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Application for Approval of Whole Chia (*Salvia hispanica L*) Seed and Ground Whole Chia as Novel Food Ingredients

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Introduction

Approval is sought under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27th January 1997 concerning novel foods and novel food ingredients for whole Chia (*Salvia hispanica L*) seed and ground whole Chia. The Regulation defines a novel food as a food which has no significant history of human consumption within the Community and which fall under the following categories… (e) "foods and food ingredients consisting of or isolated from plants and food ingredients isolated form animals, except for foods and food ingredients obtained by traditional propagation or breeding practices and having a history of safe use". Chia seeds have historically been consumed in South America but have not hitherto been used for human consumption to a significant degree within the European Community and so approval, as a novel food ingredient is required.

Chia seeds are an abundant source of the n-3 polyunsaturated fatty acid, α-linolenic acid. Alpha-linolenic acid is an essential fatty acid, which, through successive metabolic desaturation and elongation reactions gives rise to DHA and EPA. EPA and DHA are now widely recognised as having an important role in the prevention of coronary heart disease. Introduction of whole Chia seed and whole ground Chia, as a terrestrial source of α-linolenic acid, to the European diet in soft grain bread would increase the number of possible sources of n-3 polyunsaturated fatty acids available to consumers.

Chia seeds and ground whole Chia may be classified, for means of nutritional and safety evaluation, as complex novel food from a non-GM source (2.2) and consequently this dossier has been prepared in accordance with current guidelines for this category. Relevant requirements for submission to this class are as follows:

I	Specification of the Novel Food
II	Effect of the production process applied to the Novel Food
III	History of the organism used as the source of the Novel Food
IX	Anticipated intake/extent of use of the Novel Food
XI	Nutritional information on the Novel Food
XII	Microbiological information on the Novel Food
XIII	Toxicological information on the Novel Food

I. Specification of the Novel Food

Note: Certificates of analysis are included in Appendix 1

Technical information

Chia (*Salvia hispanica L.*) is a summer annual herbaceous plant belonging to the Labiatae family. Chia was a major food crop of the Aztecs and was grown in mountainous areas extending from west Central Mexico to Northern Guatemala.

Chia seeds are grown contractually on behalf of R Craig & Sons [M] Ltd in Argentina and Peru owing to the fact that sub-tropical conditions are required for growth. To date, 4 consignments of Chia seed have been received from Peru, where the crop is being commercially grown. These batches have been analyzed for proximate analysis, fatty acid profile and heavy metals. Further analyses, namely mineral and carbohydrate analysis were carried out at source and are presented below in support of our application. Certificates of analysis are included as Appendix 1.

Proximate analysis

Dry matter, protein, oil, crude fibre and ash were measured using standardized methods (Table 1.1). Proximate analysis was carried out at the Department of Agriculture and Rural Affairs, Northern Ireland (formerly the Department of Agriculture for Northern Ireland).

Table I.1. Proximate analysis of Chia seed (Mean values from 4 consignments received).

Nutrient	Mean (g/kg)	Range (g/kg)
Dry matter	922.05	917.29-926.96
Protein	211.00	208.20-213.40
Oil	322.50	314.78-325.82
Crude Fibre	277.73	249.26-300.40
Ash	48.13	45.99-49.82

Fatty Acid Profile

Chia seeds have a high oil content as can be seen from Table 1. Of the 322.50 g/kg of oil present in Chia, 58.7% is α-linolenic acid, making it the best cereal source of omega-3 fatty acids known to man. A full fatty acid profile of Chia seeds is presented in Table I.2.

Fatty acid profiling was carried out by Eclipse Scientific Group, a GLP and UKAS accredited laboratory using a standard method (SOP No AM/C/102).

Table I.2. Fatty acid profile of Chia seed (Mean values from 4 consignments received).

Fatty Acid		**% fatty acids determined**	**Range**
Myristic Acid	C14:0	0.1	0.1-0.1
Palmitic Acid	C16:0	6.7	6.6-6.7
Palmitoleic Acid	C16:1	0.1	0.1-0.1
Heptadecanoic Acid	C17:0	0.2	0.2-0.2
Heptadecenoic Acid	C17:1	0.1	0.1-0.1
Stearic Acid	C18:0	3.0	2.8-3.1
Oleic Acid	C18:1	6.9	6.6-7.0
Linoleic Acid	C18:2	18.8	18.6-18.9
Linolenic Acid	C18:3(ω-3)	58.7	58.2-59.1
Linolenic Acid	C18:3(ω-6)	0.1	0.0-0.1
Arachidic Acid	C20:0	0.3	0.3-0.3
Gadoleic Acid	C20:1	0.1	0.1-0.1
Eicosadienoic Acid	C20:2	0.1	0.1-0.1
Eicosatrienoic Acid	C20:3(ω-3)	0.1	0.1-0.1
Behenic Acid	C22:0	0.1	0.1-0.1
Docosatetraenoic Acid	C22:4	0.1	0.1-0.1
Lignoceric Acid	C24	0.2	0.2-0.2

Element Analysis

Mineral analyses were carried out by Food Control SA, Buenes Aires, Argentina and are presented below in Table I.3. Food Control SA, are members of the Union of International Independent Laboratories and are also approved by the UK Grain and Feed Trade Association (GAFTA) to issue certificates of analysis for feed ingredients.

Table I.3. Mineral Analysis

		Mean (mg/100g seed)
Sodium	Na	12.15
Potassium	K	809.15
Calcium	Ca	679.80
Iron	Fe	9.90
Magnesium	Mg	380.00
Phosphorus	P	780.00
Zinc	Zn	4.40
Copper	Cu	1.70
Selenium	Se	1.00
Chromium	Cr	0.50
Cobalt	Co	0.25
Mercury	Hg	0.01
Molybdenum	Mo	0.25
Nickel	Ni	0.25
Sulphur	S	290

With regard to the presence of potentially undesirable constituents in Chia, the seed has been analyzed for heavy metal content. The results of this analysis are presented in Table I.4 and show that heavy metals are present only at very low levels in Chia seeds. Heavy Metal analysis was carried out at the Department of Agriculture and Rural Affairs, Northern Ireland (formerly the Department of Agriculture for Northern Ireland).

Table I.4. Heavy Metal Analysis

Heavy Metal	**Ppm**
Arsenic	0.102
Cadmium	0.018
Mercury	<0.010
Lead	~0.025*

*Value only approximated as 2 samples with values less than 0.020 ppm

Carbohydrate Analysis

Determination of the make-up of the fibre fraction was carried out by Food Control SA, Buenes Aires, Argentina.

Table I.5. Carbohydrate Analysis

Fraction	
Carbohydrate	37.45
Fibre	33.91
Soluble	3.07
Insoluble	30.43

Vitamin Analysis

Determination of vitamin content of the Novel Food was carried out by Food Control SA, Buenes Aires, Argentina.

Table I.6. Vitamin Analysis

Vitamin	**Mean (mg/100g seed)**
Vitamin A	-
Vitamin C	5.4
Vitamin E	-
Thiamine	0.7
Riboflavin	0.2
Niacin	7.2
Vitamin B6	0.1
Vitamin B12	-

II. Effect of the Production Process Applied to the Novel Food

Whole Chia seeds are not processed in any way prior to their use as a food ingredient. Current agronomic practice regarding Chia seed production is detailed below, it should be noted however, that the seeds are grown contractually for R Craig & Sons [M] Ltd who have the right to specify what herbicide/pesticide treatments are used in order to comply fully with EU legislation.

The Chia seed is sown mechanically at a seeding rate of three to five kg/hectare. The seed is not treated chemically in any way prior to sowing, but a herbicide called Trifluralin (*2,6-Dinitro-N, N-dipropyl 4-(trifluoromethyl) benzeamine; α, α, α-trifluor-2,6-dinitro-N N-dipropyl-p-toluidine*) may be applied to the ground prior to sowing at a rate of two litres/hectare. No insecticide is applied. At sowing, the fertilizer consisting of diamonic phosphate is applied mechanically, localized in rows. Between thirty and forty five days following sowing, 150 kg/hectare of urea is applied, also mechanically and localized in rows.

The crop is allowed to ripen naturally. However, should there be a requirement to speed up the ripening process, Paraquat is used at a rate of one litre/hectare. Yield can vary between growing locations but is generally within the range 500 to 800 kg/hectare. The seed is mechanically harvested using a modified grain combine harvester.

A multi-residue screen for pesticide and herbicide residues was carried out on a composite sample form the four consignments of Chia seed received to date by R Craig & Sons [M] Ltd. No compounds were detected in the screen test. For full laboratory report, refer Appendix 2

Post-harvest, the seed is cleaned mechanically and not subjected to any chemical treatments. The seeds are stored in sacks within a fully enclosed warehouse facility in preparation for shipment.

In the production of whole ground Chia, the whole seeds are passed through a variable speed Christy Briton hammer mill. Manufactured by Christy Hunt Ltd, Foxhill Industrial Estate, Scunthorpe.

III. History of the organism used as a source of the Novel Food

Chia (*Salvia hispanica L.*) is a summer annual belonging to the mint family. Chia seeds were a core element of the diet of pre-Columbian civilizations, mainly the Aztecs. The species originated in mountainous areas extending from West Central Mexico to Northern Guatemala. These civilizations used this species as a raw material in making several medicinal and nutritional compounds, and even paints. These ancient peoples were obviously aware of the beneficial effects of Chia seeds, as its name comes from the Mayan word meaning 'something that makes you strong'.

Chia was one of the main crops of the pre-Columbian societies of the region, and was surpassed only by corn and beans in terms of significance in the diet. Historically, Chia seeds were roasted and ground to form a meal called 'pinole', then mixed with water to form a porridge or made into cakes. The Aztecs viewed Chia as such an important grain that it was offered to their gods during religious ceremonies. Although grown only on a very small scale, and with rudimentary technological methods, Mexican Indian descendants are still producing this ancient grain. Although not the anticipated use of the Novel Food , it is interesting to note that Chia is still used in the preparation of a popular beverage called "chia fresca", where the seeds are soaked in water and then flavoured with fruit juice and consumed as a cooling drink.

Although Chia was an important grain during the pre-Columbian age, its' cultivation decreased following the discovery of America and subsequent Christianisation. Prior to re-discovery, the growth of Chia was limited to a few hectares. Moreover, there were minimal possibilities of increasing production due to social and political factors which have resulted in the subdivision of the land into small farms, and which have brought about the corn culture, basically for preparing the popular Mexican "tortillas".

Modern science has been able to explain why ancient meso-American civilisations considered Chia a basic component of their diet and why it should be re-introduced into modern society. As a result of the lack of a reliable source of Chia, a South American company has undertaken an extensive research and development program on the seed. This has included determination of new production areas and methods

together with modern products and practices, all aimed at bringing Chia to the market as a ‘new product’. What did not take place was any genetic modification of the species; all development was targeted at improving production of the existing seed. Today Chia is grown contractually for R Craig & Sons [M] Ltd in Argentina and Peru. Being able to produce this crop in two different and distant areas is something that is unusual for a new crop, but it is a precaution that decreases climatic and political risks, and avoids concentrating the delivery season.

IX. Anticipated intake / extent of use of the Novel Food

Nutritionists now recommend that not only should the number of calories that are obtained from fat in our diet be decreased, but that we increase out intake of n-3 fatty acids. The Report of the British Nutrition Foundation (1992) advised that fat no longer be analysed simply in relation to the proportion of saturated and polyunsaturated fats, but instead the ratio of n-6:n-3 fatty acids. Currently the average diet has a n-6:n-3 ratio of 10:1 and it is recommended that this be reduced to 6:1.

There are now recommendations from several international health organisations for daily intakes of n-3 fatty acids. The British Nutrition Foundation recommends that 1.0% of our daily energy intake be in the form of Linolenic acid (C18:3, n-3). This equates to a recommended daily intake for Linolenic acid of approximately 2.5g/day.

The proposed use of Chia, is to include the whole and ground seed as ingredients in multi grain-style bread. The National Diet and Nutrition Survey of Adults Aged 19-64 years (2002) provided a breakdown of the average bread consumption by adults.

Table IX.1 Bread Consumption Figures (g/person/day) - All men and women

	95%ile	**97.5%ile**	**Average consumers**	**% of consumers**	**Average population**
Bread - White	176.6	210.7	72.9	90.6	66.1
Bread – Wholemeal	116.6	142.2	42.9	36.3	15.6
Soft Grain Bread	166.4	231.3	42.5	2.5	1.1
Other Bread	99.6	125.6	32.0	51.7	18.1
Total Bread	**204.9**	**232.5**	**102.3**	**98.5**	**100.9**

Table IX.2 Bread Consumption Figures (g/person/day) - All men

	95%ile	97.5%ile	Average consumers	% of consumers	Average population
Bread – White	199.8	231.0	89.0	92.7	50.6
Bread – Wholemeal	142.4	167.2	54.4	33.3	13.2
Soft Grain Bread	223.3	258.4	59.2	3.0	0.4
Other Bread	109.2	139.0	38.8	50.7	16.7
Total Bread	**227.9**	**259.3**	**123.6**	**99.0**	**80.9**

Table IX.3 Bread Consumption Figures (g/person/day) - All women

	95%ile	97.5%ile	Average consumers	% of consumers	Average population
Bread - White	12.70	137.9	57.1	88.6	50.6
Bread – Wholemeal	82.3	99.0	33.7	39.0	13.2
Soft Grain Bread	66.2	72.3	20.9	2.1	0.4
Other Bread	91.8	108.6	31.6	52.6	16.7
Total Bread	**158.6**	**178.4**	**82.3**	**98.2**	**80.9**

While bread consumption figures have been provided for all types of bread in Tables IX.1 to IX.3, the Novel Food shall only be included in soft grain bread. Using both whole Chia seeds and ground whole Chia, i.e. using all husk and kernel, excludes it from use in wholemeal and white breads.

Pilot studies have determined that the level of Chia seeds and whole ground Chia included in the soft grain bread mix shall be 5.0%. On this basis, daily Chia consumption figures are detailed in Table IX. 4

Table IX.4 Daily Chia consumption figures (g/person/day)

	95%ile	97.5%ile	Average
Soft Grain Bread			
All men and women	8.32	11.6	2.1
All men	11.1	12.9	3.0
All women	3.31	3.6	1.0

From these consumption figures it may be seen that mean intake of Chia is anticipated to be 2.1g/person/day. This figure is well within the level tested in the adverse effects study [Refer Section XIII].

XI. *Nutritional information on Chia*

There is an increasing consumer demand for low fat, low cholesterol products and as a result much attention has been focused on manipulating the fatty acid profile of existing foods. For example, manipulation of the lipid composition of eggs has become the focus of much recent research.

Dietary lipids are necessary as a source of energy, for transport of fat soluble vitamins and as a source of certain essential fatty acids, mainly linoleic acid (C18:2 n-3) and α-linolenic acid (C18:3 n-3). Our bodies can convert α-linolenic acid into longer chain polyunsaturated fatty acids eicosapentanoic acid (EPA C20:5 n-3) and docosahexaenoic acid (DHA C22:6 n-3). EPA can influence the process of blood clotting, leading to decreased risk from cardiovascular disease. DHA may be even more potent than EPA and has been found to reduce platelet aggregability, platelet adhesiveness and blood triglyceride levels, thus reducing the risk of cardiovascular disease. Increasing the dietary availability of these health enhancing fatty acids through fatty acid modification of commonly consumed products has been encouraged by the medical profession. The Committee of Medical Aspects of Food Policy that reported in 1995 recommended that we double our dietary intake of long chain n-3 fatty acids.

The consumption of increased proportions of n-3 fatty acids in the diet may lead to health benefits to the general population. However, the sources of n-3 fatty acids in the Western diet are limited to oily fish, green vegetables and certain vegetable oils. There is, therefore, a need to increase both the quantity and range of foods that contain these fatty acids. Marine products are the main source of n-3 fatty acids, however, it is necessary to consume three portions of fatty fish per week in order obtain the recommended daily amount of n-3 fatty acids. There is also considerable variation in the oil content of different species of fish and a present concern over the presence of dioxin residues that have been detected in fish oil.

Modern science has determined that Chia seeds have an oil content of approximately 32 percent, 62 percent of which is α-linolenic acid, making this cereal the richest non-marine source of n-3 fatty acids currently known to man. Thus by including Chia in bread products at a level of 5 percent there is the potential to provide the recommended daily amount of n-3 fatty acids through a staple food.

Chia seeds contain 21 percent protein, a level markedly greater than other nutritional grains such as wheat (14%), corn (14%), rice (8.5%), oats (15.3%), barley (9.2%), and amaranth (14.8%). Unlike most other cereals Chia seeds are not limiting in any amino acids necessary in the adult diet. Other grains are limiting in terms of two or more amino acids and in order to compensate for this, must be mixed in order to satisfy human needs [Refer Table 7.].

Chia seeds are also a rich source of vitamins B, calcium, phosphorous, potassium, zinc, and copper.

Due to the highly unsaturated nature of the oil, Chia seeds contain natural antioxidants to protect against lipid oxidation, which leads to off-flavours and rancidity. Water and methanol extracts of degreased Chia seeds have demonstrated a strong antioxidizing activity, with the most important antioxidants obtained being chlorogenic acid, caffeic acid and flavanol glycosides. Since oxidation is delayed, Chia shows a great potential within the food industry compared to other α-linolenic acid sources such as flax, which exhibit rapid decomposition due to a lack of natural antioxidants. Flavour quality and product stability are issues that greatly influence potential acceptability of products enriched with omega-3 fatty acids making this a key property of Chia.

Once the oil has been extracted from the seed, the material that remains contains 50-60 percent fibre. The seed alone possesses 5 percent soluble fibre, which appears as mucilage when it is wetted.

Table 7. Amino acid composition of Chia seeds

Amino Acid	**% of total protein**
Aspartic Acid	9.47
Threonine	4.25
Serine	6.02
Glutamic Acid	15.37
Glycine	5.23
Alanine	5.34
Valine	6.32
Cystine	1.82
Methionine	0.45
Isoleucine	3.98
Leucine	7.30
Tyrosine	3.41
Phenylalanine	5.86
Lysine	5.50
Histidine	3.19
Argenine	11.03
Proline	5.45

XII. Microbiological Information

Chia seeds from four consignments received were tested for pathogenic microorganisms and also total viable counts at Anser Laboratories, Northern Ireland. Anser Laboratories are approved by both the Department of Agriculture and Rural Development NI and the Irish Department of Agriculture to carry out such analysis. UKAS approval is pending.

There were no pathogenic organisms detected and the total viable counts were low for the Novel Food [Refer Appendix 3 for certificate of analysis].

Cytotoxicity

The bioassay carried out was based on the cytotoxic effects of mycotoxin on mammalian cells in cell culture analyzed by the method of Sanders, 1984. The BHK – 21 (C –13) Cell Assay was carried out on the Chia seeds at the Agriculture and Food Science Centre (Department of Agriculture and Rural Development, Northern Ireland). This involves preparing an extract from the seeds and examining the growth of baby hamster kidney cells when subjected to the seed extract. When a toxic compound is present, the cells are either killed or their metabolic activity reduced. The results from this assay showed that no substances inhibitory to BHK – 21(C – 13) were detected. From this work it was concluded that the Novel Food is therefore unlikely to contain Tricothernes (limit of detection via this method for T2 toxin is 4μg/kg).

Certificate of Analysis – Appendix 4

XIII. Toxicological Information

The safety of the Novel Food as a food ingredient has been established and confirmed through various studies as outlined below. It is widely recognized that there are practical problems associated with obtaining meaningful information from animal-based toxicology studies on the safety of whole foods, such as Chia. There are difficulties in relating any adverse effects that may be observed by feeding a complex food ingredient such as Chia at high levels to animals to the food itself and not other factors. For this reason the main toxicological and adverse effects studies establishing the safety of the Novel Food were carried out on human subjects.

Toxicity

Mycotoxin Screening

A composite sample from the four consignments of Chia seed received by R Craig & Sons [M] Ltd was tested for Aflatoxin B1, B2, G1 and G2, Ochratoxin, Zearalenone, DON (vomitoxin) and T2 toxin. None of the mycotoxins were detected. [Appendix 5]

Allergenicity

An extensive allergenicity study was carried out on the Novel Food at BIBRA, International Ltd., Surrey and King College, London [Appendix 6]. The allergenicity studies investigated Novel Food extract for the presence of potential cross reactive food allergens and consisted of four pieces of work, namely

i) An initial IgE binding screen using sera tested against a panel of 30 allergens by MAST and selected on the basis of their reactivity to peanut.
ii) IgE binding analysis using sera from double blind placebo food challenge individuals.
iii) Analysis of the effects of peptic digestion on IgE binding.
iv) Skin prick tests carried out in order to determine if the IgE binding observed was clinically significant.

The proteins present within the Chia extracts ranged from between 6 and 65 kD in size. The majority of Chia proteins were found to be susceptible to proteolytic digestion.

A total of 26 sera from food allergic individuals were used, covering a range of food plant sensitivities. However, given that the Novel Food is a seed, it was considered that the most appropriate approach would be to concentrate on peanut and tree nut sensitivities, which are an important group of allergens in the UK and the most likely source of cross-reactivity. Immunoblotting using sera from peanut and tree nut sensitive individuals showed binding of IgE to various Chia proteins, the majority of which were non-specific in nature.

Skin prick tests of 12 peanut and tree nut sensitive individuals revealed a low-level response in 2 individuals who were at the most broadly sensitive end of the spectrum of sensitivities. Examination of the SPT positive sera showed moderate IgE binding to a 15 kD protein. Binding of IgE to this protein band could not be excluded as non-specific and may represent a cross-reacting protein. However, this protein was shown to be susceptible to peptic digestion and therefore probably does not represent a food allergy hazard.

Adverse Effects Study

The effects of dietary intervention with Chia on selected markers of coagulation and immune function in humans was examined in a large trial conducted at the School of Biomedical Sciences, University of Ulster, Coleraine. The study was a placebo controlled dietary supplementation trial carried out on 100 male and female subject's aged between 21 and 65 from differing socio-economic groups within the Coleraine area. A 4-week dietary intervention of the Novel Food was undertaken, with the subjects being allocated at random to one of the four intervention groups. The supplements were included in a breakfast cereal and Chia intake was 2.5, 5.0 or 10.0 g per day. The control group received 4.0 g of sunflower seeds per day. Blood samples were taken at baseline and 4 weeks and assessed for haematological parameters,

plasma lipid profiles and lymphocyte sub-set typing. In addition, full anthropometric data, a lifestyle and food questionnaire and a questionnaire monitoring any possible adverse effects of the Novel Food was administered to each subject.

Overall, the results of the blood analysis indicated that there were no significant health related effects associated with even consumption of high levels of the novel food. Analysis of the adverse effects questionnaire showed a significant effect of consumption of 5.0g per day of the novel food on tiredness and fatigue. However, since this was a single response and there was no dose related effect, it was considered an anomalous result. It may therefore be concluded from this extensive study that, intake of the Novel Food, even at levels much exceeding the anticipated daily intake, has no adverse effects on human health and well being.

The full report from this study is included as Appendix 7

Canadian Study

A human feeding trial was conducted by Whitham *et al.* at the Risk Factor Modification Centre, St Michael's Hospital and the Department of Nutritional Sciences, University of Toronto to evaluate the Novel Food as a dietary supplement with the potential to lower blood pressure in individuals with type-2 diabetes. This study investigated the effects of the Novel Food on measures of glycemic control and traditional and non-traditional risk factors of cardiovascular disease in subjects with type-2 diabetes.

A randomized, single blind crossover trial using 20 subjects with type-2 diabetes was carried out. Chia was consumed by each individual at a rate of 25g chia/1000 kcals for a period of 12 weeks. This level greatly exceeds the anticipated intake of the Novel Food [Ref: Section IX]. Fasting blood samples and blood pressure measurements were taken at 0 and 12 weeks.

Using the Novel Food as a dietary supplement significantly lowered systolic blood pressure compared to the control and coagulation factors were favorably altered. C-

reactive protein, a marker of inflammation was decreased through supplementation with Chia and there were no adverse effects on glycemic control noted even at these high dose rates. In addition there were no significant changes in bleeding time, liver function or kidney parameters. [Appendix 8]

Laying Hen Trials

Two laying hen dietary intervention studies were carried out at Queen's University, Belfast. The main objectives of the studies were to compare the effectiveness of Chia as a means of modifying egg yolk lipids compared to other terrestrial and marine sources of n-3 fatty acids. In addition to measuring the effects of the Novel Food on the fatty acid profile of egg yolk lipids, bird performance and egg acceptability were monitored.

Study 1

The main aim of Study 1 was to enhance the overall fatty acid composition of egg yolk lipids by modifying the hens diet. The experimental diets were formulated to be isoenergetic and were supplemented as follows:

Control	1.5% soya oil
Marine	1.5% fish oil
Chia	14.0% whole Chia seed.

32 point of lay hens were fed the experimental diets for a period of 8 weeks. Each hen was given 120g of feed per day, therefore those birds being provided with the Chia diet were consuming 16.8g of the Novel Food per day. Normal parameters such as egg production and daily food intake were measured and in addition, sensory analysis of eggs and fatty acid profiling of egg yolk lipids was carried out.

The results showed that inclusion of Chia seed at a rate of 14% in the diets of laying hens had the potential to increase the total n-3 fatty acid content to 16% and give a n-3:n-6 ratio of 0.99. Feeding Chia to laying hens had no adverse effects on egg production, egg yolk lipid oxidative stability, egg odour/flavour or egg quality and

acceptability. There was no adverse effects if feeding Chia to the hens and it was therefore concluded that Chia is a safe and effective means of enriching eggs with n-3 fatty acids.

The full report of this work is included as Appendix 9

Study 2

Study 2 was designed to compare three terrestrial sources of n-3 fatty acids for effectiveness in the manipulation of egg yolk fatty acids, namely; whole Chia seeds, whole linseeds and linseed oil. Chia was fed at a rate of 18.2 g/day (equivalent of 15% of the diet) to 32 hens for a period of 8 weeks.

This study clearly showed that Chia was more effective than either whole linseeds or linseed oil in enhancing egg yolk fatty acid composition. Results from sensory evaluation of the eggs showed that panellists could not detect any odour or flavour differences between eggs from birds fed the three different n-3 sources and there were also no significant differences between egg quality and overall acceptability. It was concluded from this trial that Chia may be used effectively in the production of 'healthier' eggs with no adverse effects on the birds.

Human evaluation of n-3 enriched eggs

A small-scale trial designed to evaluate the bioavailability of n-3 polyunsaturated fatty acids from laying hens fed a Chia-supplemented diet was carried out at the Northern Ireland Centre for Diet and Health, School of Biomedical Sciences, University of Ulster, Coleraine. The consumption of eggs enriched with n-3 fatty acids led to a modest increase in the percentage of fatty acids in the blood plasma of the supplemented individuals.

Broiler Trials

The effect of Chia seed on the fatty acid content of breast and thigh muscle of broilers and on sensory attributes was examined at Queen's University, Belfast. The experimental diets were formulated to contain 10% Chia and were fed to birds for a period of 28d. At the end of each week birds were weighed and feed intake was measured in order to assess performance. Breast and thigh meat was removed from each bird and used for fatty acid profiling and sensory evaluation.

There were no significant differences in performance observed between control birds and those on the Chia-supplemented diet as shown in Table XIII.1

Table XIII.1 Mean performance of birds

	Control	10% Chia	SEM
Bird weight 28d	1237	1203	29.2
Bird weight 35d	1710	1692	45.5
Feed Conversion Efficiency	1.32	1.33	0.013

During sensory evaluation of the muscle tissue, no significant differences were detected between the two treatments in terms of acceptability of appearance, texture, flavour, aftertaste or meat and skin odour.

Table XIII.2 illustrates how inclusion of 10% Chia in the diet of broilers changes the fatty acid profile of breast meat. Deposition of linolenic acid (C18:3, n-3) is markedly increased in the breast meat of birds fed a diet supplemented with the Novel Food. There were no adverse effects of feeding high levels of Chia for a prolonged period on broilers.

Table XIII.2 Mean fatty acid content of breast meat

	Control	10% Chia
C14:0	0.38	0.34
C16:0	20.91	18.82
C16:1	2.88	2.49
C18:0	8.30	7.64
C18:1	29.83	26.82
C18:2	29.69	29.75
C18:3	6.32	12.67
C22:5	0.42	0.35
C22:6	1.28	1.11

Additional Information

In order to maintain their commitment to the use of Chia as a Novel Food ingredient, an extensive research and development project has been undertaken by M/S Robert Craig & Sons (M) Ltd in association with:-

(a) Loughry College, Cookstown

(b) Queen's University, Belfast

(c) The University of Ulster, Coleraine

The Company – Salba Research and Development Ltd. – has been informed by the relevant Authorities in the USA and Canada that *Salvia hispanica can be used as a food, without prior regulatory notification or approval.* [copy correspondence – Appendix 10]

Bibliography – Appendix 11

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