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Possible Use of the Carbohydrates Present in Tomato Pomace and in Byproducts of the Supercritical Carbon Dioxide Lycopene Extraction Process as Biomass for Bioethanol Production

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ABSTRACT: This study provides information about the carbohydrate present in tomato pomace (skins, seeds, and vascular tissues) as well as in the byproducts of the lycopene supercritical carbon dioxide extraction (SC-CO_2) such as tomato serum and exhausted matrix and reports their conversion into bioethanol. The pomace, constituting approximately 4% of the tomato fruit fresh weight, and the SC-CO_2-exhausted matrix were enzyme saccharified with 0.1% Driselase leading to sugar yields of ∼383 and ∼301 mg/g dw, respectively. Aliquots of the hydrolysates and of the serum (80% tomato sauce fw) were fermented by *Saccharomyces cerevisiae*. The bioethanol produced from each waste was usually >50% of the calculated theoretical amount, with the exception of the exhausted matrix hydolysate, where a sugar concentration >52.8 g/L inhibited the fermentation process. Furthermore, no differences in the chemical solubility of cell wall polysaccharides were evidenced between the SC-CO_2-lycopene extracted and unextracted matrices. The deduced glycosyl linkage composition and the calculated amount of cell wall polysaccharides remained similar in both matrices, indicating that the SC-CO_2 extraction technology does not affect their structure. Therefore, tomato wastes may well be considered as potential alternatives and low-cost feedstock for bioethanol production.

KEYWORDS: *agri-food industry wastes, alcoholic fermentation, cell wall polysaccharides, Solanum lycopersicum L., supercritical fluids, tomato pomace*

INTRODUCTION

One of the most serious problems of the food industry is the accumulation, handling, and disposal of processing wastes and byproducts. These often contain highly valuable bioactive molecules that may be conveniently extracted and marketed. For this reason, nowadays, increasing attention is being paid to the recovery, recycling, and upgrading of wastes, with positive implication for environmental and financial balances.[1]

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated vegetable crops in Mediterranean countries. A significant amount of tomatoes is consumed daily all over the world as fresh fruits, and a huge quantity of them is processed into products such as tomato juice, paste, sauce, puree, and ketchup. During processing, a byproduct, known as tomato pomace, is usually generated. This byproduct, composed of skins, seeds, and vascular tissues, represents approximately 4% of the whole fruit weight (fw); it is rich in nutrients and can be used as a potential source of carotenoids, fibers, lipids, and proteins.[2] In particular, attention is turning to lycopene extraction from tomato pomace with supercritical fluids.[3−7] The exploitation of tomato pomace could provide extra income for the tomato industry and, simultaneously, reduce the waste disposal problems and expenses.

Tomatoes and their derivatives are of particular interest due to the presence of molecules, such as ascorbic acid, folates, phenolics, polyphenolics, tocopherols and carotenoids, that exert positive effects on human health and wellness. Lycopene ($C_{40}H_{56}$), the major carotenoid of red-ripe tomato fruits, is accumulated and stored in chromoplasts; it is the molecule that shows the highest degree of unsaturation among carotenoids, having a total of 13 double bonds, with 11 of them conjugated. These conjugated double bonds are responsible for the red color and for the unique antioxidant properties of lycopene.[8]

Many health claims have been associated with lycopene consumption, including preventing different types of cancers, atherogenesis, cardiovascular diseases, macular degeneration, neurone damage, and bone calcification deficiencies, reversing male infertility, and acting in skin photoprotection in addition to acting as an antiaging compound. In addition, lycopene is thought to exert its healthy effects through nonoxidative mechanisms such as regulation of gene expression, enhancement of gap junction communication, or promotion of immune system functionality.[9] Thus, lycopene is considered to be a highly marketable natural pigment, functional, as a health-promoting ingredient, in several nutraceutical, cosmetic, and food formulations. All of this explains the strong interest in lycopene extraction from red-ripe tomato matrices by means of clean technologies. Supercritical carbon dioxide (SC-CO_2) seems to be an excellent technology for lycopene extraction from red-ripe tomatoes and represents the best alternative to conventional industrial solvent extraction.[10−14] We have recently described the optimization of biological and physical parameters for lycopene SC-CO_2 extraction from red-ripe ordinary and high-pigment tomato cultivars (cv).[15] This allowed the production of a solvent-free oleoresin enriched in lycopene dissolved in a highly unsaturated vegetable oil and a

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lycopene-extracted tomato matrix as byproduct that deserves to be analyzed and appropriately upgraded. This waste is mainly composed of primary cell wall polysaccharides, which can be used as dietary additive fiber or processed to produce bioethanol.

The main objective of this study is to provide information on the carbohydrates present in the tomato pomace and in the byproducts of the SC-CO_2 lycopene extraction process (tomato serum and exhausted matrix), to propose a possible use of these wastes as biomass for bioethanol production. The effect of SC-CO_2 extraction on cell wall polysaccharides has also been evaluated by comparison between the glycosyl linkage analyses conducted on selectively extracted polysaccharides from the tomato matrix before and after the extraction process. We also report the efficiency of Driselase digestion of cell wall polysaccharides into sugars and their fermentation into bioethanol of all studied wastes.

MATERIALS AND METHODS

Tomato Cultivation. Tomatoes were cultivated and grown to maturity as described by Ilahy et al.[16] Briefly, seeds of three ordinary tomato cultivars (Donald, Incas, and Perfectpeel) and two cultivars of high-pigment tomato hybrids (HLY 18, obtained from COIS' 94 Srl, Belpasso (CT), Italy; and Kalvert, obtained from Esasem Spa, Casaleone (VR), Italy) were germinated in alveolar boxes. One-month-old tomato seedlings were transplanted in an open field in the province of Lecce (southern Italy). Standard agronomical techniques were used for plant nutrition and pathogen prevention.[17]

Healthy, red-ripe tomato fruits, uniform in size, were visually selected and harvested. The fruits were rapidly dipped in 1% NaOCl to reduce microbial contamination, extensively washed with tap water, and immediately processed.

Tomato Processing. Large quantities of red-ripe tomato fruits (soluble solids between 5.80 and 6.20 °Brix; titratable acidity between 0.350 and 0.388%) were processed into tomato sauce as raw material to obtain a serum and a freeze-dried and milled matrix suitable for SC-CO_2 extraction as previously described.[15] Briefly, fruits of each tomato cultivar were blanched in water at 70 °C for 5 min, crushed, and sieved by a Reber 9004 N tomato squeezer (Reber, Luzzara, (RE), Italy) to obtain a tomato sauce made up of pericarp cell clumps of similar size to the sieve used (1 mm), separated from the skins, seeds, and vascular tissues (tomato pomace). The tomato sauce was packed into 1 L screw-top glass jars and pasteurized at 121 °C. The tomato pomace was immediately frozen at −20 °C and freeze-dried by using a Christ ALPHA 2-4 LSC freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for 24 h. The freeze-dried pomace was ground at 500 μm by using a laboratory mill (Retsch GmbH, Haan, Germany) to obtain a homogeneous powder. The powder was vacuum-packaged in food grade oxygen-impermeable plastic bags and stored in a freezer at −20 °C.

Freeze-Dried Tomato Matrix Preparation. For each cultivar, 5 kg aliquots of the tomato sauce were centrifuged at 27000*g* for 10 min by using a J2-21 Beckman centrifuge (Beckman Coulter, Fullerton, CA, USA) to remove water-soluble substances (tomato serum). The pellet of cv. Perfectpeel was freeze-dried by using a Christ ALPHA 2-4 LSC freeze-dryer for 24 h. The freeze-dried tomato matrix was ground at 500 μm by using a laboratory mill (Retsch GmbH) to obtain a homogeneous powder. The powder was vacuum-packaged in food grade oxygen-impermeable plastic bags and stored in a freezer at −20 °C.

The 27000*g* supernatant (tomato serum) was aliquoted and kept frozen at −20 °C until used in fermentation tests.

Supercritical CO_2 Extraction of Lycopene from Tomato Matrix. The extractions with SC-CO_2 were performed with the pilot plant described by Vasapollo et al.[10] The extractions were run by submitting about 1.3 kg of the freeze-dried and milled (500 μm) tomato matrix to a pressure of 450 bar and a temperature of 65−70 °C. The flow rate of CO_2 was 18−20 kg h^{-1}. The extraction was carried out for 3 h.

Cell Wall Polysaccharide Enzymatic Hydrolysis. Aliquots of 25 g of the freeze-dried and milled (500 μm) tomato pomace and of the SC-CO_2-extracted matrix were incubated in 500 mL of 0.1% Driselase (a mixture of exo- and endohydrolases from *Basidiomycetes* sp., Sigma-Aldrich Co., Milan, Italy) in 0.1 M sodium acetate buffer, pH 4.6, at 37 °C for 12 h under constant stirring (180 rpm). The undigested material was precipitated by centrifugation at 27000*g* for 10 min. Two additional cycles of digestion were required to improve polysaccharide saccharification.[18] The supernatants obtained after each digestion cycle were combined, and the sugar content was determined by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) as described below.

HPAEC-PAD Protocol. All samples were filtered through a 0.2 μm Spartan 13 filter (Schleicher & Schuell Microscience, Dassel, Germany) and then analyzed on a Dionex HPAEC with a CarboPac PA10 column as described by Lenucci et al.[19] The eluent flow rate was 1 mL/min at room temperature, and 20 μL samples were injected. Sugars were quantified with a pulsed amperometric detector (PAD) with a gold electrode. HPLC grade water (eluent A), 0.05 M NaOH (eluent B), and 0.8 M NaOH (eluent C) were used for monosaccharide separation, using the following linear gradient: 0−20 min, 60% A and 40% B (0.02 M NaOH); 20.1−27 min, 75% A and 25% C (0.20 M NaOH); 27.1−31 min, 100% C (0.80 M NaOH); 31.1−42 min, 60% A and 40% B (0.02 M NaOH).

The inter- and intraday variability of the method was measured by repeating the analyses three times on the same day and three times on three consecutive days using a mixture of the authentic markers (arabinose, cellobiose, fructose, galactose, galacturonic acid, glucose, glucuronic acid, isoprimeverose, mannose, rhamnose, xylobiose, and xylose). The coefficients of variation of the intra- and interday variability were calculated to be below 5 and 7%, respectively.

Sequential Extraction of Cell Wall Polysaccharides. The chemical solubilization of cell wall polysaccharides was carried out as described by Waldron and Selvendran[20] and successively modified by Piro et al.[21] Triplicate 100 mg aliquots of the SC-CO_2-unextracted and -extracted tomato matrices were sequentially extracted with (a) 50 mM cyclohexane−*trans*-1,2-diamine-*N*,*N*,*N*′,*N*′ tetraacetate (CDTA, Na salt, 10 mL), pH 6.5, at 20 °C for 6 h with shaking (CDTA-1); (b) 50 mM CDTA (10 mL), pH 6.5, at 20 °C for 2 h with shaking (CDTA-2); (c) 50 mM Na_2CO_3 + 20 mM $NaBH_4$ (10 mL) at 1.0 °C for 16 h with shaking (Na_2CO_3-1); (d) 50 mM Na_2CO_3 + 20 mM $NaBH_4$ (10 mL) at 20 °C for 4 h (Na_2CO_3-2); (e) 0.5 M KOH + 10 mM $NaBH_4$ (10 mL) at 1.0 °C for 3 h, under nitrogen; (f) 1 M KOH + 10 mM $NaBH_4$ (10 mL) at 1 °C for 6 h, under nitrogen; (g) 4 M KOH + 10 mM $NaBH_4$ (10 mL) at 20 °C for 24 h under nitrogen. At the end of the sequential extractions, an insoluble residue, usually considered α-cellulose, remained. After each extraction, the soluble polymers were separated from the insoluble residue by centrifugation (5000*g*, 10 min in a Biofuge 15 Heraeus Sepatech centrifuge). The combined alkali extracts and the insoluble residue (α-cellulose) were acidified to pH 5.0 with glacial acetic acid. The CDTA-1, CDTA-2, Na_2CO_3-1, and Na_2CO_3-2 extracts were combined and referred to as CDTA + Na_2CO_3 extract. Similarly, the 0.5, 1.0, and 4.0 M KOH extracts were combined and referred to as KOH extract. The CDTA + Na_2CO_3 and KOH extracts as well as the insoluble residue were dialyzed exhaustively in the presence of 0.05% chlorbutol (with SnakeSkin Pleated Dialysis Tubing, Pierce; M_r cutoff 10000) against several changes of distilled water for 7 days at 5.0 °C in a cold room and freeze-dried. The freeze-dried CDTA + Na_2CO_3 and KOH extracts and insoluble residue (α-cellulose) were each subjected to linkage analysis as described below.

Glycosyl Linkage Analysis. The linkage analysis of each extracted cell wall polysaccharide fraction was performed to obtain information about the overall future of tomato pericarp cell wall before and after lycopene extraction by SC-CO_2. For glycosyl linkage analysis, the samples were permethylated, depolymerized, reduced, and acetylated; the resultant partially methylated alditol acetates (PMAAs) were analyzed by gas chromatography coupled to mass spectrometry (GC-

MS) as described by York et al.[22] Initially, aliquots of each sample were permethylated using the NaOH/MeI method.[23] The methylated polysaccharides were hydrolyzed using 2 M TFA (2 h in sealed tube at 121 °C), reduced with $NaBD_4$, and acetylated using acetic anhydride/pyridine. The resulting PMAAs were analyzed on a Hewlett-Packard 5890 GC interfaced to a 5970 MSD (mass selective detector, electron impact ionization mode); separation was performed on a 30 m Supelco 2330 bonded phase fused silica capillary column. The analyses were performed by the analytical services of the University of Georgia Complex Carbohydrate Research Center.

Fermentation Tests. The tomato serum (27000*g* SN) obtained by centrifugation of Donald, Incas, Perfectpeel, Kalvert, and Hly 18 cultivars and the sugar syrups obtained after tomato pomace and SC-CO_2-extracted tomato matrix (cv. Perfectpeel) polysaccharide digestion with 0.1% Driselase were subjected to fermentation with a selected *Saccharomyces cerevisiae* strain, "Cispa 161", immobilized in sodium alginate beads. The Driselase-digested substrates were previously diluted (by adding distilled water) or vacuum concentrated to adjust sugar concentration.

Sodium alginate beads were prepared using 3% (w/v) sodium alginate dissolved in boiling hot water under vigorous stirring.[24] Cells were suspended in 100 mL of sodium alginate solution. The slurry was poured drop-by-drop from a hypodermic needle using a peristaltic pump (Miniplus 3, Gilson Inc., Middleton, WI, USA) into 100 mL of 0.05 M $CaCl_2$ with constant stirring, at room temperature and under sterile conditions. The produced spherical beads (approximately 3.8 mm in diameter, 70×10^{12} yeast cells) were washed in distilled water and immediately used. For each substrate (100 mL), 700 beads were used. The consumption of sugars and production of bioethanol were monitored over time by an HPLC system (Agilent 1100 series) equipped with a refractometric detector and an Aminex HPX-87 H (7.8 × 300 mm) column. Separation was performed at 55 °C with an eluent flow (H_2O acidified with H_2SO_4, pH 2.3) of 0.6 mL/min. All samples were filtered through a 0.2 μm Spartan 13 filter (Schleicher & Schuell Microscience) before being tested.

Statistical Analysis. Unless differently specified in the tables, results are presented as the mean value ± standard deviation of at least three independent replicated experiments (n = 3). Statistical analysis was based on a one-way ANOVA test. The post hoc method of Holm−Sidak was applied to establish significant differences between means with a confidence level of 95%. All statistical comparisons were performed using SigmaStat version 3.11 software (Systat Software Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Tomato Wastes. We have recently reported the optimization of biological and physical parameters for lycopene SC-CO_2 extraction from a freeze-dried and milled matrix composed of clumps of red-ripe tomato pericarp cells.[15] During this process, three types of wastes are produced that can be potentially used for different purposes and, in particular, for bioethanol production. The wastes are (i) a tomato pomace (composed of skins, seeds, and vascular tissues) deriving from the processing of red-ripe tomato fruits after crushing, sieving, and separation from the sauce; (ii) a serum [the supernatant obtained by tomato sauce centrifugation at 27000*g* to precipitate a compact pellet composed of clumps of red-ripe tomato pericarp cells (tomato matrix)] containing most of the soluble sugars, organic acids, and ions (named thereafter as 27000*g* SN); and (iii) the SC-CO_2-extracted tomato matrix after freeze-drying and milling (Figure 1).

We used freeze-dried and milled (500 μm) tomato pomace as biomass for bioethanol production. Table 1 reports the glycosyl residue composition of the tomato pomace, prepared from the cv. Perfectpeel, digested with 0.1% Driselase for three cycles of 12 h each, and analyzed by HPAEC-PAD. The total amount of sugars in the hydrolysate, expressed as milligrams

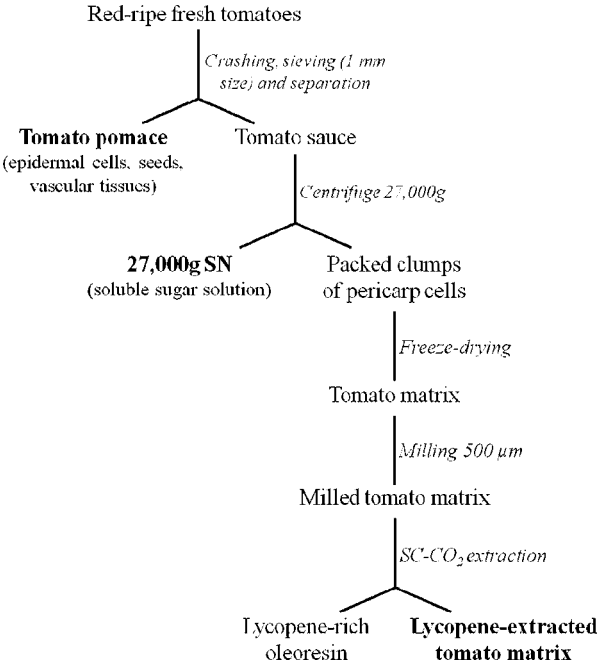

Figure 1. Wastes (bold text) generated during the production of a lycopene-rich oleoresin extracted by SC-CO_2 from a freeze-dried tomato matrix.

Table 1. Qualitative and Quantitative Glycosyl Residue Composition of Freeze-Dried Tomato Pomace Obtained from Red-Ripe Fruit of cv. Perfectpeel Digested with 0.1% Driselase and Analyzed by HPAEC-PAD[a]

sugar	mol %
rhamnose	1.2 ± 0.7
arabinose	2.9 ± 0.4
galactose	4.0 ± 0.4
glucose	40.5 ± 1.2
mannose	5.1 ± 1.2
xylose	1.1 ± 0.8
fructose	22.6 ± 2.1
isoprimeverose	3.3 ± 0.1
xylobiose	3.1 ± 0.2
cellobiose	5.8 ± 0.5
galacturonic acid	9.9 ± 1.2
glucuronic acid	0.5 ± 0.2
total (mg/g dw)	**382.9 ± 10.3**

[a]Values represent the mean ± standard deviation of three independent replicates (n = 3).

per gram dry weight (dw), was 382.9 ± 10.3 (corresponding to a concentration of approximately 6.4 g/L). With the exception of fructose and part of glucose (see the fructose-to-glucose ratio in soluble sugars 27000*g* SN, Table 2), which represent contaminants of vacuolar origin, all other sugars, including the remaining glucose, are cell wall polysaccharide components. Furthermore, the occurrence of the diagnostic disaccharide isoprimeverose (3.3 mol %) indicates the presence of a small amount of xyloglucans as cell wall polysaccharides.[25] Because the unhydrolyzed residue still contains polysaccharidic material (data not shown), it is clear that the hydrolysis of the pomace fraction must be improved by choosing a more specific enzyme mixture. However, when concentrated aliquots of the hydro-

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Table 2. Qualitative and Quantitative Soluble-Sugar Composition Determined in the 27000*g* SN from Tomato Sauce of Three Ordinary (Donald, Incas, and Perfectpeel) and Two High-Pigment (Kalvert and Hly 18) Tomato Cultivars[a]

	mol %				
sugar	cv. Donald	cv. Incas	cv. Perfectpeel	cv. Kalvert	cv. Hly 18
fructose	54.6 ± 1.0	54.3 ± 0.6	58.4 ± 2.1	53.7 ± 0.1	52.8 ± 0.3
glucose	45.4 ± 1.5	45.7 ± 0.1	41.6 ± 1.1	46.3 ± 0.2	47.2 ± 0.4
total (g/L)	**34.6 ± 1.7**	**37.2 ± 1.2**	**38.9 ± 1.8**	**46.4 ± 2.2**	**45.5 ± 2.7**
ratio	*1.2*	*1.2*	*1.4*	*1.1*	*1.1*

[a]The sugars were determined by HPAEC-PAD. Values represent the mean ± standard deviation of three independent replicates (n = 3). Italic formatting identifies dimensionless ratios.

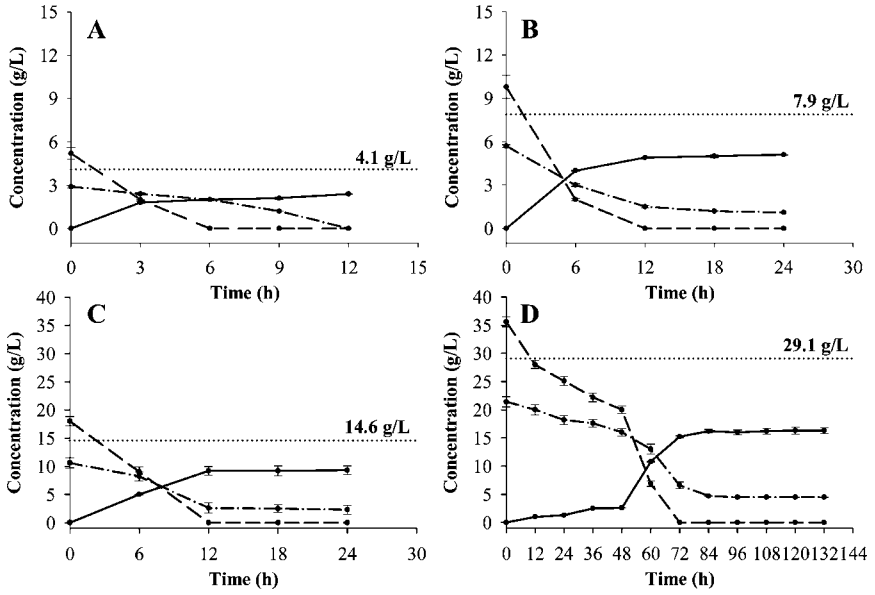

Figure 2. Time course of glucose and fructose consumption and bioethanol production during fermentation by sodium alginate bead immobilized *Saccharomyces cerevisiae* strain "Cispa 161" cells of substrates with different concentrations of sugars (A = 8.1 g/L; B = 15.5 g/L; C = 28.6 g/L; D = 57.0 g/L), obtained by enzymatic digestion of tomato pomace (cv. Perfectpeel) with 0.1% Driselase: dashed line, glucose; dash-dot line, fructose; solid line, ethanol; dotted line, theoretical yield of ethanol. Sugar amounts were monitored by HPAEC-PAD; ethanol was detected by HPLC with refractometric detector. The data represent the average of three independent replicates (n = 3).

lysate (total sugar concentrations of 8.1, 15.5, 28.6, and 57.0 g/L) were subjected to alcoholic fermentation, in the presence of *S. cerevisiae* strain "Cispa 161" yeasts immobilized in sodium alginate beads (⌀ = 3 mm), the main sugars (glucose and fructose) were fermented to bioethanol with a different kinetic (Figure 2). The amount of bioethanol produced, after 12 h of fermentation at the optimal sugar concentration (28.6 g/L), was >60% of the theoretical value, calculated by considering a maximum yield of 0.51 g ethanol/g sugar (Figure 2C).

Table 2 shows the total amount of soluble sugars determined in the tomato serum (27000*g* SN) waste obtained by centrifugation of red-ripe tomato sauce prepared from three ordinary (Donald, Incas, and Perfectpeel) and two high-pigment (Kalvert and Hly 18) cultivars. The ordinary tomato cultivars showed a total soluble sugar content significantly (P < 0.05) lower than the high pigment cultivars. The two main sugars detected were fructose and glucose in a molar ratio of 1.1 for the Hly18 and Kalvert cultivars. In the ordinary cultivars, the ratio was 1.2 for cv. Donald and Incas and 1.4 for cv. Perfectpeel. It has been reported that this ratio changes according to the cultivar and the environmental conditions of growth.[19,26]

The soluble sugar containing 27000*g* SN waste from each cultivar, which represents approximately 80% of the tomato sauce weight, was subjected to fermentation by sodium alginate beads immobilized *S. cerevisiae* strain "Cispa 161" yeast without the addition of any other growth factors. Figure 3 shows the time course of the utilization of the two sugars, fructose and glucose, for the production of bioethanol in all cultivars examined. These data clearly indicate that the 27000*g* SNs can be rapidly and efficiently fermented to bioethanol within 12 h using sugar concentrations between 17.7 and 23.3 g/L in accordance with the optimal substrate condition established for each cultivar. In all cultivars, the amount of bioethanol produced, after 12 h of fermentation, was >65% of the theoretical value. The same solutions can be either utilized as they are or biotechnologically modified as sweetener for multiple purposes.

The SC-CO_2-extracted tomato matrix represents an important polysaccharidic industrial waste. The bioconversion of this waste into bioethanol requires knowledge of the chemical structure of red-ripe pericarp tomato cell wall polysaccharides to optimize the enzymatic saccharification process. In the light of this consideration, Table 3 reports the sequential chemical solubilization of pectins, hemicelluloses, and α-cellulose (insoluble residue) from SC-CO_2-unextracted and -extracted tomato matrices. The total amount of CDTA + Na_2CO_3- and KOH-soluble polysaccharides, as well as of the

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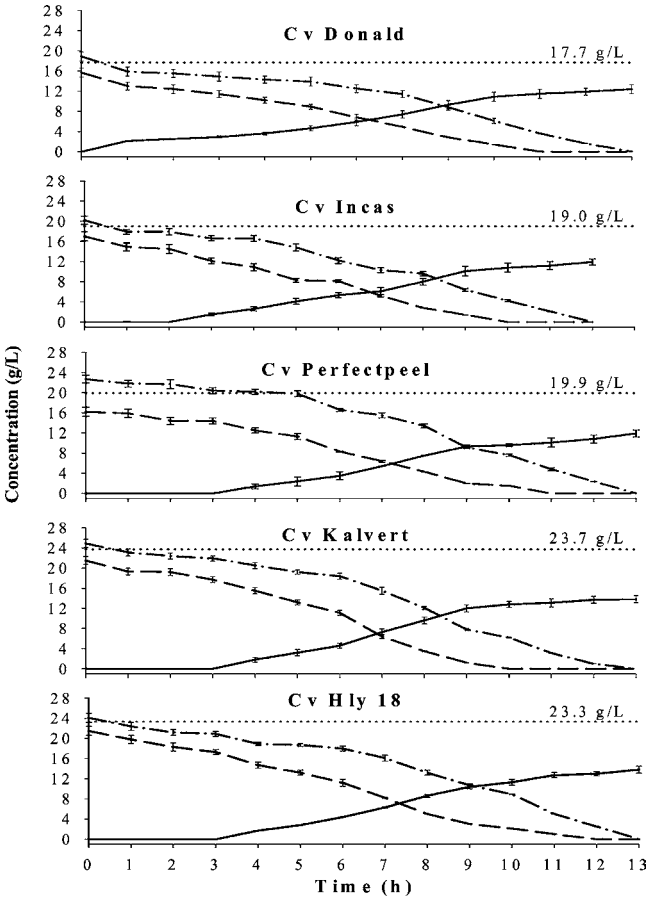

Figure 3. Time course of glucose and fructose consumption and bioethanol production during fermentation by sodium alginate bead immobilized *S. cerevisiae* strain "Cispa 161" cells of the 27000*g* SN from tomato sauce of three ordinary (Donald, Incas, and Perfectpeel) and two high-pigment (Kalvert and Hly 18) cultivars: dashed line, glucose; dash-dot line, fructose; solid line, ethanol; dotted line, theoretical yield of ethanol. Sugar amounts were monitored by HPAEC-PAD; ethanol was detected by HPLC with refractometric detector. The data represent the average of three independent replicates (n = 3).

insoluble residue, were almost identical, indicating that the SC-CO_2 treatment (450 bar, 65−70 °C, CO_2 flow rate = 18−20 kg h^{-1}) had no effect on the percentage of chemical solubilization of pectins, hemicelluloses, and α-cellulose. The percentages of cell wall matrix polysaccharides (pectins plus hemicelluloses) in the freeze-dried tomato matrix before and after SC-CO_2 lycopene extraction were 62.1 and 65.2, respectively.

Linkage Analysis and Polysaccharide Estimation. The glycosyl linkage composition of CDTA + Na_2CO_3- and KOH-solubilized polysaccharides, as well as of the remaining insoluble residue, is reported in Tables 4, 5, and 6, respectively. Additionally, because it is well established that the glycosyl linkage composition is correlated to the nature of the cell wall polysaccharides,[27] their amounts were approximately estimated as the sum of the glycosyl residues obtained from linkage analyses as summarized in Table 7, and considering all information related to type I and type II cell wall models.[28−31]

Those linkages that were not clearly attributed to the above-described cell wall polysaccharides or present in a small amount were not considered. In both matrices, 4-Gal*Ap*, 2-Rha*p*, 5-Ara*f*, *t*-GalA + *t*-Gal*p*, 4-Xyl*p*, and 4-Glc*p* were the major glycosyl residues contributing to the structure of CDTA + Na_2CO_3-solubilized polysaccharides (Table 4); other monosaccharides, <5 mol % each, were also present. Most of these glycosyl residues are characteristic of homogalacturonan and rhamnogalacturonan I (RGI) with their neutral side chains of galactans, arabinans, and highly branched arabinogalactans,[32−34] whose relative percentages, in the SC-CO_2-unextracted and -extracted matrix, are reported in Table 8. RGI is a polymer containing a backbone of repeating (1→2)-α-L-Rha*p*-(1→4)-α-D-Gal*Ap* disaccharide units, which are predominately substituted at O-4 of Rha*p* residues by neutral sugar side chains. The amounts of 4-Gal*Ap* residues in SC-CO_2-unextracted and -extracted tomato matrices (27.6 and 26.0 mol %, respectively) were almost double with respect to the mole percent of 2-Rha*p* plus 2,4-Rha*p* residues in both tomato matrices (Table 4). This indicates that the chain length of RGI is longer than that of homogalacturonan domain.

The presence of 2,4-Rha*p* is an indication of the degree of branching of RGI. In this study the mole percent of 2,4-Rha*p* remained very low with respect to the mole percent of 2-Rha*p*. Despite this, the detection of 5-Ara*f* indicates the presence of arabans as neutral side chains. High mole percentages of *t*-Gal*Ap* + *t*-Gal*p* glycosyl residues were found in both SC-CO_2-unextracted and -extracted tomato matrices, likely due to the enzymatic hydrolysis of galactan, arabinogalactan, and homogalacturonan chains that occurs during tomato ripening.[35−41] The CDTA + Na_2CO_3 treatments also solubilized a small amount of hemicelluloses (Table 8). Glycosyl residues typical of xyloglucans (4-Glc*p*, 4,6-Glc*p*, *t*-Xyl*p*, and *t*-Fuc*p*)[42,43] and arabinoxylans (AXs) (4-Xyl*p*, 2,4-Xyl*p*, *t*-Ara*f*, and 2-Ara*f*[44,45] were detected in SC-CO_2-unextracted and -extracted tomato matrices.

As far as xyloglucans are concerned, the ratios between 4,6-Glc*p* and 4-Glc*p* in SC-CO_2-unextracted and -extracted tomato matrices were 0.079 and 0.011, respectively. This unusual and markedly low ratio, with respect to the value known for dicot (3:1) and monocot (1:1) xyloglucans,[45−47] suggests that most of the 4-Glc*p* residues may derive from starch, which was not removed from the tomato matrix, to strictly respect a free-solvent protocol for SC-CO_2 lycopene extraction.[15] Assuming

Table 3. Amount and Percentage of Cell Wall Polysaccharides Sequentially Extracted with CDTA + Na_2CO_3 and KOH from SC-CO_2-Unextracted and -Extracted Freeze-Dried Tomato Matrices (cv. Perfectpeel)[a]

	SC-CO_2-unextracted matrix		SC-CO_2-extracted matrix	
extract	mg/g	%	mg/g	%
CDTA + Na_2CO_3 (pectins)	312.5 ± 2.7	36.9 ± 3.2	305.7 ± 2.2	37.5 ± 2.6
KOH (hemicelluloses)	213.7 ± 2.3	25.2 ± 2.3	226.0 ± 3.2	27.7 ± 1.1
insolubile residue (α-cellulose)	320.2 ± 3.7	37.9 ± 1.8	283.9 ± 3.1	34.8 ± 0.7
total	**846.4 ± 8.7**	**100**	**815.6 ± 8.5**	**100**

[a]The insoluble residue is also reported. Data are the mean ± standard deviation of five independent replicas (n = 5).

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Table 4. Linkage Analysis of Cell Wall Polysaccharides Solubilized with CDTA + Na_2CO_3 from SC-CO_2-Unextracted and -Extracted Freeze-Dried Tomato Matrices (cv. Perfectpeel)[a]

	mol %	
glycosyl linkage	SC-CO_2-unextracted matrix	SC-CO_2-extracted matrix
rhamnosyl		
t-Rha*p*	2.6	2.7
2-Rha*p*	13.3	12.1
2,4-Rha*p*	1.6	2.4
3-Rha*p*	1.7	1.7
arabinosyl		
t-Ara*f*	1.6	1.1
5-Ara*f*	6.2	5.0
2-Ara*f*		0.1
fucosyl		
t-Fuc*p*	1.0	0.7
glucosyl + glucuronic acid		
t-Glc*p* + *t*-GluA*p*	3.1	
galacturonic acid + galactosyl		
t-GalA*p* + *t*-Gal*p*	17.2	10.3
3-GalA*p* + 3-Gal*p*	1.6	-
2-GalA*p* + 2-Gal*p*		0.7
2,4-GalA*p* + 2,4-Gal*p*		0.7
xylosyl		
t-Xyl*p*	0.9	1.0
4-Xyl*p*	5.4	8.6
2,4-Xyl*p*	0.6	0.5
glucuronic acid		
2-GlcA*p*	0.7	0.6
mannosyl		
4-Man*p*	1.2	1.9
galacturonic acid		
2-GalA*p*	1.1	
4-GalA*p*	27.6	26.0
3,4-GalA*p*	1.1	0.8
2,4-GalA*p*	0.9	
galactosyl		
6-Gal*p*	0.8	1.6
3,6-Gal*p*	0.2	0.4
3-Gal*p*		0.7
glucosyl		
t-Glc*p*		4.6
4-Glc*p*	8.8	14.1
4,6-Glc*p*	0.7	1.6

[a]Values are the mean of three independent replicates (n = 3), and variance was <10%.

Table 5. Linkage Analysis of Cell Wall Polysaccharides Solubilised with KOH from SC-CO_2-Unextracted and -Extracted Freeze-Dried Tomato Matrices (cv. Perfectpeel)[a]

	mol %	
glycosyl linkage	SC-CO_2-unextracted matrix	SC-CO_2-extracted matrix
rhamnosyl		
2-Rha*p*	0.1	
arabinosyl		
t-Ara*f*	1.7	1.0
2-Ara*f*	0.2	
5-Ara*f* + 4-Ara*p*	0.8	0.6
xylosyl		
t-Xyl*p*	4.0	2.5
4-Xyl*p*	14.4	17.3
2,4-Xyl*p*	2.4	2.8
mannosyl		
t-Man*p*	0.9	0.5
4-Man*p*	9.9	8.7
4,6-Man*p*	2.6	2.5
galactosyl		
t-Gal*p*	6.9	6.3
2-Gal*p*	2.3	1.8
4-Gal*p*	1.1	0.8
6-Gal*p*	0.2	
2,4-Gal*p*	0.4	0.4
4,6-Gal*p*	4.2	5.6
glucosyl		
t-Glc*p*	2.7	1.6
3-Glc*p*	1.5	1.5
6-Glc*p*	1.2	1.3
4-Glc*p*	31.8	31.4
3,4-Glc*p*	0.5	0.6
4,6-Glc*p*	10.2	12.8

[a]Values are the mean of three independent replicates (n = 3), and variance was <10%.

that all 4,6-Glc*p* and one-third of 4-Glc*p* contributed to the xyloglucan backbone, the remaining 4-Glc*p* was considered to be starch. Starch is not soluble in supercritical CO_2; therefore, the starch content of the tomato matrix should not be modified by the supercritical fluid extraction. Nevertheless, the amount of starch in SC-CO_2-extracted tomato matrix was greater than in the unextracted matrix (Table 8), likely due to the high pressure (450 bar) and relatively high temperature (approximately 65−70 °C) conditions required by the extractive method that might loosen the starch granules,[48,49] allowing an improved methylation and/or subsequent TFA hydrolysis of starch polysaccharides and a better detection of glycosyl linkages.

The glycosyl linkage composition of KOH-solubilized polysaccharides was typical of hemicelluloses, which were only very slightly contaminated by pectins (Table 5). The major hemicelluloses identified were xyloglucans, AXs, and glucomannans and/or galactoglucomannans. These three distinct hemicelluloses were almost quantitatively equal as mole percentages (Table 8). Xyloglucans were identified for the diagnostic presence of 4,6-Glc*p* and 4-Glc*p* residues, which make up the backbone of the polymer and the side chains composed of *t*-Xyl*p* and 2-Gal*p*. In support of this, the enzymatic hydrolysis of SC-CO_2-extracted tomato matrix with 0.1% Driselase, followed by the detection of glycosyl residues, showed the presence of isoprimeverose among other sugars (Table 9).

Similarly to the CDTA + Na_2CO_3-solubilized polymers, we found that the ratios of substituted (4,6-Glc*p*) and unsubstituted (4-Glc*p*) glucosyl residues were approximately 1:3 and 1:2.5 in SC-CO_2-unextracted and -extracted tomato matrix, respectively. Both values are different from the typical ratio found in dicot (3:1) and monocot (1:1) xyloglucans,[45−47] suggesting that part of the 4-Glc*p* and 4,6-Glc*p* residues may derive from the hydrolysis of starch. The identified xyloglucans lack of *t*-Fuc*p* residues and the amount of *t*-Xyl*p* units were not stoichiometric with the amounts of 4,6-Glc*p* residue, and no 2-Xyl*p* residues were found to account for this difference. It is

Table 6. Linkage Analysis of Insoluble Residue (*a*-Cellulose) Remaining after Sequential Extraction with CDTA + Na_2CO_3 and KOH from SC-CO_2-Unextracted and -Extracted Freeze-Dried Tomato Matrices (cv. Perfectpeel)[a]

	mol %	
glycosyl linkage	SC-CO_2-unextracted matrix	SC-CO_2-extracted matrix
rhamnosyl		
2-Rha*p*	0.2	0.2
arabinosyl		
5-Ara*f* + 4-Ara*p*	0.3	0.1
xylosyl		
t-Xyl*p*		0.1
4-Xyl*p*	0.3	0.3
mannosyl		
t-Man*p*	0.5	0.4
4-Man*p*	11.9	10.4
2,6-Man*p*		0.3
4,6-Man*p*	2.3	1.7
galactosyl		
t-Gal*p*	2.0	2.0
2-Gal*p*	2.4	1.4
4-Gal*p*	1.5	0.6
2,4-Gal*p*	2.6	3.4
4,6-Gal*p*	1.3	1.3
2,4,6-Gal*p*	0.3	
glucosyl		
t-Glc*p*	2.2	1.5
3-Glc*p*	2.2	0.5
4-Glc*p*	64.8	70.7
3,4-Glc*p*	2.5	2.9
4,6-Glc*p*	2.7	2.2

[a]Values are the mean of three independent replicates (n = 3), and variance was <10%.

Table 7. Scheme of the Method Used To Calculate the Polysaccharide Amounts

polysaccharide	glycosyl residues considered in polysaccharide estimation
homogalacturonan and rhamnogalacturonan I	4-GalA*p* plus 2-Rha*p* and 2,4-Rha*p*
arabinan	5-Ara*f* plus 3,5-Ara*f* together with the equivalent branching of *t*Ara*f*
galactan	4-Gal*p*
arabinogalactan I	4-Gal*p* plus 3,4-Gal*p* and *t*-Gal*p* equivalent to 3,4-Gal*p*
arabinogalactan II	3,6-Gal*p*, 3-Gal*p*, 6-Gal*p* and *t*-Ara*f* equivalent to 3,6-Gal*p*
xyloglucans	4,6-Glc*p*, 4-Glc*p* equivalent to 1/3 of 4,6-Glc*p*, *t*- and 2-Xyl*p* and 2-Gal*p*, *t*-Fuc*p*
arabinoxylans	4-Xyl*p*, 2,4-Xyl*p*, 3,4-Xyl*p* and *t*-Ara*f* equivalent to 2,4-Xyl*p* plus 3,4-Xyl*p*
mannan	4-Man*p*
glucomannans	4-Man*p*, 4-Glc*p* equivalent to 1/2 4-Man*p*
galactoglucomannan	4-Man*p*, 4,6-Man*p* and *t*-Gal*p* equivalent to 4,6-Man*p*, 4-Glc*p* equivalent to 1/2 4-Man*p* plus 4,6-Man*p*

well reported that changes to xyloglucan structure occur in monocots and dicots. Enzymes that modify xyloglucan oligosaccharides have been detected in plant cell walls.[50−53] From the total amount of 4-Glc*p* (Table 5), the percentage assumed to derive from the backbone of xyloglucans, glucomannans, and/or galactoglucomannans was subtracted.

Table 8. Cell Wall Polysaccharides Sequentially Extracted with CDTA + Na_2CO_3 and KOH from SC-CO_2-Unextracted and -Extracted Freeze-Dried Tomato Matrices (cv. Perfectpeel)[a]

	mol %	
polysaccharide	SC-CO_2-unextracted matrix	SC-CO_2-extracted matrix
CDTA + Na_2CO_3		
homogalacturonan + rhamno-galacturonan I	42.5	40.5
arabinan	6.2	5.0
arabinogalactan II	1.2	3.1
arabinoxylan	6.6	9.6
xyloglucan	2.8	3.8
mannan	1.2	1.9
starch	8.6	13.6
KOH		
xyloglucan	19.9	21.4
arabinoxylan	18.5	21.1
glucomannan and/or galactoglucomannan	21.3	19.3
starch	22.1	21.6
insoluble residue		
α-cellulose	57.7[b]	64.6[b]
glucomannan and/or galactoglucomannan	23.3	20.1

[a]The amounts of insoluble residue and starch are also reported. Values are the mean of three independent replicates (n = 3), and variance was <10%. [b]The values of *α*-cellulose (4-Glc*p*) were subtracted by the amount of 4-Glc*p* present in glucomannan and/or galactoglucomannan.

Table 9. Qualitative and Quantitative Glycosyl Residue Composition of 0.1% Driselase-Digested SC-CO_2-Extracted Tomato Matrix (cv. Perfectpeel)[a]

sugar	mol %
rhamnose	0.7 ± 0.1
arabinose	1.6 ± 0.3
galactose	1.8 ± 0.4
glucose	48.3 ± 3.4
mannose	5.3 ± 1.6
fructose	20.8 ± 3.9
isoprimeverose	5.0 ± 0.3
xylobiose	1.6 ± 0.1
cellobiose	9.4 ± 1.8
galacturonic acid	4.9 ± 0.6
glucuronic acid	0.6 ± 0.3
total (mg/g dw)	**300.6 ± 28.2**

[a]The glycosyl residues were determined by HPAEC-PAD. Values represent the mean ± standard deviation of three independent replicates (n = 3).

The remaining 4-Glc*p* units were attributed to starch, which still represents slightly more than 20 mol % in both matrices (Table 8). Even in this case, all 4,6-Glc*p* was assumed to derive from xyloglucan backbone (Table 5).

The high amount of 4-Xyl*p* and 2,4-Xyl*p* residues and the presence of *t*-Ara*f* were diagnostic of AXs. The amount of *t*-Ara*f* was less than the amount of 2,4-Xyl*p* residues. The ratio of branched (2,4-Xyl*p*) and unbranched (4-Xyl*p*) xylose residues for AXs was approximately 0.16 for both SC-CO_2-unextracted and -extracted tomato matrices. This ratio indicates the

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presence of low branched AXs in tomato pericarp cell wall. The presence of low branched AXs in the primary cell wall of red ripe tomato fruit is a novel acquisition because these hemicelluloses are characteristic components of grass primary cell walls where microfibrils of cellulose bind and interlace AXs and/or glucuronoarabinoxylans (GAXs).[44] Other than xyloglucans and AXs, KOH also solubilized glucomannans and/or galactoglucomannans, identified by the presence of 4-Man*p*, 4,6-Man*p*, 4-Glc*p*, and *t*-Gal*p*. These hemicelluloses, found in both primary and secondary cell walls, have a backbone that contains the three aforementioned carbohydrates. The 4,6-Man*p* residues bear *t*-Gal*p* as a side unit at the O-6 position. Although glucomannans and/or galactoglucomannans are the preponderant hemicelluloses in the wood of all gymnosperms,[54−56] they have also been isolated from the cell walls of tobacco leaf midribs,[57] suspension-cultured tobacco cells[58] and the culture filtrate of suspension-cultured *Rubus fruticosus*,[59] tobacco,[60] and tomato cells.[61] In addition, galactoglucomannans are abundant in primary cell walls of solanaceous species.[61] Altogether our data do not agree with those reported by Seymour et al.[37] that evidenced a xyloglucomannan as the major hemicellulose of tomato cell wall.

Linkage analysis confirmed that the insoluble residue was mainly composed of the load-bearing cell wall polysaccharide α-cellulose as indicated by the prevalence of 4-Glc*p* residues (64.8 and 70.7 mol % in SC-CO_2-unextracted and -extracted tomato matrices, respectively) (Table 6). Because the insoluble residue contained also 4-Man*p*, 4,6-Man*p*, and *t*-Gal*p*, distinctive of glucomannans and/or galactoglucomannans, the values of 4-Glc*p* reported above were subtracted by the amount of 4-Glc*p* typically present in these polymers, calculated as half of 4-Man*p* plus 4,6-Man*p*. Thus, glucomannans and/or galactoglucomannans were only in part solubilized by KOH (Table 8). This strongly indicates an interaction between these hemicelluloses and cellulose microfibrils, whose nature has to be established. The presence of hemicelluloses in the insoluble residue has been reported in cell walls of different plant species.[62−64] It is important to emphasize that an increase in the synthesis of glucomannans was reported during tomato ripening.[65] Minor components of the insoluble residue (α-cellulose) were glycosyl residues from pectins and other hemicellulosic fragments.

Polysaccharide Saccharification and Fermentation. Once the structure of cell wall polysaccharides present in the red-ripe tomato matrix depleted of lycopene by SC-CO_2 was known, as a first approach we hydrolyzed the matrix by 0.1% Driselase. The glycosyl residue composition and the total amount of hydrolyzed sugars (300.6 ± 28.2 mg/g dw) are reported in Table 9. When an aliquot of the hydrolysate (28.0 g/L of sugars) was treated with *S. cerevisiae* strain "Cispa 161" yeast, the main sugars, glucose and fructose, were totally fermented to bioethanol within 12 h (Figure 4B). The amount of bioethanol produced was approximately 50% of the calculated theoretical value. At sugar concentrations >52.8 g/L, a rapid decrease in the fermentation of glucose and, more markedly of fructose, was observed with a simultaneous sharp decrease in bioethanol production (Figure 4C). When the substrate was concentrated to 91.6 g/L, no bioethanol was produced, although some fermentation of glucose and fructose was observed (Figure 4D).

The data indicate that the substrate concentration influences not only the time required for complete fermentation but also the yield of bioethanol production. Although, on the basis of our work, further optimization is required to investigate the profitability of the whole process, industrial tomato byproducts and SC-CO_2 wastes can be considered as potential alternative and low-cost feedstocks for bioethanol production. Moreover, by producing multiple products (e.g., lycopene and bioethanol), a hypothetical optimized byproducts biorefinery chain can maximize the value, energy content, and environmental benefit derived from these agro-industrial wastes, making such biomass even more competitive and profitable than that obtained from dedicated crops.

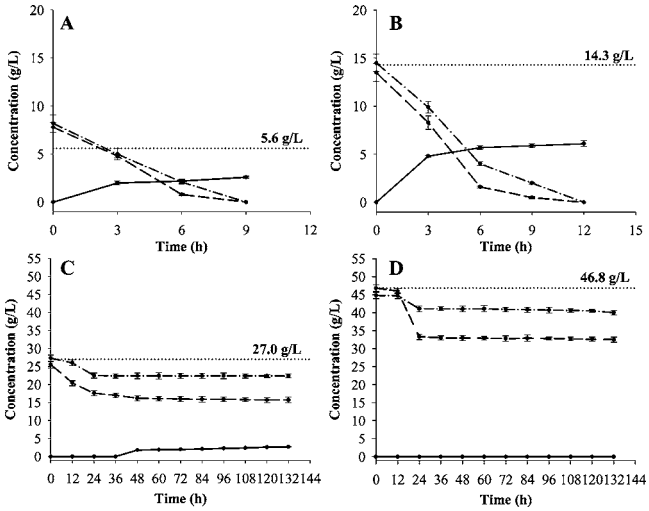

Figure 4. Time course of glucose and fructose consumption and bioethanol production during fermentation by sodium alginate bead immobilized *S. cerevisiae* strain "Cispa 161" cells of substrates with different concentrations of sugars (A = 16.0 g/L; B = 28.0 g/L; C = 52.8 g/L; D = 91.6 g/L), obtained by enzymatic digestion of SC-CO_2-extracted tomato matrix with 0.1% Driselase: dashed line, glucose; dash-dot line, fructose; solid line, ethanol; dotted line, theoretical yield of ethanol. Sugar amounts were monitored by HPAEC-PAD; ethanol was detected by HPLC with refractometric detector. The data represent the average of three independent replicates (n = 3).

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

AXs, arabinoxylans; cv., cultivar; GAXs, glucuronoarabionoxylans; HPAEC-PAD, high-performance anion exchange chromatography with pulsed amperometric detection; PMAAs,

partially methylated alditol acetates; SC-CO_2, supercritical carbon dioxide; SN, supernatant

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