Ingredients for Food and Beverage industry from a lignocellulosic source

Project number 606073

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Executive summary

Natural resources are being exhausted by the greater demand of their services and insufficient measures for their preservation. Use of waste components of agriculture as value-added products is of great relevance to preserve environment and biodiversity. Hemp is one of the oldest cultivated plants and responsible of numerous environmental benefits; resistant to pests allowing reduction of agricultural biocides use, positive crop-sequencing effect for other bioenergy crops, and fast crop growth with low husbandry costs. These factors make hemp an excellent renewable source to obtain compounds of added value in a "biomass biorefinery" approach. Thus, the LIGNOFOOD project aims at using hemp as lignocellulosic source material to obtain xylo-oligosaccharides (XOS) with the greatest prebiotic potential, and xylitol (as a by-product of the process), so that healthy products of added value can be developed with these ingredients. XOS are non-digestible dietary components that have been recently reported to stimulate growth and/or modify the metabolic activity of beneficial bacteria and improve gut health. On the other hand, xylitol is of great value because of its low caloric and low glycemic index, and used as sugar substitute.

In order to reach the project's aim, research has been carried out on: (i) Technological process development for XOS production from hemp, (ii) technological process development for xylitol production, (iii) processes integration, techno-economical feasibility and life-cycle assessments, (iv) prebiotic effect of XOS on human microflora, and (v) safety for food grade use, and food enrichment parameters for consumer acceptance.

LIGNOFOOD project's work was planned to cover all the research steps required to obtain XOS and xylitol from hemp and their food application. Firstly, protocol possibilities for the extraction of hemicelluloses (xylan) from hemp shives was researched. Secondly, production methods to obtain XOS from the extracted xylan was investigated with the use of enzymes, which were characterized, improved and immobilized in different supports. The XOS structures produced were analysed by prebiotic functionality tests. The xylan derived material has been hydrolysed to xylose. Xylose has been used to produce xyilitol by yeast biocatalysts, which have been developed aiming at high reactivity and specific co-substrate selectivity. Finally, the processes for the production of XOS and xylitol from hemp shives have been scaled-up and food products enriched in XOS and/or xylitol have been developed as proof-of-concept.

Participating SMEs will benefit from the results of the proposed research in means of know-how, patenting and licensing, and commercialization of XOS and xylitol to the food industry. Consequently, they will improve their competitiveness by gaining access to the rapidly growing market of functional foods.

Summary description of project objectives and the main objectives

Use of waste components of agriculture as value-added products (product of more value than the material in its raw state) is of great relevance to preserve environment and biodiversity, avoiding consumption of natural resources, soil degradation and competition for land use.

Project LIGNOFOOD aims at using agricultural waste products from hemp (hemp shives) as raw source to obtain compounds of healthy added-value, i.e. xylo-oligosaccharides (XOS) and xylitol. XOS are non-digestible dietary components (oligosaccharides made up of 3-10 xylose units) that have been recently reported to stimulate growth and/or modify the metabolic activity of beneficial bacterial species in the gut and improve gut health. Xylitol is a low caloric sweetener suitable for diabetics and used as sugar substitute because of its lower glycemic index than other sugars.

The goal of the LIGNOFOOD project is the revalorization of the main by-product from hemp processing, into valuable healthy ingredients for food product enrichment. To reach this goal, the following scientific and technological objectives were proposed:

- The development of a technological process to obtain XOS and xylitol from hemp.
    - Developing a pre-processing method to extract hemicelluloses from hemp shives or other parts of hemp for further processing.
    - Developing enzyme-based methods to process hemicelluloses into xylooligosaccharides (XOS) with different chemical structures and properties.
    - Finding the optimal chemical structure of XOS for the greatest prebiotic potential and impact on intestinal health.
    - Developing an enzyme-based method to obtain xylitol from by-products of the XOS production process.
- Integration of the developed methods in a combined process to obtain XOS and xylitol from hemp.
- The development of food products enriched in XOS and/or xylitol.
    - Application of XOS and xylitol to new food product developments targeting consumers concerned in maintaining their gut health, and generally interested in healthier food choices (low glycemic index, low caloric, etc).

Positive societal consequences of reaching these objectives can be foreseen on:

- Helping the sustainability of hemp processing and growing, together with its associated environmental benefits (CO_2 reduction, phytoremediation).
- Increasing the availability of healthier food solutions that will facilitate own health management and a more sustainable health care.

In the first project period, the technical objectives were related to the development of an integrated process to obtain XOS and xylitol from hemp in a sustainable and cost-effective

manner. First, it was necessary to select the most suitable pre-processing method to extract hemicelluloses from hemp shives for further processing. Then, the main objectives were to develop two enzyme-based methods: to process hemicelluloses into xylooligosaccharides (XOS) and to obtain xylitol from by-products of the XOS production process.

The production of XOS from hemp shives hemicelluloses needs chemical transformations carried out by enzymes. In the frame of project LIGNOFOOD, different enzymes were evaluated (endoxylanases, beta-xylosidases and arabinofuranosidases) in order to obtain XOS with different chemical structures and properties, and to get an optimal preparation and cost effective enzyme derivatives. In this way, the most interesting enzymes were immobilized-stabilized by multipoint covalent immobilization on highly activated glyoxyl-supports.

The xylitol production process could be accomplished by chemical means, but LIGNOFOOD aims at a cost efficient and ecologically favoured approach in which the widely used baker's yeast is employed as a biocatalyst. Even though the xylose utilization capacity of *S. cerevisiae* is negligible, its ease of handling together with its GRAS status favours its application food- and beverage-additive production. Hence, in the frame of this project xylose reductase (XR) activity will be conferred to *S. cerevisiae* by genetic engineering. The obtained strains were tested for xylitol productivity and the most promising candidate has been used for the improvement of the basal fermentation conditions. The technical process has been optimized in lab-scale bioreactor experiments.

The main technical objectives in the second period of the project focus on the one hand, on the set up of the XOS and xylitol production method at the pilot scale. In this sense, optimal and cost-effective immobilized biocatalysts of endoxylanases and β-xylosidases were designed. The use of the biocatalysts for the transformation of xylan (an agricultural waste) into xylobiose (a valuable prebiotic useful as food ingredient) and into xylose was also studied.

On the other hand, the technical objectives are related to study the optimal chemical structure of XOS for the greatest prebiotic potential and impact on intestinal health. Bifidogenic effects of XOS were demonstrated depending on type of XOS and concentration in the i-screen platform mimicking the human colon conditions containing the ex-vivo human colonic microbiota. This was demonstrated by a Bifidobacterium quantitative PCR, 16S amplicon sequencing and metatranscriptome analysis showing expression of genes involved in xylane / xylose metabolism of bifidobacteria in the colonic microbiota.

In addition, the development of food products enriched in XOS and/or xylitol is another important technical objective of the project. Finally, three food matrices were formulated with XOS and xylitol attending to XOS functionality preservation and to food quality and safety.

Description of main S&T results/foregrounds

1. Pre-treatment process of hemp shives

In the first period of the project, different existing methods for the separation of the hemicellulose fraction from lignocellulosic sources were reviewed. Several preliminary tests were carried out in order to select the most adequate method based on efficacy (% recovery), simplification, cost-efficiency and environmental impacts assuring its fit for the required purpose of XOS and xylitol production for food application.

In the second period, the scale-up of this pre-treatment of the hemp shives wascarried out, in order to obtain xylan-rich liquors by solubilisation of the hemicellulose fraction. On the one hand, 100 L of xylan liquor from hemp has been produced and supplied to project partners as a raw material for the scale-up production of XOS and xylitol. On the other hand, the design of a high-pressure reactor to perform Auto-hydrolysis at a Pilot scale was undertook.

2. XOS production and purification

A set of native and recombinant enzymes were tested in the first project period. All endoxylanases exhibited a specific activity ranged between 40 – 80 Units per mg of pure enzyme at 50ºC. All beta-xylosidases exhibited a specific activity ranged between 100-120 U/mg at 50ºC. Arabinofuranosidases exhibited a specific activity of 60-80 Unit per mg of pure enzyme. The optimal pH was always 5 and all the enzymes exhibited a good stability at 50ºC and this pH 5,0. The half-life times were usually higher than 20 hours.

In this context, the recombinant enzymes seem to be the most suitable for industrial implementation: very high amounts of easily purificable enzymes can be produced.

These enzymes were intended to be immobilized and stabilized by multipoint covalent immobilization on highly activated glyoxyl-agarose supports. Preliminary results indicated that immobilized derivatives expressed more than 75 % of catalytic activity and were fairly stable at pH 5.0 and 50ºC (with a half-life time higher than 2 months).

During the second period of the project, optimal and cost-effective immobilized biocatalysts of endoxylanases and β-xylosidases were designed. The use of the biocatalysts for the transformation of xylan (an agricultural waste) into xylobiose (a valuable prebiotic useful as food ingredient) and into xylose was also studied.

Twelve different microbial endoxylanases were immobilized and highly stabilized by multipoint covalent immobilization on highly activated glyoxyl supports. Immobilized derivatives were additionally stabilized by coating the enzyme surfaces with different hydrophilic polymers: aldehyde-dextran, glycine-dextran polyethyleneimine, etc. The most stable derivative was obtained with a commercially available enzyme (endo-1,4-beta-xylanase from *Trichoderma reesei*). The immobilized derivative of a recombinant endoxylanase from *Streptomyces halstedii* has also very interesting. Two different glyoxyl supports were evaluated: i) Agarose gels: expensive and only useful for stirred tank reactors; ii) macroporous cellulose beads: cost-effective and useful for both stirred tank and packed bed reactor. The most stable derivative was the selected commercial enzyme - cellulose beads, which preserved 56 % of catalytic activity

(regarding to soluble enzyme used for its preparation) and it was 38,000 fold more stable in experiments of thermal inactivation. The optimal conditions for xylan hydrolysis were stablished, obtained 82 % of xylobiose with small percentages of xylose and xylotriose. Multimeric β-xylosidases were also immobilized and stabilized. With these enzyme derivatives, xylobiose could be transformed in xylose in less than 2 hours under very mild conditions.

Separation processes were applied for the removal of furfural (96.08g/mol) and HMF (126 g/mol) as well as monosaccharides such as xylose (150 g/mol) and glucose (180 g/mol) from XOS fermentation syrup.

The removal of furfural and Hydroxy methyl furfural (HMF) (95%) from XOS fermentation was achieved via ion exchange with chelating resins and adsorption with activated carbon processes. Moreover, if hybrid process including nanofiltration (NF) membrane followed by ion exchange or adsorption, the removal was improved. For the removal of monosaccharides these methods was partially successful.

3. Xylitol production and purification

For the reduction of xylose to xylitol in hemp-derived technological substrate solutions a robust and efficient biocatalyst was sought. Six different *S. cerevisiae* strains that all express the NADPH-dependent enzyme xylose reductase (XR) from *Candida tenuis* (*Ct*) in its wild type form or an NADH-specific mutant of the enzyme were investigated. The strains further differed from each other in the type of promoter or in the mode of XR expression, which was either from a plasmid or from a genome-integrated expression cassette. Highest intracellular XR activities were obtained when XR was expressed from a multi-copy plasmid. The strain carrying the GPD-promoter-XR cassette in the genome showed the lowest intracellular activities. Comparison of the wild type enzyme and its variant using the plasmid-containing strains clearly demonstrated that the wild type enzyme surpasses the performance of its NADH-specific mutant. Furthermore, it was demonstrated that both ethanol and glucose, but not glycerol, are potent co-substrates for internal co-enzyme recycling. Due to cell growth deficiencies in selective medium and unstable XR activities in full medium of plasmid-containing strains, a strain with genome-integrated XR was favored to accomplish the xylitol production process. Among the strains that harbored an XR expression cassette in the genome, the one that expressed the enzyme under control of the GPD promoter gave the best performance in shaking flask tests and was thus used for optimization of xylitol production. Fed batch experiments under different aeration conditions and temperatures revealed the necessity of oxygen and a temperature of 30°C for effective xylitol production. Furthermore, the positive effect of a high biocatalyst loading (≥ 7 g_{CDW}/L) on the volumetric productivity and the positive impact of a high xylose concentration on the specific productivity of the yeast was demonstrated. Under optimized conditions, the chosen biocatalyst was able to reduce 15 g/L of xylose almost completely within 48 hours with both glucose or ethanol as co-substrates.

Data that were obtained from shaking flask experiments were used to develop a xylitol production process in a scaleable bench-top bioreactor. A one-pot biomass production and xylose reduction process was used to determine relevant conversion conditions. However,

varying cell growth in the biomass production phase led to inconsistent xylitol yields and productivities and thus biocatalyst production was decoupled from xylose conversion for the final xylitol production process. While cells were first cultivated in a medium consisting of yeast extract, peptone and glucose, tests on different media compositions and yeast extracts indicated that a combination of a yeast extract and glucose can be used for biocatalyst production in the absence of peptone. The biocatalyst was subjected to bio-reactor fermentations (final concentration ~7 g_{CDW}/L) containing ~40 g/L xylose in a medium containing yeast extract and glucose. Parameters for this model conversion were optimized. Xylose reduction by *S. cerevisiae* is known to be most efficient under glucose-limited fed-batch conditions. Our studies proposed a combination of glucose and yeast extract feeding, in order to prevent inhibitory glucose- as well as ethanol- and acetate-accumulation in the fermentation broth. Using this approach, more than 95% of the applied xylose were converted to xylitol within only 48 hours of fermentation. Yeast cells showed no loss in xylitol productivity or yields when they were immediately reused for another round of xylose reduction or stored at -80°C in 40% glycerol and then reutilized. As a cheap alternative, nutritive molasses was tested as a putative co-substrate and results revealed that it can be used at a feeding rate of 3.4 mL/h. Even without additional yeast extract feeding cells were able to utilize molasses for internal co-enzyme regeneration and xylitol production. However, sterility issues recommend rigorous sterilization of the molasses before application.

In order to test the performance of the strain in a technological, hemp-derived substrate solution, hemp shives had to be hydrolysed in a way to yield maximal xylose and minimal inhibitor (e.g. acetate and furfural) concentrations. Therefore, different chemical and hydro-thermal techniques were tested on rough-grind or milled hemp-shives with/without different concentrations of cellulases and varying incubation times. The most economical method was obtained, without subsequent enzymatic treatment. This approach yielded a primary hemp hydrolysate (HH), containing 16.9 g/L xylose, 2.4 g/L glucose and 5.6 g/L acetate. For some experiments, the primary HH was concentrated 3-4 fold by evaporation to enhance the xylose concentration in the substrate solution. Other experiments were performed using concentrated HH that underwent an additional detoxification step using $Ca(OH)_2$. Shaking flask experiments using the described IBB HH variants revealed that the biocatalyst performance suffers from highly concentrated HH and only parts of the xylose could be consumed before the process stagnated. Decreasing the amount of fed co-substrate (glucose) increased the yields moderately. However, detoxifying the concentrated HH could not bring about any benefit. Furthermore, XDH activity was observed in the employed strain when detoxified HH was used, which resulted in intracellular breakdown of the produced xylitol and thus decreased yields and pentose recoveries. As a result, it was recommended to use primary HH for the bio-reduction process. Shaking flask experiments with the technological substrate solution suggested that full xylose reduction was possible, however with reduced pentose recoveries. Bio-reactor fermentations, where stable pH, aeration and feeding conditions were ensured, resulted in >95% conversion of the present xylose (18 g/L) in 72 hours with a pentose recovery close to 100%. The underlying protocol of this successful xylose bio-reduction experiment provided the final guideline for xylitol production from hemp-derived xylose.

For the isolation of pure xylitol from the fermentation broths, several techniques were reviewed and ultimately we focused on crystallization, as this method is comparably cheap and easy to scale-up. Roughly, the final protocol includes precipitation of impurities with ethanol, concentration of the broth, the actual crystallisation step and a re-crystallization procedure to end up with a highly pure product.

4. Definition of chemical structure and characteristics of XOS for validated functionality

The prebiotic effect of different hemp-derived xylo-oligosaccharides (XOS) with different degree of polymerization and ratio of xylobiose in comparison to other existing prebiotics inulin and/or GOS was shown. XOS types and xylobiose show bifidogenic effects in the intestinal screening multi well platform simulating the human colonic microbiota conditions (i-screen platform). Different XOS types and xylobiose yield an increase in beneficial microbes, particularly *Bifidobacterium spp*. The effects are oligo-sugar type dependent as well as concentration dependent. At appropriate concentrations beneficial microbial metabolites mainly butyrate are produced. Moreover a decrease in opportunistic pathogens such as *Clostridium spp*. and *Enterobacteriaceae* (*E. coli/ Shigella*) was observed. XOS, Xylobiose and compound 3 was shown to affect gene expression of the colonic microbiota. The metatranscriptome was determined and compared to the metatranscriptome of the non-exposed negative control exposure without prebiotics thereby showing a significant difference. Expression of genes involved in xylene/xylose metabolic pathway in bifidobacteria was observed.

For functional validation, normal colon epithelial CCD 841 CoN cell line was tested to different concentrations of XOS mixture. Time and dose dependent response of the cell proliferation was determined by RTCA cell analysis system. The XOS concentrations with antiproliferative effect were identified. Effects on gene expression levels were also evaluated by microarray analysis, which required high bioinformatics performance by Gene Spring software program.

To confirm the microarray data, RT-PCR was performed from the same RNA samples and in addition, Western blot experiment was conducted (for GAPDH and BCl-2 proteins). The expression levels of the genes encoding the anti-apoptotic protein Bcl-2, antioxidant proteins GSTP1 (Gluthathione S-transferase-1), MRP1 (Multiple Drug Resistance Associated Protein-1) and NFE2L2 (Nuclear factor (erythroid-derived 2)-like 2) were determined for the different concentrations of XOS. The results obtained were in parallel with the microarray data. Western blot experiment data was also in concordance with the microarray results.

5. Application to food matrix and food grade assurance

A literature review was done for defining the main properties of XOS and Xylitol and how they could be affected by food processing. Xylooligossacharides (XOS) and Xylitol can interact with other compounds of the food matrix and modify their behavior and bioavailability .This was the premise to select the most suitable food matrices for adding XOS and Xylitol: Cookies (xylitol), Jam (xylitol and XOS), Vegetables Puree (XOS). The enriched food formulations were developed at pilot scale by replicating food industry conditions. The food formulations were characterised and the best concentration valued of each food matrix was developed in order to study its shelf life and the consumer acceptance.

The properties of XOS are depending on the degree of polymerization, long or short chain. In function of its length they would be more suitable for an application or for another. The most important parameters that have to be in mind at food processing in order to preserve the functionality of XOS are: temperature, pH, among others.

According to theses premises CTIC-CITA has been working on 3 prebiotic food matrix formulations: cookies, jam and puree of vegetables by testing different concentrations of prebiotic XOS and Xylitol. These three matrices were selected according to the analysis of scientific references.

The effect of XOS and Xylitol enrichment was studied from different point of views such as: physical-chemical, sensorial properties, prebiotic effect. After this characterization the best concentration of each food matrix was developed in order to study its shelf life. It was valued the evolution of XOS and Xylitol content, microbiological, sensorial shelf life and the evolution of the quality parameters along the whole shelf life.

In parallel, consumer preferences and acceptability of these prebiotic products were studied.

As a summary of all the results, it can be concluded:

- Both XOS and Xylitol are stable after the food processing and along 45 days and it could be recovered almost 100% of Xylitol and XOS.
- XOS improve the texture by providing more creaminess and by minimizing syneresis effect a long time. Besides, XOS acidified slightly the food matrices.
- Xylitol improves the texture by producing a juicy mouthfeel effect. However, the changes in the texture of cookies are not favorable and the cookies are softer, but acceptable.
- Xylitol in cookies is much oxidatevely stable than formula CTROL at low temperatures of analysis (140-150 ^{o}C). However, at high temperatures (180 ^{o}C) Xyilitol presents a pro-oxidant behavior.
- Sensorial: the most critical aspect is flavour, XOS provide a strong flavour that could determine the use of these compounds. It could be solved by looking for other food matrices where this flavour could be masked.

Jam enriched with xylitol/XOS and Spinach puree enriched with XOS were tested in the i-screen platform. As the xylitol and XOS are not digested by the human digestive enzymes, these food products were directly tested in the colonic microbiota. The i-screen platform containing a human colonic microbiota was exposed during different hours to jam with and without xylitol/XOS, spinach puree with and without XOS, inulin, XOS and the negative control without prebiotic. The 16S rDNA amplicon analysis results are compared to XOS and inulin exposures as positive controls and a negative control without prebiotic. Bifidogenic effects were observed as a consequence of the presence of XOS in the food products. However, pleiotropic effects of other food component affect the overall bifidogenic effect of the food as well as the colonic microbiome.

Description of potential impact and main dissemination activities and the exploitation results

Potential impact

Project LIGNOFOOD main impact is focused on helping SME participants in scouting new market niches and initiating strategic cooperation, which are the biggest challenges for SMEs. The identified LIGNOFOOD potential impacts for the consortium SMEs are the following:

- ENVIROHMEP will benefit from the know-how developed under LIGNOFOOD project regarding the optimisation of the hemp biomass pre-treatment. The company will be equipped for the production of xylan liquor in the scale of m^3 within the first semester after Project's End. Demonstration of economic feasibility of the Auto-hydrolysis process is a major target of the Pilot stage and will be validated during 2016. The company becomes potentially a commercial supplier (pioneer case in Europe) of xylan solutions to ISANATUR and other companies in the business of functional ingredients.
- ISANATUR SPAIN will implement the XOS and xylitol production process developed in LIGNOFOOD. This will bring to this company several benefits in terms of know-how, manufacture of emerging ingredients with optimized healthy properties, sales at high prices and market penetration. ISANATUR partner will benefit of direct access to a raw material that is currently scarce in Europe, if present at all.
- ZADE will be able to produce prebiotic ingredients from lignocellulosic sources such as hemp, in this way it will diversify its company portfolio.
- BIOREACTOR will gain expertise in fed-batch fermentation set-ups with the strains used in the project and the bioreactors design for future applications in the field of the biotechnology and food sectors. The company will be able to develop more expertise in bioreactors engineering issues for LIGNOFOOD new processes for XOS and xylitol production.
- HIDROLAB will extend its core engineering consultancy services by acquiring complementary skills. These skills are related to the design and coordination of boilermaker and welding workshops for the production of stirred, high-pressure vessels.

Besides the economic impacts of project LIGNOFOOD in the participant companies and throughout the European SMEs in the food ingredients and functional food sector, project LIGNOFOOD has positive environmental and societal consequences too.

In regard to the environmental impact, the use of hemp for the production of xilooligosaccharides (XOS) constitutes a new application for this crop, which will support the agriculture of hemp. Hemp agriculture has associated environmental benefits (agrochemical use reduction, CO2 reduction) that motivate the use of this raw material instead of others most commonly used for XOS production, such as corn.

A Life Cycle Assessment (LCA) of the Lignofood biotech process, from cradle to gate, was conducted according to ISO 14040. Hence, the environmental burdens of the production of XOs and xylitol within the whole value chain (from the agricultural production of raw material to the extraction of the bio-products) were estimated at two scales of production capacity (pilot and industrial plant) and for both, independent and combined/integrated production processes of

XOs and xylitol. The most critical steps and inputs were identified and measures of improvement were suggested in order to reduce the environmental footprint of both bio-products.

The Life Cycle Costing (LCC) of the process was also calculated based on both, operating costs (feedstock, energy, water and enzymes among others) and fix costs (investment, labour, maintenance, commercialisation, etc.).

Project LIGNOFOOD has a potential impact on health because the project results will increase the availability of healthier food solutions that will facilitate own health management and a more sustainable health care. Application of XOS and xylitol to new food product developments targeting consumers concerned in maintaining their gut health, and generally interested in healthier food choices (low glycemic index, low caloric, etc.) is a goal of the project.

Dissemination activities

Internal dissemination aims at making awareness within the consortium in order to accomplish the entrusted work and deadlines. The following activities have been carried out in the dissemination strategy: Internal seminars, Internal reports, Project meetings, Project website with restricted access, Mailing list, and other telecommunication tools.

External dissemination actions related to the project have been carried out too: Scientific publications, Project website, other website dissemination, Press releases, Social media, Conferences, Business meetings, trade shows and fairs, Networking with other national and EU initiatives, Networks and social platforms and outreach activities.

Exploitation of results

Expected project results emanating from project LIGNOFOOD and the exploitation strategy are summarized in the following table:

Partner	Short term interests	Long term interests
ISANATUR	Patent application of the XOS production process from hemp and purification Patent application of the integrated production process of XOS and xylitol Product Functionality assurance Functional food product formulation with XOS/xylitol Process implementation at a pilot-plant scale Small-scale production of prototypical products (xylitol/XOS)	Patents licensing Scale-up to industrial plant Product sales
BIOREACTOR	Project result 3 Partnering with potential end-user of the process being developed	Engineering services Equipment sales Attracting New clients

Partner	Short term interests	Long term interests
	Commercial agreements with ISANATUR and ZADE as the equipment provider for the pilot plant	Commercial agreements with ISANATUR and ZADE as the equipment provider for the industrial plant Commercial agreement with ISANATUR to point BIOREACTOR as the provider of equipment to lincensees of the patent owned by ISANATUR
ZADE	Biotechnological product formulation New process line in a new R&D investment New technology implementation New and innovative product addition to the current pharmaceutical product series New product development with XOS and xylitol Patent application of the XOS production process from hemp and purification, Process implementation of prototype products (xylitol/XOS) at pilot plant scale Hemp based functional food product development	Privileged conditions in the license to exploit the patents New investment for scale-up to industrial plant Product sales in new market
ENV	Project result 1, optimization for fragmentation process of biomass. Market exit for one fraction with actual low cost. Partnering with potential industrial and research agents for future developments	Scale-up to industrial plant Product sales Consolidate a Business Line related to market xylan for food purposes.

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