Characterization of Sugarbeet (Beta vulgaris, L.) Protein

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ABSTRACT

Proteins are natural components of sugarbeet root that play a negative role during the sugar processing. In this preliminary work, an analysis of the protein fraction extracted from sugarbeet root is presented. The occurrence of oxidative phenomena makes the protein isolation a difficult step. Moreover, interference compounds, such as polyphenols and carbohydrates, may affect the quantification of proteins with traditional methods of analysis. The Kjeldhal method estimated a protein content of 0.82 ± 0.03% for the two beet varieties selected. Gel electrophoresis was performed to analyze the beet protein pattern. Samples were characterized by large and small subunits with molecular weights ranging from 106 to 25 kDa, the main fractions being approximately of 61, 55, 39, 37, and 32 kDa. NMR spectroscopy may overcome the problems related to the presence of interference compounds in the beet sample (polyphenols and carbohydrates); thus an approach to estimate the effectiveness of protein extraction was proposed.

Additional Keywords: sugarbeet proteins, interference, NMR; SDS-PAGE.

Sugarbeet (*Beta vulgaris*, L.) is a source of sucrose, an important component in foods, beverages, and pharmaceutics. Sugar factories aim to improve the recovery and quality of sugar. Sugar quality, recovery yield, and crystallization time are influenced by many factors, including non-sugar components such as proteins, amino acids, and organic anions that are partially or completely removed during the purification process (Carruthers and Oldfield, 1962; Verhaart and Oldfield, 1962; Dexter *et al.*, 1967; Devillers *et al.*, 1976; Hilde *et al.*, 1983; Khelemskii and Shoikhet, 1986; Mantovani and Vaccari, 1989; Pollach *et al.*,1991.)

The complete removal of proteins from beet cossettes may be difficult to achieve during the standard industrial process of juice purification; proteins were found to be still detectable in crystalline and inverted beet sugar (Potter *et al.*, 1990). Proteins may produce foam. This aspect is not irrelevant if sugar manufacturers were to switch to (for instance) membrane technologies. To establish an identification system for sugarbeet varieties, the extraction of crude protein from leaves (Sheen, 1991) and the comparison of patterns of total and fractionated proteins from seed (Oleo *et al.*, 1992) have been carried out. Further study on protein composition might improve the sugar extraction and facilitate selection within sugarbeet breeding programs.

In this preliminary study, an innovative approach for the analysis of proteins from sugarbeet is proposed. Polyacrylamide gel electrophoresis (PAGE) and nuclear magnetic resonance (NMR) techniques were used to determine the protein profile and the quality of the extract, respectively. The NMR technique, because of its high resolution, may overcome the problem of matrix interference, thus contributing to the characterization of protein extract from a complex matrix such as sugarbeet.

MATERIALS AND METHODS

Plant samples and reagents

Sugarbeet roots were grown in experimental fields (ca. 20,000 square meters) of a sugar factory located in central northern Italy (S.F.I.R., Forlimpopoli, FC, Italy). Two commercial varieties were selected for study: Bianca (code B156) and Gabriela (code G256). The samples were stored in plastic bags at –80°C prior to protein extraction, then at –20°C during the laboratory analysis. To avoid oxidation, the samples were defrosted in a water bath under a continuous stream of nitrogen at 4°C.

Protein extraction

Proteins were isolated from beet according to the method of Ogan *et al.* (1996) with minor modifications as follows: about 150 g of beet roots were cut into small pieces, mixed in a 2:1 (w:v) ratio with 20 m*M* sodium phosphate buffer (pH 6.4) with 3% sodium metabisulfite. Samples were homogenized (Ultra Turrax T-25, Janke & Kunkel, IKA Labortechnik, Stanfen, Germany) for 10 min, then pressed and centrifuged at 2000 g for 60 min at 4°C (ALC4237R, ALC International, Milano, Italy). The beet brei was filtered through Whatman paper 41 (Whatman, Maidstone, England) and then vacuum filtered through a 0.45 mμ cellulose-acetate HA-membrane (Millipore, Bedford, MA). Proteins were precipitated at 80% ammonium sulfate saturation. After centrifugation (2000 g for 15 min at 4°C) the proteins were diluted in 2 m*M* sodium phosphate buffer (pH 6.4) and dialyzed (molecular weight cut off 4000–6000, Membrane Filtration Products Inc., San Antonio, TX) against the same buffer (4 h, 4 changes). Following dialysis, the proteins were further purified by one step ion chromatography using batch cellulose DEAE (DE52, Whatman). Two buffers, the 2 m*M* sodium phosphate buffer (pH 6.4) and the same buffer plus 800 m*M* NaCl, were used to equilibrate the resin and to elute the protein, respectively. Beet proteins were lyophilized (BVF8/RB, B. Basi, Milano, Italy) and stored at –20°C in an airtight plastic bottle.

Nitrogen and polyphenols determination

The total nitrogen content was determined with a Kjeldahl 1030 analyzer (International Pbi, Milano, Italy). Total polyphenols were measured using the Folin-Ciocalteau reagent (Singleton and Rossi, 1965).

Polyacrylamide gel electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 3% stacking and 10% resolving gels according to the method of Laemmli (1970) and Davis (1964). A vertical electrophoresis apparatus (Hoefer SE 600, Pharmacia Biotech, Uppsala, Sweden) was used at room temperature with a constant voltage of 250 V. Standard proteins were used as molecular weight markers (Bio-Rad Lab., Hercules, CA). Lyophilized beet proteins were dissolved into 2 m*M* sodium phosphate buffer (pH 6.4) and added with 5 μl loading buffer (0.25*M* Tris-HCl pH 6.8, 35% glycerol, 1% SDS, 5% 2-mercaptoethanol, and 5 mg of bromophenol blue). The protein bands were visualized by staining with 0.05% Coomassie brilliant blue R-250 in methanol/water/acetic acid (ratio 5:4:1, v:v:v) followed by destaining with water/methanol/acetic acid (ratio 7:2:1, v:v:v). Tris-glycine buffer with SDS was used for SDS-PAGE analysis (Sambrook *et al.*, 1989).

NMR analysis

Spectra of beet protein were acquired on a Bruker Spectrospin spectrometer AC-200 (Karlsruhe, Germany), equipped with a temperature control unit and interfaced with an Aspect 3000 computer. Software provided with the instrument was used for acquisition and processing of data. About 10 mg of lyophilized beet protein was dissolved in 500 µl of D2O containing 50 m*M* potassium phosphate buffer (pH 7.5) in a polypropylene micro–sample tube (1.5 ml capacity). The solution was then transferred to a 5 mm NMR tube for the spectroscopy analysis. The ^{1}H-NMR spectra were recorded at 25°C by acquiring 256 transients consisting of 16 K data points over a spectral width of 3205 Hz and an acquisition time of 2.56 sec. A relaxation delay of 3 sec was set. The spectra were calibrated assigning a chemical shift of 4.8 ppm to the residual HOD signal.

RESULTS AND DISCUSSION

Beet protein extraction and quantification

Proteins were isolated from beet roots of two commercial varieties. The Kjeldhal method estimated a similar protein content (0.82 ± 0.03%) for the two varieties. This low value may be due to browning phenomena due to oxidation of polyphenolic compounds that affect protein purification of sugarbeet brei. To avoid the browning, sodium metabisulfite was added to the beet brei. Protein determination may also have been affected by the presence of interference compounds. The Folin-Ciocalteau assay confirmed the presence of a high concentration of polyphenols that were removed by polyvinylpyrrolidone treatment.

Electrophoretic pattern analysis

SDS-PAGE analysis (Fig. 1) revealed a similar protein pattern for root extracts of the two beet varieties. Both samples were characterized by subunits with molecular weights ranging from 106 to 25 kDa, the main fractions being approximately 61, 55, 39, 37, and 32 kDa. The low MW bands might result from the breakage of large subunits as a consequence of the lyophilisation process.

Conversely, the presence of large aggregates might be due to protein dehydration (Sheen, 1991). The SDS-PAGE profiles of crystal and invert beet sugar reported by Potter *et al.* (1990) had molecular weights ranging from 16 to 92 kDa. Comparison between the patterns obtained in our study and those found in the literature suggested that several proteins contained in the beet root might pass unaltered through the extraction process of sugar. At the same time, protein hydrolysis may

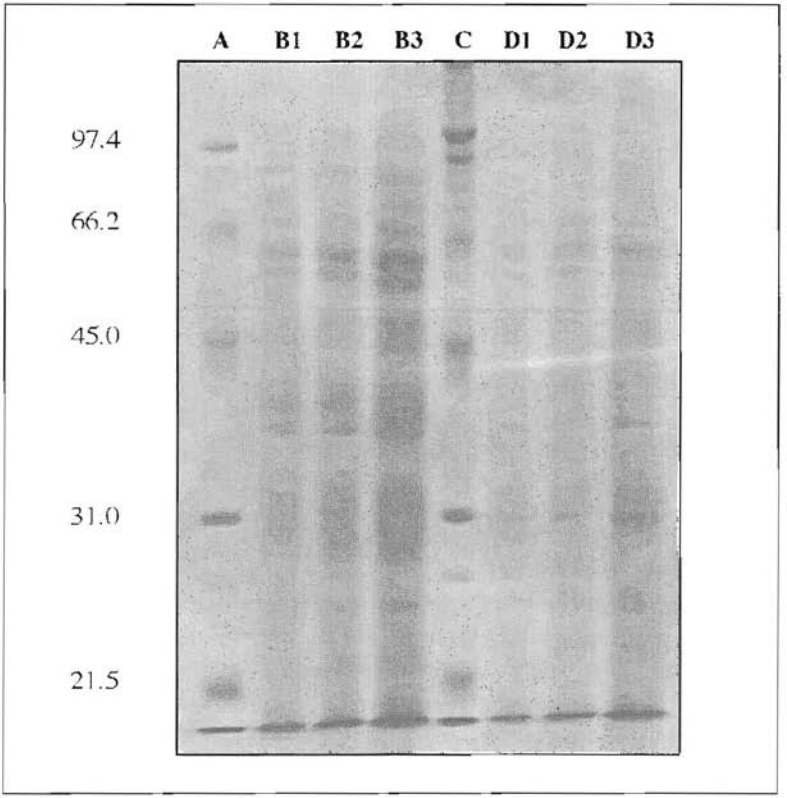

Fig. 1. SDS-PAGE profile of the protein fraction isolated from the sugarbeet roots. Coomassie blue stained 10% SDS polyacrylamide gel. Lanes A and C: molecular weight markers. Lanes B1 to B3: variety G256. Lanes D1 to D3: variety B156. Lanes B1 and D1 containing 15 mg protein; lanes B2 and D2 containing 30 μg protein; lanes B3 and D3 containing 60 mg protein.

occur due to the process temperature in the juice purification stage that goes up to 95°C and to 125°C at the evaporation step. For this reason, proteins having molecular weights larger than 92 kDa, that are not detected in purified beet sugar, are visible in the raw extract of sugarbeet. Differences in the protein profile among data from literature may also be dependent on the area of origin, genotype, and purity of the samples.

NMR analysis

The one-dimensional ^{1}H NMR spectrum (Fig. 2) showed that, apart from the sugar peaks, there is a mixture of compounds with very different

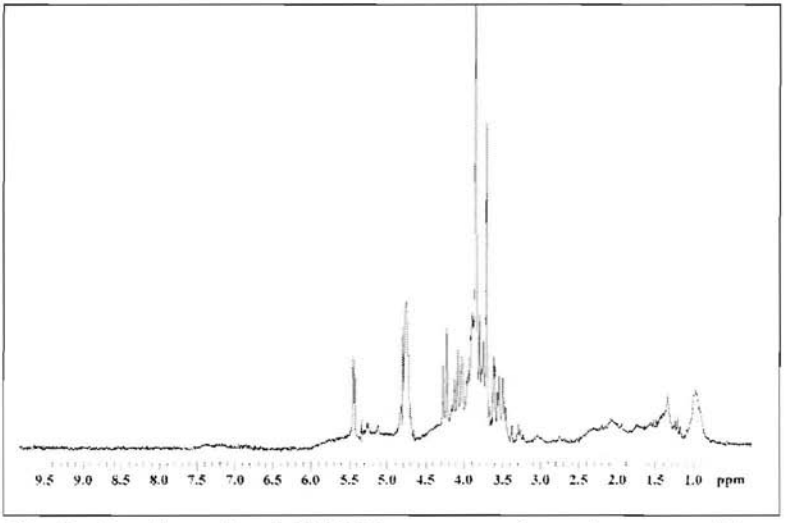

Fig. 2. One-dimensional ^{1}H NMR spectrum of protein extracted from the sugarbeet roots.

molecular weights. The signals with high intensity in the 3.5 to 4.5 ppm range can be mainly assigned to sugar molecules. Also, the doublet at 5.4 ppm was diagnostic of the α anomeric form of glucose (such as in sucrose). The signals falling outside this range were attributed to protons belonging to proteins and other water soluble substances, excluding polyphenols (the region 6.0 to 7.5 ppm is characteristic of aromatic protons) and sugars. The protein peaks were broad enough to be easily observed and they comprised the envelope of the spectra. However, some narrow peaks, not belonging to high molecular weight molecules were easily detected, for example at 3.3, 1.5, and 1.3 ppm. In particular, the hydrogen atoms belonging to the aliphatic side chain of hydrophobic amino acids (valine, leucine, etc.) have signals below 2 ppm. A definitive assignment of these signals was hindered because of a crowded spectrum with partially overlapping signals. This problem could be overcome by using high magnetic fields and multi-dimensional techniques.

The effectiveness of protein extraction could be estimated by the ratio between the sugars area (SA) and the total molecules area (TA) in samples from different purification steps. This ratio is provided by (i) integration of the doublet at δ 5.4 (the α anomeric proton of sucrose) equivalent to 1H; (ii) multiplying such area by 21 protons, i.e. the number of protons in sucrose, to obtain the sugar area (SA), and (iii) calculation of the total area (TA) of the whole spectrum, excluding the region 4.6 to 4.9 ppm

where water resonates. This total area is proportional to the content of all the substances present in the extract, mainly proteins and sugars, assuming an average number of protons for each molecule. To provide a reliable purity index, however, additional samples will need to be analyzed.

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