Abstract: In the technical field of Chemistry, encompassing products defined like FOS - fructooligosaccharides and applicable in food and or medical-pharmaceutical, veterinary and odontological industries, including the segment of supplements and functional foods, through the partial hydrolysis of inulins and similar fuctans till hydrossoluble FOS in the preferential range of DP - Degree of Polymerization from 2 to 18 or even more with reduced content of free fructose, using as catalysts for hydrolysis the citric and / or phosphoric acids as alternative to the classic hydrochloric or sulfuric acids and to the microbial enzymes, with the particularity that, through partial neutralization or simple dilution of the hydrolyzates, the catalysts now proposed, may either remain for the industrial and ulterior proposals or alternatively be removed by ion exchange resins or other means, being still possible that the citric or phosphoric acid may be conveniently diluted to the preferential range of pH 2.0 to 3.0 as moderate acidity and the hydrolysis be carried out at moderate temperatures in the range from 75°C to 90°C in the range of preferential times from 5 to 30 minutes.
"PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS"

The present invention deals with the utilization of citric acid C₆H₈O₇ and / or alternatively phosphoric acid H₃PO₄, routinely diluted and heated, as alternative catalysts to strong mineral acids such as hydrochloric or sulphuric or enzymes called inulinas and fructofuranosidas for the sake of the partial hydrolysis of inulin and similar polyfructoses aiming the preferential production of hydrolytic fragments named fructooligosaccharides – FOS with a reduced content of free monomeric fructose and a content even more reduced of degradation co-products such as hydroxymethylfurfural – HMF, a situation which is consequence from the choice of kinetic parameters for hydrolysis such as milder hydrogenionic potential or operational pH in the preferential range of pH 1.75 to 2.75 combined to milder temperatures below the ebullition of water, in the preferential range from 75°C to 90°C and heating times in the range from 5 to 30 minutes, starting from a concentration of substrate or polyfructose between 1g% and 60g%.

Fructose- or levulose-based polymers, sinoptically frutopolysaccharides, polyfructoses or fructans, may be classified into: a) inulins (or their low molecular variations – fructooligosaccharides or FOS – more properly designated fructooligomers) which occur in plants, algae, and bacteria where the linkage between the fructofuranose units is, essentially lineal, through β-2,1 links; b) levans which occur in bacteria and grasses whose inter-fructose links are β-2,6, and c) the subgroup of graminans, so said as consequence of their origin rather than the type of chemical structure, since they are highly ramified mixing both types of fructose links β-2,1 and β-2,6 and thus obtained solely from grasses. For the purpose of this invention request, using simpler language, the considerations and claims are based on inulins, since they are the best known polysaccharides and with well established industrial applications such as inulins from dahlia, chicory, yacon, asparagus, leek among other sources of increased inulin content. One particular structural feature of the molecule of native inulin or chemically integral inulin, and irrespective to the fructose units number, is the single glucose unit since the biosynthesis starts from a molecule of sucrose, namely a disaccharide of glucose and fructose. The literature
and the industrial language have been adopting the expression "FOS" or fructooligosaccharides for the oligosaccharides derived from inulin and constituted exclusively of β-2,1-linked fructose such as kestose (a frutotrisaccharide), nystose (a fructotetrasaccharide) and fructosylnystose (a fructopentasaccharide) although the term FOS, within the more correct acception from the chemical standpoint, must encompass other similar oligosaccharides such as those in which the single glucose unit remains attached and so linked to two or more fructose units or even a series of pure oligofructoses but linked by chemical unions from the β-2,6 type and hence originating from the hydrolytic fragmentation of levans. The molecular dimension of inulin or its simpler derivatives may be expressed as Degree of Polymerization – DP or GP in the Portuguese acronym, which corresponds to the number of fructose units or more properly to fructosyl residues. The DP of inulins varies between 10 and 60, and only exceptionally reaches 100. There is, however, the occurrence of DP as low as 5 or less, as it is the case of the population of fructooligosaccharids present in ripen bananas. The name inulin was adopted following the isolation of the polymer in the hot aqueous extract from the plant Inula helenium according to Rose, V., Über eine eigenthumliche vegetabilische Substanz, Gehlens Neues Allgem. Jahrb. Chem. 3, 217, 1804. It is very old – 1874 – the first report about the therapeutic properties of dahlia inulin, namely the reduction of free glucose in the urine of diabetics following the ingestion of 50 to 120 g of dahlia per day according to Külz, E. Beiträge zur Pathologie und Therapie der Diabetes, Jahrb. Tierchem., 4, 448, 1874. Differently from low DP inulins from plant origin, for instance 10 to 60, the DP of bacterial inulina may vary from 10,000 to 100,000 fructosyl units and with increased degree of ramification, in the range of 15%. A DP <40 is found for 83 and 94% of the inulin chains for chicory Cichorium intybus and Jerusalem artichoke Helianthus tuberosus, respectively. These two plants from the family Asteraceae or Compositae, together Dahlia spp. inulin, are the three main sources for the industrial processing of plant inulins. The content of inulin – wet weight - averages 17% in the two first sources whereas in dahlia the content is somewhat lower. Due to the β configuration between the fructosyl units, inulin is resistant to the hydrolysis by the action of hydrolases from human superior gastrointestinal tract and hence its classification as a non digestible fiber. Inulin is however completely hydrolyzed and fermented in the large
intestine. The catabolic fate of inulin is then as SCFAs 78% (Short Chain Fatty Acids) in the proportion of acetate : propionate : butyrate = 64 : 21 : 15, being 15% as lactate and 5% as ethanol, formate and carbonic anhydride. If computed the formation of the bacterial biomass, the fate of the generated fructose would be split into 40% of bacterial biomass, 40% of SCFAs, 15% of lactate, and 5% of minor subproducts. Being fermented in the large intestine rather than in the anterior digestive tract, inulin has a valid energetic content of only 37% from that displayed by fructose or only 1,5 Kcal/g or 6 KJ/g instead of 3,75 Kcal/g or 15,7 kJ according to Roberfroid, M. B., Slavin, J., Nondigestible oligosaccharides, Critic. Rev. Food Sci. Nutr., 40, 461–480, 2000 and Bergman, E. N., Physiol. Rev., 70, 567–590, 1990 as the energy contribution of volatile fatty acids from the gastrointestinal tract in various species still according to FASEB / LSRO “The evaluation of energy of certain sugar alcohols used as food ingredients”, Life Science Research Office, Federation of American Societies for Experimental Biology, Bethesda, MD, 1994.

The SCFAs and specially butyrate play an essential role in the integrity of the colon mucosa and in the proliferation and differentiation from epithelial cells. Inulin catabolism provokes a significant acidification of about 0,9 units in the ceccum and colon according to Sakata, T., Stimulatory effect of short chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fiber, gut microbes and luminal trophic factors, Br. J. Nutr., 58, 95–103, 1987. FOS – fructooligosaccharides with DP between 2 and 7 – besides free fructose – prepared by enzymatic hydrolysis of inulin are commercial products and they are named as Raftilose by Orafti Ltda in Belgium or Frutafit by Imperial-Suikner Unie in the Nederlands. Another FOS – frutooligosaccharides family, obtained by sucrose transfructosylation, with DP till 5, are the commercial products Neosugar, Profeed, Meioligo, and Nutraflora by Meiji Seika Ltd. from Japan and as Actilight by Béghin-Meiji Industries in Europe. FOS – fructooligosaccharides are recognized, in the USA, as GRAS – Generally Regarded as Safe. Besides being stimulatory of the colon bifidobacterial flora, FOS – fructooligosaccharides also prevent dental caries when they substitute dietary sucrose and are also implied in the reduction of serum cholesterol and triglycerides. FOS – frutooligosaccharides and more specifically the mixture of kestose, nystose, and fructosynystose, as represented in Figure 3, and
members of superior DP from the same series display about one third of the sweetener powder of sucrose but differently from the last one they are not considered caloric carbohydrates since their metabolism does occur exclusively at the end of intestine or colon where there is a bacterial microflora able to hydrolyze them, and recalling that FOS – fructooligosaccharides are not hydrolyzed by the human hydrolytic enzymes according Passos L. M. L. and Park Y. K.. Frutoooligosacarídeos: implicações na saúde humana e utilização em alimentos. *Cienc. Rural* vol.33(2), 385-390, 2003. Clinical studies have shown that the ingestion of FOS – fructooligosaccharides or inulin increases the population of colon friendly bacteria like those from the *Bifidobacterium* genus and in less extent the genus *Lactobacillus* while simultaneously there is a reduction of the bacterial population said potentially harmful or patogenic such as those from the genus *Clostridium* and other coliforms from the genera *Escherichia*, *Klebsiella* and *Enterobacter* according Gibson G.R., Beaty E.R., Cummings J.H.. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* 108 : 975-82,1995 and according to Rycroft CE, Jones MR, Gibson GR & Rastall RA. A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. *J Appl Microbiol.* 2001 91:878-87. Other benefits resulting from the ingestion of FOS – fructooligosaccharides or inulin are the increase in the production of short chain fatty acids like butyrate in the colon, enhanced absorption of calcium and magnesium, and improved elimination of toxic compounds according to Tomomatsu H.. Health effects of oligosaccharides. *Food Technology*, October: 61-5 1994. Concerning the butyric acid generated by bacteria fermenting FOS – futoooligosaccharides or inulin, it is worth mentioning that butyrate yields 70% of the energy of the colonic epithelial cells or colonocytes and without such an energy input colonocytes experience atrophy with the consequent loss of the integrity of colonic barrier of mucosa what allows the harmful translocation of bacteria, including those pathogenic according to Cummings J.H. & Macfarlane G.T.. Role of intestinal bacteria in nutrient metabolism. *Clin. Nutr.* 1, 16:3-11, 997. The butyrate, additionally, reverts the resistance to apoptosis or programmed death in cancerous colonic cells according to Bornet FR & Brouns F. Immune-stimulating and gut health-promoting properties of short-chain fructo-oligosaccharides. *Nutr Rev.* 2002 60:326-34 and increases the immunogeneity, and,
in combination with interleucin 2, causes a complete clearance of colon carcinoma induced in rats according to Perrin P., Cassagnau E. & Burg C.. An interleucin 2 / sodium butyrate combination as immunotherapy for rat colon cancer peritoneal carcinomatosis. Gastroenterology 107:1697-1708, 1994, being still worth of mention that FOS – fructooligosaccharides are also useful as prebiotics in the feeding of porks and poultry according to Fishbein, L., Kaplan, M., Gough, M. Fructooligosaccharides: a review. Vet. Hum. Toxicol., Washington, v.30 (2), 104–107, 1988. Bifidobacteria, once fed with these oligofructoses or oligosaccharides from fructose, secrete a peptide which is inhibitory for most of the pathogenic microorganisms that cause acute diarrhea according to Wang, X., Gibson, G. R., Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine, J. Appl. Microbiol., 75,373–380, 1993. The possibility of inulin and FOS – fructooligosaccharides preventing malignant neoplasias or cancer has been extensively tested in rats and mice pre-exposed to DMH - dimethylhydrazine or its alkanoyl derivative AOM – azoxymethane that targets the colon preferentially and where DNA is damaged, so generating the starting process of carcinogenesis. In this starting process, ACFs – aberrant cryptic dumpfts appear in the mucosa, and in the subsequent weeks, the tumors. As compared to controls, animals received inulin or oligofructoses as prebiotics or Bifidobacterium sp. or Lactobacillus spp. as probiotics or still the mixture of both, as symbiotics. The results have shown that inulin reduced the risk and quantitative occurrence of ACFs and long term tumors, and hence the colon cancer and in this case the inulin of higher molecular weight were more efficient, the symbiotics being even more efficient than the isolate administrations according to Magnuson, B., Carr, I., Bied, R. P., Ability of aberrant crypt foci characteristics to predict colonic tumor incidence in rats fed cholic acid. Cancer Res., 53, 4499–4504, 1993. Inulin solubility in water is limited to a maximum of 10% at room temperature but it increases significantly with heating while FOS – fructooligosaccharides solubility in water may reach 80%. Concerning the sweetener power, inulin is slightly sweet namely 10% of the sucrose value while FOS reach 35%. Inulin displays synergy with most of the jelly-forming compounds such as gelatin, alginate, k- and i-carragenans, gellam gum, and maltodextrins. Inulin improves the stability of foams and emulsions like in aerated desserts or mousse, ice
creams and sauces according to Franck, A., Coussement, P., Multi-functional inulin, *Food Ingd. Anal. Int.*, October, 8–10, 1997. Some of the food technological applications for inulin and FOS – fructooligosaccharides are: dairy products, frozen desserts, *pasta* and jellies, bread and similar, breakfast cereals, fruit-based products, meat, meat- and chocolate-based products. The pursued effects are body and palatability, foams and emulsions stability, substitution of sucrose, synergy with synthetic sweeteners, texture and melting point, retention of moisture, substitution of fats, and resistance to heat. The detection of the chromatographic profile of the subpopulation of inulins and oligofructoses (FOS) is carried out through liquid chromatography of high efficiency in ionic exchange columns and pulse amperometric detection, a technique established as HPLAEC-PAD. Within this method, total chicory inulin displays, besides glucose, fructose and sucrose, a total of 60 peaks. Conversely, the partial depolymerization of inulin, for example using citric or phosphoric acid as defended in this patent request, results in chromatograms as exemplified in Figures 6 and 7. The dietetic fibers are plant materials, most of them originating from cell walls and whose chemical nature are carbohydrates, which differ in physical, chemical, and physiological properties but which are not digested by human enzymes, but this may occur thanks to enzymes from the ceccum and colon microflora. Therefore, those fibers have the following properties: are natural components from edible plant cells; carbohydrates from the chemical standpoint; resistant to hydrolysis by human enzymes at the level of stomach and duodenum and jejunе; amenable to hydrolysis and partial or total fermentation in the colon according to Kritchevsky, D., Dietary fibre, *Annu. Rev. Nutr.*, 8, 301–328, 1988.

Despite the extensive establishment in the literature about the acid hydrolyses of polysaccharides, the strong acids hydrochloric HCl and sulfuric H₂SO₄ are in progressive disuse thanks to new hydrolytic tools produced by the modern Biotechnology, namely the hydrolytic and specific enzymes such as amylase, cellulases, hemicellulases, and pectinases, amongst other, despite their more elevated costs. There is, however, a recent report of the use of sulfuric acid for the hydrolysis of inulin in an autoclave – 121°C; 15 min – according Saha, B.C., Enzyme and Microb. Technology, 39(5), (2006) 991-995. With no additional help than the Merck Index – 10th Edition, hydrochloric acid HCl even in its more concentrated form is up
to 38% and in this condition may provoke severe burns besides the delivery of toxic vapor fumes, a fact that limits its utilization by man given the risk of ocular damage, dermatitis and photosensibility in the contact within the industrial environment. Another limitation, when the recovery of HCL is intended, is the formation of azeotrope with water, getting worse thanks to the elevation of the boiling temperature to 108.6°C due to the 20.2% HCl content. However, HCl is still a classic analytical reagent for the renitent peptide linkage of proteins, and curiously, when at pH around 2, it is an ingredient of human gastric juice, provided its action in this acidity range is found only in the stomach. In the same bibliographic source, the sulfuric acid H₂SO₄ allows concentrations in the range of 93 to 98%, and under these conditions, is extremely corrosive and deeply akin of water, what explains the facility for the dehydration and carbonization of wood and other biological substrates enriched in water and hydrogen, as it is the case of the polyfructoses. Pouring water on this concentrated acid allows to strong exothermic reaction and water mass ejection, reason by which it is recommended, when carrying this operation, an inverted operation, namely the careful and slow addition of acid on the water. Sulfuric acid is corrosive for all parts of the body and the inhalation of vapors, in the case of the concentrated acid saturated with the sulfurous anhydride which gives origin to the full acid, causes serious pulmonary damage, and the contact with the eyes may provoke complete loss of the vision besides the skin necrosis. Even the contact with diluted solutions, if frequent, have caused dermatitis. Due to the shortly reasons now exposed, the utilization of hydrochloric and sulfuric acid in the laboratorial and industrial environments asks for deep care. They are designed as strong acids due to the quick and complete dissociation of protons when in aqueous media, and hence they are not defined in terms of pKa(s):

\[
\text{HCl} \quad \rightarrow (\text{H}_2\text{O}) \quad \rightarrow \quad \text{H}^+ + \text{Cl}^- \\
\text{H}_2\text{SO}_4 \quad \rightarrow (\text{H}_2\text{O}) \quad \rightarrow \quad 2 \text{H}^+ + \text{SO}_4^{--}
\]

Thanks to these hydrogenionic strengthness, these acids, even in diluted solutions, provided there is no control of heating and time of reaction, promote the degradation of free sugars — and even more easily of fructose, a ketosugar — till furfural (case of pentoses such as arabinose and xilose) or till hidroxymethylfurfural — HMF (case of hexoses such as glucose, fructose, and mannose). For these reasons, they may be
utilized for the industrial preparation of hydroxymethylfurfural – HMF, namely a three times-dehydrated hexose, which is an important raw material for the chemical industries of aldehydes, glycols, ethers, aminoalcohols and acetals, but being HMF highly undesirable if present, as a degradation product, in the preparation of fructose or their fructooligosaccharides – FOS, when hydrolyses are carried with mineral acids. Herein there is no emphasis to the third common and strong mineral acid, nitric acid HNO₃, since its concentrated form accumulates and liberates the mutagenic and yellow-red vapors of the nitrogen dioxide NO₂ and nitrogen tetroxide N₂O₄, which are considered the most insidious gases, reason by which nitric acid upper concentration is only 65%. The present invention thus explores acid catalysts or proton donors more easily administered – the case of citric and phosphoric acids – however without loss of a particular hidrogenionic capacity to be explored for the hydrolytic breakage of more labile polysaccharide links as it is the case of polyfructoses inulins and similar levans. This is exactly the fundament of our inventive activity or innovation act, provided the establishment of ideal conditions for the main objective – production of FOS – fructooligosacchrides - may be attained through the careful manipulation of the remaining kinetic parameters such as effective pH of each acid during the hydrolyses, heating temperature, and time of reaction.

Citric acid C₆H₈O₇ is a product of industrial fermentations using selected starins of the fungus *Aspergillus niger* and it can be also extracted from fruits like orange and apple, corresponding to a triacid with pKₐ1 = 3.13, pKₐ2 = 4.76 e pKₐ3 = 6.40, namely, sequentially dissociating 3 protons per each molecule under dissociation, the first of them more acidic according to the following simplified equation:

\[
\text{HOOC-CH₂-CH₂-COOH} \rightarrow \text{OOC-CH₂-CH₂-COO⁻} + 3 \text{H⁺}
\]

Citric acid is also widely found in the plant and animal kingdoms once it is the entry metabolite for the Krebs or tricarboxylic acid cycle which is present in the
metabolism of almost all live organisms according to any Biochemistry text book or the universal knowledge. As pure substance it is a crystalline solid and at 20°C is 59.2% soluble in water, a value which further increases to 84% in boiling water. According to the Merck Index, diluted solutions of citric acid are pleasant to taste and hence its indication as acidulant for foods, beverages, medicines, co-reagent of effervescent tablets and even as synergistic anti-oxidant and in a even better application in the co-formulation of fructooligosaccharides – FOS, as defended in this request of invention, since this citric catalyst does not deserve compulsory removal after inulin hydrolysis. Conversely, acid hydrolyzates from more common polysaccharides such as starches, when utilizing sulfuric or hydrochloric acids, usually demand a surplus step, namely the removal of the acid string catalyst either as insoluble precipitates (e.g., calcium sulfate for the sulfuric acid) or through de use of anion exchange resins (e.g., case of sodium chloride for HCl).

Phosphoric acid, more precisely orthophosphoric acid H₃PO₄, is a commercial product with a net content of 85% w/v, density between 1.69 and 1.71, syrupy, stable, relatively inert at room temperature, and corresponding to a triacid with pKₐ1 = 2.15, pKₐ2 = 6.82 e pKₐ3 = 12.38 as logarithmic values from the dissociation constants for their hydrogens as protons, namely, it also liberates, like citric acid, 3 sequential protons for each molecule in aqueous media:

\[
\begin{align*}
H₃PO₄ & \rightarrow H^+ + H₂PO₄^- \\
H₂PO₄^- & \rightarrow H^+ + HPO₄^{2-} \\
HPO₄^{2-} & \rightarrow H^+ + PO₄^{3-}
\end{align*}
\]

The Merck Index attributes a pleasant acid taste to phosphoric acid when conveniently diluted, namely, like the citric acid, it displays organoleptic properties compatible with beverages, medicines, and foods, reason by which the main author of this invention (J.D.Fontana) already made use of phosphoric acid for the controlled hydrolysis of starches pursuing either glucose or maltooligosaccharides as it is detailed in the Brazilian patent request to INPI (National Institute of Industrial Property) PI 0002001-0 published on Revista Propriedade Industrial, number 1617, from January 2, 2002, page 132. This procedure obviously corresponds to another type of substrate than inulins (as well as to other hydrolytic products) and encompasses the application of much more severe kinetic parameters since starch is
by far more resistant to acid hydrolysis than inulins. Salts of phosphoric acid are widely spread in living beings under the form of nucleic acids, coenzymes and phosphorylated sugars, besides mineral phosphates as such, which assimilated by humans, animals, and microorganisms from several plant sources since they constitute the main buffering system for blood and plasma. There is no attribution to phosphoric acid, even in its concentrated form, of the risks and damages previously appointed to sulfuric and hydrochloric acids, although the concentrated solution may irritate skin and mucosa. The processing of phytobiomass polysaccharides such as inulins, as herein defended with citric and/or phosphoric acids, is thus advantageous since once finished the hydrolysates, these catalysts do not deserve necessary removal as is the case of hydrolyzates obtained with sulfuric or hydrochloric acids. It may be recalled that the citric and phosphoric acids are acceptable and widely used as acidulants and/or flavor increasers as reported in the Merck Index, 10th edition, entries 7228 and 2297, and pages 330 and 1059, respectively. In fact, one may found citric or phosphoric acids or their salts in commercial foods such as the yogurts Original from Yoplait USA Inc., from Minneapolis, Minnesota, USA and Creamy Fruit Blends from Dannon Co. Inc., USA which are sold in the United States and even in national dental creams like Sensitive and Prevent Anti-Plaque both from Colgate-Palmolive Ind. & Com Ltd., from Sao Bernardo do Campo, Sao Paulo, Brazil.

Concerning the hydrolysates of inulin and similar polyfructoses, these may be partial or totally hydrolyzed till fructose and inulooligosaccharides, respectively, utilizing two alternatives: A. enzymatic hydrolysis, and B. acid hydrolysis. For the sake of enzymatic hydrolyses the most employed inulinases or β-fructofuranosidases are those obtained from de mold genus *Aspergillus*. Given the mechanisms for the enzymatic reactions and the nature of the hydrolysis products there are two types of inulinases: exoinulinases which successively release residues of fructose, and hence hydrolyze the molecule of inulin randomly, generating inulotriose, inulotetraose, and inulopentaose, and the subsequent series, namely fructooligosaccharides – FOS as main hydrolysis products. The mold *Aspergillus*, for example, produces the two types of inulinases (exo- and endo-inulinases) according to W. Jing, J. Zhengyu, J. Bo and A. Augustine. Production and separation of exo- and endoinulinase from *Aspergillus*
ficuum, Process Biochemistry 39 (1), 5-11, 2003. When there is no interest in the production or co-production of free fructose, the enzymes of such mold require a pre-step of purification of the desirable endo-inulinases according to J. Zhengyu, W. Jing, J. Bo and X. Xueming. Production of inulo-oligosaccharides by endoinulinas from Aspergillus ficuum. Food Research International 38 (3) 301-308, 2005. Conversely, when fructose is the target free sugar, the exo-inulinases are then subject to pre-purification like in the case of Aspergillus fumigatus to increase also their thermostability according to Gill P.K., Manhas R.K. and Singh P. Hydrolysis of inulin by immobilized thermostable extracellular exoinulinas from Aspergillus fumigatus. Journal of Food Engineering 76 (3): 369-375, 2006. The industrial production of enzymes, and furthermore their isolation and/or purification are high cost-procedures as matter of the universal knowledge since it deals with a noble and less stable molecular population, namely proteins with enzymatic activity. Since these procedures involve selection of microorganisms, genetic manipulation or even transgenesis, costs of the final products, the enzymes, are proportionally elevated and hence by far higher than those for mineral and simple catalysts, although citric and phosphoric acid are really innovations as defended in this patent request. As an example, the company Sigma-Aldrich, one of the world leading company for several reagents, practiced, in June 2007, the following prices for 3 products produced by its associate BioChemika Ultra: US$ 107.50 / 50 mg of crude powder of inulinases from Aspergillus niger containing 25 units of enzyme / mg; US$ 18.70 for 50 g (a 1.000 times bigger mass) of 99.5% pure citric acid; and only US$ 38.10 for 250 mL of 85% phosphoric acid. Another source of inulinases are the yeasts and mainly those from the genus Kluyveromyces. In K. marxianus the secreted inulinases content may be increased by 5-6 times when using caproylated inulin as inducer according to Fontana J.D., Baron M., Diniz A.C.P., Franco V.C. "Microbial inulinas secretion using chemically-modified inulins". Applied Biochemistry and Biotechnology 45-6: 257-268, 1994 as reported by the main author of the present patent request. The use of mutants of this same yeast also ensures an increased inulinas activity according to Campos, D. et al (1990). "A multipotential hydrolytic reactor using the yeast Kluyveromyces marxianus". Appl. Biochem. Biotechnol. 24:25, 511-519. The third source of inulinas are the bacteria. From 32 isolates from soil, 20 have been
characterized as Flavobacterium multivorum as producers of inulinas or β-fructofuranosidases active either on inulin or sucrose according to Allais, J.J., Kammoun, S., Blanc, P., Girard, C. and Baratti, J.C. Isolation and characterization of bacterial strains with inulinas activity. Applied and Environmental Microbiology 52 (5), 1086-1090. Another source of enzymes able to hydrolyze fructans, the levanases, is the genus Bacillus. The enzyme from B. subtilis has been cloned and overexpressed in Escherichia coli, maintaining the hydrolytic capacity on levan, inulin, and sucrose according to Wanker, E., Huber, A. and Schwab, H. Purification and characterization of the Bacillus subtilis levanases produced in Escherichia coli. Applied and Environmental Microbiology 61(5), 1953-1958, 1995. An alternative for the preparation of FOS – fructooligosaccharides other than the partial acid or enzymatic hydrolyses of inulin, is the transfructosylation by means of enzymes. For this, the enzyme transfructosylase from Aspergillus oryzae is incubated with a high concentration of sucrose in the range of 60%. The low water activity allows residues of fructose to migrate to other molecules of sucrose thus generating preferentially tri- and tetrasaccharides from the type kestose (G1-F2; monoglucose-difructose) and nystose (G1-F3; monoglucose-trifructose) according to Sangeetha P. T., Ramesh M. N. and Prapulla S. G.. Fructooligosaccharide production using fructosyl transferase obtained from recycling culture of Aspergillus oryzae CFR 202. Process Biochemistry 40(3-4),1085-1088, 2005. However, the step of preparation of transfructosylase is also of high cost. A product from the hydrolytic action of microorganism enzymes completely different from fructose and their oligosaccharides also may be obtained using bacterial enzymes from the genus Arthrobacter and from another taxonomically uncharacterized genus, although differentiated by the RAPD-PCR Random Amplification of Polymorphic DNA – Polymerase Chain Reaction. Such hydrolysis product is the DFA III or difructose anhydride III or the lowest DP FOS but under a dehydrated form. It is the case of inulobiose, a disaccharide, which, in the hydrolysis reaction, undergoes a dehydration between the two fructose residues according to the report of one of the authors of the present patent request as in Fontana J.D., Rogelin R., Kaiss J., Hauly M.C.O., Franco V.C., Baron M. PCR protocol-based and inulin catabolism-based differentiation of inulinolytic soil bacteria. Applied Biochemistry and
Biotechnology 45-6: 269-282, 1994. These fructose anhydrides, of less sweetener power than free fructose, may also arise as secondary products during the acid hydrolysis of polyfructoses or inulins, depending on the severity of the kinetic parameters.

Sucrose is extremely labile to acid hydrolysis according to J. O’Brien, Journal of Food Science 61(4) (1996), 679-682 and hence it is a model of acid lability to inulins, a string of successive β-fructosyl units. Sucrose, in fact, is the initial disaccharide of any integral inulin molecule. Hence, from the standpoint of innovation in the field of acid hydrolysis of polyfructoses like inulins, it is advisable to think about the utilization of milder mineral acids as catalysts, and is exactly this possibility what is considered in this patent request when using citric or phosphoric acid as catalysts. Concerning the acid hydrolysis of inulin the available literature is somewhat scarce due to either the advances of the biotechnological pathways or due the high lability of free fructose to acids thus generating HMF – hydroxymethylfurfural thanks to a triple dehydration of free fructose what is easier than with free glucose according to Antal, M.J., Mok, W.S.L. and Richards, G.N. Mechanism of formation of 5-(hydroxymethyl)-2-furaldehyde from fructose and sucrose. Carbohydrate Research 199(1), 91 - 109, 1990. Given this unconvenience in the action of strong catalysts such as sulfuric and hydrochloric acids, the alternative applying milder hydrogenionic acids such as the citric and/or phosphoric is desirable and innovative. Still dealing with the state-of-art, the strong hydrolysis of Jerusalem artichoke inulin was carried out looking for the production of inulobiose according to Dickerson A. G. and Moor J.. Purification of inulobiose obtained by acid hydrolysis of inulin. Carbohydrate Research 39(1), 162-163, 1975. The same substrate has been the target of acid hydrolysis pursuing the production of ethanol according to Kim K. and Hamdy M.K.. Acid-hydrolysis of Jerusalem-artichoke for ethanol fermentation. Biotechnology and Bioengineering 28(1): 138-141, 1986. Hydrolyses with various acids, resins, and with inulinases from Saccharomyces fragilis were comparatively applied to Jerusalem artichoke pursuing the production of ethanol according to Fleming S.E. and GrootWassink J.W. Preparation of high-fructose syrup from the tubers of the Jerusalem artichoke Helianthus tuberosus L. CRC Crit Rev Food Sci Nutr.12(1):1-28, 1979. The
enzymatic method resulted in lower HMF content and color. Alternatively to the
conventional acid hydrolyses, inulin from Jerusalem artichoke or from chicory may
be transformed into fructose and HFCS – High Fructose Corn Syrups, through
percolation of in columns filled with strong cation exchange resins combined to the
heating of the acidic eluates according to Yamazaki, H. and Matsumoto, K..
Production of fructose syrup United States Patent 4,613,377; September
23, 1986. Another alternative of inulin hydrolysis till fructose is the utilization of
zeolites or complex silicates. According to the authors the hydrolysis is preferential to
release free fructose with a minimal co-production of degradation products according
then to Abasaeed A. E. and Lee Y. Kinetics of inulin hydrolysis by zeolite LZ-M-8.
Hung. J. Ind. Chem. 24, 149-154, 1996. It is not the objective of the present patent
request the production of fructose syrups from inulin or levan. What is intended and
in fact already consolidated by us is the innovative and preferential production of
larger fragments of inulin, namely, the FOS – Fructooligosaccharides, using citric and
/ or phosphoric acid catalysts. In the case of any exceptional co-production of fructose
due to the severity of one or more kinetic parameters, hydrolyzates will require the
post-treatment with activated charcoal or other “color” absorbents in order to remove
the parallel co-product, HMF or hydroxymethylfurfural as the dehydration of part of
the free fructose will be unavoidable. Taking in account all considerations already
exposed for both strong and milder acids, it is obvious, adopting the same kinetic
condition of pH or acid concentration, that the co-generation of HMF will be greater
in the case of sulfuric or hydrochloric acids as compared to citric or phosphoric acids.

Still regarding the state-of-art and more particularly regarding the
applications of FOS – fructooligosaccharides, according to the US Patent 5,843,922,
a special nutrition medium for bifidobacteria has been prepared when submitting a
carbohydrate substrate containing a fructosyl unit to the action of rapid flow and
thermolysis in the presence of various mineral and organic acids. Such a substrate is
identified as sucrose but there is also mention to fructose, raffinose, and staquiose, the
second latter being oligosaccharides containing glucose, galactose, and fructose.
There is no mention to inulin or other fructans as substrates. Regarding acid catalysts
for the step of transfructosylation – a technique opposed to hydrolysis – which
converts sucrose – but not inulin – into oligofructoses such as kestose and nystose,
there is mention to citric, tartaric, benzoic, lactic, and phosphoric acids, besides potassium and sodium salts of phosphoric acid. The ideal temperature of such process of transfructosylation is 125°C to 145°C – by far above the ebullition of water and even more above the ideal range of temperature defended in the present patent request – besides an additional step of adding crystalline sucrose to the reaction medium. Given all these conditions there is no parallel between that US Patent and our proposal. In fact, in the US Patent 5,843,922 the preparation of the substrate is better described and compared to the popular practice of “sweet cotton”, which ensures anhydrous sugar, a lower melting point and hence less degradation in the time course of the reaction. As summarized by the authors, the proposed pyrolysis of sucrose leads to a mixture of fructosylated products which includes 1-kestose, 6-kestose, neokestose, fructooligosaccharides and anhydride of fructose according to Whistler, R. L. and BeMiller, J. N. Preparation of oligosaccharides and products therefrom. *US Patent* 5,843,922, December 1, 1998. Still progressing with a specific bibliographic search, FOS – fructooligosaccharides were incorporated to a solution of artificial sweeteners such as aspartame or acelsulfame K, and other plant inputs such as tea, maintaining the pH in the range 3.0 to 3.5 thanks to the addition of phosphoric, citric or malic acids. After 4 weeks at room or controlled (32.2°C, 21.1°C or 4.4°C) temperatures the residual percentages of inulin, FOS, and fructose were measured.

The slow and progressive hydrolysis of fructosylated substrates and the emergence of accumulated free fructose ensure the sweetener power of the beverage according to Aldrich J. A.; Hanger L. Y.; Ritter G., *US Patent* 6,713,116, March 30, 2004. This case – different from that postulated by us – has as scope the generation of fructose, in long term (weeks) under low temperatures, as the main product. This kind of optimized preparation of fructose from inulin, excluding or minimizing the co-production of HMF – hydroxymethylfurfural and DFAs – difructose anhydrides, is also possible utilizing diluted solutions of phosphoric through boiling at pH 2 as reported by the main author of this patent request as in Hauly M.C.O., Bracht A., Beck R and Fontana J.D. Fructose and fructose-anhydrides from dahlia inulin. *Applied Biochemistry and Biotechnology* 34-5: 297-308, 1992. In accordance to researchers from the Taualipas University, Mexico, the monomer fructose may also be obtained from polymeric inulin from agave *Agave Americana* through hydrolysis
with sulphuric acid at 1% and 100°C. The same group of researchers, in the subsequent year, examined the effect of the concentration of phosphoric acid, the temperature, and time of hydrolysis to obtain agave oligosaccharides. They concluded that phosphoric acid, in the kinetic conditions employed, was not necessary since a maximum of oligosaccharides was obtained by simple heating (water) of agave juice at 80°C for 90 minutes, a completely different condition from our patent request using citric or phosphoric acid, according to Ayala, R. C. G., Jacques, C., Leon, J. A. R.. RESPYN – Revista de Salud Pública y Nutrición, edición especial 1-2004; http://www.uanl.mx/publicaciones/resryn/especiales/ee-1-2004/51.htm; access on July 5, 2007 and according to Gomez-Ayala R. C., Ramirez J. A., Jacques C., Vázquez M. and Téllez- Luis S. J. Extraction of inulin oligosaccharides from Agave Americana. 2005 IFT - Institute of Food Technologists, July 15-20 - New Orleans, Louisiana, poster 54E-10 and http://ift.confex.com/ift/2005/techprogram/paper_28819.htm; access on July 5, 2007. Our laboratorial experimentations, partially illustrated in the designs and figures presented and explained below, have demonstrated the opposite, namely: the careful control of the kinetic parameters (concentration of substrate, effective pH of hydrolysis for the citric or phosphoric acids, temperature of hydrolysis, and time of reaction) may efficiently modulate the production of FOS – Fructooligosaccharides from inulins, also ensuring fructose as non-dominant co-product and trace amounts of HMF. Furthermore, although HMF – hydroxymethylfurfural is an undesirable co-product for most of the industrial applications of FOS, there are cases where its presence along with fructosugars is highly wanted as the case of Balsamic Vinegar from Modena according to Theobald, A., Muller, A. & Anklam, E., Determination of 5-hydroxymethylfurfural in vinegar samples by HPLC, J. Agric. Food Chem., 46(5), (1998), 1850-1854. Still regarding the state of the technique when using phosphoric acid, it is worth mentioning its use to selective hydrolysis of the hemicellulose fraction from the whole ligno-(hemi)-cellulose complex from sugar cane and sorghum bagasses as well as the partial hydrolysis of cassava starch for the production of maltooligosaccharides as result of the conditions of the thermopressurization of aqueous - < 1% - phosphoric acid as reported by the main author of this patent request as in Fontana J.D., Correa J.B.C., Duarte J.H., Barbosa A.M., Blumel M. Aqueous phosphoric-acid hydrolysis of

Concerning the state-of-art under a wide concept, namely the vision of FOS – Fructooligosaccharides like a food product and nutritional or medicinal tool with aggregated value, the data bank of INPI – The National Institute of Industrial Property (Brazil) registers the Patent PI0404152-6 dealing with a food based in soy and FOS; the Patent PI0401407-3 dealing with a mixture of seven fibers, one of them being inulin and/or FOS; the Patent PI0202602-3 covering the production of FOS – fructooligosaccharides from sucrose with enzymes from yeast; the Patent PI0115311-0 referring to a nutritional composition for a better immunological condition and based on vitamins, salts, inulin and FOS; the Patent PI0108828-9 dealing with a prebiotic for the increase of immunological defense, preferentially a FOS; the Patent PI9609619-5 involving the use of FOS and analogues based on other common oligosaccharides arising from xylose and galactose, namely XOS and GOS, for the treatment of medium otitis; the Patent PI0108341-4 referring to inulin products with improved nutritional properties being one fermentable and other not; the Patent PI9913658-9 dealing with inulin fractions of different molecular weights; the Patent PI9902934-0, a process for the preparation of inulin from chicory and their products and the Patent PI9700887-7, sinoptically converting the preparation of inulin derivatives. There is no mention in these 10 patent requests to citric or phosphoric hydrolyses of inulin for the obtention of FOS – fructooligosaccharides, what, obviously, does not generate any conflict with the present patent request, namely the refinement of the utilization of non conventional mineral and/or organic acids, also compatible with the human and animal metabolism and whose main scope is the preparation of FOS – fructooligosaccharides from inulin and similar polyfructoses. Under the same focus, the USPTO – United States Patent and Trade Office registers the US Patents 5,127,956 and US 5,254,174 sequentially dealing with the method of preparation of a mixture of carbohydrates containing fructose, glucose and fructooligosaccharides – FOS from aqueous extracts of Jerusalem artichoke and chicory, adjusting the pH with carbonic anhydride or phosphoric acid after the alkalinization with lime Ca(OH)₂ and physical operations such as filtration and
hyperfiltration but without any alteration of the native chemical structures, namely, without intention or mention to hydrolytic processes – intention which is clear in our patent request – and still the extraction of tubercles of Jerusalem artichoke or chicory roots pursuing the native inulin and their natural FOS, both populations being then separated by physical means. The Patent US 4,613,377 deals with the preparation of fructose-enriched syrups and FOS – Fructooligosaccharides but using cation exchange resins, namely, other type of catalysts than citric or phosphoric acid. The US Patent 7,084,131 covers the preparation of polydisperse polysaccharides treating inulin with enzymes or combined with citric acid, but with or without milder heating between 10°C and 41.1°C and hence without intention of inulin hydrolysis due to the extremely low temperature. The intention is to prevent the flocculation or clumping of native inulin thanks to the presence of the cold acid. The US Patent 6,982,093 just explores the liberation of fibers under the form of a tablet of chewing gum of controlled viscosity. The US Patent 6,855,358 describes the process of preparation of a sterilized beverage with previous ozonization, added by a terpenoid which activates the adenylate cyclase, and by a nutraceutical, in the case, one FOS and also hidroxi-citric acid, namely the formulation of distinct ingredients, without any hydrolytic intention. The US Patent 6,461,650 deals with the preparation of beverage supplements, these ones with the addition of citrate and / or calcium phosphate, and optionally one fructooligosaccharide ensuring a final pH between 2.5 and 4.5 in the previously referred drink. There is no mention of the words “hydrolysis” or “heating” - a situation completely different of our request - where hydrolysis and heating are conclusive remarks. Conversely, US Patent 5,334,516 corresponds to a method for the preparation of ramified FOS – Fructooligosaccharides utilizing enzymes, namely biotechnological tools.

In our patent request, the utilization of acids with moderate potency – ensuring partial hydrolysis through the modulation of the kinetic parameters such as acid pH, temperature and time of hydrolysis – has as an intentional scope the fragmentation of the native inulin molecule till simpler subfractions or FOS – Fructooligosaccharides, either according to their molecular dimension – DP between 2-3 to 18 or more, or ensuring complete hydrossolubility, what obviously also increases the sweetening power which is practically null in the
native inulin. Since fructose sweetening power is ca. 70% superior to that of sucrose, FOS sweetening power is intermediate between those values for inulin and fructose.

In fact, it does not exist any correlation or overlap between our patent request and both the national and foreign patent requests mentioned above. In other words, the partial hydrolysis of inulin and other polyfructoses, with the intention of the production of smaller fragments (2-3 to 18 monomeric units) is original thanks to the selective choice of the catalysts, citric or phosphoric acids. Moreover it offers innovation in this field of knowledge since this technology also offers the concomitant utilization of the catalysts, besides the qualitative guarantee of already known FOS – Fructooligosaccharides, whose industrial application is well consolidated. One initial advantage of the process of hydrolysis herein described is the possibility of working with a wide and flexible range of substrate from 1% to 50% or just in the limit of the polysaccharide wetting with the dilute acid(s) since in the first minutes of heating inulin already experiences a dramatic solubilization in the reactional water medium. Another advantage of utilization of citric or phosphoric acids for the hydrolysis is their permanence in most of the hydrolyzates as such, after dilution, and/or after partial or total neutralization with weak (ammonia; lime; zinc oxide or hydroxide) or strong (potassium, sodium) hydroxides. In the first case – hydrolyzates dilution – citric or phosphoric acids will act as taste (sweetening) enhancers. In the second case – neutralization of hydrolyzates – the resulting mixture with the essential triad for fermentation C,N,P (FOS and ammonium phosphate, for instance) is already done. Salts of citric or phosphoric acids, as examples, are ordinarily present – along with fructose or HFCS or FOS – in commercial products such as yogurts like “Original” from Yoplait and “Creamy Fruit Blends” from Dannon, as one of us (J.D.Fontana) supermarkets found them in San Diego, California, in December, 2004. In both mentioned yogurts, the sweetening power is ensured by the presence of HFCS – High Fructose Corn Syrups, besides calcium phosphate, citric acid, and/or sodium citrate. The formulation of these products with FOS replacing HFCS would be so undoubtedly advantageous as proposed in this patent request. It is also worth stating that the medicine “Emetrol” from McNeil-PPC, Inc. / Pfizer which is based in a mixture of fructose, glucose, and phosphoric acid to which the cure of nauseas from stomach irritation is attributed (http:// www.
pfizer.com/product.aspx?id = 408; access on July 6, 2007). Furthermore, natural sources of citric acid and citrates such as orange, lemon, an orange are recognized having corrective action even for envenomation with uranium according to Ortega, A. et al., Treatment of experimental acute uranium poisoning by chelating agents. Pharmacology and Toxicology 64 (1989) 247-251. In fact, phosphoric acid and their salts are present, like chemically bound components, in the noblest human molecular population: the DNA and RNA (nucleic acids), as activator for several sugars (glucose-6-P; fructose-bi-phosphate), besides the more important coenzyme of human body, ATP - Adenosine TriPhosphate, the cell "energetic coin". Parallely, citric acid as its anion citrate, is the feeding metabolite for the Krebs cycle, and practically present in all living beings. These short considerations and the healthy and "body-friend" behavior of the citric and phosphoric acids, specially when buffered by the corporal liquids, are available in any Biochemistry textbook, and for instance, in Stryer, L. Biochemistry, W.H. Freeman & Co., New York, 3rd Edition. This circumstance, namely the direct utilization of the phosphoric or citric catalysts is effectively innovative as compared to the hydrolyses carried out with the classical and stronger sulfuric or hydrochloric acids since the latter require a post-hydrolysis treatment for their removal as insoluble salts (e.g., calcium sulphate) or through ion exchange resins (e.g., HCl -> NaCl). The same circumstance is not – obviously-derived from the state-of-art since the authors of the present request are also exploring the extreme lability of inulin employing milder acids such as phosphoric and citric. In fact the inventiveness concerning one of the catalysts – phosphoric acid – has been already explored by us in the Brazilian Patent INPI 000-2001-0, J.D.Fontana, "Phosphoric hydrolysis of starches" although under more severe kinetic conditions. Fructose from inulin may be obtained by the action of exoinulinases or strong acids, in the second latter case with the inconvenience of the formation of appreciable amounts of the undesirable co-product HMF or hydroxymethylfurfural. Conversely, the production of FOS may be attained – partially – with the use of endoinulinases, an expensive alternative - or even with milder sulfuric or hydrochloric hydrolyses but with the inconvenience of a surplus step of catalyst removal. However, the production of FOS – with less or no HMF at all – and without the need of catalyst removal is only attainable with the proposed milder acids,
phosphoric or citric {or any other organic acids with the appropriate pKa(s)}. And this fact is beyond the actual state-of-art or known techniques since both acids are "body friend" and completely physiological to human and animal beings, as well as for useful industrial microorganisms. This new methodology or technology – phosphoric or citric hydrolysis of inulin – is thus not evident from the state-of-art. The potential of the new methodology for several industrial applications is clearly evident. FOS or FrutoOligoSaccharides – with or free from phosphate or citrate – are interesting substrates for a larger range of industrial applications. Concerning some of these industrial applications, FOS are ideal substrates for the beneficial human (and animal) microflora composed of Bifidobacterium spp. e Lactobacillus spp, since the formers (as prebiotics) reinforce the natural flora of the mentioned probiotics when administered in the form of special foods (e.g., yogurts) and beverages lightly sweetened. Additionally, since FOS – obtained through phosphoric or citric hydrolyses – contain some content of free fructose they are pleasantly sweet in order to be employed as artificial sweeteners even in odontologic products without the unconvenience of provoking caries as the case of glucose syrups. Concerning the improvements and advantages in relation to the existent techniques such as enzymatic hydrolyses, the preparation of FOS or fructooligosaccharides with phosphoric or citric acids are by far more economic since the procedure is simpler and the catalyst are less costly. There is no doubt (e.g., http://en.wikipedia.org/wiki/Coca-Cola_formula; July 3, 2007) that the formula or industrial recipe for the most famous soft drink of the world – Coca Cola or Coke – includes harmless acidulants like the phosphoric acid (actual substitute for the citric acid), besides sweeteners like HFCS – High Fructose Corn Syrups or the cheaper sucrose in 3rd World countries. In fact, the label of "Coca Cola Classic 12 oz" commercialized at San Diego, California on May, 2007, indicated, besides other ingredients, the phosphoric acid and HFCS. FOS or Fructooligosaccharides from phosphoric or citric hydrolyses could be an interesting and new alternative.

From the bibliographic material and arguments herein presented, it may be deduced that the utilization of phosphoric and citric acids for the partial depolymerization of inulin (and related polyfructoses) to hydrossoluble FOS with DP – Degree of Polymerization form 2-3 to 18 or more as concisely explained in
“CLAIMS 1 to 15” is not evident from the state-of-art, thus contemplating the requirements of originality, innovation, and industrial application. Furthermore, taking into account the factor economy of process as determinant for the industrial application, for the obtention of pH 2 or 3 namely the more productive phosphoric or citric hydrolysis to FOS it is enough to add only 0.05 mL of pure phosphoric acid (85% w/v) or 0.35 g of pure citric acid to 1 liter of reactional medium. The respective pHs (in pure water) are 2.45 and 2.52. The respective amounts when using a solution of 5g% of purified inulin rises to only 0.4 mL of phosphoric acid and 1.4 g of citric acid. These moderate proportions can be further reduced to a ratio acid catalyst : inulin simply adopting effective pH of hydrolysis in the range of 2.75 and enlarging the reaction time or slightly increasing the temperature. In the same way, taking advantage of the normal conditions in the industrial environment, namely high availability of steam, hydrolyses may be carried in the temperature of water ebullition thus economizing the acid catalyst input through pHs in the range from 3 to 4.

From the advantages herein appointed on behalf of phosphoric and citric acids – safe and part of the human metabolism – as the most important fundament for the present patent request, it is obvious that a series of other organic and inorganic acids – equally safe and metabolically significant – may also be used for the partial hydrolysis of inulin provided their pKa1 are in the useful range as explained in the following Table. They also add strategic, energetic, and metabolically useful anions to the produced FOS from inulin. Amongst them are the acids other than citric and also belonging to the previously mentioned cycle of Krebs: α-ketoglutaric, isocitric, fumaric, malic, oxaloacetic, and succinic. Other acids, also advantageous, and pertaining to other metabolic pathways of the living beings are: glyceric, gluconic, glucuronic, glutaric, lactic, malonic, pyruvic, tartaric and even the carboxylic acids from the series acetic, propionic and butyric or hidroxi-butyric. Still able of inclusion in this large set are 2- and 3-glycerophosphorics, and pyrophosphoric H4P2O7 acids, the latter, in fact, being converted into phosphoric acid when dissolved in hot water. For the sake of better understanding of this enlarged proposition for the partial hydrolysis of inulin, such alternative acid catalysts are ordered in the following Table according their values of pKa(s), the key physicochemical parameter for such kind of mild hydrolysis:
<table>
<thead>
<tr>
<th>ACID</th>
<th>pKa or pKa₁</th>
<th>pKa₂</th>
<th>pKa₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>phosphoric</td>
<td>2.15</td>
<td>6.82</td>
<td>12.38</td>
</tr>
<tr>
<td>citric</td>
<td>3.13</td>
<td>4.76</td>
<td>6.40</td>
</tr>
<tr>
<td>(pyrophosphoric</td>
<td>0.9</td>
<td>2.00</td>
<td>6.60</td>
</tr>
<tr>
<td>glycerophosphoric (3 e 2)</td>
<td>1.37-1.42</td>
<td>3.42-3.55</td>
<td>7.1</td>
</tr>
<tr>
<td>oxaloacetic</td>
<td>2.22</td>
<td>3.89</td>
<td>13.03</td>
</tr>
<tr>
<td>α-Ketoglutaric</td>
<td>2.47</td>
<td>4.68</td>
<td>-</td>
</tr>
<tr>
<td>pyruvic</td>
<td>2.50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>malonic</td>
<td>2.83</td>
<td>5.69</td>
<td>-</td>
</tr>
<tr>
<td>glucuroonic</td>
<td>2.96</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>tartaric</td>
<td>2.98</td>
<td>4.34</td>
<td>-</td>
</tr>
<tr>
<td>fumaric</td>
<td>3.03</td>
<td>4.44</td>
<td>-</td>
</tr>
<tr>
<td>lactic</td>
<td>3.08</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>isocitric</td>
<td>3.29</td>
<td>4.31</td>
<td>6.40</td>
</tr>
<tr>
<td>malic</td>
<td>3.40</td>
<td>5.11</td>
<td>-</td>
</tr>
<tr>
<td>glyceric</td>
<td>3.55</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>gluconic</td>
<td>3.86</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>succinic</td>
<td>4.16</td>
<td>5.61</td>
<td>-</td>
</tr>
<tr>
<td>glutaric</td>
<td>4.31</td>
<td>5.41</td>
<td>-</td>
</tr>
<tr>
<td>β-hidroxibutyric</td>
<td>4.39</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>acetic</td>
<td>4.76</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>propionic</td>
<td>4.83</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>butyric</td>
<td>4.86</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

All these acids, differently from the stronger sulfuric and hydrochloric
will allow the preferential production of FOS or fructooligosaccharides from inulin,
additionally avoiding the co-generation of the inconvenient HMF or
hydroxymethylfurural. Smaller amounts of fructose will be also formed. The action
of phosphoric, citric, and the remaining milder acids will provoke the “randomized”
breakage of fructosidic linkages thus favoring the formation of FOS (or free fructose),
hypothetically according to the following mechanism:
inulina = \( I \{\text{gluc-O-(fructose)}_n\} + (H^+ / \text{HOH}) \rightarrow I \text{gluc} + (\text{fructose})_m \)
(fructose)_m + (H^+ / HOH) → (fructose)_x + y free fructose
y free fructose + H^+ → z hydroxymethylfurfural + H_2O
where the arrows and ....... decrescently indicate the intensity of the reaction; n = a maximum of 60 or the native inulin integrity; l = the whole inulin molecule and still the single glucose unit; m = till 18 or more, namely the population of formed FOS or fructooligosaccharides; x = 2 to 11, the final population of FOS; y = the reduced content of free fructose; z = the still more reduced content of hydroxymethylfurfural, being n > m > x > y > z.

The understanding of the technique of phosphoric or citric hydrolytic fragmentation of inulin (an related polyfructoses) till hydrossoluble FOS or fructooligosaccharides as dominant reaction products and the defense of the list of claims from this patent request is additionally clarified with designs and figures numbered from 1 to 8.

Figure 1 displays a simplified representation of the chemical structure of the native inulin molecule which initiates by a glucose residue (sucrosyl unit) following the addition of successive fructosyl residues, from 11 to 60, and then the molecule finalizes by a fructose unit. Hence, inulin – differently from other natural polysaccharides such as starch, cellulose, hemicelluloses, pectins, and gums – does not bear a reducing end. As an example of a first laboratorial trial, Figure 2 displays a $^{13}$C-Nuclear Magnetic Resonance (NMR) spectrum for inulin isolated and purified from dahlia roots utilizing a hot water extraction provided a mild buffering at pH 7. It followed a precipitation of inulin by cooling, ressolutibilization in warm water and reprecipitation with 3 volumes of ethanol, new redissolution in warm water followed by percolation through a column of anion exchange resin, being the second latter steps destined to the removal of acidic contaminants (residual hemicellulose) and color in accordance with the methodology developed by one of the authors of the present patent request as in Hauly M.C.O., Bracht A., Beck R and Fontana J.D. Fructose and fructose-anhydrides from dahlia inulin. *Applied Biochemistry and Biotechnology* 34-5: 297-308, 1992. The step of ethanol precipitation also ensures the removal of sesquiterpenelactone, implied in the generation of contact dermatitis. The purified inulin sample, 50 mg/mL, was dissolved in δ-DMSO. The resonance signals were registered as chemical shifts (δ or ppm) in a Brucker 400 MHz
spectrometer and designated as C-1 = 61.6 ppm, C-2 = 103.1 ppm, C-3 = 77.6 ppm, C-4 = 75.0 ppm, C-5 = 82.0 ppm and C-6 = 61.9 ppm according us in Fontana J.D., Baron M., Diniz A.C.P., Franco V.C. Microbial inulinase secretion using chemically-modified inulins. *Applied Biochemistry and Biotechnology* 45-6: 257-268, 1994. These signals are typical for the anhydrous and interlinked β-D-fructofuranose residues while the signals of low intensity, collectively named as (g) refer to the single glucosyl unit: C-1 = 92.2 ppm, C-2 = 71.8 ppm, C-3 = 73.2 ppm, C-4 = 70.3 ppm, C-5 = 72.8 ppm and C-6 = 61.6 ppm, a trustable feature ensuring native inulin integrity. In the following figures from 4 to 8, the quality of the employed inulin was exactly the one depictured in Figure 2.

Figure 3 depicts the structural features for 3 FOS – Fructooligosaccharides, the simplest of the series, kestose (trisaccharide), nystose (tetrosaccharide), and fructosylnystose (pentasaccharide), which correspond to hydrolytic fragments of inulin and respectively bearing one, two, or three fructosyl units, besides the initial sucrose molecule. It is worth saying, that the designation “FOS” – *lato sensu* - also encompass those glucose-free fragments such as inulobiose, inulotriose, inulotetraose, and so on.

Figure 4, from a second laboratorial work, is a TLC – Thin Layer Chromatography on silicagel 60 (Merck) developed with the mixture isopropanol : ethyl acetate : water 5:1:2 as mobile phase and with partial runs of 1/3, 2/3, and 3/3 of the front line and developed with orcinol : sulfuric acid : methanol at 100°C for 5 minutes. This figure illustrates the profile of FOS – Fructooligosaccharides when one hydrolyzes purified inulin from dahlia roots (5g%) with phosphoric acid at pH = 2.5 at 85°C during 15 minutes (15), 30 minutes (30), and 45 minutes (45), indicating that the modulation of a single kinetic parameter – time of hydrolysis – already allows to govern the quantitative relation of FOS > fructose (cases 15 and 30) or the opposite (FOS < fructose; 45). A similar strategy for FOS > fructose may also be governed by the other parameters (pH itself or temperature of hydrolysis), as shown for hydrolysis of inulin with phosphoric or citric acids at pHs from 1.75 to 3.75 in the range of 50°C to 950°C (as better explained in Figure 5). In Figure 4, (f) and (g) denotes for free fructose and glucose, respectively. GP refers to the Degree of Polymerization. It is remarkable that phosphoric or citric hydrolyses of inulin may be effectively addressed
to the preferential preparation of FOS – Fructooligosaccharides since when addressed to a higher fructose content (lane 45), some amount of the co-product HMF – hydroxymethylfurfural turns clearly visible in the front zone of the chromatogram. Its companion spot most probably is a DFA (dfructose anhydride).

Figure 5 is a bar graphic comparing the effect of the kinetic parameters such as temperature and time of hydrolysis once fixed the hidrogenionic potential at pH = 2.5 and their respective capacities for the modulation on the qualitative nature of the products from the hydrolyses of dahlia inulin with phosphoric or citric acids when the substrate is used at a concentration of 5g%. According to the intended innovation in this patent request – the preferential production of FOS or FrutoOligoSaccharides – is obviously that, for each range of temperature, namely, 75°C, 85°C ou 95°C, either in the phosphoric or in the citric hydrolyses, the formation of FOS is preferential in (8x2 =) 16 assays, except for those two – at 95°C and during 25 minutes – where fructose shows predominance with respect to FOS. Incidentally, those two exceptional conditions – which are not the scope of this patent request – also led to the formation of some HMF – hydroxymethylfurfural – undesirable in a inulin hydrolysis, with the attenuating condition that a less expensive practice as activated charcoal is able to remove the contaminant HMF. Concerning the reaction yield reported to the initial inulin input, the diluted phosphoric acid guarantees in the times of 25 min at 85°C and of 15 min at 95°C percentages of hydrolyses up to 80%, being the most of the products – 76% and 63%, respectively – FOS or frutooligosaccharides and being the remaining fructose since HMF is no longer detected under these conditions of hydrolysis. The same approach is attained with citric acid although with somewhat reduced yields – 64% or 76%, but even so FOS correspond to 78% and 74% of the hydrolysis products.

Figure 6 derives from another practical example of laboratory work, namely a high performance liquid chromatography or HPLC in a column of 10 micra microparticles of silica gel derivatized with amino groups and provided by Spectraphysics. Twenty microliters of a citric hydrolyzate of inulin at 10% obtained at pH 2.5 during 5 or 15 minutes at 85°C were applied to the column and elution proceeded with 70% acetonitrile at a 1 mL/min flow rate and the monitoring was carried out with DRI– differential refraction index. It is shown that in both conditions
5 and 15 minutes — inulin is converted, by citric acid, into a family of FOS — FructoOligoSaccharides with DP — Degree of Polymerization — 3 to 18 or more (considering the analytical capacity of the referred column), still emphasizing that fructose, with respect to FOS concentration, contributes with a maximum of 25% and a minimum of 5%.

Figure 7, resulting from another practical laboratory work, is also — like Figure 6 — a HPLC but carried out with samples arising from phosphoric acid hydrolysates under the same conditions of those described in Figure 6. The qualitative profiles for FOS are quite similar in both figures. In 8 of 9 assays, FOS predominates.

In the nineth — 25 minutes of hydrolysis at higher temperature — FOS and fructose contents are more or less equivalent.

Figure 8 depicts the result of the last practical laboratory work, namely the fermentation of a phosphoric hydrolyzate of inulin obtained at pH = 2.5 during 15 minutes at 85°C. The hydrolyzate was inoculated with 10 x 10⁵ CFU — Colony Forming Units (initial optical density = 0.60 at A650 nm) of a mixture of probiotic microorganisms: Lactobacillus acidophilus LA-5, Bifidobacterium BB-12 and Streptococcus thermophilus - BioRich from Chr. Hansen S/A, Hershølm, Denmark. Such hydrolyzate was adjusted to 2% (as total sugars), neutralized to pH 6.5 with ammonia and supplemented with MRS medium, and the cultivation of the bacterial triad was carried out for 48 h at 42°C. The supernatants of the cultures — 20 microliters - were analyzed in a Rezex-ROA-AO column from Phenomenex using 8 mMolar H₂SO₄ as mobile phase and 0.5 mL/min flow rate and using a RID monitor.

It is noticeable that the microbial population converted the FOS population (besides free fructose) into a mixture of acetic acid > lactic acid very fast. This confirmed the expectation that the present patent request may be extended towards the industrial fermentation of the resulting FOS or frutooligosaccharides regarding the segment of foods and beverages, and also possibly medicinal and veterinary products, and even more preferentially food supplements and foods called functional or nutraceuticals, since the experiment has clearly demonstrated the fermentation capacity of phosphoric (or citric, not show) hydrolyzates of inulin by the beneficial flora of human / animal intestines.
CLAIMS

1. "PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS" characterized by the fact that, utilizing the fructopolysaccharides in concentration up to 50g% w/v or in the limit of its solubility by heating, the catalysis being done through the citric acid or phosphoric acid, diluted and heated, in a concentration of acid to attain an effective pH in the range between 1.5 and 4.0, and heating in the range of 50°C to 100°C during a period from 1 to 90 minutes regarding the production of a population of fructooligosaccharides as dominant products in the sugary hydroxylate and with DP – Degree of Polymerization varying from 2 to 18, besides a minor content of fructose.

2. "PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS" according to claim 1, characterized by the fact that the polyfructoses utilized as substrates for hydrolysates are obtained from bulbs, subterranean stems, roots, rhyzophores, and tubercles of dahlia, chicory, yacon, Jerusalem artichoke or any other plants producing inulins such as those from the families Asteraceae / Compositae, Liliaceae and Gramineae, besides bacterial levans.

3. "PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS" according to claim 1, characterized by the fact that the pH for hydrolysates is in the range between 2.25 and 2.75, and more preferentially at pH 2.5.

4. "PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS" according to claim 1, characterized by the fact that the preferential range of temperature is in the range from 75°C to 95°C.

5. "PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS", according to claims 1 and 4, when the temperature is increased till water ebullition or with the application of more heated steam, compensating the pH of hydrolysis to a milder range from 3 to 4, and reducing the time of hydrolysis to the range between 1 and 4 minutes.
6. "PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS" according to claim 1, characterized by the fact that the preferential range of hydrolysis is between 5 and 25 minutes.

7. "PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS" according to claim 1 and also claims 3, 4, 5 and 6, characterized by the fact that the process combines the kinetic parameters under a preferential way to obtain fructooligosaccarides in the range of DP – Degree of Polymerization between 2 and 18 or even more, namely pH 2.5 for the citric hydrolysis between 85°C and 95°C during 15 to 20 minutes and, in the same pH value, for the phosphoric hydrolysis between 80°C and 90°C during 5 to 20 minutes.

8. "PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS", according claim and also to claims 2, 3, 4, 5, 6 and 7, characterized by the fact that citric or phosphoric hydrolyzates, once carried out the respective dilutions with water and the consequent decrease of the acidity or through partial or total neutralization, are utilized as such for the sake of direct and ulterior fermentation or the utilization for human or animal consumption as canned products, dairy, candies, alcoholized beverages or not, low caloric foods and dietetics and still other similar formulations such as food supplements, functional foods, nutraceuticals and odontological inputs, without other post-treatments except for those appropriate for their combination to other nutrients or ingredients.

9. "PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS", according to claim 1 and also claims 3, 4, 5, 6, 7 and 8, characterized by the fact that the citric or phosphoric hydrolyzates are partially neutralized to the range of pH between 2.8 and 6.9 using weak bases such as ammonium, calcium or zinc hydroxides or strong bases such as sodium or potassium hydroxides before their incorporation to foods, beverages and dental creams or in the destination for subsequent fermentation steps.
10. “PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS”, according to claim 1 and also claims 2, 3, 4, 5, 6, 7 and 8, characterized by the fact that citric or phosphoric acids are previously eliminated from the hydrolyzates through percolation or treatment with anion exchange resins before the application in foods and beverages and still in fermentative processes.

11. “PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS”, according to claim 1 and also claims 3, 4, 5, 6, 7, 8, 9 and 10, characterized by the fact that the hydrolysis of inulin and/or other similar polyfructoses is carried out with mixture of citric and phosphoric acid, diluted, heated, and in any proportion of acid catalysts.

12. “PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS”, according to claim 1 and also claims 3, 4, 5, 6, 7, 8, 9, 10 and 11, characterized by the fact that the citric or phosphoric hydrolyzates, specially those obtained under more severity of kinetic parameters as acidity and temperature or longer time of hydrolysis, are treated with activated charcoal or other absorbing phases for the removal of colored by-products and hydroxymethylfurfural.

13. “PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS”, in accordance with claim 12, characterized by the fact that the target of the activated charcoal or other post-treatment absorbents are citric or phosphoric hydrolyzates in which there are predominance of free fructose over the FOS – fructooligosaccharides.

14. “PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS”, in accordance with claim 1 and also with claims 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13, characterized by the combination of the fructooligosaccharides, exempted or not from free fructose, and produced by citric or phosphoric acids and said as prebiotics, to microorganisms prebiotics from the genera Bifidobacterium,
*Lactobacillus*, and similar for the formulation of symbiotics destined to human or animal feeding.

15. "PROCESS OF CITRIC OR PHOSPHORIC PARTIAL HYDROLYSIS OF INULIN FOR THE OBTENTION OF FRUCTOOLIGOSACCHARIDES – FOS" in analogy to claim 1 but utilizing an alternative of hydrolysis through catalysis with other organic acids than citric or phosphoric and whose acidities as expressed by the physicochemical parameter pKa₁ display minimal and maximum values from 2.2 to 4.86.
Fig. 1
Fig. 2
Fig. 3

kestose  nystose  fructosynystose
Fig. 5

![Graph showing the effects of different acids on HMF, fructose, and FOS production.](image)

- **Citric Acid**
- **Phosphoric Acid**

Legend:
- □ HMF
- ■ fructose
- □ FOS
Fig. 6

- Fructose
- Degree of polymerization - DP

Concentration vs. minutes

- 15 minutes line
- 5 minutes line
Fig. 7

- 15 minutes
- 5 minutes

fructose

degree of polymerization - DP
Fig. 8

![Graph showing metabolites over time.](image)

- **FOS**: 25 units
- **fructose**: 5 units
- **lactic acid**: 10 units
- **acetic acid**: 15 units

**Legend**:
- **Culture**
- **Hydrolyzate**

**Time (minutes)**: 0 - 22

**Concentration**