

# SATIN 289800 Final Report

## Final Publishable Summary Report

### Executive Summary

The SATIN study was designed to facilitate exchange of experience and knowledge between industry and academia and inform the development of products that promote weight loss, healthier eating and reduction of weight-related disease. With 7 SMEs and 4 commercial partners we integrated and developed advanced technologies to process and screen novel food products with the aim of producing new high quality foods to help Europeans achieve a balanced diet.

In the first phase of the project we assessed and exploited ingredients and matrices to develop products with enhanced appetite suppression effected by targeting multiple mechanisms underpinning satiety. These were evaluated through *in vitro* and *in vivo* methodologies and produced for clinical studies. In WP1 we integrated advanced technologies to screen novel food structures through *in vitro* models to isolate and refine products according to their satiating potential. This yielded biologically relevant *in vitro* assays to generate a platform to enable the screening of a wide range of food ingredients and processing conditions before *in vivo* testing. Subsequent to optimization of the platform, 30 pure ingredients (reference compounds and ingredients under consideration for inclusion in food products prepared in WP2) and 6 food products (the lead candidate product from each producer in WP2 and identified for inclusion in WP4 studies) were examined. A comprehensive bibliographic search of scientific databases was also completed to identify plant extracts which have previously been identified as having satiating properties, whether at *in vitro*, *in vivo* or at human scale. Twenty-seven plant extracts or natural compounds were identified, some of which were included in prototypes developed in WP2.

In WP2 we developed novel food processing technologies that combine active ingredients and changed food structure to produce a range of novel satiety enhancing products, and having completed *in vitro* screening, produced 80 different prototypes that passed through safety analysis, early sensory evaluation and consumer testing. Flavour release and texture building, factors that impinge on satiation/early satiety, were then assessed in the 6 lead products. For the acute human intervention studies in WP4 the 6 selected foods/drinks were produced by WP2 partners despite some delays in production up-scaling. In WP3 we demonstrated the effects of prototype products on biomarkers of satiety and on nutrient bioavailability using *in vivo* studies and validating new *in vitro* approaches. Two studies were conducted using three diets enriched with three differing ingredients and the biological measures yielded were used to validate short-term batch and long-term simulation *in vitro* models. While no effect was seen for main objectives in *in vivo* study outcomes (appetite, body weight, key biomarkers) the ingredients had very distinct effects on gut microbiota which validated the *in vitro* models.

In the second phase we examined the effects of exposure to diets enriched with satiety-enhancing foods on appetite, energy intake, nutrient availability and body weight. In WP4 we demonstrated the effects of final food products on within-meal satiation, post-meal satiety and / or reduced appetite and biomarkers of satiety, and demonstrated the enduring effects of one individual food product on satiety and the potential to induce weight loss in a 12 week trial. Using the data from Phase 1, and published literature, 6 prototypes were selected and the methodological platform was adapted to their potential mechanisms of action and seven studies were conducted (six acute). Two products demonstrated appetite suppressing effects and one was included in a medium term (12 week) trial. In WP5, we aimed to examine the long-term consumer and health benefits of adhering to a diet containing existing satiety enhancing products. However, due to the paucity of effective products available within and outside SATIN, the study was redesigned to examine three products with prior proven effects on appetite to be tested at three clinical centres. In WP6 we looked to validate health claim endpoints and commercialize technologies (*in vitro* platform) and products yielded including a data collection app.

## Summary Description of Project Context and Objectives

### PROJECT CONTEXT

Despite considerable advances in the i) measurement of appetite expression and its biomarkers, and increased understanding of ii) the biological processes underpinning satiation and satiety, and iii) the impact of nutrient composition and the physical characteristics of food on eating behaviour, few satiety enhancing products have entered the European market. Traditional product development, reformulation, and incorporation of satiety enhancing ingredients have largely failed to convert this theoretical knowledge into products that are effective and appealing. This has driven the need to find more innovative ways of enhancing the satiating properties of food.

The aim of the SATIN project was to develop food products that regulate appetite by reducing hunger, accelerating within meal satiation and enhancing between meal satiety. The intention was to accomplish this by employing novel food processing methods to modify food structure to produce functional foods for weight management. SATIN's goal was to produce and help commercialise finished products whose biomarkers of appetite and nutrient bioavailability will be well characterised. A new collaboration between SMEs, Industry and Academic to optimise innovation and integrate advanced technologies was needed to achieve this. The project also examined whether a diet containing satiety enhancing products is a legitimate approach to weight management. Through the development of healthier foods, with added functional benefits, SATIN offered an important contribution to the reformulation of the diet of European consumers to promote health. SATIN also sought to educate consumers and other stakeholders about satiety to optimise the impact of outcomes. Furthermore, the project provided substantive support to SMEs.

### OVERALL OBJECTIVE

Ultimately, the objective of the project was to generate a range of commercial satiating products and bring them to market thus broadening the range of appetite-regulating products currently available to the consumer. A specific focus was to generate less elitist and cheaper healthy products that are more accessible to all. With this approach the project had the potential to impact on a wider population, enhancing standard of living and lifestyle and helping to address health inequalities. Introducing the novel food processing technologies identified in the project to mainstream manufacturing to enable production of commercial products could also offer additional economic impact through strengthening of the EU economy.

### PHASES

The project had five research and development work packages (WP) in two phases. **WP1, WP2 and WP3 in phase 1** were structured concurrently. Both **WP2 and WP3 supported WP4 and WP5 in phase 2**. Across the full duration of the project there were two more work packages **WP6 and WP7**.

**WP 1. Selection of improved satiating food components by in vitro screening.** Industrial and academic partners collaborated to produce an *in vitro* screening model of the gut to identify potential satiety enhancing food structures, screen specific ingredients, and model nutrient bioavailability *in vitro*.

**WP2. Sensory factors and food structures in satiation and satiety.** Industrial partners developed novel food processing techniques to alter food structure, assess sensory-satiety in early consumer testing and produce a range of food products in a variety of matrices for human intervention studies.

**WP3. Microbiota, gut function and biomarkers of appetite and related health claims.** Industrial and academic partners assessed the effects of foods on nutrient bioavailability, investigating novel biological mechanisms associated with gut microbiota, analysing biomarkers of satiety and long-term improvements in health.

**WP4. Satiety and consumer health.** Academic partners assessed the satiating effects of food products in short and medium term satiety studies of individual products to demonstrate their enduring effect on appetite and weight loss inducing potential sufficient to substantiate health claims.

**WP5. Proof of concept – lasting health benefits for consumers.** Academic partners examined the long term benefits of satiety-enhancing products on weight regain and improving indicators of health

**WP6. Dissemination and exploitation.** Industrial partners and SMEs worked to commercialise any resulting products and other IP.

**WP7. Project co-ordination and management.** The University of Liverpool co-ordinated and managed the project throughout its duration.

## INDIVIDUAL PROJECT OBJECTIVES

To address the aims and produce the intended impact, SATIN was designed with eight distinct objectives.

**Objective 1: Integrate advanced technologies to screen novel food structure through *in vitro* models to isolate and refine products according to their satiating potential.**

*Commercial and academic partners in WP1 developed and validated new in vitro gastrointestinal models that analyse satiety biomarkers to assay novel ingredients, refine prototype food structures, and assess nutrient bioavailability to assist product development in WP2.*

**Objective 2: Develop novel food processing technologies that combine active ingredients and change food structure to produce a range of novelty satiety enhancing ingredients.**

*Commercial partners in WP2 developed food products using novel processing techniques. These were delivered ready for clinical assessment assisted by in vitro modelling developed in WP1.*

**Objective 3: Produce finished food products that pass through safety analysis, early sensory evaluation and consumer testing.**

*Commercial partners in WP2 consumer- and safety- tested effective formulation to produce final products for clinical evaluation in WP4 and WP5.*

**Objective 4: Demonstrate the effects of prototype products on biomarkers of satiety and on nutrient bioavailability using *in vivo* studies and validating new *in vivo* approaches.**

*Commercial partners further modelled changes in gut microbiota and associated biomarkers in vitro in WP3 to inform in vivo analysis in WP1. Academic partners examined the effects of prototype foods on gut function and other mechanisms underpinning biomarkers of satiety and nutrient availability in vivo in WP3 to inform biomarker analysis in WP4 and WP5.*

**Objective 5: Demonstrate the effects of final food products on within-meal satiation, post-meal satiety and / or reduced appetite and biomarkers of satiety.**

*Academic partners assessed the acute and enduring effects of satiety-enhancing products using a range of validated protocols and measures in WP4. Biomarkers of satiety taken in these studies were analysed in WP3.*

**Objective 6: Demonstrate the enduring effects of individual food products on satiety and their potential to induce weight loss.**

*The enduring effects of satiety-enhancing products on appetite and body weight were assessed by academic partners in WP4 in medium term clinical studies. The effect of energy deficit induced by caloric restriction and structured exercise on product efficacy were also determined. Biomarkers of satiety taken in these studies were analysed in WP3.*

**Objective 7: Demonstrate the long-term consumer and health benefits of adhering to a diet containing satiety enhancing products.**

*The effect of satiety-enhancing products on long-term weight regain prevention was assessed in **WP5** in a 12-week multisite intervention. Biomarkers of health outcomes were taken during the intervention and analysed in **WP3**.*

**Objective 8: Validate health claim end points and commercialise technologies and products.**

*All commercial partners collaborated to disseminate findings to key stakeholders, including health claim regulators, and exploit resulting project IP to innovate food processing in Europe and market creditable appetite controlling food products in **WP6**.*

## **THE CONSORTIUM**

The SATIN consortium consisted of seven SMEs and four commercial partners ensuring that advanced technologies developed to process and screen novel food products were applied to the food industry and improved European economic competitiveness. The safety and efficacy of products developed were rigorously examined by seven leading academic research teams to ensure consumers had new high quality processed foods to help them achieve a balanced diet.

## Description of Main S & T Results and Foregrounds

### WP1: Selection of improved satiating food components by *in vitro* screening.

#### Task 1: Development of an *in vitro* food component screening platform

Robust, biologically relevant *in vitro* assays have been developed and combined to generate a platform to enable the screening of a wide range of food ingredients and processing conditions before *in vivo* testing. This approach will also allow identification of the components responsible for the health promoting properties of foods and thus facilitate targeted improvement of the composition of food. Beyond the life of the project this platform will be attractive and available to the function food industry.

#### *Subtask 1.1: Establishment of a dynamic gut model for the evaluation of digestive/fermentative processes and the bioavailability of active ingredients and processed food products.*

The SHIME technology platform is a dynamic gut model consisting of five compartments, respectively simulating the stomach, small intestine, ascending colon, transverse colon and descending colon. The SHIME allows sequential simulation of gastric (pepsin-HCl, low pH) and small intestinal (pancreatic enzymes and bile salts) digestive processes. This enables evaluation of the effect of digestive and metabolic processes in the stomach and small intestine on the final biological activity profile of test products. As the aim of the project was to develop a range of different food matrices containing the active ingredients, the modelling capacity of the SHIME was extended towards more elaborate processing of complete food matrices. To achieve this, the platform was upgraded by inclusion of a mouth step and also a dynamic dialysis step to simulate the absorptive processes occurring in the small intestine. The SHIME setup was also been equipped with a dialysis system (between the small intestinal and colon compartment) to remove small digested compounds, simulating the removal of small nutrients that normally occurs in the small intestine. Validation of the modified SHIME platform was achieved using several reference compounds (pure ingredients with known active compounds or as ingredients within the final food matrix).

#### *Subtask 1.1a: Solubility and availability of ingredients in the intestinal tract for absorption*

The ability of ingredients to influence satiation / satiety depends on their stability to digestive processes and availability along the GI tract for absorption. A static gastrointestinal digestion system was established to assess stability, whilst bioavailability and the cell response to a wide range of satiety ingredients was examined by means of a cell-based *in vitro* intestinal model using the Caco-2 cell line. Pure reference ingredients and positive controls were examined to validate the models.

Stability was assessed through HPLC-MS analysis of aliquots (soluble fraction following centrifugation) taken at different stages of the digestive process. Differing stability of ingredients was identified. Additionally, the system was able to differentiate between the different fates of a number of bioactive compounds in a single reference ingredient, with some reaching the small intestine step to different degrees but others not surviving initial digestive phases being identified only in the raw ingredient and not in aliquots from the static system.

Furthermore a bioavailability cell-based assay, using a Caco-2 co-culture system to model absorption and intestinal permeability was fully optimized. In this assay cells are separated from the intestinal digest (soluble fraction from static or dynamic gut models) by a dialysis membrane. The integrity of cell monolayers was examined by transepithelial electrical resistance and confirmed.

#### *Subtask 1.2: Chemosensors and GI hormone secretion cell based assays*

An *in vitro* cell based platform was optimised and validated with reference controls. This platform comprised of primary assays, GI hormone secretion read out assays and secondary assays.

Primary assays consist of enteroendocrine cell lines which endogenously express chemosensor receptors and which are able to secrete GI hormones upon stimulation with food components. Immortalised cell lines with the ability to secrete detectable levels of the most relevant satiety hormones (GLP-1, CCK and PYY) upon

stimulation with food ingredients, were identified and parameters optimised for cell culture.

GI hormone secretion read-out assays consist of recombinant cell lines expressing a specific GI hormone receptor and a luminescent or fluorescent functional readout, able to detect the GI hormones secreted upon stimulation of primary assays. Three recombinant cell lines expressing: the human GLP-1 receptor (GLP-1R), the human cholecystinin receptor (CCKAR) and the human PYY receptor (NPY-2R) were generated. To select a final pure and best performing clone, all the cells underwent sequential limiting dilutions, functional clone pool analysis, isolation and retesting of individual clones and experimental protocol optimization. The correct pharmacology was also verified by using specific receptor agonists and antagonists to obtain EC50 and IC50 values, respectively, in full agreement with the literature data. In all the three cases, robust and well performing assays were obtained.

After establishing robust functional assays for the three GI hormones, primary and read-out assays were functionally couple to generate the bioassay systems. A qPCR analysis was undertaken to determine the expression of receptors for food components and optimal conditions to detect hormone secretion were established. Unfortunately, it wasn't possible to detect PYY secretion in the medium of cells despite incubation with many different stimuli. As such it wasn't possible to develop a cell-based bioassay for PYY for use in **Phase 1** of the project.

3. Secondary assays consist of recombinant cell lines expressing specific chemosensors and a luminescent or fluorescent functional readout, able to detect the interaction of food components on GI chemosensor receptors. The secondary assays generated for SATIN contain the following receptors of interest:

- GPR120, activated by long chain fatty acids (LCFA)
- GPR43, activated by short chain fatty acids (SCFA) (sugar metabolites)
- TGR5, activated by bile acids
- GPR93, activated by peptides

In addition to the cell-culture based assays described above, an ex-vivo gut tissue based GI hormone secretion assay panel using Ussing Chamber technology was optimized to ensure tissue integrity and survival and fine-tuned to verify the best procedure for sample collection and storage. The most reliable and accurate method to analyse satiety hormones (GLP-1, CCK and PYY) in the Ussing Chamber medium were also investigated. Samples taken at different time points from both sides of the Ussing Chamber set-up (serosal and mucosal) were analyzed using ELISA and RIA assays. Optimal assay conditions were established to test the samples from artificial digestion.

#### Task 2: Identification of new food ingredients by means of *in vitro* screening

A comprehensive bibliographic search of scientific databases was completed to identify plant extracts which have previously been identified as having satiating properties, whether at *in vitro*, *in vivo* or at human scale. Twenty-seven plant extracts or natural compounds were identified. Based on the level of scientific data available, the availability of the plant and the safety and legislative information available, this list was narrowed down to 20 extracts. Individual bibliographic reports, focused on the satiating effects and potential role in the satiety cascade, were prepared for each extract and shared with partners to enhance their knowledge in this area. Characterization of the same plant extracts using HPLC and HPLC-MS methodology was also achieved. To further aid the food manufacturers in the SATIN consortium, the stability of the active compounds present in the plant extracts was also determined in model beverages following heat treatment under different processing conditions. The library of 20 extracts was also screening on the high throughput *in-vitro* cell based platform established in **subtask 1.2**. On the basis of data created a number of partners incorporated botanical ingredients from this library in their prototypes generated in **WP2** using novel processing techniques. In this instance ingredients were exposed to the full screening technology platform (SHIME system + cell line reports) as outlined below.

#### Task 3: Determination of the influence of food processing and food matrix effects on the activity profile of food ingredients

A number of reference ingredients with known satiety properties were initially tested to optimize the screening technology platform (i.e. SHIME + cell line reporters). The ability to exclude compound toxicity was confirmed. Following the standardization a new set of lead compounds were tested independently to ensure

that ability to discriminate between the effect of the food matrix and of the food matrix plus the active ingredient.

Subsequent to optimization of the platform 30 pure ingredients (reference compounds and ingredients under consideration for inclusion in food products prepared in **WP2**) and 6 food products (the lead candidate product from each producer in **WP2** and identified for inclusion in **WP4** studies) were examined. (Details of the selection process applied for the food products is provided later in the summary; **WP4**).

Ingredients and food products were artificially digested through the dynamic gut model. Both short-term (single passage through the gut model) and long-term (repeated simulations to reflect products with pro-longer mechanism of action in the gut e.g. fibres) SHIME experiments were applied. The samples collected from the different SHIME compartments, at different treatment times, were delivered to the **WP1** members, who developed the *in vitro* platform, to i) examine fermentation outcomes; ii) test their capability to induce an *in vitro* secretion of GLP-1 and CCK; iii) evaluate their capacity to stimulate chemosensor receptors and iv) analyse their stability and bioavailability. In each case the detected parameters were used to prepare a ranking based on the profile of the test products

#### Task 4: Evaluation of results and selection of combined food components and processing treatments for further study in human intervention trials

To aid the selection of food products for inclusion in clinical trials in **WP4** the results obtained from analysis of the 6 food products in **Task 3** were carefully analyzed and a final comprehensive **WP1** prototype ranking based on the original 6 lead compounds was compiled and is demonstrated below.

MATRIX	INGREDIENT	CELL LINES (AXXAM)	SHIME (ProDI)	SOLUBILITY, AVAILABILITY (UMUR)	USSING CHAMBER (BioAct)
Yoghurt/Pudding [NIZO]	Satiagel ADG 38 [Cargill]	1	5	2	1
Tomato Juice [CTAEX]	Polydextrose [Cargill]	2	3	4	4
Orange Nectar [Coca-Cola]	NAXUS LC [BioActor]	3	2	4	3
Pineapple Nectar [Juver]	Kemfe [Naturex]	4	4	4	5
Bread [BioActor]	NAXUS MC [BioActor]	5	1	2	/
Fish balls [CTC]	Viscogum [Cargill]	5	6	1	1

(The rank order is from 1 to 6; with 1 being the best position).

### WP2: Sensory factors and food structures in satiation and satiety

#### Task 1: Modification of existing whole foods (natural raw materials) through the use of specific processing techniques

Existing whole foods (natural raw materials), such as fruits and vegetables/drinks, dairy foods/drinks, meat/fish (meal components), beverages, breads and nutritional bars, that are representative of typical foods/drinks of the daily European diet were modified by means of specific processing techniques including homogenization, fermentation, restructuring, heat treatment, inclusion of raw fibres, blending, protein modification, inclusion of cultures with potential satiating effects and gastro-protective coating techniques. Over 80 different prototypes were generated.



## Task 2: Assessment of sensory satiation and early satiety effects of modified existing whole foods (natural raw materials)

The release of flavor during food consumption has been used as an indicator for the potential of sensory satiation. It is hypothesized that, during the consumption of a meal, aroma molecules reach the olfactory epithelium retronasally and activate brain areas leading to sensory-related satiation. In addition to sensory perception, other physical parameters during gastrointestinal passage such as meal viscosity, coagulation, and emulsion stability, influence food intake. To assess the satiating potency of the prototypes developed in **task 1** their *in vivo* flavour release profiles were assessed by atmospheric pressure chemical ionization-mass spectrometry (APCI-MS technology; satiation effects) and their food textural properties were assessed by *in vitro* stomach digestion (SIMPHYD; early satiety effects).

APCI-MS technology measurements were performed in triplicate by 7 volunteer participants using a fixed eating/drinking protocol for each product application in terms of duration in mouth, duration of swallowing, amount of chews etc. The results from the triplicate measurements were averaged and analysed. In the SIMPHYD platform the rheological characterization resulted in the measurement of viscosity or force in time for respectively, liquid and semi-solid/solid food applications under gastric conditions following a representative *in vivo* digestion protocol. Responses were ranked per measurement from 1 (highest performance) to 6 (lowest performance). As it was not possible to compare the different product matrices directly due to the versatility of the matrix characteristics, a ratio was calculated between the response from the specific product matrix and its corresponding blank or placebo. This ratio was then compared between the different product matrices and translated into a ranking from 1 to 6. These rankings were combined with results from **WP1** to aid with the selection of food products to include in **WP4** clinical trials.

PRODUCT	WP2 TESTING	
	FLAVOUR RELEASE	TEXTURE BUILDING
Yoghurt/pudding [NIZO] Satiagel ADG 38	6/6	1/6
Tomato Juice [CTAEX] (only matrix effects tested, not active ingredient Polydextrose)	6/6	6/6
Orange Nectar [Coca Cola] NAXUS LC	6/6	2/6
Pineapple Nectar [Juver] Kemfe	6/6	6/6
Bread [Bioactor] NAXUS LC (2.5 fibre incorporation)	4/6 Factor 2.4 lower compared to blank	6/6
Fish balls [CTC] Viscogum	5/6 Factor 1.28 lower compared to blank	6/6



### Task 3: Application of various potential satiating/satiety enhancing ingredients in a range of food products

A list of ingredients for use in foods / process modeling was composed by ingredient producers in the consortium and discussions with SME's were initiated around their specific food processing technologies and their technical functionality for ingredient addition to particular food applications. This information assisted in identifying potential ingredients for addition to specific food applications.

The criteria set for ingredient selection were:

- Nature of the study: in vitro/ ex-vivo screening assays vs. human trials: taste/ palatability, applicability in food, others
- Food applications: dairy, fish/ meat, bakery, fruits and vegetable juices, beverages
- Processing techniques that food production units bring into SATIN
- Nature of the ingredients/ compounds: carbohydrates, fibers, proteins, lipids, non/low caloric sweeteners, botanicals
- Food safety of the ingredients/ food matrix through modeling (GRAS/ novel food status, if applicable)
- Proven efficacy of ingredients, evidence of efficacy and site of action/ food matrix through modeling
- Anticipated mode-of-action: nasal/ oral functioning (e.g. flavor release), gastric effects (e.g. emptying rate, stomach distension), upper intestinal effects (e.g. digestion kinetics, ileal brake, transit time), gut fermentation. Processing modifications and application of appetite regulating ingredients are expected to lead to food products addressing multiple triggers in the satiety cascade.

Physico-chemical characterization, nutritional values and sensory acceptance of the products have all been examined. Two distinct sensory analyses were carried out: (i) Compared to placebo and (ii), sample acceptance. In both cases, 10 trained panelists evaluated the products.

Of these prototypes the lead candidate from each producer in **WP2** was identified for consideration for inclusion in the 6 slots available in **WP4** acute clinical trials. Of the original 6 products identified, four were selected to progress to clinical studies, reformulation was recommended for one product and one was withdrawn. To optimize clinical trial capacity, partners were encouraged to generate new products, leading to further activity under **Task 3**. Ultimately one reformulated product and two new products were developed.

### Task 4: Production of food / drinks for human intervention studies

For the acute human intervention studies in **WP4** the 6 selected foods/drinks were produced by **WP2** partners. Some delays in production up-scaling were encountered and impacted on **WP4** time lines. One product was recalled due to manufacturing issues which rendered product batches unusable due to microbial contamination of spillage on the exterior of product containers. The trial was restarted with new product and the producer covered the financial cost to SATIN. Consistent with the study outcomes (reported in **WP4**), two products were selected for inclusion in the **WP4** MT trials, with **WP2** partners responsible for their continued production.

PRODUCT	Academic partner for medium term human intervention trial
Tomato juice from concentrate [CTAEX] incl. Polydextrose (Cargill)	UNILIV
Yoghurt / pudding [NIZO] incl Satiagel ADG 38 (Cargill)	UNILEEDS

In both cases delays in production were encountered. These largely related to problems encountered with the upscaling of product provision from **WP2** partners for the **WP4** trials.

*Yoghurt/pudding (NIZO)*: The short shelf life of the product proved particularly challenging. Additionally, recruitment for the intervention trial proved more difficult than initially anticipated. Consequently, the trial demanded additional production runs far exceeding those anticipated

*Tomato juice (CTAEX)*: Whilst the producers of the selected study product (CTAEX) did not have the capacity to meet production requirements to progress the SATIN project so they released production details to Cargill (satiety ingredient supplier) to search for an alternative producer. Cargill reviewed the potential to generate the required product in their internal pilot plant. Test runs and small scale-up suggested that production could be achieved internally at Cargill facilities. Yet, despite Cargill's best efforts, various problems progressively extended the study start date until, due to restricted timelines remaining until the end of SATIN, the project reached a point where it was illogical to further pursue preparations for the trial designed to examine the medium term impact of the tomato juice product. Activity was refocused on the examination of the acute impact of tomato juice using the limited existing stocks (see **WP4**).

### **WP3: Microbiota, gut function and biomarkers of appetite and related health claims**

#### **Task 1: Generation of objective biological criteria to select candidate ingredients, food matrices and changes in food structure for WP2.**

This task began with scoping exercises to ascertain i) the ingredients available for use within SATIN (consortium partners and the Industrial Advisory Board) and ii) the available food processing techniques / capabilities. The physical characteristics of available ingredients were also captured in the scoping exercise allowing the ingredients to be mapped on to likely food and beverage matrices. Using the satiety cascade as a theoretical model, the likely GI tract target for specific ingredient-food matrix combinations was also examined. Following discussions with academic and commercial partners this process was formalised and the criteria for ingredient selection agreed to include:

1. The food application (beverages, dairy etc)
2. The processing technique applied
3. The nature of the ingredient
4. Food safety of the matrix
5. Ingredient efficacy
6. Likely mode of action

#### **Task 2: Effects of nutrient bioavailability and mechanism of action of new and improved dietary products**

Well-designed *in vitro* simulation technologies provide an important and cost-effective approach for assessing the fate of dietary components in the gut that complement *in vivo* studies. *In vitro* systems either focus on one gut compartment or simulate passage through all gut compartments. In order to obtain the most physiologically relevant information, both approaches were applied in this task. Data were coupled to that obtained in the short term human dietary trials (**task 3**) as a means of validating the *in vitro* approach.

##### **Subtask 2.1: Short-term batch experiments**

The pure ingredients being considered by partners in **WP2** for inclusion in prototype products, and a selection of ingredients within selected food matrices underwent short-term simulation to compare the intestinal fate of the product. Samples were collected from the simulated ascending colon to analyse colon acidification, gas production, SCFA, lactate and ammonium production, concentration of bifidobacteria and lactobacilli. Moreover, samples from the colon compartment were also collected to evaluate the GI hormone secretion and chemosensor activation profiles (**WP1**).

Examination of isolated ingredients in **subtask 2.1 WP3** is equivalent to activity reported under **subtasks 3.1 and 3.2 of Task 3 in WP1** and has been reported in the relevant location.

### *Subtask 2.2: Long-term simulation experiments*

In line with **WP2**, a selection of the ingredients considered in the short term experiments were studied in food matrices in long term studies. In order to be able to discriminate between the effect of the food matrix and of the food matrix plus the active ingredient both of these have been tested independently. For food matrices, mouth and dialysis steps (developed in **WP1**) were added in the technology platform and optimized for the particular food types (e.g. increased mechanical breakdown to stimulate chewing of solids).

Long-term simulation experiments also focused on the food matrices +/- active ingredients examined in the **WP3** human dietary trials. Products were artificially digested the SHIME technology platform and samples were collected from each colon compartment to measure the parameters identified in **task 3 WP1**.

- Actistar led to a positive effect on all of the analysed parameters
- NAXUS MC did not have a strong supportive effect on Lactobacilli, especially at mucosal levels
- Viscofiber led to an increase in ammonium concentration in the distal colon (possible sign of high selectivity) and did not support mucosal lactobacilli and luminal bifidobacteria.

The table below summarizes the rankings from all of the long-term SHIME experiments conducted (**WP1** and **WP3**). Rankings were determined according to the approach detailed in **Task 3 WP1**.

The final ranking of all of the prototypes examined in **WP3** was found to be consistently higher than the prototypes analyzed in **WP1** (with the exception of Bread including NAXUS LC).

Rank	Food matrix	Active ingredient	
1	Bread	ActiStar	WP3
2	Biscuits	ActiStar	WP3
3	Bread	NAXUS MC	WP3
4	Bread	NAXUS LC	WP1
5	Bread	Viscofiber	WP3
6	Orange Juice	NAXUS LC	WP1
7	Tomato Juice	Polydextrose	WP1
8	Pineapple Nectar	Kemfe	WP1
9	Yoghurt/Custard	Satiagel ADG 38	WP1
10	Fish balls in tomato sauce	Viscogum	WP1

GI hormone secretion, and chemosensor activation profiles were also examined in the samples obtained from the colon compartments. The approach for assigning scores and rankings is described in **Task 3 WP1**.

The table below summarizes the rankings (derived from analysis on the cell-based platform) from all of the long-term SHIME experiments conducted (**WP1** and **WP3**) to provide a final overview of all of the tested prototypes. Despite CCK analysis being omitted from ranking for all of the prototypes examined in **WP3** (due to toxicity in the assay), they are still ranked higher than the majority of the prototypes analysed in **WP1**.

Prototype rank vs blank	Food matrix	Active ingredient	Score
1	Yoghurt/Custard [NIZO]	Satiagel ADG 38 [Cargill]	85
2	Bread [BioActor]	NAXUS MC [BioActor]	58
3	Bread [Aberdeen]	Viscofiber [Naturex]	50
4	Bread [Aberdeen]	Actistar [Cargill]	37
5	Biscuits [Aberdeen]	Actistar [Cargill]	28
6	Orange Nectar [Coca-Cola]	NAXUS LC [BioActor]	26
7	Tomato Juice [CTAEX]	Polydextrose [Cargill]	18
8	Pineapple Nectar [Juver]	Kemfe [Naturex]	15
9	Fish balls [CTC]	Viscogum [Cargill]	3
Prototype rank vs matrix	Food matrix	Active ingredient	Score
1	Yoghurt/Custard [NIZO]	Satiagel ADG 38 [Cargill]	33
2	Bread [Aberdeen]	Actistar [Cargill]	29
3	Bread [Aberdeen]	Viscofiber [Naturex]	25
4	Tomato Juice [CTAEX]	Polydextrose [Cargill]	12
5	Biscuits [Aberdeen]	Actistar [Cargill]	10
6	Orange Nectar [Coca-Cola]	NAXUS LC [BioActor]	8
7	Pineapple Nectar [Juver]	Kemfe [Naturex]	2
8	Bread [BioActor]	NAXUS MC [BioActor]	0
8	Fish balls [CTC]	Viscogum [Cargill]	0

The GI hormone secretion profiles illustrated above will be further through specific comparison to *in vivo* profiles from **WP3** human dietary trials (**Task 3** below) to verify the validity of the *in vitro* platform developed in **WP1**.

### Task 3: Short-term human dietary intervention studies

Two studies were undertaken within this task. **Study 1** incorporated a resistant starch product (Actistar) into the diet of overweight/obese participants after weight loss diet and compared to the effect of the natural fibre maltodextrin. Different matrices were used during the study. Some products (biscuits, chocolate pudding mix and bread mixes) for control and resistant starch diets were provided and raw product was also incorporated into cereal and cereal bars in order to guarantee consumption across the intervention at a daily dose of 22-26g RS per day. The presence of RS in all test products was confirmed using an AOAC approved method for the determination of RS

Twenty- four participants underwent a free-living, randomised, single blinded study in which participants were provided with all food and drinks during 4 dietary periods. Maintenance Diet (M) for 3 days, 21 day weight-loss period (WL) followed by two subsequent 10 day periods on alternative weight maintenance diet using resistant starch 3 (RS) or Control (C). Diets were matched on calorific content and volunteers were previously screened to ensure suitability for the study. Body weight, body composition, blood pressure, appetite questionnaires, blood, urine and faecal samples were collected at the end of each dietary period for measurement of outcomes. The possibility of using a wireless motility device (SmartPill™) to assess gut pH and stomach- rectal transit time and of using a hydrogen/ methane breath analyser to measure of carbohydrate fermentation, were both investigated. One female participant withdrew from the assessment of transit time due to swallowing problems. 1 male performed the test using RS3 and C breakfast and no complications were reported.

An average of 21g/d of Actistar was consumed by participants. However, this dose did not determine changes on short and long term “hedonic” appetite nor energy intake. A reduction of body weight was observed after WL diet (30%prot, 30%fat, 40%CHO, 100%RMR) and maintained after RS and C diet. However, no significant differences were found between these two maintenance diets. Similar changes were

observed in body composition. Improvements in fasting metabolic parameters were also observed after WL diet. Specifically a reduction in Total Cholesterol, LDL-Cholesterol, and triglycerides was observed. Fasting Glucose values showed a statistically significant reduction after WL and after RS diets.

**Study 2** incorporated raw ingredients into bread mixes, sauces, soups and puddings to complete the study menu at a dose of 6g per day of Beta-glucan (Viscofiber®) and 15g per day of Arabinoxylan (MC-Naxus®). The study design comprised 27 days of dietary intervention in which participants received, after an initial 4-day ad libitum diet and a 3-day maintenance diet (M), either (1) 10 days of MC-Arabinoxylan weight loss diet (Diet A) and 10 days of a Control weight loss diet (Diet C) (randomised order), or (2) 10 days of a Beta-glucan weight loss diet (Diet B) and 10 days of a Control weight loss diet (Diet C). Twenty subjects (BMI 27-42 kg/m<sup>2</sup>) in the study were included after medical screening and dietary interventions were provided using Beta-glucan Vs Control and Arabinoxylan Vs Control. Body weight, body composition, blood pressure, appetite questionnaires, blood samples, urine samples and faecal samples were collected at the end of each dietary period for measurement of outcomes. Gut pH and transit was assessed using a wireless motility device (SmartPill™) in three participants (2 males and 1 female; diet A and C group).

Body weight, body composition, blood pressure, appetite questionnaires, blood samples, urine samples and faecal samples were collected at the end of each dietary period for measurement of outcome. A summary of the results is shown below:

- Improvements in BMI and blood pressure were similar for A (AXOS-containing) and B (beta-glucan-containing) weight loss diets relative to the control (C) weight loss diet (over 10 day periods).
- Decreases in total plasma cholesterol and fasted glucose were seen for the B weight loss diet relative to the initial M diet. Changes in these parameters were not significant for the AC comparison.
- A significant contribution of AXOS and beta-glucan fibres to satiety during the weight loss period was not observed.
- No evidence was found for gastrointestinal discomfort from the added ingredients at the dose given.

#### Task 4: Analysis of biomarkers to assess physiological responses

Samples of faeces, urine and blood were collected from both studies.

##### Subtask 4.1: Gut hormones

Four hundred plasma samples (**Study 1**) and seven hundred and eight plasma samples (**Study 2**) were preserved using protease inhibitor cocktail, ABSF and DPPIV and stored at -70°C freezer until analysed for Insulin, Leptin, Active glucagon like peptide-1, Ghrelin, PYY, and glucose-dependent insulinotropic polypeptide (GIP) using mesoscale (MesoScale Discovery®).

In **Study 1**, during a test meal challenge, insulin responses were lower after WL, RS and C (insignificance order) and no difference was observed in glucose response to the meal. A suppressive effect was observed in GIP levels after WL, RS and C meals. No other effect was observed on the peptides studied.

In **Study 2**, in measurements taken following test meals, AUCs for insulin, ghrelin and blood glucose changed significantly relative to the initial M diet, but did not differ between A and C or between B and C diets. The AUC for GIP was however significantly lower for the A than for the C diet.

##### Subtask 4.2: Impact on gut microbiota and metabolites

A total of 173 faecal samples from **Study 1** and 217 samples from **Study 2** were collected and processed. Samples were examined for microbiota composition by qPCR analysis, measurement of metabolites (especially short chain fatty acids and nitrosamines) and amplified 16S rRNA genes (in DNA samples).

In **Study 1** faecal samples were obtained from all subjects on days 5, 8, 26, 29, 37, 40, 47 and 50.

SCFAs: the average ratios of major faecal SCFA acetate, propionate or butyrate were not significantly altered across the diets. However the percentages of total SCFA represented by the branched chain fatty acids iso-butyrate and iso-valerate were significantly lower in the RS dietary period compared to the control period. This suggests that the presence of ActiStar® 11700 in the diet lowered the extent to which amino acids are fermented by gut microbes.

*N-nitroso compounds*: faecal NOC were highly variable between individuals on all four diets and no significant changes in faecal NOC were observed between different dietary treatments. Hence, none of the study diets led to increased formation of these potentially harmful compounds in the colon.

*Microbiota analysis by targeted qPCR and by 16S rRNA V4 sequencing*: at the phylum level, Firmicutes dominated the microbiota of all but four subjects, accounting for  $72.5 \pm 10.7$  % of the total community and the faecal microbiota of most subjects was also characterised by a prevalence of both Bacteroidetes ( $17 \pm 9.1$  %) and Actinobacteria ( $6.7 \pm 8.1$  %). Data obtained from qPCR from the same samples were in excellent agreement with the results of high throughput sequencing. Both approaches detected a significant increase in representation of *Ruminococcus* spp., especially *Ruminococcus bromii*, when subjects were consuming the RS3 diet. Inter-personal variation was found to be mostly attributable to microbial community differences at higher taxonomic ranks, i.e. family and genus, with phylum and class level composition remaining consistent between subjects. The microbial response after ActiStar® 11700 consumption was characterised by both the enrichment and reduction of specific species within the gut microbiota. The majority of OTUs exhibiting a significant response to dietary RS belonged to the important gut bacterial genera *Ruminococcus* and *Eubacterium*, with additional changes occurring within the *Bifidobacterium*, *Faecalibacterium*, *Alistipes* and *Lachnospiraceae* groups.

In **Study 2** samples were collected on days 4, 8, 16, 18, 25 and 28.

SCFAs: Weight loss diets (30% protein, 40% carbohydrate) led to a slight decrease in % butyrate and a slight increase in % propionate and in branched chain fatty acids among total SCFA in faecal samples relative to the initial M diet (15% protein, 55% carbohydrate). This was unaffected by the inclusion of beta-glucan or Arabinoxylan in the weight loss diets.

*Microbiota analysis by 16S rRNA V4 sequencing.*

$\beta$ -glucan

- $\beta$ -glucan consumption led to elevated levels of *Blautia* within the faecal microbiota, an important gut genus of which a number of species exhibit acetogenic activity leading to acetate production.
- The  $\beta$ -glucan-induced *Blautia* increase was attributable to the significant enrichment of two specific operational taxonomic units classified as unknown *Blautia* species.
- Levels of the butyrate-producing genus *Roseburia* were also enriched by  $\beta$ -glucan consumption, due to the significant increase of one individual *Roseburia*-classified OTU.

*Arabinoxylan*

- Consumption of arabinoxylan had a substantial and significant impact on the composition of the faecal microbiota, significantly increasing and reducing levels of the major gut phyla Actinobacteria and Firmicutes respectively.
- Arabinoxylan supplementation was associated with a major bifidogenic effect, in which one bifidobacterial OTU was significantly enriched in the majority of subjects to account for over 17% of the total faecal microbiome,
- Species-level identification revealed the enriched bifidobacterial OTU is likely to represent *Bifidobacterium adolescentis*, a probiotic species with many health-promoting effects.

### WP1-WP3: *in vitro-in vivo* validation

The data generated from **WP3** analysis provided important information on gut function and mechanism of action of the ingredients examined in the short term dietary intervention studies necessary to validate the SATIN *in vitro* platform.

In **Study 1** RS was able to enrich the concentration of starch fermenters and butyrate producers. Short- and long-term *in vitro* studies showed that RS increased SCFA and lactate production implying that especially saccharolytic micro-organisms were enriched. In terms of microbiota composition, RS showed a small bifidogenic effect and an increase in butyrate producers. Moreover, most of the species that increased their concentration upon treatment with RS *in vivo* belonged to the *Firmicutes* phylum. Data on qPCR showed that, *in vitro*, the same phylum was increased.

In **Study 2** with arabinoxylan and B-glucan the following *in vivo* results were obtained:

- B-glucan: increased species involved in acetate and butyrate production
- Arabinoxylan: increased *Bifidobacterium* spp.

*In vitro* data provided additional information to support these findings:

- B-glucan: enhanced acetate, propionate and butyrate production. Moreover, species that increased their concentration upon the treatment with the test product *in vivo* belonged to the *Firmicutes* phylum. Data on qPCR showed that, *in vitro*, the same phylum was increased
- Arabinoxylan: led to an increase in butyrate but also acetate and lactate, which could imply that *Lactobacilli* and/or *Bifidobacteria* were enhanced. *In vitro*, two different arabinoxylan were tested: a medium chain (MC) and a long chain (LC) arabinoxylan. With the MC compound, *Lactobacilli* increased whereas *Bifidobacterium* concentrations stayed approximately the same. With the LC arabinoxylan *Bifidobacterium* concentrations increased during the treatment.

The three ingredients were also tested in the cell-based assays of the *in vitro* platform in **WP1** in different conditions:

- Actistar, beta-glucan and arabinoxylan were tested as pure ingredients,
- Actistar and beta-glucan were tested also as digested ingredients, collected after a short term SHIME
- Arabinoxylan (Long and Medium chain), which was included in one of the SATIN prototype products, was also tested as a digested ingredient, collected from different SHIME compartment after long term experiment.

While the pure ingredients showed a stimulating effect on different biosensor receptors (GPR120, GPR93 and GPR43) and on GI secretion (CCK, GLP-1), the samples collected from the artificial intestine compartments didn't display any significant effect, with the exception of Arabinoxylan LC, which showed a weak activity on GPR120, GPR93 and CCK secretion assays, but only from samples derived from some tracts of the intestine.

In general only weak stimulation of cell-based assays was identified for the tested ingredients. Consequently their pro-satiety properties *in vivo* were expected to be low. As such outcomes from the *in vitro* platform were in agreement with and confirmed by the *in vivo* studies (validation of negative effect).

A validation of positive effects of ingredients detected on the *in vitro* cell-based platform, in predicting pro-satiety effects *in vivo*, may be better achieved by testing a higher number of samples in *in vivo* designs.

#### *Subtask 4.3: Inflammatory markers and metabolic related molecules*

Samples provided by 19 participants in **Study 1** and 20 participants of **Study 2** were analysed for



inflammatory markers TNF, IL-1B, IL6, IL8, IL10, IL12p70 and C- reactive protein in plasma.

In **Study 1** samples were taken at four time points (8, 29, 40 and 50 days). No significant differences were observed for any of the biomarkers and groups analysed, but a shift in the balance of inflammatory markers was found with higher level of pro- inflammatory interleukins (IL-6 and IL-8) and lower amount of anti-inflammatory ones (IL-10) identified when RS was applied immediately after WL diet.

In **Study 2** samples were taken at time points covering each of the three diets (Maintenance, control and Fibre) of the study.

- Tumour necrosis factor alpha (TNFalpha) is a pro-inflammatory biomarker. In the plasma of participants involved in the BG diet, levels in all study phases were found to be similar to those of normal-weight people (1-4 pg/mL). However, statistically significant differences in TNFalpha levels were identified between phases in the AX diet. In this case, volunteers showed elevated concentrations of TNFalpha during maintenance and control diets, which were decreased to normal levels after including AX in their diet.
- IL-1B and IL-8 are pro-inflammatory. Both ingredients decreased the level of IL-B after C phase. However only the AX diet produced a significant increase in IL-8 compared to any other phase in the study.
- IL-6 is consider to be a pro-inflammatory cytokine in obesity and together with IL-8 is considered to be one of the most important inflammatory biomarker in the disorder. Consistent with this, study participants showed an increased level of IL-6 compared to the normal range (< 5 pg/mL). Significant differences were only identified between phases in the BG diet, where levels were elevated in the control phase compared to the maintenance phase and restored in the BG phase. However, in all phases values were still above normal range.
- IL-10 is a key anti-inflammatory cytokine. In all cases, measured levels were below normal range. These findings are consistent with reports of an unbalance of inflammation (more pro- and less anti-inflammatory biomarkers than normal-weight people) in the obese population. In the BG diet levels of this anti-inflammatory interleukin were reduced in the control and BG diet phases compared to the maintenance phase. By contrast levels were comparable in the maintenance and AX diet phases in the AX study but reduced in the control phase.

In summary, both ingredients (Betaglucan and Arabinoxylan) are able to change concentrations of several biomarkers involved in inflammation. However, changes were not consistent with both anti-inflammatory and pro-inflammatory biomarkers being increased or decreased at the same time, making interpretation difficult.

#### **WP4: Satiety and consumer health**

##### **Task 4.1: Generation of objective biological and behavioural criteria to select candidate ingredients, food matrices and changes in food structure for WP2**

In discussion with representatives from **WP1** and **WP2**, **WP4** partners compiled a list of the available parameters to assess product selection for **WP4** and **WP5** studies. From this list the key criteria driving selection were identified as a) indication of efficacy from published literature and **WP1** and **WP2** studies; b) not being subject to patents held outside the SATIN consortium or industry advisory board; c) seeking prototypes that covered a range of different food categories; d) seeking prototypes that targeted different parts of the GI tract and e) seeking prototypes that addressed a range of eating occasions. A data gathering exercise was undertaken with **WP2** partners to obtain details of the specific parameters for their prototype food products. Contributing to knowledge exchange between academic and industrial partners, the satiety cascade was incorporated as a theoretical model to examine the potential of specific ingredient-food combinations to target particular parts of the GI tract.

##### **Task 4.2: Development of methodological platform for human dietary trials**

**WP4** and **WP5** partners developed protocols and training regimes incorporating objectives, inclusion and exclusion criteria for the participants, overall study designs and a description of study visits detailing the agreed approaches to assess the key components of acute dietary trials.

#### *Subtask 4.2.1: Satiety/satiety and food intake*

A single-blind, randomised crossover design (for non-fermentable food products) or between measures design (for fermentable products) was established to examine whether satin food products can enhance satiety and reduce appetite and/or accelerate satiation in the short-term. Between-meal satiety and/or within-meal satiation measures were incorporated through administration of test meals in the laboratory and subjective measures of appetite sensations were also integrated into the design.

#### *Subtask 4.2.2: Food hedonics (Explicit and implicit motivation)*

It was agreed that control over hedonic eating would be assessed through the use of a battery of behavioural and psychometric tools including eating behaviour trait questionnaires (Three Factor Eating Questionnaire, Binge Eating Scale, Power of Food Scale, Cravings Questionnaire) and computer-based tools (the Leeds Food Preference Questionnaire; LFPQ). As validation of the LFPQ has only been achieved with UK specific food products, **WP4** partners agreed that its potential use would be restricted to the two UK-based testing sites. However, it was agreed that eating behaviour trait questionnaires and the Control of Eating Questionnaire (COEQ) would be applied at all **WP4** testing sites.

#### *Subtask 4.2.3: Circulating appetite signalling peptides and energy metabolism*

A number of differing methodologies were identified for incorporation in **WP4** short term studies to assess biomarkers of satiety and satiation. Methodologies were selected in accordance with the principle that biomarkers should be valid (with established links to physiology), reproducible, feasible (assessable within the timeframe of studies) and ultimately both ethical and minimally invasive. In this respect plasma analysis of gut peptides was not deemed justified in short term clinical studies.

#### *Task 4.3: Development of methodological platform to identify consumer benefits*

Satiety and satiation may provide further benefits to the consumer beyond those offered by appetite control, such as effects on the pleasure of eating, mood and motivational states. To gain an understanding of the real world consumer benefits of the use of satiety enhancing food products developed in SATIN, questionnaire and experimental paradigms were collated to collect relevant real world data. A number of potential benefits of satiety directly relevant to weight control were identified through this review including 1) providing appetite control strategies for consumers generally and for those who are highly responsive to food cues; 2) offering pleasure and satisfaction associated with low-energy/healthier versions of foods without feeling 'deprived'; 3) reducing dysphoric mood associated with hunger specially during energy restriction; and 4) improved compliance with healthy eating or weight management efforts.

#### *Task 4.4: Short term evaluation of novel dietary components*

Six food products representing the lead candidate from each producer in **WP2** were identified for inclusion in the 6 slots available in **WP4** acute clinical trials. These products were selected to

- Cover a range of food categories
- Cover a range of eating occasions
- Target different parts of the GI tract

Each product was assessed on all component assays of the SATIN *in vitro* platform. From the resultant data **WP1** and **WP2** partners created rankings (as discussed earlier in the report) to aid with product selection for **WP4** trials. This data was supplemented with results provided by **WP2** producers relating to sensory acceptance, food safety and allergen profiles. As directed by **WP6**, prior art/ freedom to operate checks were also completed for each of the 6 products. The complete data set is summarised in the table below.

Of the original 6 products identified, one was withdrawn prior to the selection process due to issues encountered with sensory acceptance. Academic partners in **WP4** reviewed the *in vitro* data in conjunction with literature (published and proprietary) relating to potential mechanism of action for the remaining five food products/ingredients. This literature provided insight into the relevant platform components to be considered in each case. As a consequence of the review process, four products were selected to progress to **WP4** short term clinical trials and reformulation was recommended for the final product on the basis of low level activity on relevant platform assays. Partners were also encouraged to generate new products to occupy the remaining available clinical trial slot.

The reformulated product and two new products underwent relevant *in vitro* tests to support their inclusion in **WP4** acute trials and the selection process was repeated with two products being selected to complete the full quota for the short-term trials. Results of this additional testing is also demonstrated below.

Products were assigned to academic testing sites (2 per testing site; see below) **WP4** academic partners were responsible for generating bespoke protocols, based on known mechanism of action and within the confines of the agreed methodological protocol for **WP4** short-term trials. Protocols were reviewed, adapted and approved by **WP4** academic partners before trials commenced.

Product	Test Site	Start Date	End Date	Delay
Yoghurt/Pudding (Satiagel ADG38)	UNIVLEEDS	September 2014	December 2014	Synchronisation of supply and demand between limited lab capacity and small production facility
Tomato Juice (Polydextrose)	UNILIV	October 2014	April 2015	Ingredient supply, nutritional analysis
Orange Nectar (Naxus LC)	UCPH	January 2015	February 2015	Initial trial halted November 2015
Pineapple Nectar (Kemfe)	UNIVLEEDS	October 2014	December 2014	Reformulated product
Fishballs (Viscogum)	UNILIV	September 2014	April 2015	No
Pineapple Nectar (Viscofibre)	UCPH	March 2015	April 2015	New product

Initial WP4 Decision Making Table

	WP2 PRODUCER	WP2 TESTING		WP1 TESTING						WP2 PRODUCER		Producer/ Ingredients supplier	OUTCOME
	ORAL	MOUTH	STOMACH	DUODENUM	COLON			SUITABILITY					
PRODUCT	SENSORY ACCEPTANCE	FLAVOUR RELEASE	TEXTURE BUILDING (Viscosity)	USSING CHAMBER	CELL LINES Ingredient	CELL LINES Matrix	CELL LINES Combination	SHIME	SOLUBILITY, AVAILABILITY	FOOD SAFETY	ALLERGEN PROFILE	IP	
Yoghurt/pudding [NIZO] Satiagel ADG 38	√	X	1/6	√ 1/6 [15.3pM]	√ 1/6 [33]	√ 2/6 [38]	√ 1/6 [85]	N/A (5/6) [3.5]	√ 2/6 [18/30]	√	√	√	Selected - Leeds
Tomato Juice [CTAEX] Polydextrose	√ (8 from 9)	X	N/A	√ 4/6 [3.1 pM]	√ 2/6 [12]	√ 4/6 [2]	√ 4/6 [18]	√ 3/6 [11.8]	√ 4/6 [13/30]	√	√	√	Selected - Liverpool
Orange Nectar [Coca Cola] NAXUS LC	√	X	2/6	√ 3/6 [7.3pM]	√ 3/6 [11]	√ 5/6 [2]	√ 3/6 [26]	√ 2/6 [13.3]	√ 4/6 [17/30]	√	√	√	Selected - Copenhagen
Pineapple Nectar [Juver] Kemfe	√ (6 from 7)	X	X	√ 5/6 [1.7pM]	√ 4/6 [2]	√ 3/6 [5]	√ 5/6 [15]	√ 4/6 [3.8]	√ 4/6 [17/30]	√	√	√	Under modification
Bread [Bioactor] NAXUS LC	Issues with acceptance	1/6 [2.4x lower]	X	NOT TESTED	√ 5/6 [0]	√ 1/6 [70]	√ 2/6 [58]	√ 1/6 [16.4]	√ 2/6 [18/30]	√	√	√	Withdrawn
Fish balls [CTC] Viscogum	√ (3.7 from 5)	2/6 [1.28x lower]	X	√ 1/6 [18pM ingredient only]	√ 5/6 [0]	√ 5/6 [0]	√ 6/6 [3]	√ N/A (6/6) [-2.7]	√ 1/6 [20/30]	√	√	√	Selected - Liverpool

WP4 Decision Making Table Following Reformulation

PRODUCT	WP2 PRODUCER	WP2 TESTING		WP1 TESTING						WP2 PRODUCER		Producer/ Ingredients supplier	OUTCOME
	ORAL	MOUTH	STOMACH	DUODENUM	COLON			SUITABILITY					
	SENSORY ACCEPTANCE	FLAVOUR RELEASE	TEXTURE BUILDING (Viscosity)	USSING CHAMBER	CELL LINES Ingredient	CELL LINES Matrix	CELL LINES Combination	SHIME	SOLUBILITY, AVAILABILITY	FOOD SAFETY	ALLERGEN PROFILE	IP	
Yoghurt/pudding [NIZO] Satiagel ADG 38	✓	X	1/6	✓ 1/6 [15.3pM]	✓ 1/6 [33]	✓ 2/6 [38]	✓ 1/6 [85]	N/A (5/6) [3.5]	✓ 2/6 [18/30]	✓	✓	✓	Selected - Leeds
Tomato Juice [CTAEX] Polydextrose	✓ (8 from 9)	X	N/A	✓ 4/6 [3.1 pM]	✓ 2/6 [12]	✓ 4/6 [2]	✓ 5/6 [18]	✓ 3/6 [11.8]	✓ 4/6 [13/30]	✓	✓	✓	Selected - Liverpool
Orange Nectar [Coca Cola] NAXUS LC	✓	X	2/6	✓ 3/6 [7.3pM]	✓ 3/6 [11]	✓ 5/6 [2]	✓ 4/6 [26]	✓ 2/6 [13.3]	✓ 4/6 [17/30]	✓	✓	✓	Selected - Copenhagen
Pineapple Nectar [Juver] Kemfe	✓ (6 from 7)	X	X	✓ 5/6 [1.7pM]	✓ 4/6 [2]	✓ 3/6 [5]	✓ 3/6 [35 - updated]	✓ 4/6 [3.8]	✓ 4/6 [17/30]	✓	✓	✓	Selected - Leeds
Fish balls [CTC] Viscogum	✓ (3.7 from 5)	2/6 [1.28x lower]	X	✓ 1/6 [18pM ingredient only]	✓ 5/6 [0]	✓ 5/6 [0]	✓ 6/6 [3]	✓ N/A (6/6) [-2.7]	✓ 1/6 [20/30]	✓	✓	✓	Selected - Liverpool
Pineapple Nectar [Juver] Ginseng/Viscofibre	✓	NOT TESTED	NOT TESTED	N/A NOT TESTED		CCK only GLP-1 toxicity [20]	CCK only GLP-1 toxicity [25]	N/A NOT TESTED	NOT TESTED	✓	✓	✓	Selected - Copenhagen

### Abridged results of short term human dietary trials

After discussion among **WP4** and **WP5** partners it was agreed that 1) Yoghurt/Pudding (Satiagel ADG38), and 2) Tomato Juice (Polydextrose) sufficiently met the inclusion criteria for inclusion in **WP5**. A summary of study outcomes for each product on which this decision is based is provided in the table below:

Product	Test Site	Primary Outcome	Secondary Outcome	Further Comments
Yoghurt/Pudding (Satiagel ADG38)	UNIVLEEDS	Significant reduction in Ad libitum intake of active product	Trend for lower total energy intake  Trend for increased SQ	
Tomato Juice (Polydextrose)	UNILIV	Reduction in intake at ad libitum lunch after 4 weeks consumption of active product.	No effect of active product on total daily intake.  Trends for reduced hunger and increased fullness.	
Orange Nectar (Naxus LC)	UCPH	No effect of active product on ad libitum intake		
Pineapple Nectar (Kemfe)	UNIVLEEDS	No effect of active product on ad libitum intake		Trend for greater ad libitum intake of dinner meal after control product
Fishballs (Viscogum)	UNILIV	No effect of active product on ad libitum intake		
Pineapple Nectar (Viscofibre)	UCPH	No effect of active product on ad libitum intake		Control product rated more liked and less bitter  More reporting of GI adverse events after active product

With continued problems encountered with the upscaling of tomato juice/soup provision from **WP2** partners for the **WP4** medium term trials a review of **WP4** was conducted. With **Task 4.5.2** (Eating behaviour during energy deficit from physical activity) continuing as planned, the project was deemed able to meet its overall objectives. However, **Task 4.5.1** (*Eating behaviour during energy deficit from imposed dietary restriction*) was no longer feasible. Consequently, activity was reprioritized to enable the deliverable associated with **Task 4.5.1** to be achieved.

#### *Subtask 4.5.1: Eating behaviour during energy deficit from imposed dietary restriction*

Consistent with the contingency plans stated in the DOW, activity in **WP4** was refocused on earlier tasks (**Task 4.4** Short term evaluation of novel dietary compounds in human dietary trials) and the experimental framework they provided was used to generate the deliverable associated with **Task 4.5.1**.

Specifically, analysis of the short term trial (4 weeks duration) conducted during the previous reporting period and examining the impact of polydextrose (PDX) supplemented tomato soup in a normal weight male population, demonstrated large effect sizes for PDX-induced reductions in food intake and increases in satiety (reduced hunger and increased fullness). However, the sample size of the study prevented any firm conclusions being drawn. Expansion of recruitment to this trial enhanced the potential of identifying beneficial effects of a novel SATIN food product on appetite, intake and consumer benefits of satiety.

A randomized crossover between subjects design with two conditions was used to investigate food intake, feeding behavior and appetite following intake of a polydextrose containing tomato soup and a tomato soup control with similar sensory and nutritional characteristics. The product or control was consumed as the first part of a lunch on study days in the Study Centre as a fixed load meal. It was followed one hour later by an *ad libitum* buffet lunch. The effect on appetite was assessed using monitoring of subjective motivation to eat over the study day, as well as intake at a subsequent *ad libitum* simultaneous choice test meals presented over the remainder of the day. Participants visited the study centre on four occasions; initially to undertake a routine screening tests, with three subsequent study visits to consume the four daily meals and snacks and complete appetite ratings (feeding days). A wash-out interval of at least seven days was included between condition/visit one and two with visit three falling on the 28<sup>th</sup> day of the dosing period.

#### Summary of key findings

- **Ad libitum intake after product:** no significant difference but a trend for reduction at dinner and a medium effect size at lunch were observed.
- **Ad libitum intake at end dosing:** no significant difference but a large effect size at dinner was identified
- **Total energy intake:** no difference
- **Appetite sensations:** no significant difference but large effect sizes for hunger and fullness were observed immediately after intake
- **Food craving (consumer benefits):** a large effect size for reduction in intensity was identified
- **Adverse symptoms:** no difference
- **Overall:** Increased sample size strengthened the findings from the first phase of data collection and analysis continues to suggest that polydextrose may reduce energy intake and appetite. Indications for an effect on craving intensity have also emerged. However, the sample size remains too small to allow any firm conclusions to be drawn.

#### *Subtask 4.5.2: Eating behaviour during energy deficit from physical activity*

A longitudinal parallel group with acute cross-over design was used, with participants being randomised following screening to receive the Active or Control yoghurt pudding throughout the intervention period. Participants were required to consume their respective product four times per week throughout the 12-week intervention period. Compliance was monitored by having participants completing weekly records



documenting the date and time of each yoghurt/pudding consumption and a confirmation that the entire portion was consumed. In addition, the intervention consisted of a personalised 12-week exercise programme designed to elicit an energy deficit of 500 kcal per day (2,500 per week) at moderate intensity (70-80% of heart rate maximum). All exercise was supervised by the research team and recorded using a heart rate monitor (Polar RS400, Kempele, Finland).

To assess effects on measures of appetite control (i.e. satiety, satiation, energy intake, peptide biomarkers and food hedonics) a randomised mixed cross-over design was used. Participants attended two probe days at the start of the intervention and two probe days at the end of the intervention. Across the probe days the procedure was identical apart from the yoghurt/pudding served as part of breakfast. To reduce order effects the probe days were counterbalanced across participants.

To assess the effect on weight, body composition and health markers, participants attended two measurement sessions, one at the start of the intervention and one at the end of the intervention. Participants attended this session following an overnight fast and after having avoided alcohol and moderate-to-vigorous physical activity for 24-hours prior to the measurement session and caffeine for 12-hours prior to the measurement session. At the end of the intervention, biomarkers of appetite (e.g. insulin, GLP-1, PYY) will be measured in the postprandial period (**WP3**) in response to the Active compared to the Control product (**Task 5.6**).

### Summary of key findings

#### *Intervention outcomes*

1. **Weekly weight change:** Changes in body weight were objectively assessed weekly in the research unit throughout the intervention period. There were no differences in change in body weight between the Active and Control group across the intervention period when the data were analysed according to intention to treat and completer population.
2. **Body mass and composition change:** Body composition was assessed using air displacement plethysmography at baseline and post-intervention. There were no differences in change in body mass or fat mass between the Active and Control group when the data were analysed according to intention to treat and completer population. The Control group gained more fat free mass and reduced their percentage body fat to a greater extent than the Active group in both the intention to treat and completer population analyses.
3. **Waist and hip circumference change:** Hip and waist circumference were assessed in the research unit at baseline and post-intervention. There were no differences in change in hip and waist circumference between the Active and Control group when the data were analysed according to intention to treat and completer population.
4. **Health markers:** There was no effect of intervention week or intervention group on systolic or diastolic blood pressure. The intervention reduced participants' resting heart rate and fasting blood glucose, and increased their cardiovascular fitness. The Control group had a greater reduction in their fasting blood glucose at the end of the intervention compared to the Active group in both the intention to treat and completer population analyses.
5. **Psychological wellbeing:** Psychological wellbeing was assessed at baseline, at the end of the first and tenth week of the intervention and at post-intervention using the Beck Depression Inventory II, Perceived Stress Scale and the State Trait Anxiety Inventory. The Control group (intention to treat and completer population) had higher BDI-II scores at baseline compared to the Active group, indicating mild levels of depression in this group. This reduced to the same level as the Active group following the first week of the intervention. Levels of State Anxiety increased following the first week of the intervention in the intention to treat analyses only. There was no effect of the intervention or group on Perceived Stress Scale or Trait Anxiety scores.

### Probe day outcomes

1. **Appetite sensations:** There was no effect of yoghurt/pudding condition at baseline and post-intervention on ratings of hunger, fullness, desire to eat or prospective consumption. At baseline, the Active group reported higher levels of fullness compared to the Control group.
2. **Gastrointestinal sensations:** There was no effect of yoghurt/pudding condition or group at baseline and post-intervention on ratings of nausea and bloatedness.
3. **Energy intake:** There was no effect of condition or group on overall probe day energy intake. At the ad libitum lunch, the Control group consumed more energy in the control condition compared to the Active group across baseline and post-intervention probe days. Participants consumed fewer calories of chocolate from the ad libitum snack box in the Active condition at baseline and post-intervention. At post-intervention, participants also consumed fewer calories from the yoghurt in the snack box in the Active condition.
4. **End of day questionnaire:** At baseline, participants reported reduced appetite for something sweet in the EoDQ in the Active condition compared to the Control condition. This finding was not replicated at post-intervention. When analysed across time, there was a main effect of condition with appetite for something sweet being lower in the Active compared to the Control condition. At baseline, the 24-hr CoEQ showed that participants reported higher levels of Craving Control in the Active condition compared to the Control condition, this effect was not replicated at post-intervention.
5. **Blood glucose response:** At baseline, blood glucose levels were higher in the Active compared to the Control condition at 15 and 30 minutes post-consumption of the yoghurt pudding. At post-intervention, blood glucose levels were higher in the Active compared to the Control condition 15-minutes post consumption of the yoghurt pudding. There was no interaction with week or group.

### Task 4.6: Biomarkers of satiation and satiety associated with novel dietary components

Venous blood samples were collected into 10ml syringes and then transferred to EDTA containing Monovette tubes. The tubes contained a mixture of inhibitors to prevent degradation of the peptides to be assessed. Samples were drawn at eight time points during the morning of the probe day at 0 minutes and after breakfast at +15 minutes, +30 minutes; +60 minutes; +90 minutes; +120 minutes; +180 minutes and +230 minutes for the measurement of metabolic and appetite peptide levels. After collection, samples were centrifuged for 10 minutes at 4°C and 4000 rpm. Samples were immediately pipetted into Eppendorf tubes and stored at -80°C awaiting analysis. Peptide analysis was done using the MesoScale Discovery platform. Concentrations of Ghrelin were assayed in one assay and the remaining peptides in a custom-made kit. All analysis were done in duplicate and the mean value was used.

### Summary of key findings

1. **GLP-1:** At baseline, there was no effect on condition on plasma levels of GLP-1. At post-intervention, there was a trend ( $p=0.054$ ) for an effect of condition with lower plasma levels of GLP-1 in the Active compared to the Control condition. There was no effect of intervention on fasting GLP-1 levels.
2. **Insulin:** There was no effect of condition on plasma levels of insulin at baseline or post-intervention. There was a main effect of exercise intervention but not yoghurt/pudding group on fasting insulin levels, with lower levels of fasting insulin post-intervention compared to baseline.
3. **GIP:** At baseline, there was a main effect of condition with lower plasma levels of GIP in the Active compared to the Control condition. There was no effect of condition at post-intervention. There was no effect of the intervention on fasting GIP levels.
4. **Ghrelin:** There was no main effect of condition on plasma levels of ghrelin at baseline or post-intervention. There was no effect of the intervention on fasting ghrelin levels.
5. **PYY:** There was no main effect of condition on plasma levels of PYY at baseline or post-intervention. There was no effect of the intervention on fasting PYY levels.
6. **Leptin:** There was no effect of the intervention on fasting leptin levels.

## WP5: Proof of concept: lasting health benefits for consumers

### Task 5.1: Development of a methodological platform for long-term intervention study

During the preparations for the intervention study (discussed under **task 5.2** below), issues were encountered in securing sufficient satiety products to support the whole diet approach on which the methodological platform was established. On this basis, the methodological platform was amended:

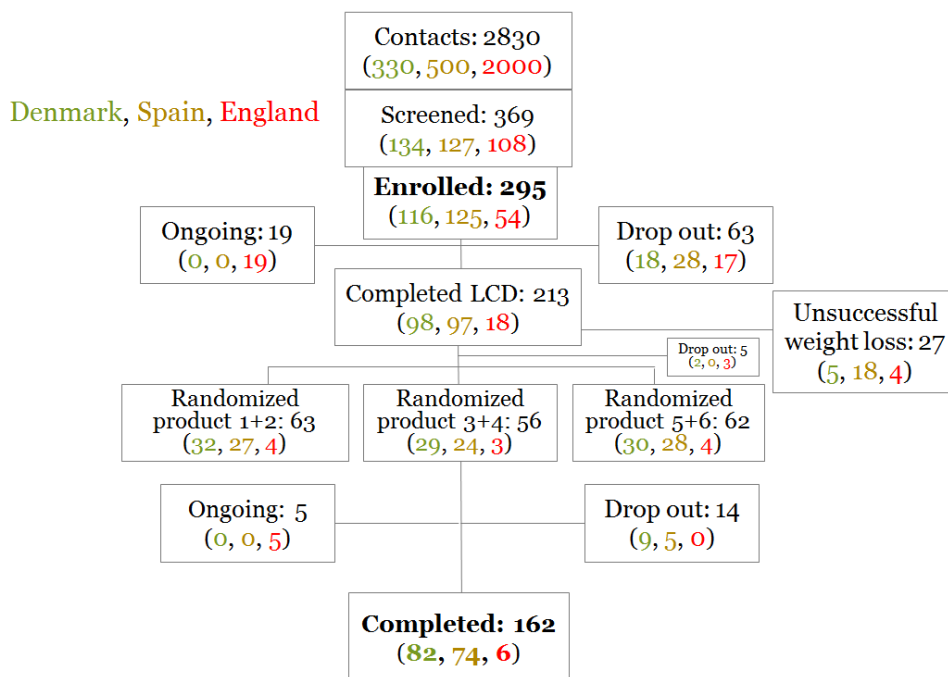
- Rather than a single intervention in which participants are allowed to choose freely from a number of intervention products, three sub-studies were conducted, each testing one specific intervention product or its control.
- The duration of the intervention was reduced from 24 to 12 weeks (following a period of weight loss)
- Participant numbers were reduced to three hundred subjects (50 in each of the six arms)
- The weight-loss period (LCD) was reduced from ten to eight weeks

### Task 5.2: Long term human intervention study

The study was conducted as a 12 week double blinded parallel randomized multicentre study, with six arms (three different products with proven satiety enhancing effect and matching control products). Before being eligible for the intervention period; an initial weight loss of a minimum of 8 % was required. This was achieved by administration of a low calorie diet (LCD) for 8 weeks managed by biweekly dietician supervised group sessions. Failure to reach a minimum of 8% weight loss resulted in exclusion from the study. Prior to the randomized weight loss maintenance intervention, a 7-10 days run-in period for diet stabilization was included. During the intervention participants were advised to follow a general healthy weight loss maintenance diet in accordance with national dietary guidelines and further randomized to include a study specific products either with (active) or without (control) satiety enhancing properties. Main study visits for the assessment of study outcomes (body weight, waist circumference, hip circumference, sagittal diameter, body composition, blood pressure, consumer benefit tests etc.) were performed before the LCD, before the maintenance intervention and at the end of the intervention. Every four weeks during the maintenance period, body weight, waist and hip circumference were measured and in addition the participants met individually with a dietician a total of three times. On two occasions at the beginning of the maintenance period and one at the end, appetite probe days were conducted for assessments of the acute and sustained effect of the study specific products on appetite. Additionally, several consumer benefit questionnaires were applied during the whole course of the study.

A total of 295 participants with an initial body mass index of 27 to 35 kg/m<sup>2</sup> and a fat mass of no less than 23% were enrolled in the study on January 3<sup>rd</sup> 2017. A total of 68 participants dropped out during or right after the LCD period and 27 were excluded due to <8% weight loss. A total of 181 participants were eligible to be included in the 12 week weight loss maintenance intervention. On January 3<sup>rd</sup> 2017, 162 participants completed the whole study period and 5 were ongoing in the intervention period. Recruitment and study progress and the number of study visits completed so far are presented below.

Flow chart of the study. Numbers are shown overall and divided by study sites



At the start of recruitment at the Spanish site, an unexpectedly high numbers of participants failed to the  $\geq 8\%$  weight loss. Consequently, only 79 participants qualified to initiate the maintenance period despite 97 subjects completing the LCD period. The challenges were discussed by the **WP5** partners on conference calls and e-mails. Ultimately the formula diet providers were contacted by the **WP5** partners, and the success rate of participants accomplishing the weight loss in Spain increased markedly when more savoury flavours of the formula diet were provided.

At the start of recruitment at the English site a much slower inclusion rate than anticipated was experienced. Several internal **WP5** conference calls were performed in order for the sites to exchange experiences from recruitment. In attempt to reduce the risk for lack of power in the study, the work package decided to extend recruitment at the English site.

Consistent the monitoring plan prepared as a part of the methodological platform of the study, each study site was monitored by an external monitor. Remote audit before initiation of the study was performed via the trial master file hosted on a site enabling sharing of documents (<https://vocal-external.liv.ac.uk/>). After initiation of the study, the external monitor carried out site monitoring in Spain in April 2016, in Liverpool in July 2016 and in Copenhagen in October 2016. Paper CRFs and reports from the electronic data entry site (EasyTrial) were monitored. Any deviations were registered during the monitoring and corrections and adjustments requested for subsequent data collection.

During data collection, source data were collected using paper CRFs according to the guidelines of ICH-GCP as far as it was possible. Along with the practical execution of the study, source data from the paper CRFs was entered electronically in EasyTrial – an online web-based system for the management of data obtained in a clinical trial ([www.easytrial.net](http://www.easytrial.net)). Only source data entered before January 3<sup>rd</sup> 2017 are presented in this report. Thus, data collected but not yet entered is not presented explaining why, in some instances, the number of completed procedures etc. does not add up to the number of participants completing the visits. During October 2016 primary data cleaning was initiated and will be updated as the trial continues.

Preliminary analysis of a subsample of those completing the maintenance period suggests the potential for a sustained decrease in appetite (decreased appetite measured as lower total energy intake over the course of the appetite probe days) to result in less body weight regain during the maintenance period. From the preliminary analyses of the results available so far, it seems that the body weight increases 0.23 kg per each 1 MJ higher total energy intake at the appetite probe day after the maintenance period compared to the total energy intake at the control appetite probe day before initiating the maintenance period (P=0.039).

### Task 5.3: Verification of sustained effects of novel food products on satiety/satiation and associated biomarkers

Utilising the methodology developed in **WP4 (task 4.2)** enduring effects of novel food products on satiety/satiation, food intake and food hedonics were examined in all subjects participating in the long-term intervention study in accordance with the amended **WP5** protocol. Probe days were conducted at baseline (with control product for all subjects at the end of the run in period - a minimum of 7 days), at the beginning of the intervention and after 12 week's intervention (For both of the latter days the subjects were provided with products according to their randomisation).

Appetite was measured at 8 hour appetite probe days. Participants were served standardised meals including the study products according to the randomization. Energy intake (kilo joule and gram) and subjective appetite sensations (visual analogue scales) were assessed to measure the acute and sustained effect of the study specific products on appetite.

Participants were asked to keep their food and fluid intake as well as activity levels similar on the day prior to each study visit. They were also asked not to consume alcohol, take part in vigorous exercise and were provided with a diary to record intake and activities undertaken from 1700 the day before each study session. Participants arrived at the study centre at a pre-arranged time (from 0900 to 0940) in a fasted state having consumed nothing but water from 2400 the night before each session and first completed visual analogue scales (VAS) pertaining to their current appetite. Participants were then provided the fixed breakfast and were asked to consume the entire breakfast within 20 minutes. After intake, further appetite ratings and additional palatability ratings were completed. Participants were then provided with hourly appetite VAS and a 500g bottle of water. They were asked to abstain from eating or drinking anything except the water provided by the researcher which they could re-fill with additional water.

Four hours after breakfast commenced, participants returned to their testing cubicle and completed appetite VAS before being served the ad-libitum lunch. Further appetite and sensory VAS were then completed before participants were provided with hourly VAS to complete and a 500g bottle of water. The ad libitum dinner was provided four hours after commencement of the ad libitum lunch after which participants were given a snack box which contained the EoDQ to complete before retiring for the evening. They were asked to return the snack box (including packaging from any of the foods consumed) the next day. Upon completion of all sessions participants were debriefed and reimbursed for their time. The exact timing of provision of the study products was dependent on the proposed mechanism of action for each product. At the baseline probe day (start of the maintenance period) all participants received the control product from their respective randomisation.

Energy intake (mean±SD) at the ad libitum eating occasions in the appetite probe days across the maintenance period is shown below for the study population as a whole and subsequently divided by products according to randomization.

*Ad libitum energy intake (mean±SD) for products 1 and 2 across the maintenance period*

	Baseline (all control)	Baseline (randomized)	End of maintenance
Lunch (g)	325.2±122.0	332.2±117.9	334.3±145.0
Lunch (KJ)	1718.2±696.2	1753.9±617.0	1727.2±755.0
Dinner (g)	302.8±107.6	285.6±98.7	287.9±135.0
Dinner (KJ)	2193.5±848.1	2085.8±818.9	2049.3±714.6
Snack box (KJ)	2455.9±1863.2	2089.7±1635.1	2518.1±2014.2

#### Task 5.4: Consumer benefits

From the consumer perspective satiety enhancing products should produce enduring effects on appetite. These should be sufficient to counteract the physiological consequences of energy restriction and the psychology of deprivation associated with chronic energy restriction.

A platform of consumer benefit questionnaires were applied before the LCD, before the maintenance intervention and at the end of the intervention measurement. Most of the questionnaires were completed by the participants at home prior to the visits. However, three of the questionnaires were answered at the study site as a part of the visit. Three consumer benefit computer tests (experimental paradigm tasks) were also applied at these visits. Nine of the consumer benefit questionnaires were applied as a part of the product pick up visits during the maintenance period of which the three was answered at the study site during the product collection. Additionally, a questionnaire assessing appetite and experience of the study program was applied at all visits throughout the study period after initiating the LCD.

The experimental paradigm tasks were performed in front of a computer screen and took between 5-10 minutes for completion.

- Attentional bias was measured using a visual probe task. During the task, two task pictures were presented simultaneously (one appetitive and a matched control picture) before both pictures disappeared and a probe appeared in the location of one of these pictures. Faster reaction times to congruent probes (behind the appetitive picture) are indicative of attentional bias.
- Automatic approach responses were measured using a stimulus response compatibility task. During this task a single picture (appetitive or matched control) was presented in the centre of the screen with a manikin above or below it. Participants were instructed to make the manikin approach one group of pictures and avoid the other according to block instructions.
- Behavioural control was measured using the Go/No-Go task, which assesses the ability to withhold pre-potent responses in the presence of appetitive cues. During the Go/No-Go task participants were first instructed to respond as rapidly as possible to appetitive cues and withhold responses to neutral cues. In the next block the instructions were reversed; withhold responses to appetitive, respond as rapidly as possible to neutral cues.

Consumer benefits were also assessed by a questionnaire based approach, informed by Hetherington et al (2013) and containing the following list of questionnaires:

- End of Day Appetite Questions
- Control of Eating Questionnaire (7 day and 24h)

- Additional Question from Broader conception of self-control
- Liking and Pleasantness of Food
- Three Factor Eating Questionnaire - TFEQ a.k.a the Eating Inventory
- Food Thought Suppression Inventory
- Positive and Negative Affect Scale (PANAS)
- Hospital Anxiety and Depression Scale (HADS)
- Dutch Eating Behaviour Questionnaire (DEBQ)
- The Power of Food Scale
- The Mindful Eating Scale
- Eating self-efficacy
- Weight self-efficacy Scale
- Self-Report Measure of Quality of Life (EQ-5D)
- Quality of Life: IWQOL (Impact of weight on quality of life)-Lite
- Warwick-Edinburgh Mental Well Being
- Extended Satisfaction with Life Scale
- Self-Consciousness Scale
- Craving For Sweet Foods

Questionnaires handed out at the same time were combined into one document and handed out to the participants as one questionnaire. The participants were not presented with the different titles and breakdown of the component questionnaires.

Scores (mean±SD) from a representative sample of consumer benefit questionnaires for the study population as a whole across the LCD and intervention period are shown below. Whilst data collection and analysis are incomplete it is impractical to model the full platform of consumer benefit data.

	Baseline (before LCD)	After LCD	After Intervention
<b>HADS</b>	10.1±6.2	7.0±5.1	8.1±6.1
<b>PoF</b>	33.4±13.0	31.1±10.4	30.5±10.8
<b>Mindful Eating</b>	28.9±6.3	28.0±5.1	27.1±5.7
<b>IWQoL</b>	57.6±20.2	49.0±15.2	44.5±13.8

HADS=Hospital Anxiety and Depression Scale; PoF=Power of Food Scale; IWQoL=Impact of weight on quality of life.

#### Summary of key findings

- Reductions in HADs, POF, Mindful eating were observed. However, these appear marginal (perhaps not significant issues for the study population at outset compared with more severely obese samples)
- A more substantive change in the Impact of Weight on Quality of Life was noted. However, this is not a complete data set not has any statistical test been completed.



## Potential Impact and main dissemination activities and exploitation results

### Key Features of SATIN

Despite 40 years of appetite research the European consumer lacks a range of functional foods with proven effects on appetite expression. The SATIN project aimed to address this with i) a distinct theoretical concept – the satiety cascade – to generate evidence based concepts; ii) the development of a novel *in vitro* model to increase the number of concepts likely to succeed in clinical studies, iii) a robust platform of clinical studies with clear endpoints designed to demonstrate efficacy, and iv) a means of delivering end products to consumers with substantiated health benefits.

The satiety cascade, developed by Blundell and colleagues, was the chosen model by which the consortium conceived new product prototypes to target key appetitive processes, to produce beneficial reductions in hunger and meal size and increase within meal satiation and post meal satiety. Products considered included breakfast items, snacks, beverages, and meal items to ensure satiety enhancing options were developed for most eating occasions. Products were specifically designed to reduce energy intake and promote weight loss, and collectively produce prolonged and sustained changes in appetite and eating behaviour which alone or in combination should prevent weight regain. The involvement of industry allowed SATIN to have access to a wide portfolio of ingredients, processing techniques, product expertise, and the necessary sensory and consumer science to develop a portfolio of products. Considerable progress was made developing products, some of which may eventually reach the market.

The second important feature was the a unique *in vitro* modelling system designed to combine human gut cell lines and a dynamic artificial gut model and used to select active ingredients and test how novel food structures enhance or diminish their potential impact on key satiety systems. SATIN specifically set out to develop a methodological platform, lasting beyond the duration of the project, to increase the efficiency of the translation of concepts into actual products, by validating then exploiting an *in vitro* model. The model has been successfully developed and in part validated and is now available for commercial research. In time this should reduce the number of ineffective products entering clinical trials by allowing a greater range of products to be tested in a valid simulation of the human gut, benefitting industry and consumers.

Prior to SATIN few studies examined the sustained effects across the day or if the chronic effects of these test products produce meaningful changes in appetite sufficient to produce tangible health benefits. Within SATIN a programme of controlled gold standard clinical studies robustly characterising the effects of products was developed. This methodology took on board the requirement of regulators to prove health claims while providing clear support to industry in developing products that have the potential to meet these standards and may in turn inform the interpretation of those standards.

Finally, a key feature of SATIN was the focus on, and route to, the development of commercial end points. Through proactive IP management from project outset, the SATIN IP management system offered levels of confidentiality that i) allowed groups of commercial partners to co-ordinate their activities without disclosing commercially sensitive IP to the whole consortium, but overall ensured ii) no conflict of interest developed across the whole consortium in terms of ingredient or intended product type and iii) existing background IP was used effectively to generate the widest possible range of potentially effective products. In more general terms the commercial partners provided SATIN with considerable consumer insight and the project specifically set out to examine the utility of a satiety based approach to long-term weight management. This provided a clear route to market for distinct products and advanced our understanding of the benefits of a satiety based approach to weight management for consumer health with wide commercial benefits.

## Impact Summary

SATIN aim to achieve impact in the following ways:

1. Increase current scientific knowledge and build on the technical capacity of and expertise within European SMEs,
2. Produce economic benefits that come from the development of new foods with altered structure, novel food processing techniques, and identify new dietary strategies and novel functional ingredients/formulations, and;
3. Improve consumers' access to foods that have the potential to produce beneficial changes in diet and improve the health status of the European population in line with public health policy.

This project strove to develop food products that helped regulate food intake during a meal by accelerating satiation, enhancing satiety, and/or reducing food intake. A dietary-based approach would enable consumers to effectively control their own caloric intake and maintain their health, and new and improved products would enlarge the range of foods available to help consumers achieve this. The project guaranteed food safety and yielded products concepts, which if developed, could be used by a broad sector of the public to assist weight management through lifestyle changes such as caloric restriction and physical activity. This was achieved by developing foods using novel processing methods to modify food structure by a consortium of commercial enterprises. The effects of these foods on biomarkers of satiety and on nutrient bioavailability in human trials was assessed in a series of studies and preparation for the commercialisation of resulting IP was made. The SATIN project aimed to address the expected impacts by engaging at all levels with key groups such as end-users, policy communities and other stakeholders as detailed below and in the associated deliverables.

**Adiposity and its Consequences:** Overweight and obesity pose the most serious risk to health in Europe from non-communicable. In Europe adult rates of overweight and obesity have steadily climbed in both males and females and even though rates may have levelled off in some European countries the burden of cardio-metabolic disease associated with it continues to rise. The problem is exacerbated in certain geographic regions, disadvantaged socioeconomic groups and specific ethnic populations all of whom generally suffer poorer health. Even moderate levels of adiposity (overweight) significantly increase the risks of developing non-communicable diseases particularly type-2 diabetes. Moreover, abdominal adiposity in the overweight and obese (fat distribution around the waist) is associated with chronic diseases such as cardiovascular disease, stroke, specific cancers, musculo-skeletal disorders, and reproductive and fertility issues as associated health costs. The impact of obesity and obesity-related disease in turn has an incredible impact on individuals' quality of life and psychosocial wellbeing. However, even modest weight loss can produce significant improvement in health outcomes. *IMPACT: The SATIN project has developed food product concepts for use in conjunction with diet and exercise, which if further developed and commercialised, should help European consumers prevent weight gain, aid weight loss and help prevent weight regain.*

**Consumer Diet and Weight Gain:** The causes of weight-gain are multi-factorial, combining biological vulnerability, specific behavioural and lifestyle factors, along with current and past life events, and the situational factors that make up the 'obesogenic' environment. The physical and nutritional aspects of the modern urban environment, particularly the ready availability of cheap energy-dense, high fat, sugar, and salt (HFSS) foods in the forms of meals, snacks and beverages, contribute to passive and active overconsumption and consequently weight gain and obesity. Perhaps the most significant change has been to our diet, and the economic trends in consumption and the contribution to obesity are well documented. This dramatic change in habitual diet is particularly reflected in an increase in the amount of energy-dense, HFSS foods and beverages consumed. A major shift in the food culture is required to reverse weight gain trends. A necessary step in that is to provide consumers with healthier low fat, less energy-dense, low FSS

alternatives. However, in addition to these, consumers require foods with specific functionality that can satisfy appetite even during periods of reduced caloric intake and increased energy expenditure for effective weight management. **IMPACT:** *The SATIN project used the most advanced food processing technologies and leading international expertise in appetite and weight management to generate product concepts nearly ready for commercialisation for European consumers.*

**Weight Management and Successfully Controlling Appetite:** The overweight often invest considerable time, money and effort trying to control their weight. The multibillion dollar global weight management industry is well established and ever growing. Unfortunately, neither popular diet plans nor widely available commercial products appear to produce lasting solutions. However, the energy balance equation, routed in diet (energy intake) and activity (energy expenditure), provides a useful starting point for understanding the fundamentals of weight control - an intake of energy surplus to requirements promotes weight gain. Changing energy balance is fundamental to weight management. Systematic reviews of clinical evidence suggest that more meaningful weight loss can only be achieved through recommended adherence to a 600 kcal deficit diet or a low-fat diet. This represents a substantial change in energy balance. Individuals experience difficulty making the necessary behavioural adjustments to sustain this deficit and yield significant weight loss. Difficulties result from the strong biological forces that maintain weight status and the negative psychological consequences such as feelings of deprivation associated with caloric restriction. Increased preoccupation with food, relentless thoughts of eating and serious difficulty with dietary adherence when confronted with food are the consequences of more severe caloric restriction. For the consumer, targeting appetite could be a useful means of managing feelings of diet-induced hunger and deprivation. **IMPACT:** *The SATIN project developed a number of appetite-fulfilling, satiety-enhancing food products concepts with the potential to help European consumers resist the strong environmental and situational cues to over consume preventing weight gain/regain and to assist weight loss.*

**A European-based Approach:** In order to achieve the ultimate objective of the project (the development of safe and effective products to enhance within-meal satiation, strengthen post-meal satiety, and/or reduce appetite using novel processing techniques to alter the structure of food) an approach which is both cross sector and multidisciplinary was required. The SATIN consortium contained key 7 SMEs and 4 other industrial partners with specialised experience in novel processing, changing food structures, consumer testing, consumer behaviour, up-scaling production, regulatory affairs and food safety, and consumer markets from around Europe. These commercial bioscience and technology innovation organisations, combined with leading European food companies and academic and industrial partners, had unrivalled experience in appetite regulation, nutrition, exercise and energy expenditure, nutrient signalling, gut physiology and function, microbiota, fermentation, body composition, dietary interventions and weight management. **OUTCOME:** *These partners maximised the ability of the consortium to 1) fulfil project objectives and 2) maximise the outcomes of the project including benefits to industry, consumers, policy makers, public and stakeholders, across Europe.*

**Impact on the European Food Industry:** The exploitation of mechanisms by which foods with novel structures affect human eating will produce clear benefits to European food producers and their customers. By understanding how novel food structures can enhance satiation and satiety and using these to mitigate the psychological effects of perceived deprivation, even under conditions of dietary and /or exercise-induced energy deficit, the food industry will be able to exploit novel processing technologies to produce a new generation of functional foods for consumers wishing to control their appetite and their body weight. **IMPACT:** *Through ongoing dissemination the research provides the food industry with invaluable data on the consumer benefits of satiety-enhancing products and designing products or programmes to help consumers deal with difficulties achieving successful weight management.*

The European food industry is committed to improving the diet and health of European consumers but the economics of production and marketing are still largely geared to promoting a HFSS diet. For the food industry to achieve its goal of supporting health and well being, and to meet specific commitments made to both member states and European regulatory and policy makers, a new generation of alternative healthier products have to be developed. In addition, foods with specific health benefits need to be developed to help consumers' actively manage their weight. The project systematically developed and tested novel foods taking those with proven biological and behavioural activities into clinical studies. IMPACT: *The project has demonstrated how synergy between food development technology and in vitro testing can produce novel products which can then quickly enter into an in vivo testing phase and move to market ultimately aiding industry in meeting its commitments and consumer adopting healthier behaviours.*

**Regulatory Issues:** From 2008 across Europe it has been required that nutritional health claims are supported by scientific evidence demonstrating that the claims are meaningful and accurate, and can thereby help consumers in making healthy diet choices. These data are submitted by industry to the European Food Safety Agency (EFSA) for review. To date, few satiety or weight control claims have been approved by EFSA due to insufficient or inadequate supporting data. Consequently, despite the demand, European consumers face a lack of credible dietary options for appetite and weight control. OUTCOME: *Through dissemination the consortium is informing the European food industry on optimal research design to support proven appetite and weight-related health claims and has been in contact with EFSA to discuss the scientific basis of current regulatory guidance.*

**Other benefits to food industry:** The food industry is the leading manufacturing sector in Europe in terms of turnover, value added, employment and number of companies. The European food industry is made up of a relatively large number of companies dominated by a large number of SMEs. For the European food industry, innovation and knowledge diffusion are keys to improving competitiveness. Functional foods still constitute worldwide the fastest growing sector in the food industry and recently gained substantial market presence. IMPACT: *SATIN has helped to i) increase research capacity in functional food development and novel food processing technology, ii) stimulate collaboration between European SMEs and build partnerships with European ingredients makers and food producers, iii) develop the work between academia and industry in identifying and developing novel targets and technologies, and iv) facilitate knowledge exchange between the public and private sector in appetite, physiology and gut function, and diet, health and weight management through exchanges between members and the training of young scientists.*

**Impact on Public Health and Diet:** A food-based approach to weight management is the most appropriate means of preventing weight gain across the European population. According to recent WHO figures 600 million adults are clinical obese. Replacement of fat or sugar in foods by ingredients having a lower caloric value is a strategy that can help in restoring the imbalance between caloric intake and exercise. Medical weight loss interventions can be effective for individuals but are expensive and limited as a population-wide solution. To deal with current overweight and obesity within the population as a whole, a significant change in the food environment is required. SATIN has made a contribution to this by assessing the impact of novel satiety-enhancing foods on eating behaviour, diet, gut physiology and function, and on indicators of health. The use of satiety-enhancing products in medium term studies and weight control interventions within SATIN has enabled us to examine if such food can increase either the efficacy or adherence to lifestyle interventions in European consumers. IMPACT: *The project has communicated about satiety and appetite control with medical and public health professionals and to the public in the context of healthy eating and obesity awareness. Moreover, through the creation of novel structures the SATIN project has produced food product concepts reformulated to reduce fat and sugar content while maintaining their key sensory attributes and consumer appeal.*

## DISSEMINATION

A broad range of dissemination activities have been undertaken in SATIN, with input from all partners, using a variety of media to engage different stakeholder groups. These numerous activities have been highlighted as News on the project website and are included in the dissemination log in the ECAS database. The activities recorded includes presentations, interviews, TV and radio broadcasts, posters, media briefings and articles in the popular press.

### Website

satin-satiety.eu

The SATIN website was quickly established at the start of the project. The website has evolved throughout the project and been regularly updated with Newsletters, Publications, News. The Consortium has also benefited from a secure intranet, which is an essential project repository containing uploaded Periodic Reports, Meeting Reports, Publications, Deliverables and Template Documents.

### Conference presentations

SATIN scientists at all levels have presented their research findings at a wide range of national and international scientific conferences throughout the 5 years, covering topic areas including obesity, appetite regulation and gut hormones, satiety and appetite control. Oral and poster presentations, especially those highlighting ground-breaking findings, often appeared hand-in-hand with press releases, radio and TV interviews and podcasts, enabling wide dissemination to a variety of stakeholders.

### Public engagement

The consortium as a whole has been active in the area of Public Engagement throughout the 5 years of the project, including participation in international events such as the British Science Festival and European Researchers' Night, including Explorathon 2016 in Aberdeen.

### Policy Engagement

The EU Platform for Action on Diet, Physical Activity and Health represents a main stakeholder group with which SATIN sought to engage. This is a forum for European-level organisations, ranging from food industry representatives to consumer protection NGOs, committed to tackling issues in diet and physical activity. Its emphasis is on prevention of chronic disease through dietary and lifestyle change. Through collaboration with the FP7 project Full4Health, SATIN was promoted (along with Full4Health and NeuroFAST) in a briefing document for the 'Platform' meeting in September 2012. This document was incorporated as a research highlight into the 'What is new?' section delivered by Philippe Roux, Deputy Head of Unit, European Commission, DG Health and Consumers, Health Determinants at the meeting.

SATIN was again presented by the Full4Health co-ordinator in an address to both the EU Platform for Action on Diet, Physical Activity and Health and the High Level Group of Member States on Nutrition and Physical Activity (European Government Representatives) at their adjacent meetings in February 2013. The presentation had the title 'Research targeting food reformulation in the regulation of hunger and satiety'.

At the Platform meeting in September 2015 focused on food reformulation, the SATIN project was directly invited to participate.

### Industry Engagement

The SATIN study was designed to facilitate exchange of experience and knowledge between industry and academia. Consistent with this, the SATIN project has been promoted directly to industrial scientists e.g. Food Matters Live, Vitafoods.

### Other

Scientific audiences were engaged through a conference jointly sponsored by SATIN and Full4Health at Association for the Study of Obesity conference, “Satiety – From Origins to Application” in March 2015.

Dissemination activities will be supported by a number of journal articles (recently published or planned) and designed for both general and specialist audiences e.g. *Current Obesity Reports*.

### **EXPLOITATION**

SATIN has generated a significant amount of new knowledge but the lead-in time to fully exploit this could be considerable. Technologies (in vitro platform and a data collection app) represent project outcomes currently closest to market. However, they require further research (TRL5) which is expected to take place under the further research plans beyond the life of SATIN.

## Address public website and contact details

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