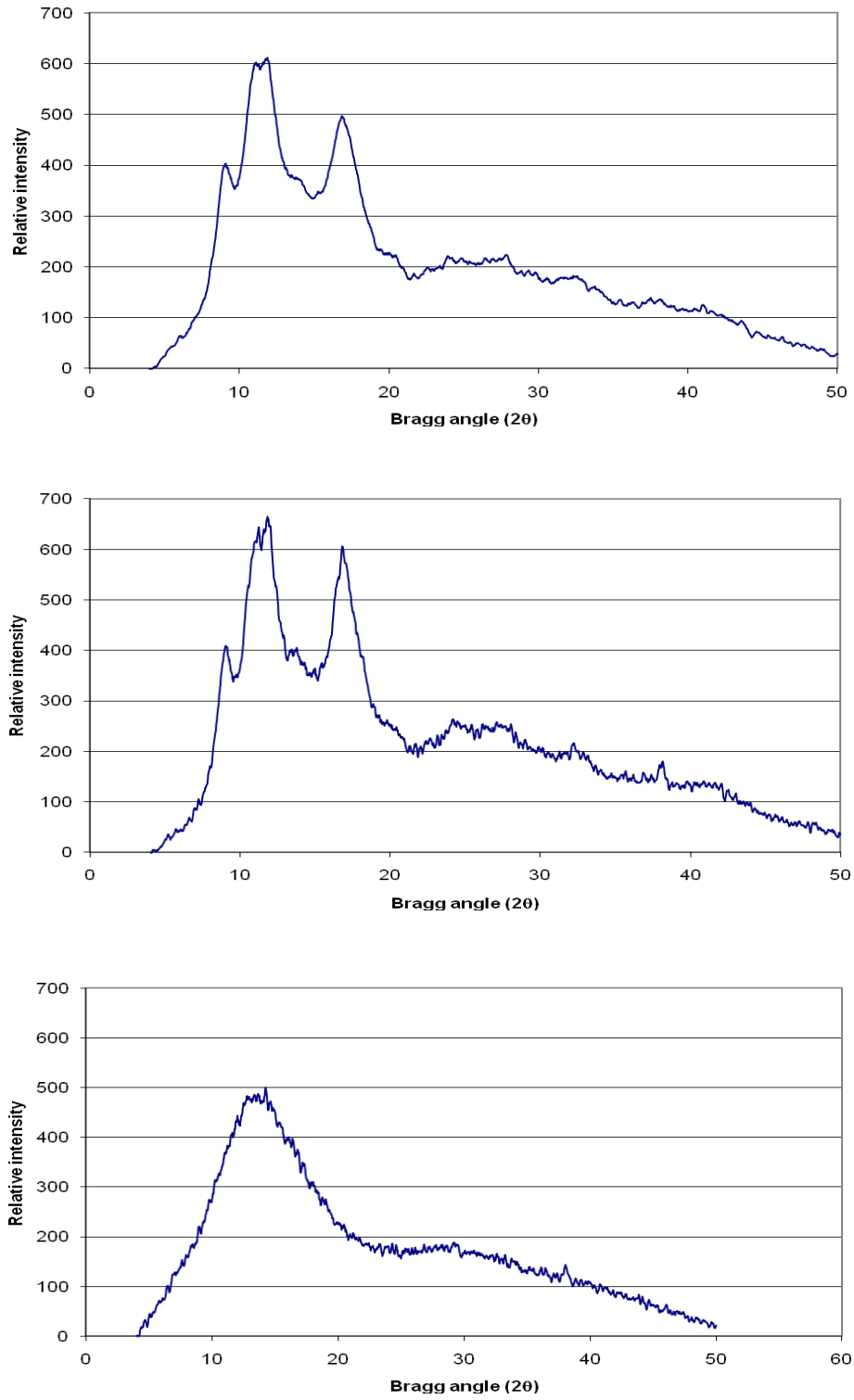

Figure C4: X-ray diffraction patterns for wall materials used in encapsulation of AA: CAPSUL® (upper), K4484 (centre) and Hi CAP™ 100 (lower).

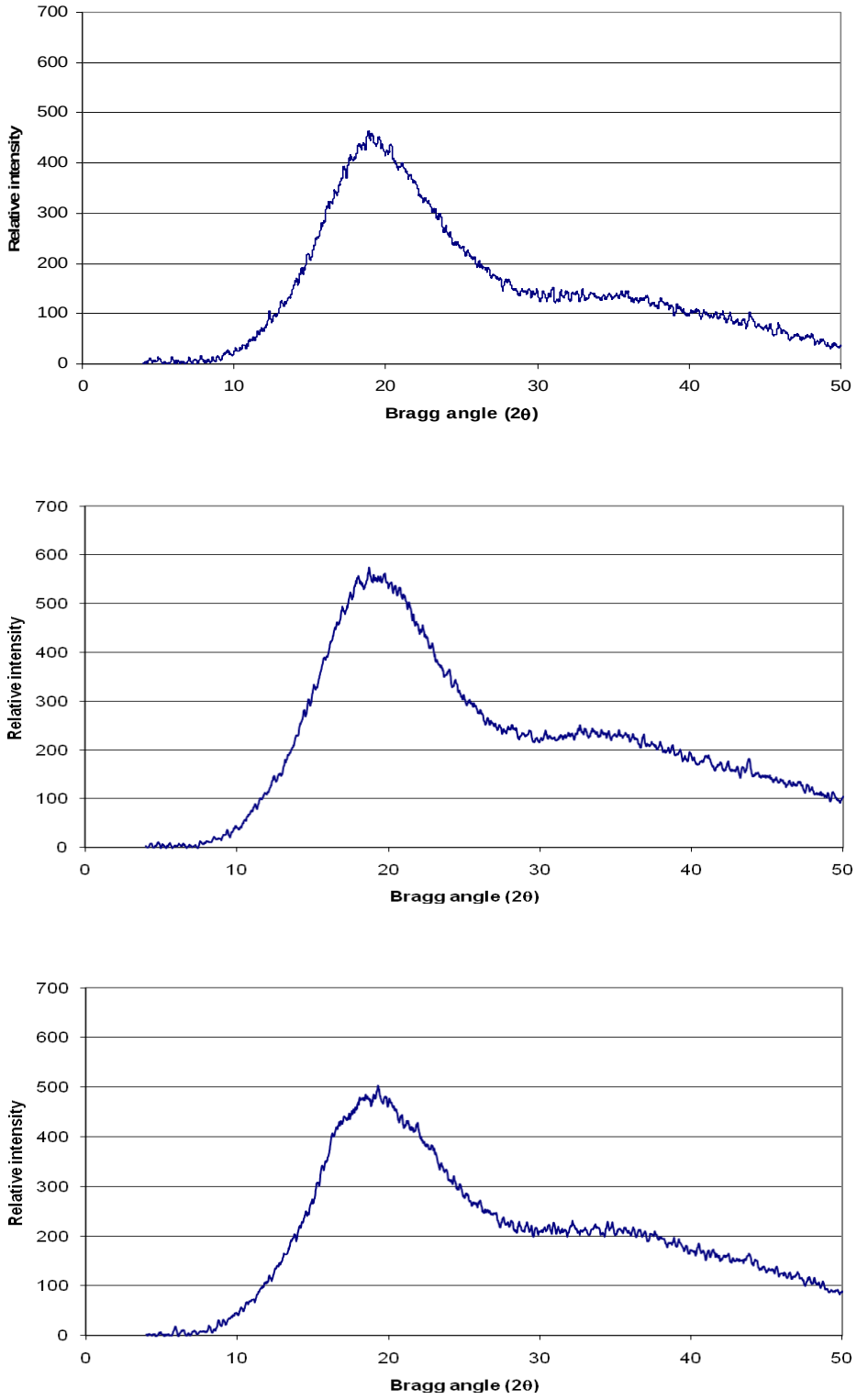

Figure C5: X-ray diffraction patterns for microcapsule preparations incorporating AA: F17+GA (upper), F17+Instant 449 (centre) and F17+GA+Hi Maize™ 1043 (lower) (combinations 1, 2 and 3 respectively).

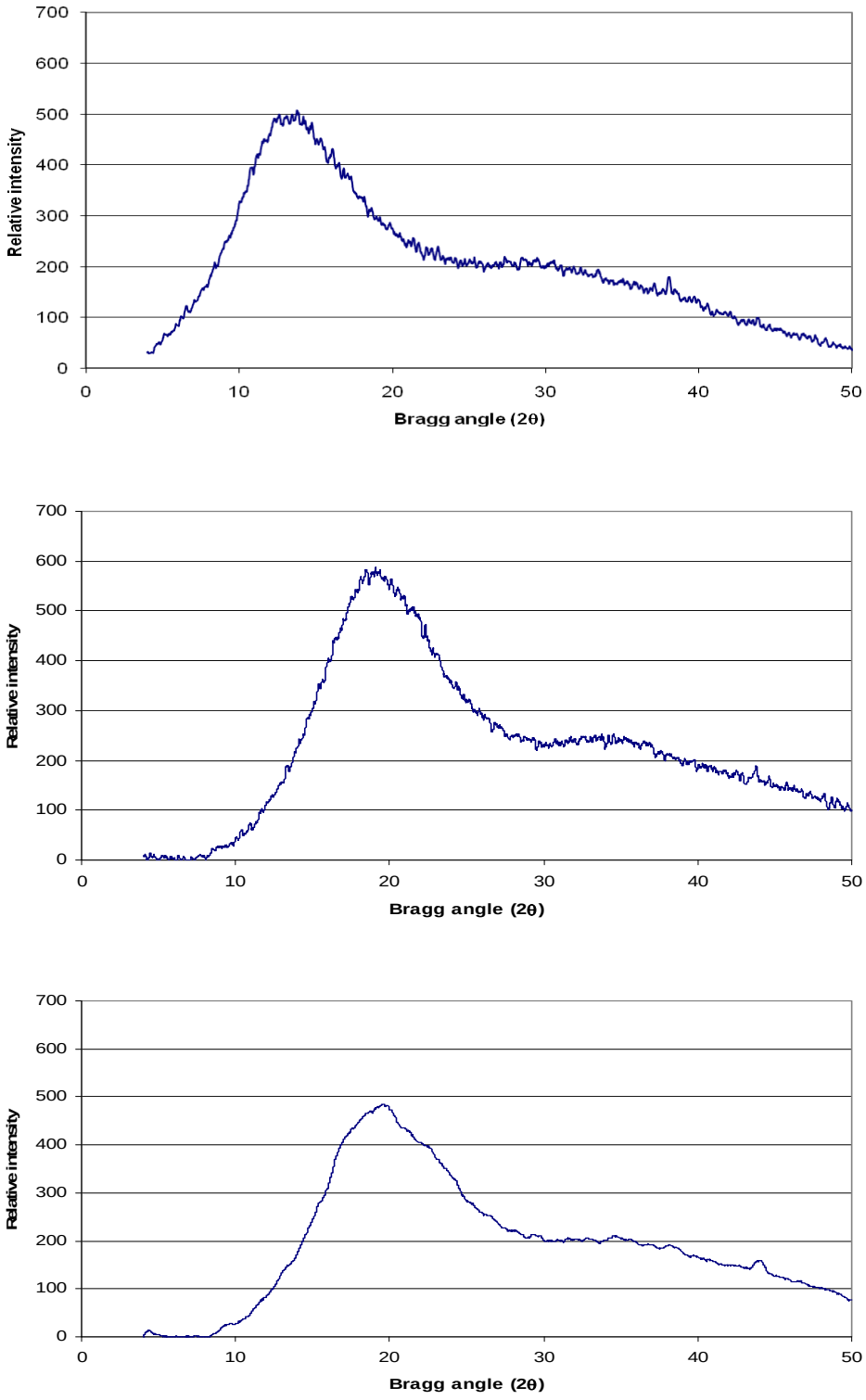

Figure C6: X-ray diffraction patterns for microcapsule preparations incorporating AA: F30+GA (upper), F30+Instant 449 (centre) and F30+GA+Hi Maize™ 1043 (lower) (combinations 4, 5 and 6 respectively).

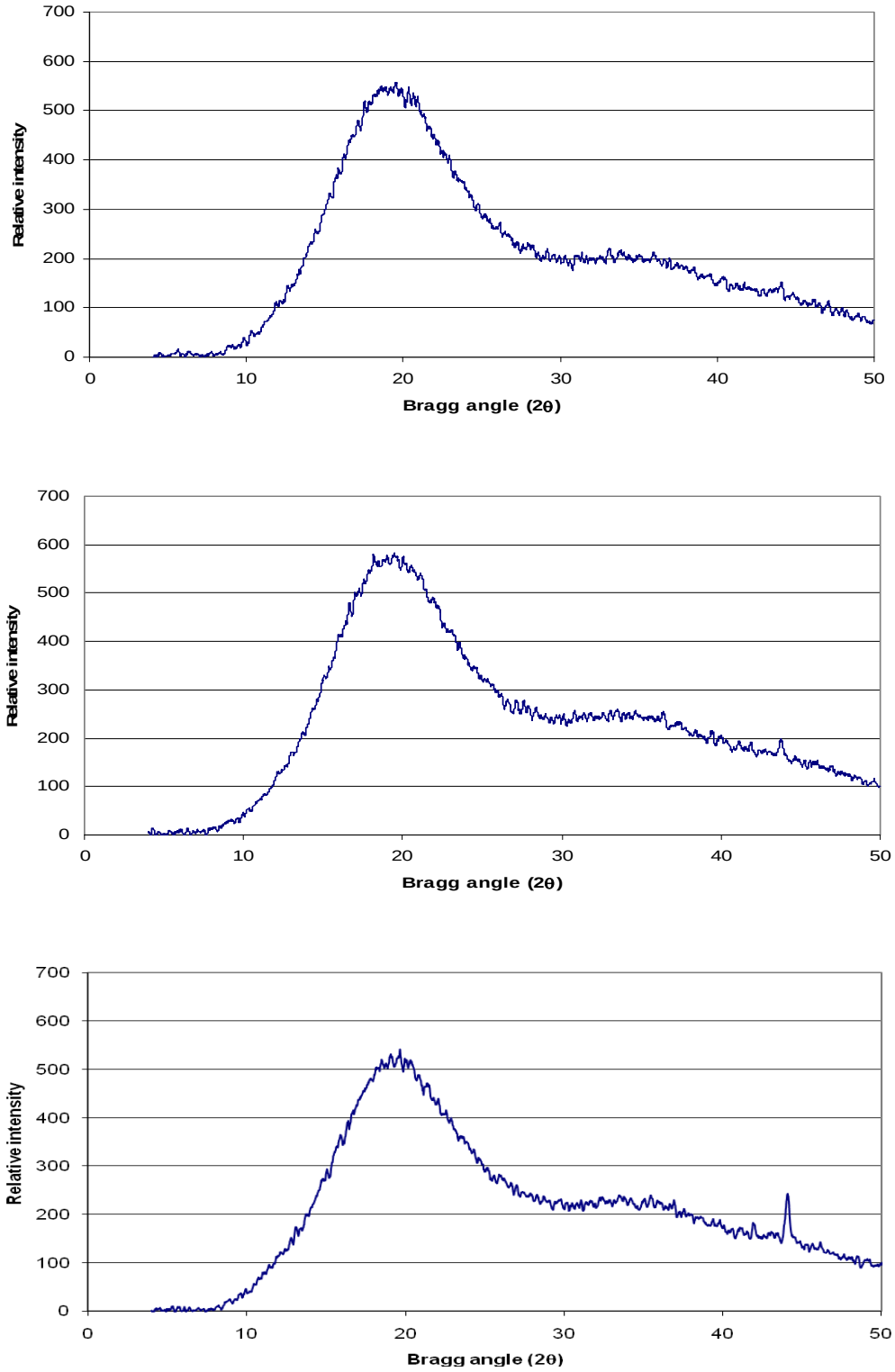

Figure C7: X-ray diffraction patterns for microcapsule preparations incorporating AA: F17+Instant MAPS (upper), F30+Instant MAPS (centre) and CAPSUL (lower) (combinations 7, 8 and 9 respectively).

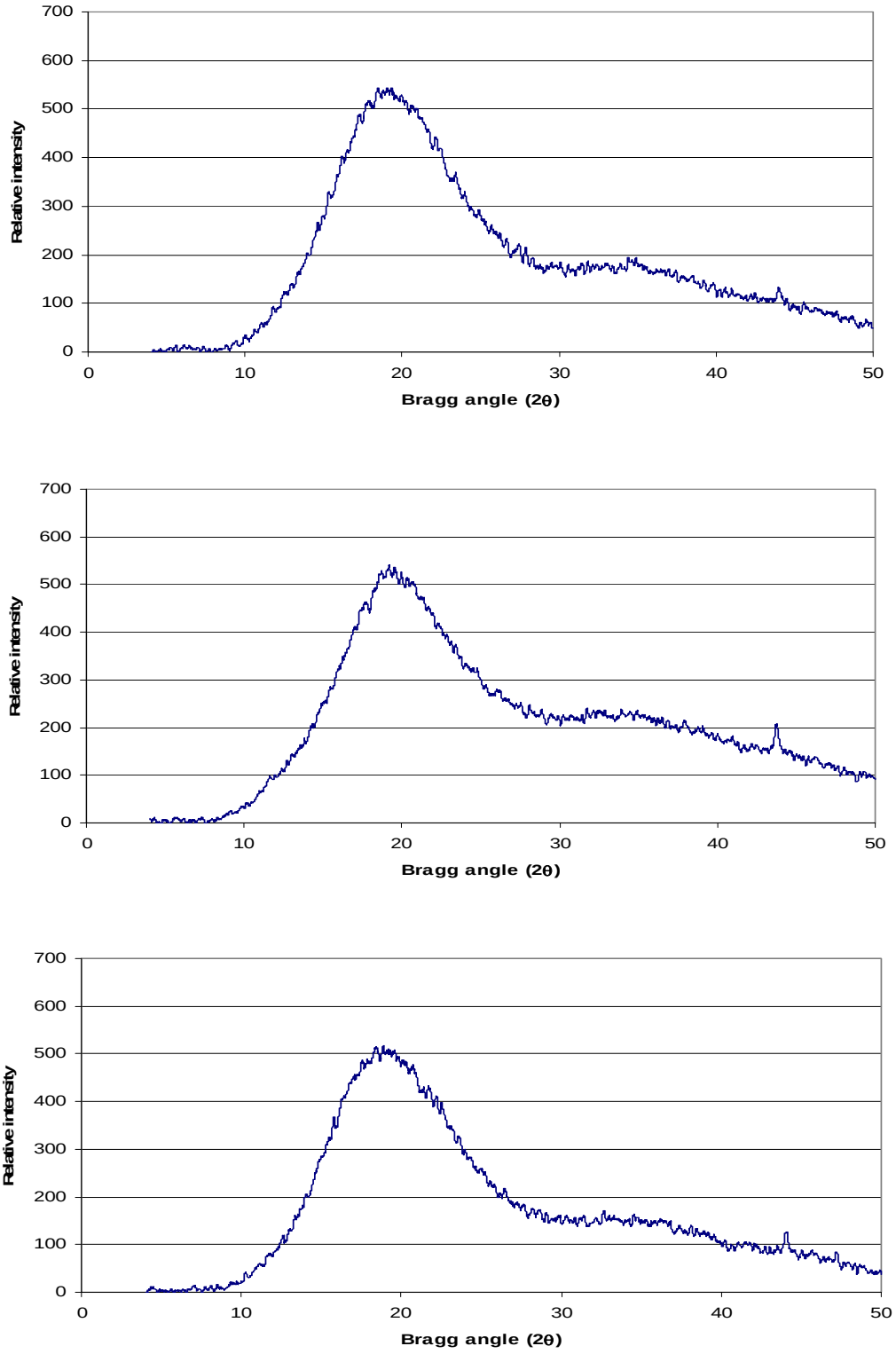

Figure C8: X-ray diffraction patterns for microcapsule preparations incorporating AA: F17 (upper), Hi Cap 100 (centre) and K4484 (lower) (combinations 10, 11 and 12 respectively).

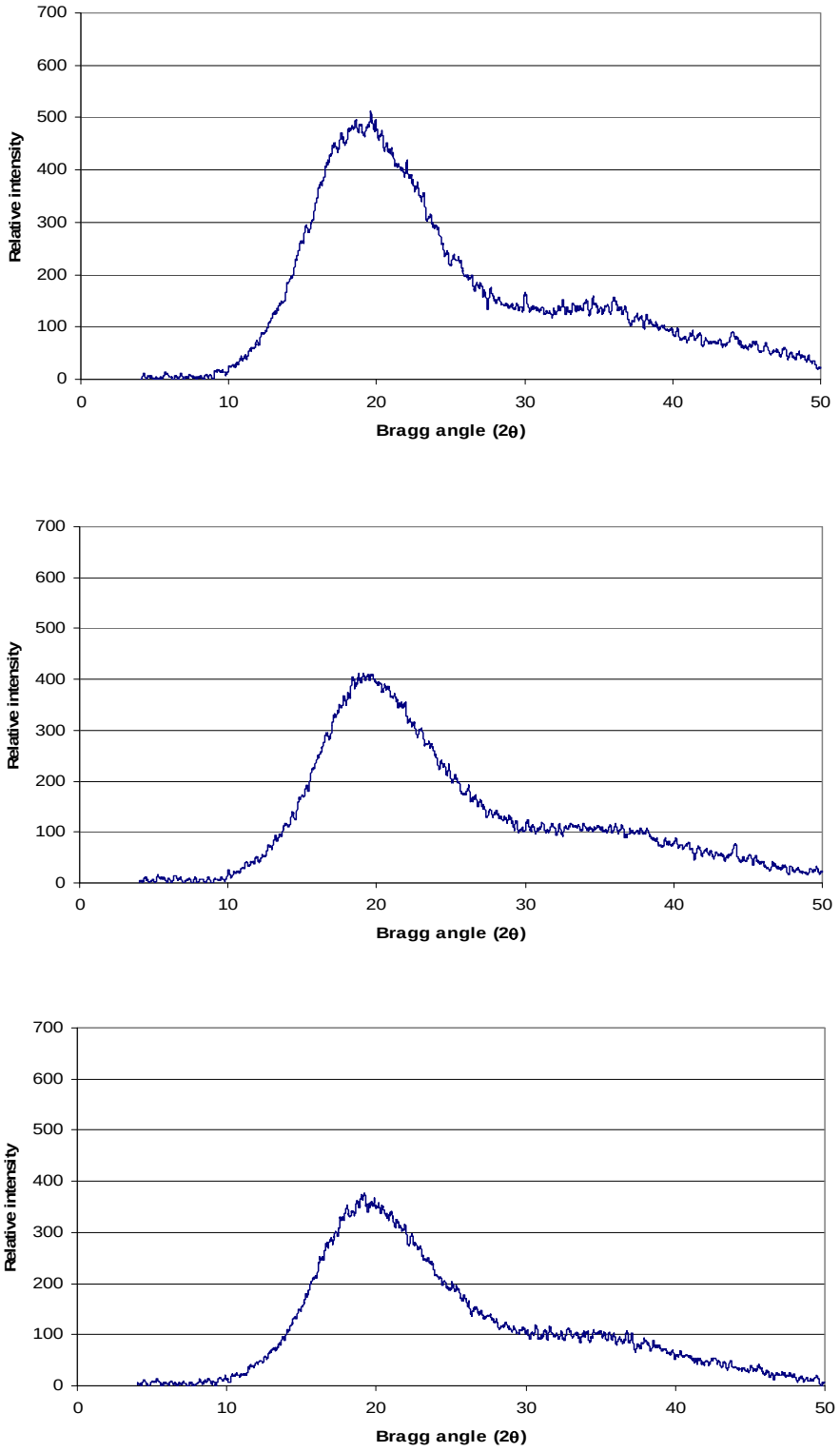

Figure C9: X-ray diffraction patterns for microcapsule preparations incorporating AA: K4484+Hi Maize™ 1043 (upper), F17+GA with 18%AA (centre) and F17+GA with 36%AA (lower) (combinations 13, 14 and 15 respectively).

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Microencapsulation, Ascorbic acid, Vitamin stability

19. ABSTRACT

A trial to evaluate a range of microcapsules prepared using various encapsulating agents individually and in combination has been completed. The aim was to provide a means of protecting ascorbic acid (AA) from harsh conditions of elevated temperature over extended storage periods, and to obtain a basis for the selection of encapsulating agents for the AA fortification of specialised food products. Microcapsules were prepared using a pilot scale spray dryer; good yields and high recoveries of AA were obtained. The resultant materials were fine powders of relatively uniform particle size, spherical with varying degrees of indentation but no evidence of mechanical damage. Samples of the microcapsules were stored at temperatures of 20, 30, 37 and 48 °C for periods of up to 15 months. There were significant variations in AA retention between treatments and encapsulating agents. It is recommended that six encapsulating agent combinations and loading levels be further evaluated to determine their potential for the fortification of selected food products.