



Omega-3 Fatty Acids from Vegetarian Sources

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Introduction

The public are becoming increasingly aware of the health effects of essential omega-3 fatty acids. These fatty acids have been shown to be beneficial in many aspects of health including the development of the central nervous system and eye development in children. They have also been shown to be factors in the prevention of coronary heart disease and the treatment of inflammatory conditions such as rheumatoid arthritis and Crohn's disease

Supplementation of a natural drink i.e. orange juice or fruit smoothies would provide an appetising source of these fatty acids. Undoubtedly the best sources of these fatty acids are fish oils, but the taste and odour may be unpalatable in such drinks. Also the use of fish oil precludes the vegetarian section of the customer based, potentially limiting the appeal of any Omega-3 containing products. Good vegetable sources of these fatty acids are walnuts and linseeds. Both contain α -linolenic acid (ALA) which is converted in the human body into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the omega-3 fatty acids found in fish oils. It is possible that walnuts or linseeds could be incorporated into a palatable drink, with significant health benefits comprised from natural products

Foods high in omega-3 acids are susceptible to oxidation and the development of off-flavours. This project investigated the extent of oxidation of walnut and linseed ALA, in experiments designed to mimic the storage conditions of such products. The concentration of ALA was determined by gas chromatography-mass spectrometry, following conversion of the ALA into a 4, 4-dimethylxazoline



(DMOX) derivative, due to the instability of native ALA in the GC-MS process. Experiments were carried out over the natural shelf-life and use-by life of a fresh fruit drink product i.e. 7 days.

Materials and Methods

All chemicals used in this experiment were obtained from Sigma-Aldrich (UK). Walnuts and pasteurised orange juice were own-brands obtained from the Co-operative stores group, and Golden linseeds were obtained from Holland and Barrett Ltd.

Lipid extraction from Walnut and Linseed

2 g of nuts/linseeds were ground in a pestle and mortar in the presence of liquid nitrogen. 6 ml hexane/isopropanol (3:2, v/v) was added and incubated at room temperature for 1 h with constant stirring. The samples were left to settle and the supernatant was removed. The remaining nut material was washed with 4 ml of hexane/isopropanol. Supernatants were combined and 7 ml of 6.8% sodium sulphate was added. The solvent layer was then removed, dried and the pure oil was weighed. ALA standards also underwent the same extraction procedure, to note any loss during the extraction.

Analysis of Omega-3 content and stability

The following experiments were carried out to determine Omega-3 content of purified oils from linseeds, walnuts, and the extracted ALA standard. All experiments were carried out at 4 time points (day 0, day 2, day 5 and day 7). Experiments were incubated at 4 °C to mimic cold storage conditions of juice products.



Omega 3 source (500 µl)	SDW	Orange juice	10 % vitamin C solution
ALA standard	5 ml	-	-
ALA standard	-	5 ml	-
ALA standard	-	-	5 ml
Walnut oil	5 ml	-	-
Walnut oil	-	5 ml	-
Walnut oil	-	-	5 ml
Linseed oil	5 ml	-	-
Linseed oil	-	5 ml	-
Linseed oil	-	-	5 ml

Following derivitisation and GC-MS analysis further experiments were devised to determine the effect of pasteurisation on Omega-3 stability.

500 µl of extracted oils or ALA standard were added to 2.5 ml of Orange Juice plus 2.5 ml of 10 % Vitamin C solution. 1 ml of this mixture was then added to a thin walled micro-centrifuge tube (to aid in heat transfer) and then either refrigerated immediately, or heated at 80 °C for 15 s in a dry heating block to mimic a standard pasteurisation process. Total fatty acid content was then extracted and analysed using GC-MS as before.



Preparation of DMOX derivates

(Adapted from www.lipidlibrary.co.uk)

10 mg of the pure oil extraction was added to 0.25 g of 2-amino-2-methyl-1-propanol and placed in a heating block at 190 °C overnight. 5ml of diethyl ether/isohexane (1:1 v/v) was added, plus 5 ml of sterilised distilled water (SDW). The organic layer was removed and a further 2 ml of solvent was added. The organic layers were then combined and 1 g of sodium sulphate was added, and incubated at room temperature for 1 h. This was then passed through a 3 cm sodium sulphate purification column. Purified samples were then dried and re-suspended in 1 ml hexane.

GC-MS analysis

Samples were analysed on a Hewlett-Packard Gas chromatography mass spectrometer, with a temperature ramp rate of 10 °C per minute, to a maximum temperature of 350 °C. 1 µl of sample was analysed. DMOX derivates of Omega-3 fatty acids were not present in the GC-MS compound database, and so correct identification of the compounds was carried out using analysis of molecular weights (278.43 for ALA). For non-derivitised fatty acids, an internal library search was conducted.

Concentrations of ALA from different sources were determined by comparing GC-MS peak percentages against a calibration curve derived from pure ALA standard, giving percentage ALA of total pure oil.



Results

Fatty acid profiles of linseeds and walnuts

Total extracted fatty acid profiles were analysed using GC-MS for both linseed and walnut oils. Mass profiles were analysed using the internal software database. The results are presented below

Linseed oil

Hexadecanoic acid

11, 14, 17 - Eicosatrienoic acid

Heptadecanoic acid

9, 12, 15 Octadecatrienoic acid (ALA)

7, 10, 13 Hecadecatrienoic acid

9,12 Octadecanoic acid

Oleic Acid

5 Octadecanoic acid

9, 12, Octadecanoic acid

Walnut oil

Tetradecanoic acid

Hexadecanoic acid

9 Hexadecanoic acid

Octadecanoic acid

5, 8, 11, Heptadecatrienoic acid

9, 12, 15 Octadecatrienoic acid (ALA)

5, 8, 11, 14, Eicosatetraenoic acid

9, 12, Octadecadienal

11, 14, 17 - Eicosatrienoic acid



To determine accuracy of the library searches, standards of ALA were run and mass measurements and retention times were calculated and matched to the retention times of peaks present in the walnut/linseed analysis.

Shelf life of Omega 3 Fatty Acids

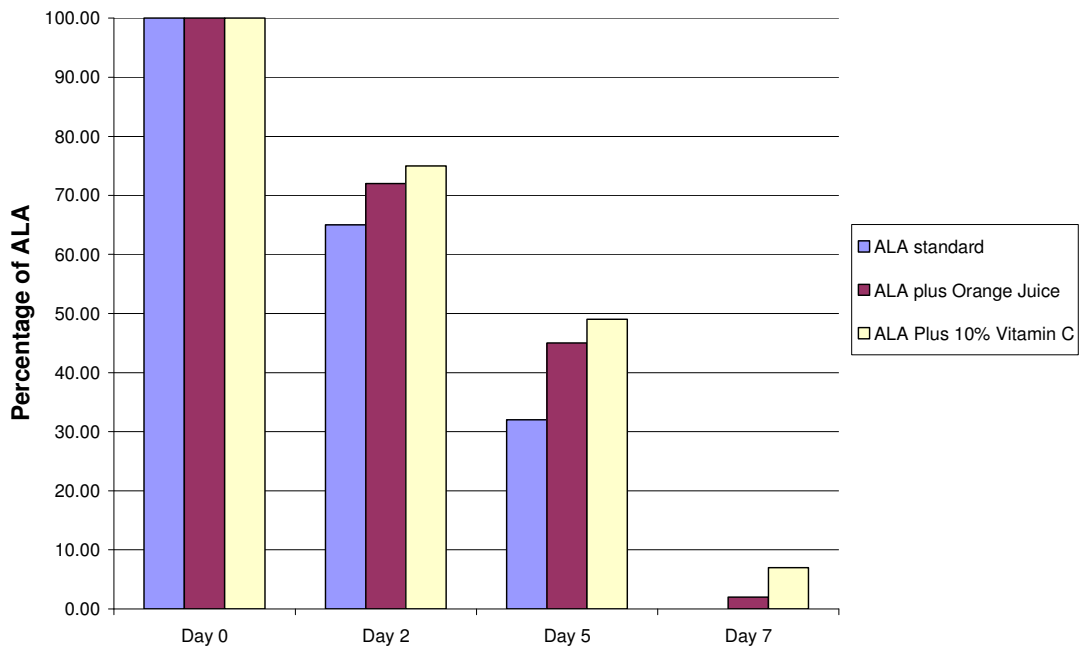
Experiments were carried out at days 0, 2, 5 and 7. Results are presented as percentage of day 0 ALA remaining as detected by GC-MS analysis

The analysis of pure ALA over time, showed an immediate decrease of ALA levels by day 2 of the experiment. Levels continued to fall and by day 7 no ALA could be detected. With the addition of orange juice ALA levels still fell but at a slower rate and ALA could still be detected at day 7. With the addition of Vitamin C solution the rate of decrease was lower still, reaching a final level of 7% at day 7.

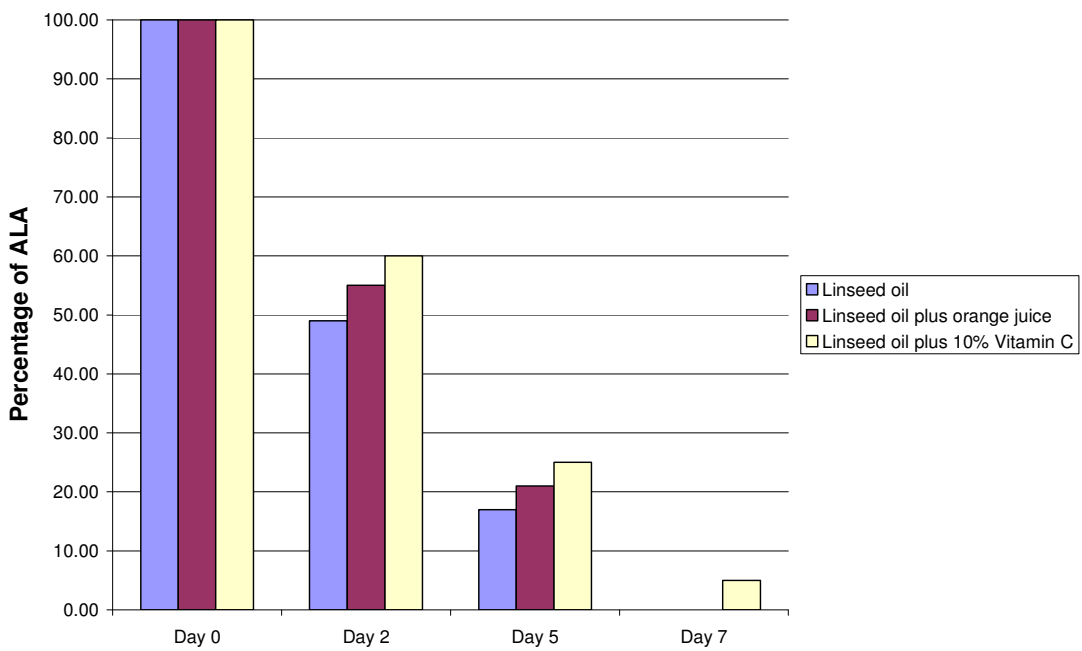
ALA extracted from both walnuts and linseeds followed a similar pattern to pure ALA, with the addition of orange juice and vitamin C decreasing the rate of decline. However the overall decline rates were much lower than seen with pure ALA with an approximate decline of 50 % for linseed ALA and 40 % for walnut ALA compared with an approximate reduction of 30 % for pure ALA by Day 2. By day 7 only ALA from linseed oil plus vitamin C could be detected. (All results shown in fig 1)



A



B



C

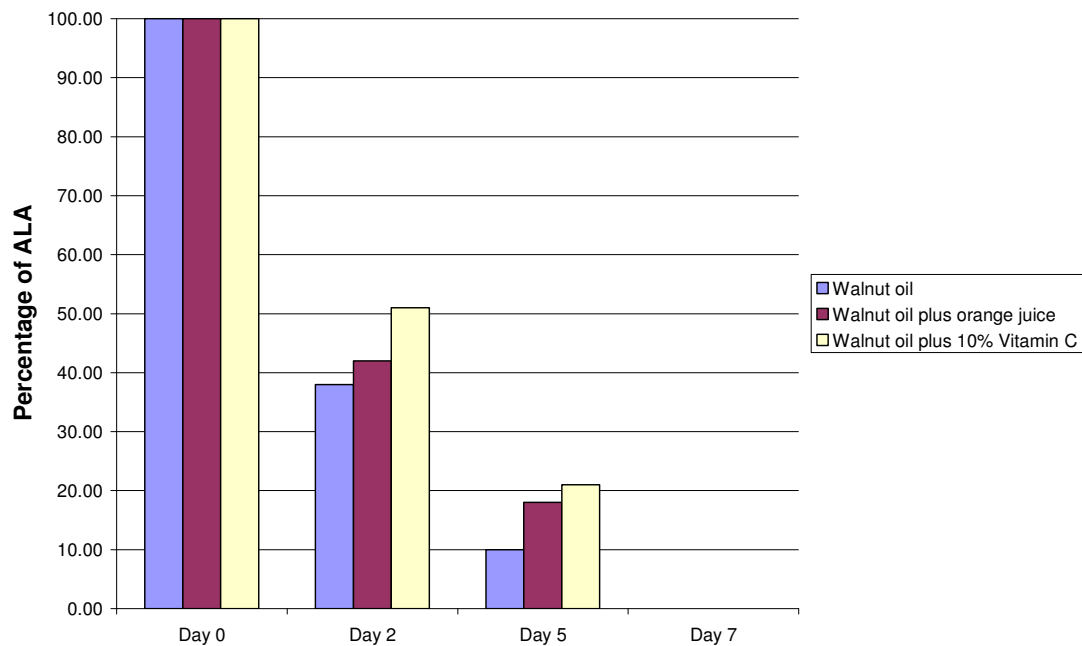


Figure 1. GC -MS analysis of ALA concentration over time. A) Pure ALA. B) ALA extracted from Linseed oil. C) ALA extracted from walnut oil.

From these results it was clear that ALA from linseed oil had a greater shelf-life than ALA from walnut oil. Also the addition of vitamin C and orange juice had a protective effect on the degradation of ALA, possibly through the prevention of oxidation.

Effects of Pasteurisation on ALA concentration.

To determine the effect of pasteurisation on the shelf life of ALA, experiments were devised to mimic a simple pasteurisation process. extracted linseed oil was added to orange juice and vitamin C solution and percentage ALA monitored over time.



As shown in figure 2 pasteurisation had an immediate effect on the levels of ALA present in the artificial juice product, with a decrease of approximately 60%. Levels of ALA were undetectable after day 2 in pasteurised samples.

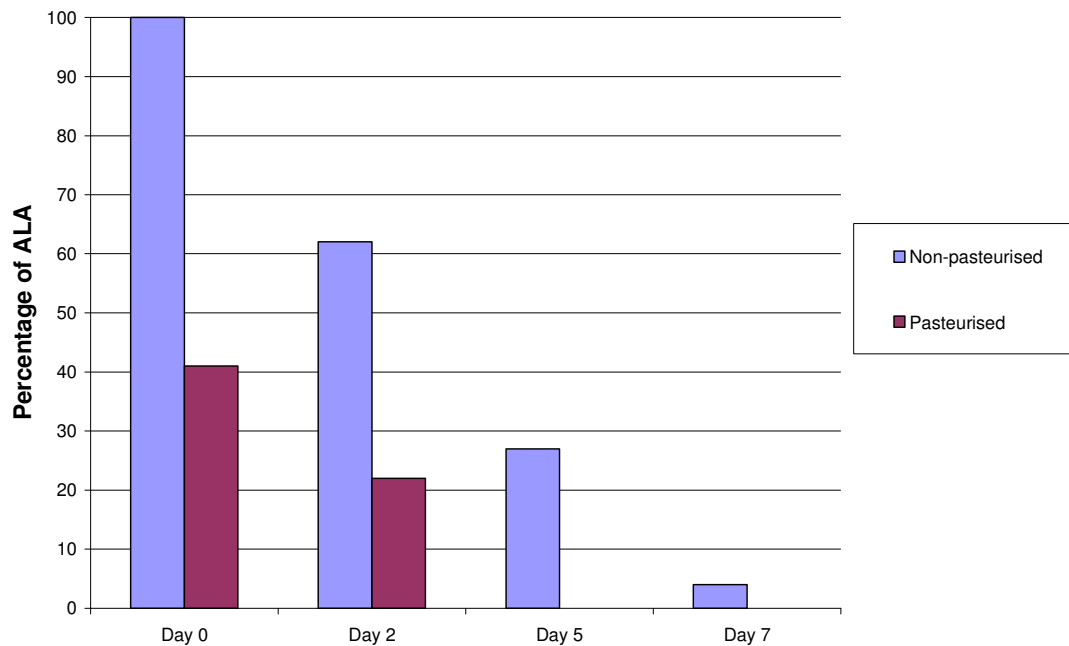


Figure 2. Effect on pasteurisation on ALA levels in vitamin C enriched orange juice.

Conclusions

- We have successfully developed a method for accurately monitoring the concentration of ALA in extracted oils using GC-MS. This method can also be used to give a fatty acid profile for both linseed and walnut oils using mass spectra analysis.
- ALA was successfully extracted from the total oil content of both walnuts and linseeds and used to enrich an orange juice product.
- Levels of ALA decreased over time in the orange juice products, with the addition of vitamin C solution lowering this rate of decrease, possibly due to anti-oxidant effects. ALA extracted from linseeds had a lower decline rate than ALA extracted from walnuts
- Pasteurisation had a detrimental effect on the levels of ALA in vitamin C enriched orange juice, indicating that ALA from linseed oil is unstable, even at relatively low temperatures (80 °C). This reduction would obviously need to be taken into account when producing any product containing ALA (Omega-3). Possible solutions include adding the extracted oil to the fruit products after pasteurisation, although obviously this would need to be carried out aseptically to reduce the possibility of introducing bacterial contaminants. An alternative method would be to produce a non-pasteurised omega-3 containing product, although this would obviously reduce the products shelf life. However results have indicated



that any omega-3 containing product should be consumed within 7 days, to retain any health benefits from the product.



