

Appendix 5

ORGANOLEPTIC, PHYSICAL AND CHEMICAL STABILITY OF DHA-RICH OIL FROM *SCHIZOCHYTRIUM* SP. MICROALGAE (DHA-S OIL) IN WHITE BREAD

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SUMMARY

This study was designed to show the stability of DHA-rich oil from *Schizochytrium* sp. microalgae (referred to herein as DHA-S) in fortified white bread compared to control bread produced with vegetable shortening. The DHA-S oil replaced part of the vegetable shortening commonly used in table bread formulations in order to obtain 32 g slices of bread fortified with 50 mg DHA (approximately 150 mg DHA per 100 g serving of bread). Commercial loaf breads were produced following the sponge dough procedure with the aim of obtaining samples for organoleptic evaluation, chemical analyses, crumb colour and texture analyses for 14 days storage at room temperature, the typical commercial shelf-life of table bread. Bread produced using DHA-S oil resulted in a 32 g slice with 50 mg DHA and had almost identical properties as the control bread produced using vegetable shortening. Addition of DHA-S oil did not cause any significant difference in trans-fat or sterol content compared to control. Addition of DHA-S oil did not significantly affect optimum water absorption but reduced mix time between 10–15%. All breads changed in texture as expected, throughout 14 days storage, the major changes occurred after 3 days.

INTRODUCTION

This study was designed to show the stability of DHA-rich oil from *Schizochytrium* sp. microalgae (DHA-S oil) in fortified white bread as compared to control bread produced with vegetable shortening under commercial conditions.

The DHA-S oil replaced part of the vegetable shortening commonly used in table bread formulations in order to obtain 32 g slices of bread fortified with 50 mg DHA (approximately 150 mg DHA per 100 g serving of bread). Commercial loaf breads were produced following the sponge dough procedure with the aim of obtaining samples for organoleptic evaluation, chemical analyses, crumb colour and texture analyses for 14 days storage at room temperature, the typical commercial shelf-life of table bread.

In addition, this work sought to show that reaction products, identified by various competent authorities during the Novel Food Ingredient approval process, were not produced to a significant degree during the bread making process. Sterols, whilst recognized to be insignificant in terms of respective maximal intakes of DHA-rich oil, (resulting in no more than 25 mg of all sterols per portion) are also extremely difficult to analyse. Consequently, total conventional sterols were analysed to demonstrate general oxidative stability. Bread making itself does not involve the high-temperature frying conditions usually associated with oxysterol formation.

EXPERIMENTAL

Baking Procedure

A Straight Dough Baking Procedure was utilized to evaluate and subsequently optimize ingredients in bread making following Method 10-10B from the American Association of Cereal Chemists (AACC, 2000). The method was devised for evaluating bread-wheat quality and a variety of dough ingredients were utilized for evaluation of DHA-S oil in the bread making process. Commercial bread flour with 10.7% protein (N x 5.7) and 0.4 % ash, from commercial sources was utilized for all of the bread making trials.

Following ingredient evaluation and optimization using Straight Dough Baking Procedure (AACC Method 10-10B, 2000), a Sponge Dough Baking Procedure (AACC Method 10-11, 2000) was applied to obtain commercial loaves for organoleptic, chemical and physical analyses throughout 14 days storage at room temperature. Breads were weighed and allowed to cool down for 45 min at room temperature before packaging in sealed plastic bags.

Organoleptic Analysis

Untrained panellists (25 to 30 individuals) evaluated the colour, flavour, texture and overall acceptability of the control bread and the DHA-S oil bread containing 50 mg DHA per slice during the 14 days storage. Breads were evaluated using a hedonic scale of 4 to -4 (4 = like extremely, 0 neither like nor dislike; -4 dislike extremely). Panellists evaluated breads in individual booths in a Sensory Evaluation Laboratory at day 1, 3, 5, 10 and 14. Before evaluation, each slice of bread was cut into four pieces. Panellists were instructed to rinse their mouth with water after each bread sample evaluation.

Physical Analysis

Texture

Determination of bread firmness throughout 14 days storage at room temperature was objectively evaluated according to AACC method 74-09 (2000) with a texture analyser (model TA.XT2, Texture Technology Corp., Scarsdale, NY) equipped with a cylindrical probe.

Colour

Crumb colour was determined with a Minolta Colour Meter (Model CR-300, Minolta Camera Co., Ltd. Osaka, Japan). L, a, b were obtained and colour index E determined by the following equation $E = (L^2 + a^2 + b^2)^{1/2}$

Chemical Analysis

Fatty Acid Profile Determination

Sixteen slices of bread from sixteen separate loaves were analyzed for fatty acid profile including DHA, EPA and DPAn-6. Aliquots of the bread were lyophilised and composite dried samples prepared. Fat in aliquots of the dried bread composites was transesterified *in situ* with 1.5 N HCl in methanol, in the presence of toluene and an internal standard (methyl tricosonate). The resultant fatty acid methyl esters (FAME's) were extracted with toluene. The FAME's were separated, identified, and quantitated by gas-liquid chromatography (HP 5890 Series II, Agilent Technologies) equipped with a flame ionization detector (GLC-FID). Chromatography was carried out using a capillary column (SP-2380 0.32 mm x 30 m, Supelco Co., Inc., Bellefonte PA). FAME's in the extracts were separated, identified, and quantified by GLC-FID using a split injection (20:1 split). A three-point internal standard calibration curve was used for quantification using commercially available reference standards (NuCheck Prep, Elysian, MN).

Sterols Analysis

Oil was extracted from the bread samples using a chloroform/methanol extraction method. The extracted oil was then subjected to sterol analysis using ISO 122228: 1999.

Trans Fatty Acids

An aliquot of the extracted oil was also used to determine trans-fatty acids (18:1 and 18:2) by GLC using AOCS Method Ce 1f-96.

Statistical Analysis

Data were subjected to statistical analysis using SAS (SAS Institute Inc. 2001). Differences among means were compared using LSD at a level of significance of 0.05.

RESULTS AND DISCUSSION

Organoleptic Analysis

The results of organoleptic analysis are presented in Table 1. There were no significant differences between DHA-S oil fortified bread and control bread for any of the measured organoleptic properties.

Physical Analysis

Colour

Addition of DHA-S oil did not significantly affect lightness (L), a, b and colour indexes (E) determined at the centre portion of the bread crumb. The colour remained the same throughout 14 days storage (Table 2).

Texture

Bread crumb firmness progressively increased during storage. The major change in firmness occurred after the third day of storage at room temperature. The 14 day old storage had firmness values at least three times greater and was higher than the average firmness after one day storage. This important difference throughout the 14 days is attributed to bread staling, Table 3.

Chemical Analysis

Fatty Acid Profile

The fatty acid profile of the bread produced using DHA-S oil is shown in Table 4. The DHA-S oil bread contained approximately 50 mg DHA per 32 g serving (approximately 150 mg DHA per 100 g bread) as confirmed using gas chromatography. In addition to the designed increase in DHA in the DHA-S bread, docosapentaenoate n-6 (DPA n-6) also increased as expected. With the exception of the increase in DHA and DPA n-6, there were no other significant differences in the fatty acid profile of fortified vs. control bread. Some minor changes in fatty acid profile were noted in the fatty acid profile of the DHA-S oil bread when compared to the vegetable shortening control bread (e.g., palmitate, stearate, oleate); however these changes were attributed to replacing vegetable shortening with DHA-S oil.

Trans-Fatty Acid and Sterols

DHA-S oil contains no detectable trans-fatty acids; therefore, no additional trans-fatty acids resulting from the use of DHA-S oil in fortified bread were anticipated. Trans-fatty acid analysis from DHA-S fortified bread and control bread were determined by the Mylnefield Laboratories, Scottish Crop Research Institute (under the supervision of Dr. William Christie). Total trans-fatty acids (C18:1 and C18:2) were significantly higher in the control bread (175.2 mg/ 100 g serving) compared to the DHA-S oil bread (144.3 mg/ 100 g serving). Trans-fatty

acids were evident in both DHA-S and control breads due to the fact that partially hydrogenated vegetable shortening was used in the formulation. More trans-fatty acids in the control bread were also expected due to the fact that some of the partially hydrogenated vegetable shortening used in the control bread formulation was replaced by DHA-S oil in the fortified bread.

Comparison of total fat, total trans-fatty acid content and total sterols of control and DHA fortified bread are depicted in Fig 1. Control and DHA-S oil bread showed non-significant differences between total oil and total sterols, and as previously mentioned, total trans-fatty acids were significantly higher in the control bread since some of the partially hydrogenated shortening used in the control was substituted with DHA oil in the DHA fortified bread.

REFERENCES

American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th Edition, St. Paul, MN, USA

Table 1. Effect on organoleptic properties of table breads (control bread and DHA-S oil bread containing 50 mg DHA) stored for 14 days.

Days of Storage	Colour				Flavour				Texture				Overall Acceptability			
	1	3	6	13	1	3	6	13	1	3	6	13	1	3	6	13
Organoleptic Scores Hedonic scale of 4 to -4 (4 = like extremely, 0 neither like nor dislike; -4 dislike extremely)																
Control	2.56 ab	3.16 a	2.16 b	2.52 b	2.76 a	3.24 a	2.84 b	2.64 b	2.80 b	3.20 ab	2.92 a	2.40 b	2.80 ab	3.08 a	2.72 b	2.36 b
DHA-S 50 mg DHA per 32g serving	2.56 ab	3.00 a	2.40 b	3.16 b	3.64 a	3.44 a	3.28 ab	3.24 b	2.80 b	2.72 b	3.28 a	3.12 b	3.28 ab	3.04 a	2.92 ab	2.96 b

Different letters mean significant differences (p<0.05)

Table 2. Effect on crumb colour scores of table breads, (control bread and bread containing 50 mg DHA) stored for 14 days.

Treatment	Colour	Day 1	Day 3	Day 4	Day 10	Day 14
Control	Lightness	67.86 a	70.89 a	68.16 a	70.93 a	69.60 a
	A	-2.42 ab	-2.64 bc	-2.45 a	-2.46 b	-0.84 c
	B	12.82 ab	12.90 c	12.93 b	13.92 cd	14.32 abc
	Colour Index	69.11 a	72.11 a	69.43 a	72.33 ab	71.07 a
DHA-S Oil	Lightness	65.97 a	68.24 ab	59.32 a	69.85 ab	68.01 a
	A	-2.55 b	-2.65 bc	-1.89 a	-2.39 b	-0.84 c
	B	13.23 ab	12.76 c	13.77 ab	13.55 d	13.30 c
	Colour Index	67.33 a	69.47 ab	61.05 a	71.19 ab	69.33 a

Different letters mean significant differences ($p < 0.05$)

Table 3. Effect on crumb texture analysis of table breads (control bread and bread containing 50 mg DHA) stored for 14 days

Treatment	Day 1	Day 3	Day 4	Day 10	Day 14
<i>50 mg DHA/omega 3</i>					
Control	180.8 ab	244.4 a	477.4 b	490.6 a	435.9 a
DHA-S Oil	176.6 ab	228.8 a	461.9 b	434.4 a	396.1a

Different letters mean significant differences ($p < 0.05$)

Table 4. Fatty acid profile of DHA-S oil enriched bread and control bread (expressed per serving of 32 g) after 14 days storage.

	Control Bread	DHA-S Bread
Fatty Acid (mg/serving)		
50 mg/serving		
Dodecanoate	19	21
Myristate	25	30
Myristoleate	6	5
Palmitate	162	153
Palmitoleate	24	20
Stearate	104	82
Oleate	167	138
Linoleate	138	136
Arachidate	3	2
Linolenate	16	14
Eicosenoate-11	4	3
Eicosadienoate-11,14	0	0
Behenate	0	0
Eicosatrienoate-11,14,17	0	2
Arachidonate	0	0
Erucate	0	0
Eicosapentaenoate EPA	0	4
Lignocerate	1	2
Nervonate	0	0
Docosapentaenoate DPA N-6	0	20
Docosapentaenoate N-3	0	0
Docosahexaenoate DHA	0	51
Omega 6	138	156
Omega 3	16	71
Saturated	313	290
Monounsaturated	201	166
Polyunsaturated	154	227

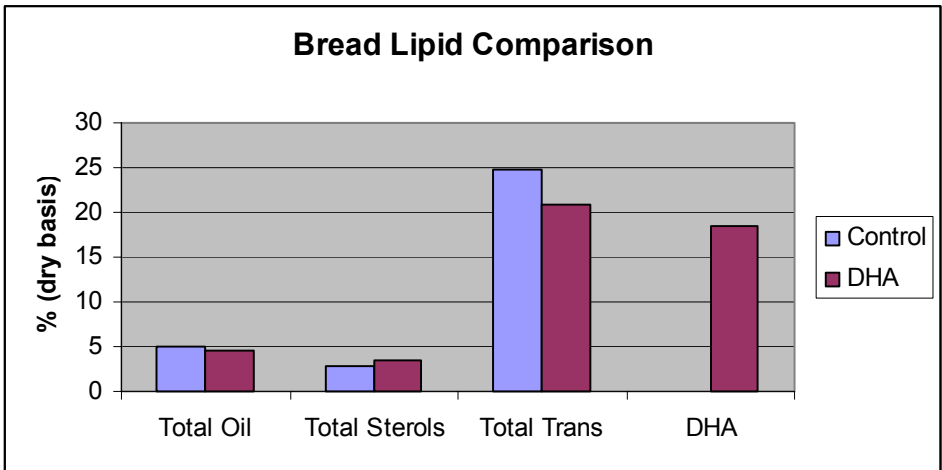


Fig 1. Lipid composition of DHA and Control breads.