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A juice extractor can simplify the downstream processing of plant-derived biopharmaceutical proteins compared to blade-based homogenizers

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ABSTRACT

The production of biopharmaceutical proteins using plant-based systems has recently become economically competitive with conventional expression platforms based on microbes and mammalian cells, but downstream processing remains a significant cost factor. Here we report that, depending on the protein expression level, production costs for biopharmaceuticals made in plants can be reduced by up to 30% if a juice extractor is used instead of a blade-based homogenizer or blender. Although the extraction efficiency is lower, combining extraction and solid–liquid separation into a single operation reduces the extract volume by 80%, which achieves savings of ~60% for downstream consumables and labor. Additionally, juice extraction can easily be scaled-up to process several tons of biomass per day and its continuous mode of operation simplifies downstream processing steps because the volume of storage tanks and the duration of hold times are reduced. The juicer setup is also compatible with flocculation and to some extent with leaf blanching, which increase the efficiency of extract clarification and product purification, respectively. The use of juicers can therefore significantly increase the competitiveness of plant-based production platforms.

1. Introduction

The advantages of plant-based expression systems for the manufacture of biopharmaceutical proteins include low costs, ease of process scale-up and product safety [1–3]. The relevance of product safety became evident when the Israel-based company Protalix Biotherapeutics began producing glucocerebrosidase (for the treatment of Gaucher disease) in carrot cells [4] and outcompeted the previous market leader Genzyme because the latter had to cease their production due to virus contamination [5,6]. The upstream capacity can be increased easily by providing larger greenhouses or by open field cultivation. In contrast, downstream processing (DSP) is a major cost factor for plant-based production systems [7,8], mainly reflecting the large number of unit operations required for clarification due to the high particle burden in extracts prepared by blade-based homogenizers, hereafter described as blenders [7,9,10]. The scalability of DSP equipment can also be limited by technical and economic constraints, e.g. the size of a blender is restricted by the power and maximum speed of the motor supplied with the device, which depends on the cost/benefit ratio to the manufacturer. These constraints ultimately reduce the economy of scale because scale-up becomes associated with increasing costs. Even before technical constraints apply, numbering-up may become necessary to prevent lag times and delays during processing that result from discontinuous blending steps. To avoid these drawbacks, we tested the ability of a continuously operational juice extractor to extract protein-containing plant sap from transgenic tobacco leaves expressing two model proteins (the monoclonal antibody 2G12 and the fluorescent reporter protein DsRed), thereby integrating extraction and initial solid–liquid separation in a single device. We investigated the compatibility of this method with blanching and flocculation, two techniques that facilitate the purification of recombinant proteins and the clarification of plant extracts, respectively [11,12]. We also used a previously described cost model to evaluate the impact of juice extraction on production costs compared to the use of a conventional blender [8].

Abbreviations: CCCE, counter current continuous extraction; DoE, design of experiments; DSP, downstream processing; NTU, nephelometric turbidity unit; POI, protein of interest; TSP, total soluble protein.

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2. Materials and methods

2.1. Plant cultivation and protein extraction

Transgenic tobacco plants expressing monoclonal antibody 2G12 and the fluorescent protein DsRed were cultivated in a greenhouse as previously described [10]. Leaves were harvested 50 days after seeding, and the intact leaves were blanched as previously described [11] if this step was included in the design-of-experiments (DoE) setup. Proteins were then released from the tissue using either a blender [10], or a bench-top juice extractor (8006 Nutrition Center Masticating Juicer, Omega, Harrisburg, PA, USA) running at 150 W and 80 rpm. The proteins were released from 100 to 150 g of leaf biomass either by blending for 3 × 30 s (with 30-s breaks) in three volumes (3 mL g^{-1}) of extraction buffer (50 mM phosphate, pH 7.0, 500 mM sodium chloride, 10 mM sodium bisulfite), or by processing in the juice extractor with an average residence time of 30 s. Protein re-extraction from spent solids was carried out as described for blending, using three volumes of extraction buffer per gram of solids.

2.2. Flocculation and filtration

Before bag filtration [10], the flocculant Polymin P (BASF, Ludwigshafen, Germany) was added to the extracts in concentrations determined by an IV-optimal DoE consisting of 29 runs. The DoE included blanching temperatures of 20, 45 and 65 °C tested in combination with pH values of 6.0, 7.0 and 8.0 as well as various flocculant concentrations in the range 0–8 g L^{-1}. Details of the DoE procedure are provided elsewhere [13].

2.3. Protein quantitation

The concentrations of total soluble protein (TSP) and DsRed were determined using the Bradford method [14] and fluorescence detection, respectively, as previously described [12]. The concentration of 2G12 was determined by densitometric analysis of immunoblots after separation by LDS-PAGE and detection using a goat α-human heavy and light chain antibody conjugated to alkaline phosphatase (109-055-003, Dianova, Hamburg, Germany) to catalyze the colorimetric reaction of nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP). Authentic 2G12 antibody was used to generate a standard curve. The DsRed/TSP and 2G12/TSP concentration ratios were used to calculate the purity of the two target proteins in all samples.

3. Results

3.1. Juicer extraction increases the protein concentration but reduces the overall yield

Transgenic tobacco leaves expressing 2G12 and DsRed were harvested from greenhouse-grown plants as previously described [10]. The concentrations of TSP, DsRed and 2G12 in the extract were 2.9, 2.4 and 1.4 times higher, respectively, when juice extraction was used instead of blending (Fig. 1a). Furthermore, the extract volume was reduced by 80% when juice extraction was used instead of blending. In contrast, the yield per unit biomass was 40–65% lower for juice extraction (Fig. 1b) and was not affected if the biomass was processed in multiple juicing cycles because no additional extract was released. Accordingly, we found that the quantity of proteins that could be re-extracted from solids leaving the juicer was 3 ± 1-fold (n = 3) higher than the quantity re-extractable from solids in the blender.

3.2. Continuous operation of the juicer increases the process throughput

In contrast to batch-wise processing with a blender (Fig. 1c), it was possible to operate the juicer continuously (Fig. 1d). Using a bench-top scale setup, this translated to a processing rate of 200 g biomass min^{-1}, whereas the blender only achieved a throughput of 60 g biomass min^{-1} (100 g min^{-1} if break times were omitted). In our pilot-scale production facility, proteins are extracted discontinuously from 200 kg leaf biomass using blenders in 20-kg batches every 20 min, or an average of 1 kg min^{-1}. With a leaf packing density of 150 kg m^{-3} this corresponds to a volumetric processing speed of 0.4 m^3 h^{-1}, which is surpassed by a factor of five even when using small pilot-scale juicers (~2.0 m^3 h^{-1}; e.g. CROCODILE 200 T, Heger, Herrenberg, Germany).

3.3. The juicer integrates extraction and solid–liquid separation

The juicer setup also integrated extraction and initial clarification because it removed the majority of insoluble leaf debris from the extracted plant sap, whereas a blender disperses the homogenized tissue as particles in the extract [9,10]. We found that juice extraction yielded a residual 0.03 ± 0.02 g of solids (wet mass) g^{-1} biomass (0.04 ± 0.03 g mL^{-1} extract; n = 3), whereas blender extraction yielded 0.28 ± 0.05 g solids (wet mass) g^{-1} biomass (0.07 ± 0.01 g mL^{-1} extract; n = 3).

3.4. Juicer and blender extracts behave similarly during flocculation or blanching

We also investigated the compatibility of juice extraction at different pH values with blanching and flocculation, which can facilitate subsequent DSP steps [11,12]. We found that blanching at >40 °C and flocculation with >2 g L^{-1} Polymin P (BASF, Ludwigshafen, Germany) reduced the extract turbidity after bag filtration by more than 85% in the pH range 6–8 (Fig. 2a) and that turbidity declined with increasing pH. The corresponding DoE models were of good quality (Table 1) and predicted a broad turbidity minimum for intermediate blanching temperatures (~42 °C) and flocculant concentrations (~4 g L^{-1}). In contrast, concentrations of only <2 g L^{-1} were required for blender extraction to minimize the turbidity after bag filtration but the reduction was also ~85% for this setup resulting in ~150–200 NTU [12].

Blanching at 65 °C reduced the TSP concentration of juice extracts by ~90% (Fig. 2b). This effect was largely independent of flocculant concentration or pH and matched the reduction of ~92% that was achieved at the same temperature using blender extraction [11]. After blanching at 65 °C, the concentration of DsRed in the leaf extracts was reduced by 50% compared to controls at 20 °C (Fig. 2c) whereas reductions of 30% have been reported for blender extraction [11]. Similarly, the concentration of 2G12 was reduced by 60% after blanching at 65 °C (Fig. 2d). In contrast to the reduced yields, the purity of DsRed and 2G12 increased ~3.5 and ~1.3-fold after blanching compared the non-blanched juice extract (Fig. 3) reaching 0.60 and 0.006 mg mg^{-1} for DsRed and 2G12 respectively. A similar purity of 0.64 has been reported for DsRed after blanching in blender extract [11].

3.5. Blending is more expensive than juicer extraction

We found that the juicer extract volume was 80% lower than the blender extract volume, but the yield was also ~50% lower. Despite its limited compatibility with blanching, similar bag filtrate turbidity can be expected after flocculation so the subsequent depth filter equipment should have a similar capacity. Using this information, we updated a previously published cost model for