Received: 09/10/2024 Revised: 13/02/2025 Accepted: 11/06/2025 Published: 18/07/2025

Journal of the Institute of Brewing
EST 1895

ORIGINAL ARTICLE

DOI 10.58430/jib.v131i2.74

Improving wort filtration with extruded cassava flour: insights from macromolecular analysis

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Abstract

Why was the work done: The high starch content of cassava flour makes it a potential adjunct for beer production, but its poor filtration performance limits its utilisation.
How was the work done: This study investigated how the inclusion of extruded cassava flour (30%) affected the filtration of malt wort by analysis of the content and molecular weight distribution of macromolecules - starch, β-glucan, arabinoxylan, and protein - in both wort and fine particles from spent grain.
What are the main findings: Extrusion enhanced the hydrolysis of starch, β-glucan, and protein, leading to a lower soluble starch and β-glucan content, higher total nitrogen levels, and reduced high molecular weight fractions in extruded cassava flour (ECF) wort. These changes also reduced the viscosity of the ECF wort compared to native cassava flour (NCF) wort. Additionally, more low and medium molecular weight polysaccharides were found in fine particles from wort from the ECF mash separated from spent grain. Accordingly, these polysaccharides were less likely to aggregate with gel-protein, thereby improving filtration speed. Scanning electron microscopy and particle size analysis supported these findings, showing that fine particles in wort from the ECF wort had a looser, more porous structure and a smaller particle size.
Why is the work important: This study provides practical insight into how extrusion can improve the filtration efficiency of cassava flour used as an adjunct, broadening its potential use in the production of beer.

Keywords

extrusion, cassava flour, wort filtration, wort viscosity, gel-protein complexes, adjunct.

Introduction

Beer is rich in nutrients including proteins, amino acids, carbohydrates, vitamins, and minerals (Bamforth 2002). Its widespread appeal is supported by high production volumes, diverse flavours, and competitive pricing. However, with the rising cost of barley malt, brewers are increasingly seeking alternative adjuncts to reduce expense and diversify beer offerings. Cassava (*Manihot esculenta*), globally the third most produced tuber crop, thrives in tropical and subtropical regions such as Africa, Asia, and the Americas. Its abundant supply, high starch content (*ca*. 80% dry basis), and economics render it a compelling adjunct for brewing. The use of cassava can increase fermentable sugars in wort, boost alcohol content and beer stability, and enhance flavour profiles (Chisenga et al. 2019; Li et al. 2021).

However, issues with wort filtration increase when adjuncts supplement barley malt. Hug and Pfenninger (1980) highlighted the challenges of filtering wort produced from mashes with 30–50% barley malt and 50–70% cassava. Poor filtration can impact both the quality and quantity of wort, reducing beer yield and production efficiency. Key factors contributing to inefficient wort separation include: (i) residual starch in the mash, consisting of poorly degraded small granules and soluble starch (high molecular weight dextrin), which increase wort viscosity forming a dense, viscous layer that clogs filter pores (Li and Maurice 2013; Zhu et al. 2015), (ii) non-starch polysaccharides, particularly β-glucan and arabinoxylan from cell walls, which are solubilised and degraded during mashing (Jin et al. 2004; Tomasi et al. 2017). β-glucan tends to gel and precipitate, while arabinoxylans can create highly viscous solutions, especially when interacting with β-glucan, both of which hinder filtration (Jin et al. 2004) and (iii) gel-protein aggregates with undegraded starch, β-glucan, and arabinoxylans to form large protein complexes, which obstruct wort filtration by forming a sediment in the filter bed, adhering to filter pores, filling gaps between grain particles, and - potentially - sealing the pores (Barrett et al. 1973; Buhler 1996).

To mitigate wort filtration issues with adjuncts, commercial exogenous enzymes can be added during mashing (Lu and Li 2006; Desobgo et al. 2010). Alternatively, modifying the adjunct, through extrusion can also filtration. Extrusion - a high-temperature, short duration process that applies high pressure and shear force – results in starch gelatinisation and protein denaturation (Ye et al. 2018). Additionally, extrusion increases the water extraction of β-glucan and arabinoxylans, altering their degradation during mashing and affecting their levels in the wort (Sharma and Gujral 2013; Fadel et al. 2018). The use of extrusion modified adjuncts has been shown to enable switching from double to single mashing (Ma et al. 2016), increasing extract yield, reducing wort viscosity (Briggs et al. 1986; Dale et al. 1989), and improving filtration speed (Cadenas et al. 2021).

Research on the causes of filtration issues with cassava adjuncts are limited. This study explores the factors that improve wort filtration rates with extruded cassava flour (ECF) compared to native cassava flour (NCF). A comprehensive analysis of key factors influencing wort filtration is presented, focusing on the content and molecular weight distribution of macromolecules (starch, β-glucan, arabinoxylans, and protein) in the wort and fine particles separated from spent grains. The findings offer insight into the use of extruded cassava in brewing.

Materials and methods

Materials

South China No. 9 cassava flour was obtained from the Chinese Academy of Tropical Agricultural Sciences (Hainan, China). Thermostable α-amylase (CAS: 9000-90-2, 40,000 U/g), protease (CAS: 42613-33-2, 50 U/mg) and D-(+)-xylose were purchased from Macklin Inc. (Shanghai, China). Amyloglucosidase (CAS:9032-08-0, 100,000 U/mL) from Rhawn (Shanghai, China), pancreatin (CAS:8049-47-6, 8 × USP) from Merck (Darmstadt, Germany) and xylanase (CAS: 9025-57-4, 5,000 U/g) from Ruiyang Biotechnology Co. Ltd (Jiangsu, China). Dimethyl sulfoxide (DMSO, HPLC grade) and pullulan standard (2.12×10^5 g/mol) were obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA). All other reagents were analytical grade.

Extrusion

Cassava flour was extruded using a co-rotating twin-screw extruder model UVTE36–24, manufactured by Changsha Chuangxiang Food Technology (Hunan, China). The extruder had a length-to-diameter ratio of 24:1 with a die diameter of 5 mm. The extruder operated across five temperature zones set at 40, 50, 60, 87, and 113°C. The moisture content was adjusted to 23% (dry basis) using an automatic water inlet device. The screw speed was set to 148 rpm, with a feed rate of 10 kg/h. After extrusion, the cassava flour was dried at room temperature (25°C) until a moisture content of 10%. It was then ground and sieved through a 0.18 mm mesh before being stored in a sealed bag.

Mashing process

The barley malt (Commander) - moisture 4.1 ± 0.3%, protein 10.8 ± 0.4%, Kolbach index 37.2 ± 0.2%, diastatic power 330 ± 3 °WK) - was ground using a two-roller disc mill (Künzel, Mainleus, Germany) with a gap width of 0.8 mm. Cassava flour was used to replace 30% of barley malt in the mashing process. For the preparation of extruded cassava flour (ECF) mash, 140 g of barley malt was mixed with 60 g of ECF with 800 mL of preheated distilled water (50°C), followed by heating with continuous stirring. The temperature profile was: 50°C for 1 h, 63°C for 1 h, 70°C for 30 min, and 78°C for 10 min to stop the mashing process. The heating rate was 1°C/min.

For native cassava flour (NCF), gelatinisation and liquefaction were necessary before mashing with malt. A mixture of 60 g of NCF and 30 U/g thermostable α-amylase was combined with 280 mL of preheated water, incubated at 90°C for 20 min, and then heated to 100°C for 10 min. The mixture was cooled to 63°C prior to combining with the malt mash. Concurrently, 140 g of malt with 520 mL water was heated at 50°C for 1 h. The liquefied NCF was added to the malt mash, and the temperature raised to 63°C for 1 hour, followed by the steps as used for extruded cassava flour.

Filtration rate

Wort and spent grain were separated using a modified transparent Buchner funnel (Zhu et al. 2015). Following the mashing process, the mash was transferred to the filtration unit and allowed to stand for 20 min to allow the precipitation of malt husks and the formation of a filter layer. The volumes of wort were recorded at 10 min intervals over 80 minutes. Filtration was terminated when no further wort runoff was observed.

Separation of fine particles from spent grain

Spent grain was sieved through a 0.5 mm mesh and washed with wort obtained by gravity filtration. The mixture of fines particles and wort was then centrifuged at 5,000 × *g* for 20 min. The supernatant (wort) was collected, and the spent grain was rinsed again. This procedure was repeated three times. The centrifuged wort and precipitated fine particles were collected separately. The fines were lyophilised, ground, sieved using a 0.5 mm mesh, and stored in a sealed bag.

Wort viscosity

Wort viscosity was measured by a falling ball viscometer (VISCOBALL, Fungilab, Spain). A suitable density ball was chosen and the time (t) recorded of the ball falling through the wort at 20°C. The wort viscosity was calculated by Kinetic viscosity formula:

$$V = K \cdot (d1 - d2) \cdot t \qquad (i)$$

Where V is wort viscosity, K is viscosity constant, d1 is the density of ball, and d2 is the density of wort at 20°C.

Extraction of macromolecules from wort

Extraction of soluble starch from wort

Soluble starch in the wort was extracted following the procedure of Yu et al (2020). Wort (2 mL) was mixed with 8 mL of 95% (v/v) ethanol and stood for a minimum of 30 min to precipitate soluble starch. The mixture was centrifuged at 6,000 × *g* for 10 min and the supernatant discarded. To remove protein and β-glucan, the precipitate was treated with protease and lichenase. 95% ethanol was added and centrifuged at 4000 × *g* for 10 min to collect the precipitate which was dried.

Extraction of β-glucan from wort

The β-glucan was extracted according to the method of Tomasi et al (2017) with minor modifications. Wort (20 mL) and 10 g of ammonium sulphate was held at 4°C for 48 hours to precipitate β-glucan. After centrifugation at 1,500 × *g* for 10 min the supernatant was decanted. The precipitate was resuspended in 5 mL of 50% (v/v) ethanol, with the volume adjusted with ethanol to 45 mL. The mixture was centrifuged at 1,500 × *g* for 5 min, with the ethanol washing step repeated twice.

The precipitate was suspended in 0.1 M sodium acetate buffer (pH 5.0). Thermostable α-amylase (3.75 mg) was added and incubated at 80°C for 90 min. Amyloglucosidase (1.4 µL) was added and incubated at 60°C for 12 h. The mixture was centrifuged at 1,500 × *g* for 15 min. Supernatant (15 mL) was decanted and combined with 0.45 mL of 0.5 M sodium phosphate solution. Pancreatin (1 mg) and 20 mg of xylanase were added and the solution incubated at 40°C for two hours. β-glucan was precipitated with 22.5 mL of absolute ethanol for 12 h, and centrifuged at 1,000 × *g* for 10 min. The precipitate was dried at 40°C for 6 h.

Extraction of arabinoxylan from wort

Arabinoxylan was extracted from wort using previously published methods (Viëtor et al. 1993; Andersson et al. 2009). Wort (1 mL) was mixed with 4 mL of absolute ethanol and held at 4°C for 12 h. The mixture was centrifuged at 5,000 × *g* for 10 min, and the supernatant discarded. The precipitate was resuspended in an 80% (v/v) ethanol and centrifuged using the same conditions. The precipitate was treated as above for extraction of β-glucan from wort, except that xylanase was replaced with 100 µL of lichenase (1,000 U/mL, Megazyme), and the mixture was incubated at 40°C for 2 h. After this, 135 mL of absolute ethanol was added and the mixture kept on ice for 2 h for the arabinoxylan to precipitate. The precipitate was dried and stored for further analysis.

Extraction of protein from wort

Protein extraction from wort was according to Niu et al (2018) with slight modifications. Ammonium sulphate (80% saturation) was added to wort and kept at 4°C for 12 h. The mixture was centrifuged at 8,000 × *g* for 10 min. The precipitate was dissolved in distilled water and dialysed at 4°C for 24 h using a 3.5 kDa molecular weight cut-off membrane. The protein extract was freeze dried.

Protein was dissolved at 150 µg/mL in distilled water for subsequent procedures. This solution was mixed with 25 µL of 'reducing' buffer containing 0.08 M Tris-HCl buffer (pH 6.8), 1% (w/v) sodium dodecyl sulphate (SDS), 2% (v/v) β-mercaptoethanol, 5% (v/v) glycerol, and 0.025% (w/v) bromophenol blue

Extraction of macromolecules from fine particles

Extraction of starch from fine particles

Using the method of Yu et al (2017), fine particles containing 4-6 mg of starch were incubated with Tricine buffer (250 mM, pH 7.5, with 0.9 U/mL protease) at 37°C for 2 h to remove protein, and then centrifuged at 4,000 × *g* for 10 min. The precipitate was suspended in 0.45% (w/v) sodium bisulphite solution for 30 min and centrifuged at 4,000 × *g* for 10 min. Lichenase (0.5 mL) was added to the precipitate, and the mixture was incubated at 40°C for 1 h to remove β-glucan. The mixture was centrifuged at 4,000 × *g* for 10 min, and the precipitate dried.

Extraction of β-glucan from fine particles

β-glucan was extracted using EBC method 4.16.1 and that of Marconi et al (2014). Fine particles were defatted with 2-propanol:petroleum ether (2:3) in a Soxhlet extractor for 6 h and dried overnight. Defatted sample (1 g) was mixed with 5 mL of 50% (v/v) ethanol and heated in a boiling water bath for 15 min. On cooling to ambient temperature, 5 mL of ethanol was added and centrifuged at 1,000 × *g* for 10 min. The supernatant was discarded, and the ethanol participation step was repeated twice. The precipitate was resuspended in 20 mL of sodium acetate buffer and (as for β-glucan extraction from wort) thermostable α-amylase, amyloglucosidase, pancreatin and xylanase added.

Extraction of arabinoxylan from fine particles

Arabinoxylan was extracted according to Gruppen et al (1991). Fine particles (200 mg) were mixed with 100 mL of saturated barium hydroxide solution (containing 260 mM sodium borohydride) at 20°C for 16 h. The mixture was centrifuged at 5,000 × *g* for 20 min and the precipitate extracted with 50 mL of the (above) solution for 1 h, followed centrifugation. The supernatants were pooled, neutralised using acetic acid, and dialysed against a 0.2 M sodium acetate buffer (pH 5) and distilled water. The extract was freeze-dried.

Protein extraction from fine particles

Using the method of Park et al (2021), samples (500 mg) were extracted with 10 mL of 2% (w/v) SDS with magnetic stirring for 1 h at room temperature. The mixture was centrifuged at 3,000 × *g* for 15 min, and 30 µL supernatant mixed with 10 µL of reducing buffer and heated in a water bath for 10 min.

Analyses of wort and fine particle extracts

The starch content in wort and fine particles was measured using the Total Starch (α-amylase and amyloglucosidase) assay (Megazyme International, Wicklow, Ireland). The soluble starch in wort was precipitated with 95% (v/v) ethanol and centrifuged at 6,000 × *g* for 10 min. For fine particles, the sample was washed with 80% (v/v) ethanol to remove glucose and maltodextrin, followed by centrifugation at 4,000 × *g* for 10 min. The supernatant of the wort and fine particles were discarded. The precipitate was dispersed by adding 1.7 M sodium hydroxide, and the pH of the mixture was adjusted to 5.0 using a sodium acetate buffer (600 mM, pH 3.8). Thermostable α-amylase and amyloglucosidase were added into the tube, and the mixture was incubated at 50°C for 30 min to degrade starch to glucose. This was quantified with the glucose oxidase-peroxidase reagent, and the absorbance determined at 510 nm using a microplate reader (Multiskan Sky, Thermo Fisher Scientific, Waltham, USA).

β-glucan

β-glucan was determined in wort (EBC Method 8.13.1) and fine particles (EBC Method 4.16.1), using the β-Glucan kit from Megazyme. β-glucan in wort (5 mL), was precipitated with ammonium sulphate (2.5 g) at 4°C for 20 h. The mixture was centrifuged at 1,000 × *g* for 5 min, and the supernatant was discarded. The precipitate was resuspended in 10 mL of 50% (v/v) ethanol and centrifuged, with the ethanol-washing step repeated once. The precipitate was resuspended in sodium phosphate buffer (20 mM, pH 6.5). Lichenase and β-glucosidase were added to degrade the β-glucan into glucose, which was quantified using the glucose oxidase-peroxidase reagent.

For the measurement of β-glucan in fine particles, the sample was washed twice with 10 mL of 50% (v/v) ethanol and centrifuged at 1,000 × *g* for 10 min. The precipitate was resuspended in sodium phosphate buffer (20 mM, pH 6.5) and incubated for 10 min in a boiling water bath. On cooling to room temperature, lichenase and β-glucosidase were added and β-glucan determined as for wort.

Arabinoxylan

The arabinoxylan content was quantified using a colorimetric method (Douglas 1981). Fine particles (5 mg) was combined with 2 mL of distilled water and 10 mL of an extraction solution (110 mL glacial acetic acid, 2 mL hydrochloric acid, 5 mL phloroglucinol (20% w/v), and 1 mL glucose (1.75% w/v). For wort, 1 mL with distilled water (1 mL) and 10 mL of extraction solution was prepared. The reaction tubes were heated for 25 minutes in a boiling water bath. The arabinoxylan content was quantified at 510 and 552 nm using a standard curve from different concentrations of D-(+)-xylose.

Protein

The soluble nitrogen content of wort and the protein content of the fine particles was determined using the Kjeldahl method. A conversion factor of 6.25 between nitrogen and protein was used.

Molecular weight distribution of polysaccharides

The distribution of the molecular weights of the polysaccharides (starch, β-glucan, and arabinoxylans) was determined by size exclusive chromatography, coupled with a multi-angle laser light scattering detector (BI-MWA, Brookhaven Instruments Corporation, Holtsville, NY, USA) and a Waters 2414 refractive detector (Waters, Hicrom Ltd. UK). Purified polysaccharides were dissolved in an eluent gradient (90% DMSO containing 50 mM lithium bromide) at a concentration of 4 mg/mL by heating at 80°C for 2 h. The solution was centrifuged at 10,000 × *g* for 10 min, and the supernatant filtered through a 0.45 µm syringe filter. Samples (100 µL) were injected to three size exclusive chromatography columns (Styragel HMW7, Styragel HMW6E and Styragel HMW2, Waters, USA) using a manual injector (7725i, Rheodyne, CA, USA). Pullulan was used as the internal standard to calculate the molecular weight of polysaccharides. The molecular weight distribution was cumulative expressed as a function of the molecular weight.

Molecular weight distribution of protein

Sample (20 µL) was loaded to a polyacrylamide gel electrophoresis (PAGE) system, comprised of a 4% (w/v) stacking gel and a 12% (w/v) resolving gel. Electrophoresis was performed using the DYY-8C system (Liu Yi Biotechnology Co, Beijing, China) at a constant current of 100 mA for 2 h. After electrophoresis, the gel was stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 for 4 h and then destained for 12 h using a solution of methanol, acetic acid, and water (1:1:8). A pre-stained protein marker with a molecular weight range of 17 to 180 kDa (Real-Times Biotechnology Co., ltd. Beijing, China) was used as a reference to determine the molecular weight of the bands.

Scanning electron microscopy (SEM)

SEM was used to examine the micromorphology of native and extruded fine particles separated from spent grain. The dried fine particles were scattered on a double sided tape to a sample holder and coated with a thin layer of gold. The microstructure of samples was examined at a magnification of 1,000 and 5,000 x.

Particle size distribution

Wet fine particles from centrifugation were used for particle size distribution using a Mastersizer equipped with a Scirocco 2000 unit (Malvern Instrument Ltd, Malvern, UK). Samples were suspended in distilled water with an obstruction value of about 15%, and a pump speed of 2,000 r/min. D10, D50, and D90 represented the particle sizes corresponding to 10, 50, and 90% of total volume in the particle size distribution. Additionally, the surface-weighted D[3,2] and volume-weighted D[4,3] mean particle diameters were recorded. The span, calculated as (D90 - D10)/D50, quantified the width of the particle size distribution, with a smaller span value indicating a narrower distribution.

Statistical analysis

All indicator measurements were repeated three times to ensure accuracy. Analysis was via Kruskal–Wallis non-parametric analysis of variance test followed by Dunn's test as a post-hoc test for multiple comparison ($P < 0.05$) using SPSS software version 25.0 (IBM. USA) and with graphing using Origin 2021 (OriginLab Crop, Northampton, MA, USA).

Results and discussion

Filtration

Wort filtration is key step in brewing, impacting on wort quality, yield volume, and profitability. The rate of filtration of worts from native cassava flour (NCF) and extruded cassava flour (ECF) are reported in Figure 1. The yield and filtration rate of NCF wort were lower than ECF wort for the same filtration time, even when thermostable α-amylase was used with NCF. The wort filtration rate with ECF was comparable to that of barley malt when thermostable α-amylase was added to the mash with high sorghum substitution levels (Goode et al. 2002).

At the end of filtration with the transparent Buchner funnels, the spent grain from worts of NCF and ECF was visually different (Figure 2, A1 v B1). With NCF spent grain, fine particles settled within the filter bed, obstructing the filtration channels . In contrast, the filtration channels in ECF spent grain consisted

Figure 1.

Filtration rate of worts with extruded and native cassava flour (ECF and NCF) adjunct.

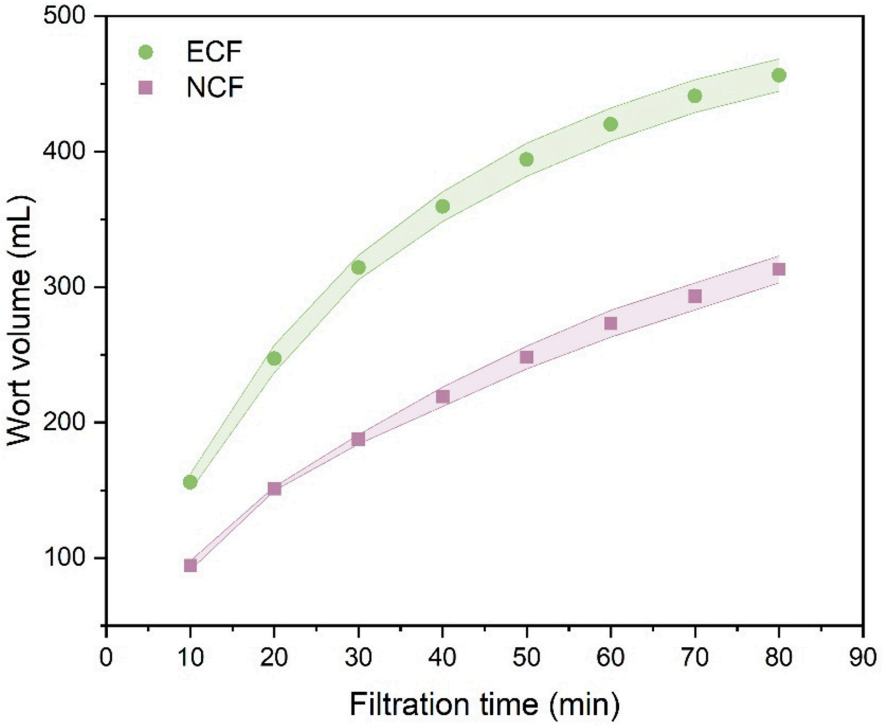
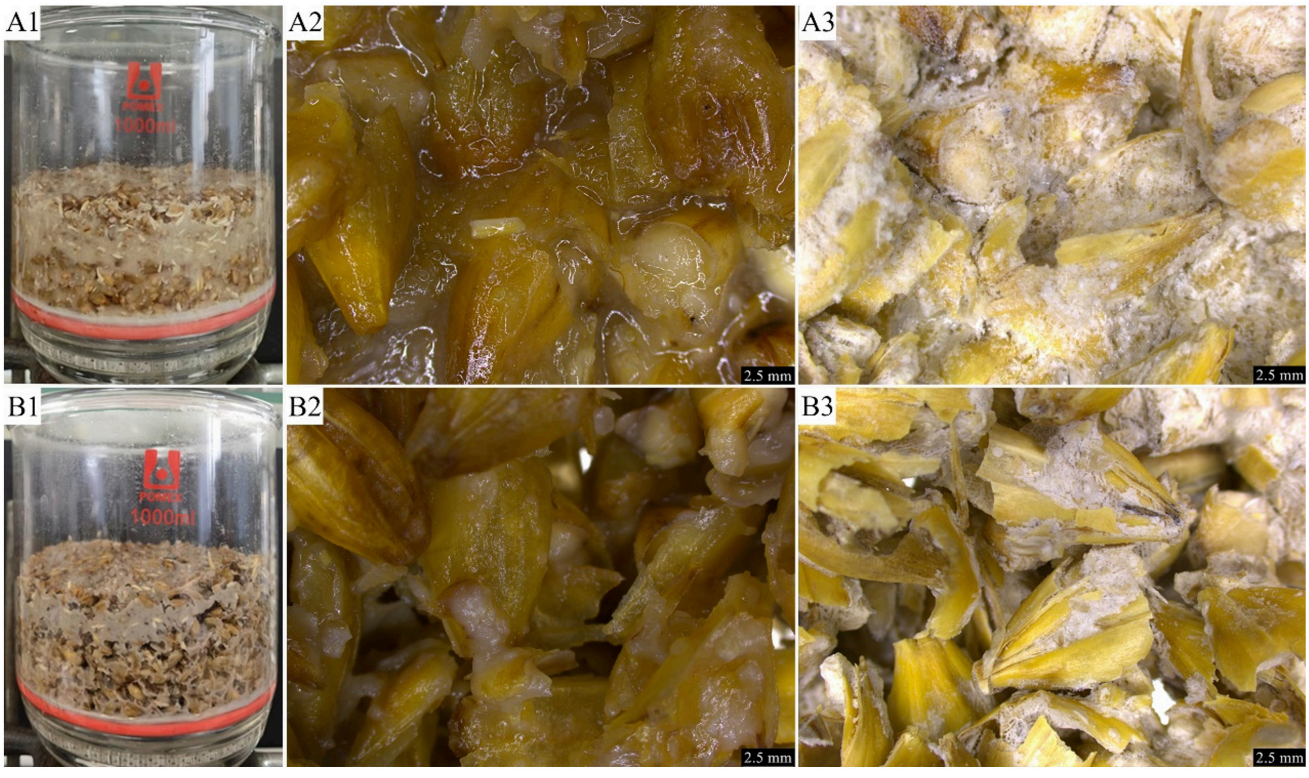


Figure 2.

Filtration device and spent grains of native (A) and extruded (B) cassava flour. Spent grain in Buchner funnels (A1, B1); Images of spent grain (A2, B2 - magnification x 600). Images of freeze-dried spent grain (A3, B3 - magnification x 600).



of coarse husks, with fine particles dispersed within the pores, but without clogging of the filtration voids. Additionally, in the lyophilised NCF spent grain (Figure 2A3), fine particles adhered tightly to the husk, causing it to clump together and form a closed structure. Conversely, the freeze dried ECF spent grain (Figure 2B3) had fewer fine particles attached to the surface of the husk, exhibiting a looser structure facilitating wort filtration. These analyses suggest that the extrusion treatment improved the wort filtration rate and altered the structure of the filter layer. Accordingly, the effect of extrusion on the filtration performance using cassava as an adjunct was analysed.

Properties of wort

Soluble starch in wort

During the mashing process, amylase breaks down starch into fermentable sugars and soluble starch residues (dextrin), some of which remains in the spent grain. Soluble starch contributes to wort viscosity and can impact the filtration performance (Sadosky et al. 2002).

As shown in Figure 3A, the concentration of soluble starch decreased from 15.1 mg/mL in NCF wort to 13.8 mg/mL in ECF wort ($P < 0.05$). This reduction in soluble starch in the ECF wort suggests a higher degree of degradation compared to NCF. Extrusion proved effective in altering the starch structure by decreasing the molecular weight, relative crystallinity, double helix degree, branching, and shortening the chain length of amylopectin, which facilitates the degradation of starch by amylase (Wang et al. 2022). In our previous study, ECF was used in the mashing process, leading to a higher extract yield and increased production of fermentable sugars (Qi et al. 2023). Another factor in the reduced soluble starch content of ECF wort is that starch in cassava associates with pulp and proteins, soluble carbohydrates and fats, forming bound starch granules (Saengchan et al. 2015). The extrusion treatment of cassava flour promotes the release of bound starch granules, as high temperature, shear force, and pressure disrupts the structure of cassava flour. Consequently, the released, gelatinised starch was more susceptible to degradation, resulting in a lower level of soluble starch in ECF wort.

Size exclusive chromatography using a multi-angle laser light scattering-refractive detector was used to determine the molecular weight distribution of soluble starch in NCF and ECF worts. Figure 3B details the cumulative average molecular weight (MW) and the minimum-maximum MW range (Table 1). In the NCF wort, soluble starch had a molecular weight range of 3.5×10^3 to 1.5×10^5 g/mol and a MW of 1.2×10^4 g/mol. Following extrusion, the soluble starch in the wort had a reduced molecular weight range of 1.2×10^3 to 5.2×10^4 g/mol and a lower MW of 4.74×10^3 g/mol.

Soluble starch is primarily produced by α-amylase, acting on amylose and amylopectin to generate dextrins (Vriesekoop et al. 2010). Compared to the more flexible amylose, amylopectin is branched and helical forming a crystalline structure which is prone to fracture during extrusion, reducing molecular size and molecular weight (Li et al. 2014). The viscosity of NCF wort was 1.62 ± 0.01 mPa·s, while the viscosity of ECF wort was lower at 1.34 ± 0.02 mPa·s ($P < 0.05$). The extrusion of cassava flour decreased both the content and molecular weight of soluble starch in the wort, contributing to lower viscosity and improved filtration speed.

β-glucan in wort

The viscous and gel-forming β-glucan comes from barley cell walls and is linked with increased beer viscosity (Kupetz et al. 2015). The β-glucan content of barley malt, NCF, and ECF is reported in (Supplementary information) Table S1. In the NCF wort, the β-glucan content (Figure 3A) was 63.5 mg/L but with extrusion (ECF) the level was reduced significantly to 25.1 mg/L ($P < 0.05$). The activity of β-glucanase which degrades β-glucan, is sensitive to temperature, losing about 50% of its activity at 45°C after 40 minutes and becoming completely inactivated at 65°C within 2 minutes (Muller 1995). The extrusion process resulted in the better solubilisation and degradation of β-glucan in the cassava adjunct at 50°C for 1 hour during mashing. These findings align with those of Home et al (1993), who reported that the concentration of β-glucan in wort is higher when mashing at 65°C compared to 35-55°C, due to the limited activity of β-glucanase at the higher temperature.

Figure 3.

Changes in macromolecules in NCF and ECF worts. (A); Cumulative values against the molecular weight of soluble starch (B), β-glucan (C), and arabinoxylan (D) in NCF and ECF worts. NCF - native cassava flour; ECF - extruded cassava flour. Values with different letters indicate a significant difference between means (P < 0.05).

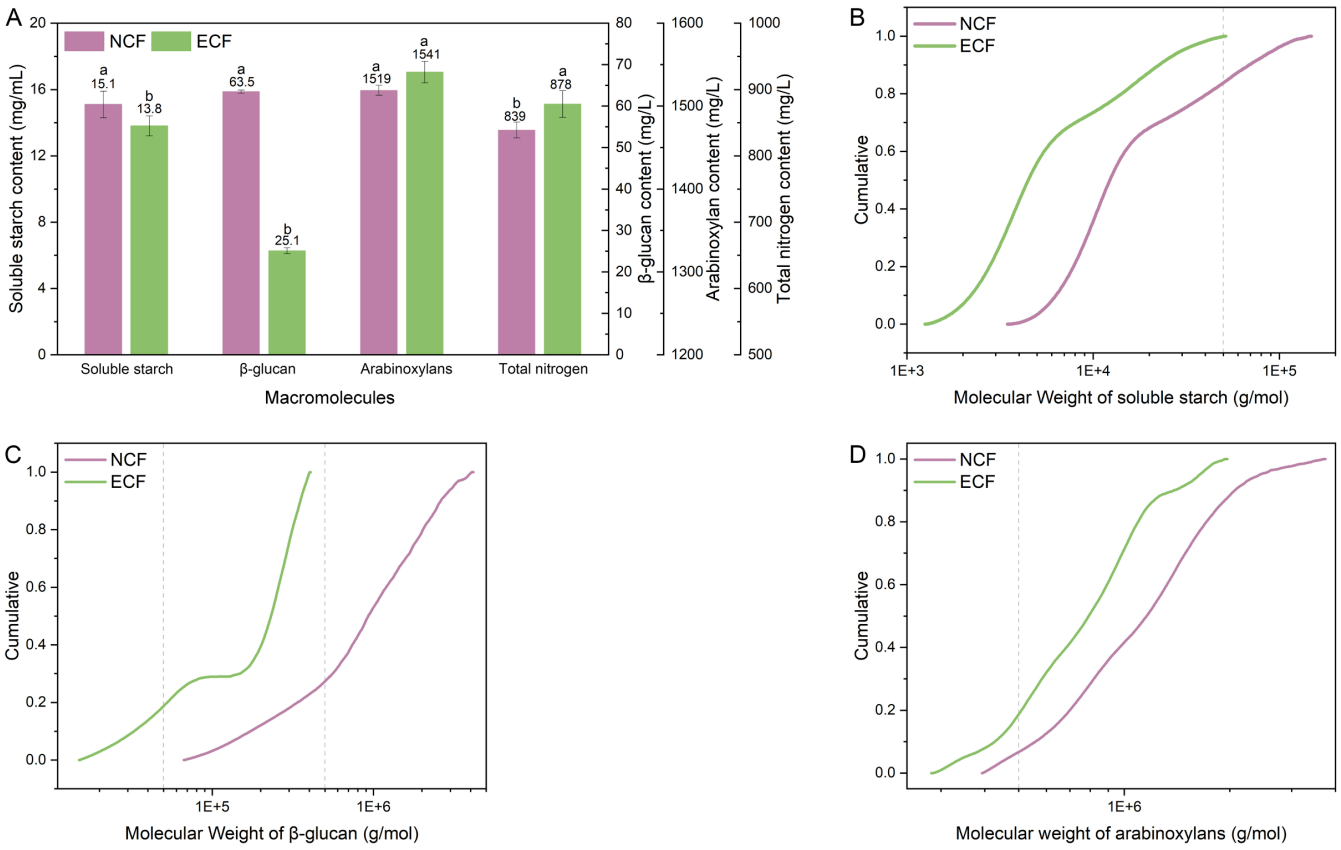


Table 1.

Molecular weight parameters of worts - native cassava flour (NCF) and extruded cassava flour (ECF).

Parameters	Soluble starch		β-glucan		Arabinoxylans	
	NCF	ECF	NCF	ECF	NCF	ECF
MW (g/mol)	$(1.2 \pm 0.1) \times 10^{4a}$	$(4.74 \pm 0.08) \times 10^{3b}$	$(4.7 \pm 0.6) \times 10^{5a}$	$(9.2 \pm 0.3) \times 10^{4b}$	$(9.84 \pm 0.05) \times 10^{5a}$	$(6.9 \pm 0.6) \times 10^{5b}$
Min MW (g/mol)	$(3.5 \pm 0.2) \times 10^{3a}$	$(1.2 \pm 0.1) \times 10^{3b}$	$(6.7 \pm 0.2) \times 10^{4a}$	$(1.5 \pm 0.2) \times 10^{4b}$	$(3.9 \pm 0.2) \times 10^{5a}$	$(2.8 \pm 0.2) \times 10^{5b}$
Max MW (g/mol)	$(1.5 \pm 0.5) \times 10^{5a}$	$(5.2 \pm 0.8) \times 10^{4b}$	$(4.2 \pm 0.8) \times 10^{6a}$	$(4.1 \pm 0.6) \times 10^{5b}$	$(3.7 \pm 0.8) \times 10^{6a}$	$(2.0 \pm 0.4) \times 10^{6b}$
LMW (%)	83.5 ± 1.8^{b}	99.9 ± 0.0^{a}	–	18.6 ± 0.2	–	–
MMW (%)	16.5 ± 1.8^{a}	0.1 ± 0.0^{b}	35.8 ± 0.6^{b}	81.4 ± 0.2^{a}	6.70 ± 0.06^{b}	25.0 ± 0.6^{a}
HMW (%)	–	–	64.2 ± 0.6	–	93.30 ± 0.06^{a}	75.0 ± 0.6^{b}

All values are expressed as arithmetic mean ± standard deviation (n=3). Values with different letters between NCFP and ECFP for same paremeter indicate a significant difference between means (P < 0.05). NCF - native cassava flour; ECF - extruded cassava flour; HMW - high molecular weight; MMW - medium molecular weight; LMW - low molecular weight. LMW: $<5 \times 10^4$ g/mol; MMW: 5×10^4 to 5×10^5 g/mol; HMW: $>5 \times 10^5$ g/mol.

In addition to concentration, the molecular weight of β-glucan is an important factor in wort filtration. The concentration of β-glucan in the wort was relatively low (Figure 3A, < 100 mg/L), but a difference in molecular weight distribution was observed. Extrusion resulted in a decrease in the MW of β-glucan, from 4.7×10^5 g/mol to 9.2×10^4 g/mol (Figure 3C, Table 1). The molecular weight fractions influencing filtration are categorised into three groups: high molecular weight (> 5×10^5 g/mol), medium molecular weight (5×10^4 to 5×10^5 g/mol), and low molecular weight (< 5×10^4 g/mol) (Sadosky 2008). In the NCF wort, the range of molecular weights of β-glucan was 6.7×10^4 to 4.2×10^6 g/mol, with medium and high MW accounting for 35.8 and 64.2%. In contrast, the molecular weight of β-glucan in the ECF wort ranged from 1.5×10^4 to 4.1×10^5 g/mol, with low and medium MW accounting for 18.6 and 81.4% of the total. Notably, there was no low MW β-glucan fraction in the NCF wort and no high MW fraction in the ECF wort. Low molecular weight β-glucan has a minimal impact on wort viscosity compared to high MW β-glucan, which tends to aggregate with other components, creating a viscous solution slowing filtration (Jin et al. 2004). Therefore, the absence of high molecular weight β-glucan in ECF wort contributed to decreased wort viscosity and increased filtration speed.

Arabinoxylan in wort

Arabinoxylan is a non-starch polysaccharide found in barley cell walls which are extracted with hot water to form viscous solutions. In beers, arabinoxylan levels are approximately ten times higher than those of β-glucan, and their impact on wort viscosity and filtration is comparable to β-glucan (Li et al. 2005). The arabinoxylan content of barley malt and cassava flour is reported in (Supplementary information) Table S1. Mashing converted arabinoxylan into a water soluble fraction, with 1519 mg/L in NCF wort and 1541 mg/L in ECF wort (Figure 3A).

The extrusion of cassava flour required different mashing schemes with double mashing for NCF and single mashing for ECF. During the mashing stage at 50°C for 1 hour, arabinoxylans in the extrudate were more effectively solubilised. As the mashing temperature increased, the activity of endoxylanases declined but arabinoxylans were solubilised at these higher temperatures, increasing their concentration in the wort (Li et al. 2005). The level of arabinoxylans could also reflect insufficient enzyme activity in cassava flour when substituting 30% of malt, reducing arabinoxylan degradation. Despite the similar arabinoxylan content in both worts, the viscosity of ECF wort was lower, prompting further analysis of the MW distribution of arabinoxylans.

The molecular weight distribution of arabinoxylan from NCF and ECF worts are reported in Figure 3D. The MW of arabinoxylan in NCF wort was 9.84×10^5 g/mol, which decreased to 6.9×10^5 g/mol after extrusion. The molecular weight distribution of arabinoxylan in NCF wort ranged from 3.9×10^5 g/mol to 3.7×10^6 g/mol, whereas arabinoxylan in NCF wort after extrusion ranged from 2.8×10^5 g/mol to 2.0×10^6 g/mol. The percentage of medium and high molecular weight arabinoxylans in NCF and ECF wort was respectively 6.7 and 93.3%, and 25.0 and 75.0%. The reduction in both MW and the proportion of high molecular weight arabinoxylans in ECF wort contributed to the decreased wort viscosity, thereby enhancing filterability.

Protein in wort

Nitrogen compounds in wort are required for successful fermentation and reflect the raw materials and the mashing process (Fumi et al. 2009). The total nitrogen content of worts was 839 mg/L for NCF and 878 mg/L for ECF (Figure 3A). This marginal increase may reflect protein denaturation during extrusion due to high temperature and shear forces. Denatured proteins expose new sites for enzyme action, enhancing protein degradation (Zhang et al. 2017). However, the mashing procedure differed, with the extruded adjunct having a protein rest, allowing proteases to hydrolyse the denatured proteins. In contrast, when adjuncts are not extruded and have a protein rest, protein is less available (Poreda et al. 2014). This result was consistent with previous studies where the total nitrogen in the wort with extruded adjuncts was increased (Grujić 1999; Jiang et al. 2023).

Molecular weight is a key parameter that indicates the extent of protein hydrolysis (Xie et al. 2014). To gain further insight into protein degradation,

sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed on proteins from NCF and ECF worts. Both worts showed prominent bands in the 27 to 40 kDa range, but the bands in ECF wort were generally denser and darker, while those in NCF wort were lighter, particularly at 40 kDa (Figure 4A). This suggests that protein degradation was more extensive in the mash containing ECF and malt. The 40 kDa band corresponded to protein Z, which is known to be relatively resistant to the brewing process and is commonly found in both wort and beer (Hao et al. 2006). The weaker band for protein Z in NCF wort may be due to protein precipitation, as at mashing temperatures > 70°C, protein Z undergoes precipitation and aggregation with macromolecules (polysaccharides), affecting the filtration performance (Huston Jr et al. 1986; Hennemann et al. 2019). The high levels of soluble starch, high molecular weight β-glucan, and high molecular weight arabinoxylan in NCF wort are likely to result in increased protein aggregation, impeding filtration.

Properties of fine particles

Wort filtration is affected not only by the properties of the wort but also by the characteristics of the spent grain. During wort filtration, malt husks serve as medium in the process. As the wort flows through, fine particles with diverse structures and chemical composition deposit, creating a heterogeneous filter cake (Hennemann et al. 2019). Understanding the characteristics of fine particles would help to understand their impact on filtration. Accordingly, fine particles within the filter cake were separated and analysed for their physicochemical properties that could affect filtration. The composition of fine particles separated from spent grains of NCF and ECF are reported in Supplementary information Table S2.

Morphology of fine particles

SEM micrographs of fine particles isolated from spent grain after mashing with NCF and ECF as adjuncts are reported in Figure 5. Here, the fine particles separated from spent grain of extruded cassava flour (ECF) were irregular in shape, varied in size, and were widely dispersed. These particles had rough, loose, and porous surfaces that may provide effective filtration channels for wort. Similar fine particles were reported when β-glucanase was added during mashing (Barrett et al. 1975). In contrast, the fine particles separated from spent grains with NCF as an adjunct tended to aggregate, forming larger, block structures with smooth, dense surfaces lacking visible pores (Figure 5B). This dense structure would likely impede wort flow, reducing filtration speed. These SEM images of fine particles separated from spent grains with an adjunct of extruded or native CF, provide an explanation for the enhanced filtration rate with ECF.

Starch in fine particles

Fine particles are composed of starch, β-glucan, arabinoxylan, and protein, which all effect wort filtration. Figure 6A shows that the starch content of fine particles separated from spent grains of NCF and ECF as adjunct was 8.7 and 8.3%, respectively.

Figure 4.

Electrophoretic separation of proteins - (A) NCF and ECF wort; (B) NCFP and ECFP fine particles. NCF - native cassava flour; ECF - extruded cassava flour; NCFP - fine particles separated from spent grain mashed with native cassava flour; ECFP - fine particle from spent grain mashed with extruded cassava flour.

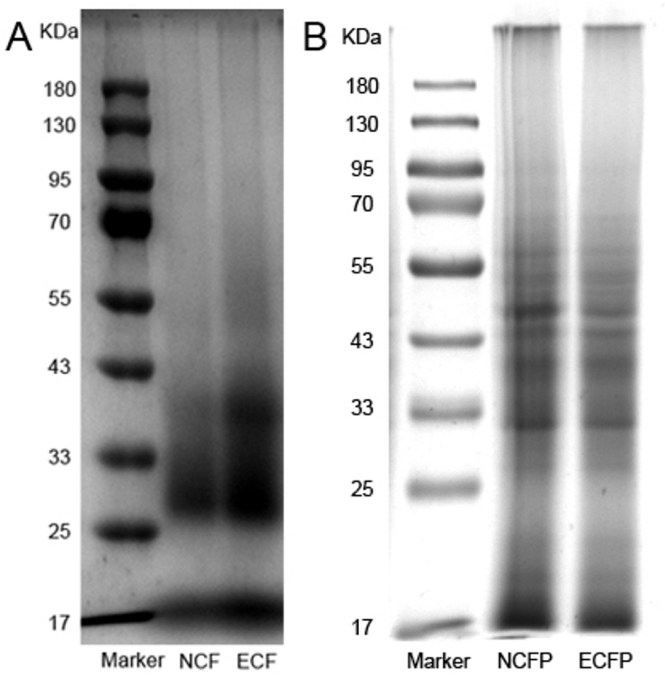
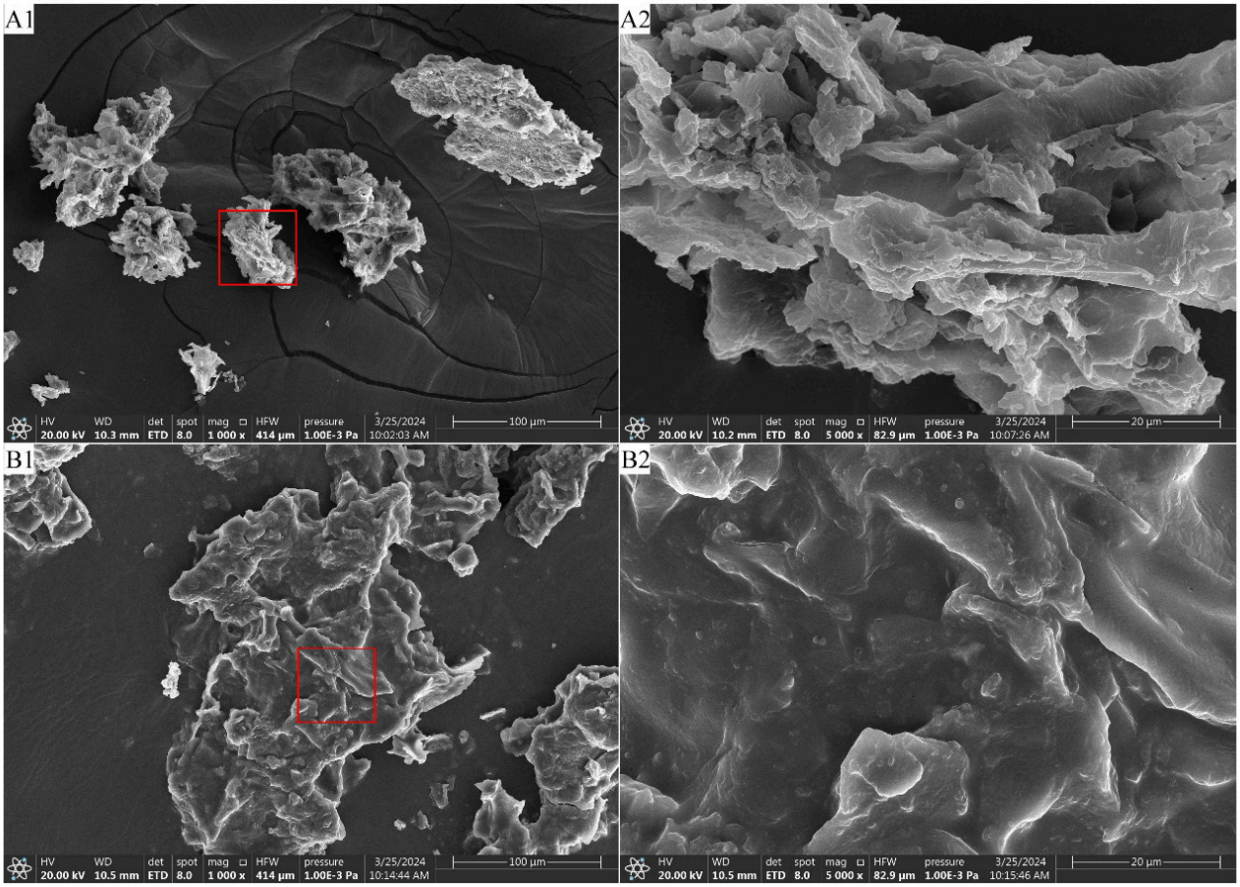


Figure 5.

Scanning electron micrographs (A) ECFP; (B) NCFP. A1/B1 - magnification x 1,000. A2/B2 - enlargement of rectangular area in A1 and B1, magnification x 5,000). NCFP: fine particles from spent grain mashed with native cassava flour; ECFP: fine particles from spent grain mashed with extruded cassava flour.



During the NCF mashing process, gelatinisation and liquefaction steps (with 30 u/g of thermostable α-amylase) were used to degrade cassava starch into fermentable sugars during the amylase rest. Despite this, fine particles separated from spent grains of ECF had a higher starch content indicating a more efficient starch utilisation. This is likely to be due to the extrusion process, which causes gelatinisation and breakdown of starch, reducing its molecular weight (Ye et al. 2018).

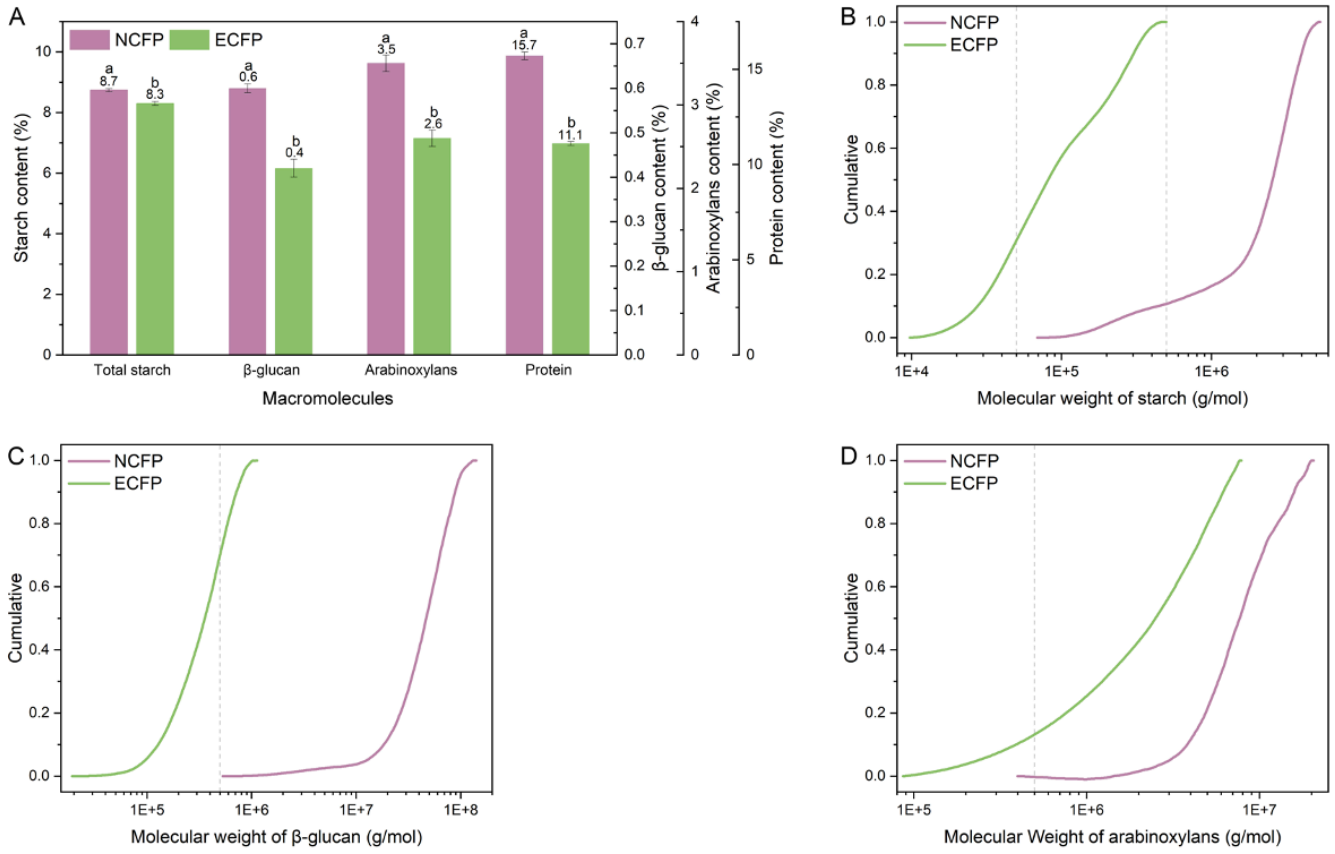
The molecular weight distribution of starch in fine particles is shown in Figure 6B. The MW of starch in fine particles separated from spent grains of NCF and ECF as adjunct were 1.1 × 10^6 g/mol and 6.0 × 10^4 g/mol, respectively. The molecular weight of fine particles separated from spent grains of NCF ranged from 6.9 × 10^4 to 5.3 × 10^6 g/mol, whereas the molecular weight of fine particles separated from spent grains of ECF were smaller, ranging from 9.7 × 10^3 to 5.0 × 10^5 g/mol. Indeed, the high MW fraction was absent in fine particles of ECF spent grains, indicating a reduced tendency to bind to hemicellulose and proteins, which enhances wort filtration performance in ECF wort (Barrett et al. 1975; Li et al. 2013).

β-glucan and arabinoxylan in fine particles

Approximately 70% of β-glucan is extracted into the wort, while the remaining 30% remains as an insoluble fraction in spent grain (Jin et al. 2004). The incomplete degradation of endosperm cells wall can lower extract yield and negatively affect beer quality (Lu et al. 2006). The β-glucan content in fine particles from NCF spent grains was 0.6%, whereas extrusion reduced the β-glucan content in fine particles from ECF spent grains to 0.4% ($P < 0.05$) (Figure 6A). The MW of β-glucan in fine particles from ECF spent grains was lower at 2.4 × 10^5 g/mol compared to 2.6 × 10^7 g/mol of NCF fine particles.

Figure 6.

Content of macromolecules in NCFP and ECFP fine particles. (A); Cumulative values plotted against the molecular weight of soluble starch (B), β-glucan (C), and arabinoxylans (D) in NCFP and ECFP. Values with different letters indicate a significant difference between means ($P < 0.05$). NCFP: fine particles from spent grains mashed with native cassava flour; ECFP: fine particles from spent grain mashed with extruded cassava flour.



The molecular weight distribution of β-glucan in fine particles from NCF spent grains ranged from 5.3×10^5 to 1.4×10^8 g/mol, with no observed low molecular weight and medium molecular weight fractions. In contrast, β-glucan in fine particles from ECF spent grains ranged from 1.9×10^4 to 1.1×10^6 g/mol, comprising 0.7% low molecular weight, 71.8% medium MW, and 27.5% high MW fractions.

Unlike β-glucan, only about 12-20% of arabinoxylans are solubilised in wort, with the remainder in the spent grain (Hennemann et al. 2019). The arabinoxylan content in fine particles from NCF spent grains was 3.5%, with extrusion enhancing the solubility of arabinoxylans which decreased to 2.6% in fine particles from ECF spent grains (Figure 6A). The molecular weight of arabinoxylans in fine particles from NCF spent grains ranged from 4.0×10^5 to 2.1×10^7 g/mol, while fine particles from ECF spent grains ranged from 8.70×10^4 to 7.9×10^6 g/mol. The MW of fine particles from NCF spent grains ranged was 6.3×10^6 g/mol while that of ECF spent grains decreased to 1×10^6 g/mol.

Like β-glucan in fine particles from NCF spent grains, the high molecular weight component arabinoxylan was 100% (Table 2). In terms of fine particles from ECF spent grains, the medium molecular weight and high molecular weight accounted for 13.5 and 86.5% of the molecular weight of arabinoxylan. Both soluble β-glucan and arabinoxylan increase wort viscosity, while their insoluble fractions bind small particles, such as undegraded starch granules and proteins, forming lumps that block filtration channels (Barrett et al. 1975). The elevated proportion of high molecular weight β-glucan and arabinoxylan present in fine particles from NCF spent grains would contribute to the formation of blocking structures (Figure 5B), reducing the filtration of NCF wort.

Table 2.

Molecular weight parameters of fine particles - native cassava flour (NCFP) and extruded cassava flour (ECFP)

Parameters	Starch		β-glucan		Arabinoxylans	
	NCFP	ECFP	NCFP	ECFP	NCFP	ECFP
MW (g/mol)	$(1.1 \pm 0.9) \times 10^{6a}$	$(6.0 \pm 0.4) \times 10^{4b}$	$(2.6 \pm 0.1) \times 10^{7a}$	$(2.4 \pm 0.1) \times 10^{5b}$	$(6.3 \pm 0.5) \times 10^{6a}$	$(10.0 \pm 0.2) \times 10^{5b}$
Min MW (g/mol)	$(6.9 \pm 0.5) \times 10^{4a}$	$(9.7 \pm 0.2) \times 10^{3b}$	$(5.3 \pm 0.2) \times 10^{5a}$	$(1.9 \pm 0.3) \times 10^{4b}$	$(4.0 \pm 0.7) \times 10^{5a}$	$(8.70 \pm 0.06) \times 10^{4b}$
Max MW (g/mol)	$(5.3 \pm 0.7) \times 10^{6a}$	$(5.0 \pm 0.7) \times 10^{5b}$	$(1.4 \pm 0.5) \times 10^{8a}$	$(1.1 \pm 0.1) \times 10^{6b}$	$(2.1 \pm 0.5) \times 10^{7a}$	$(7.9 \pm 0.5) \times 10^{6b}$
LMW (%)	–	32.9 ± 2.2	–	0.7 ± 0.1	–	–
MMW (%)	9.9 ± 0.9^{b}	67.1 ± 2.2^{a}	–	71.8 ± 0.7	–	13.5 ± 0.4
HMW (%)	90.1 ± 0.9	–	100^{a}	27.5 ± 0.7^{b}	100^{a}	86.5 ± 0.4^{b}

All values are expressed as the arithmetic mean ± standard deviation (n=3). Values with different letters between NCFP and ECFP for same substance indicate a significant difference between means ($P < 0.05$). NCFP and ECFP - fine particles separated from spent grains of native and extruded cassava flour as adjuncts; HMW - high molecular weight; MMW - medium molecular weight; LMW - low molecular weight; LMW $<5 \times 10^4$ g/mol; MM 5×10^4 to 5×10^5 g/mol; HMW $>5 \times 10^5$ g/mol.

Protein in fine particles

At 65%, protein is major component of spent grain (Celus et al. 2006). The content and protein separated by electrophoresis are presented in Figures 6A and 4B. A higher protein content (15.7%) was found in fine particles from NCF spent grains, compared to 11.1% in fine particles from ECF spent grains. The lower protein content in fine particles from ECF spent grains, coupled with a higher soluble nitrogen concentration in ECF wort, suggests that extrusion promotes more efficient protein hydrolysis. The molecular weight distribution of proteins in fine particles from ECF spent grains was like that from NCF spent grains, but the bands in the ECF fine particles appeared lighter overall. For fine particles from NCF spent grains, the bands of 30 and 45 KDa were clearer than those of fine particles from ECF spent grains. Hordeins, representing about 60% of the total protein, are storage proteins in barley (Connolly et al. 2013). Based on the electrophoretic mobility, hordein is categorised into four groups: B, C, D, and γ. B-hordeins (30–45 KDa) make up about 70–80% of hordeins, and tend to aggregate into an impenetrable complex or gel-protein, with D-hordeins (>100 KDa) and glutelin through intra- and intermolecular disulphide bonding limiting wort filtration (Silva et al. 2008; Connolly et al. 2013). The gel-protein in fine particles from NCF spent grains can also aggregate with undegraded starch, β-glucan, and arabinoxylans, forming large protein complexes. These complexes adhere to the walls of filter pores, potentially leading to complete blockage of the pores (Buhler 1996).

Particle size distribution of fine particles

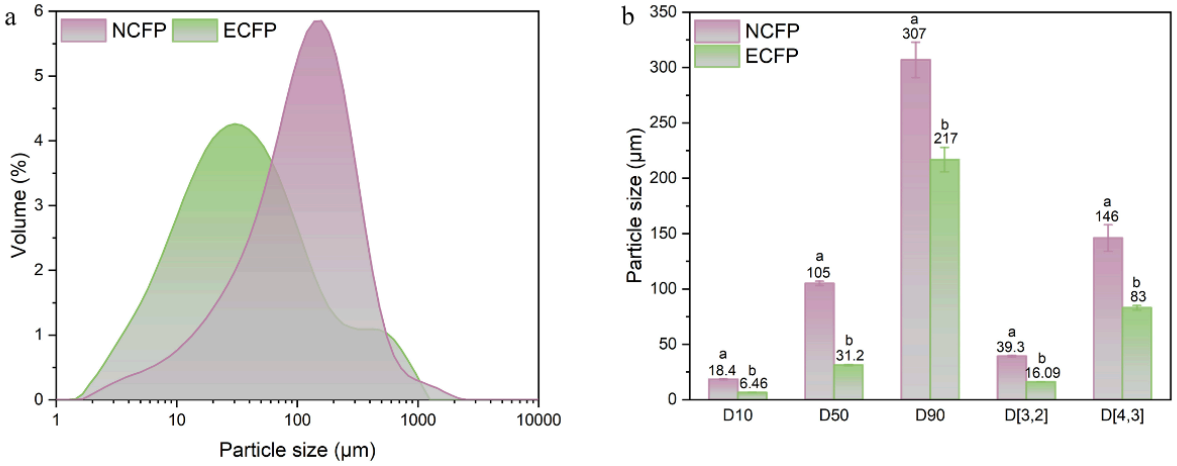
The curves of particle size distribution of fine particles separated from spent grains of NCF and ECF are presented in Figure 7A with D10, D50, D90, D[4,3], and D[3,2] shown in Figure 7B. The particle size distribution of fine particles from ECF spent grains differed markedly from those from NCF spent grains. Fine particles from NCF spent grains had a narrower distribution with a span value of 2.74 ± 0.09, compared to a broader span value of 6.7 ± 0.3 for fine particles from ECF spent grains. However, the D10, D50, D90, D[4,3], and D[3,2] values were higher for fine particles from NCF than those from ECF spent grains. This aligns with analysis, which indicated that the aggregation of macromolecules led to an increase in the particle size of fine particles from NCF spent grains, resulting in the clogging of filtration channels and hampering the filtration of NCF wort.

Conclusions

This study examined the efficiency of wort filtration and the effect of extrusion of cassava flour used an adjunct. The focus was on the content and molecular weight distribution of macromolecules in both wort and fine particles recovered from spent grain. Extrusion was found to significantly increase filtration speed, with wort from extruded cassava flour (ECF) having lower levels of soluble starch and β-glucan with higher levels of total nitrogen suggesting enhanced hydrolysis of starch, β-glucan, and protein.

Figure 7.

Particle size distribution (A) and (B) D10, D50, D90, D[3,2], and D[4,3] of NCFP and ECFP. NCFP and ECFP - fine particles separated from spent grains of native and extruded cassava flour as adjuncts; D10, D50, and D90 - the maximum diameters of the 10%, 50%, and 90% of the total volume of particles; D[3,2]: surface weighted mean; D[4,3]: volume-weighted mean.



Additionally, the lower MW and greater low molecular weight fractions of soluble starch, β-glucan, and arabinoxylan contributed to a reduction in wort viscosity from 1.62 mPa·s with native cassava flour (NCF) to 1.34 mPa·s in ECF wort. Additionally, protein Z was less likely to aggregate with low molecular weight and medium molecular weight fractions of polysaccharides in ECF wort, improving wort filtration performance. With the fine particles from ECF spent grains, residual macromolecule levels, MW, and high molecular weight fractions of polysaccharides were lower. Gel-protein, aggregated with starch, β-glucan, and arabinoxylan, formed fewer large complexes in fine particles from ECF spent grains due to the lower high molecular weight fractions. SEM and particle size distribution analyses confirmed that fine particles from ECF spent grains had a loose more porous structure and smaller particle size compared to fine particles from NCF spent grains. In conclusion, this study demonstrates that extrusion is effective in enhancing wort filtration rates by reducing high molecular weight fractions of macromolecules, thereby lowering the viscosity of ECF wort and minimising the formation of gel-protein complexes in fine particles from ECF spent grains.

Author contributions

Mingming Qi: Conceptualisation, investigation, methodology, formal analysis, writing (original draft).
Lijun Jiang: Investigation, methodology, writing (review and editing).
Jialin Song: Validation, data curation, writing (review and editing).
Feng Han: Methodology, formal analysis, writing (review and editing).
Mei Xu: Software, methodology.
Yueming Li: Software, formal analysis.
Chengye Ma: Writing (review and editing).
Shanfeng Chen: Writing (review and editing).
Hongjun Li: Conceptualisation, supervision, project administration, funding acquisition, writing (review and editing).

Acknowledgment

This study was supported financially by the Key Technology Research and Development Program of Shandong Province (2022CXGC010604).

Conflict of interest

The authors have no conflicts of interest to declare.

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