Development of chickpea beverages through enzymatic treatments: from rapid visco analyzer to pilot plant production

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Declarations

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Research highlights

- Rapid Visco Analyzer was used to evaluate hydrolysis under pilot plant conditions
- Treatments with amylases reduced viscosities of chickpea beverages
- Combining enzymes led to desirable physicochemical attributes than single enzymes
- Laboratory results were validated on a pilot plant scale.
- A formulation for chickpea beverages with 3% protein content was proposed.

Abstract

Plant-based beverage production generally involves enzymatic treatments to overcome technological challenges. Thus, this research aimed to evaluate the potential use of the Rapid Visco Analyzer (RVA) to simulate pilot plant (PP) conditions and determine the enzyme(s) and enzyme-concentrations to be used in PP for beverage production using chickpea flour. α-Amylase-TA, maltogenase-MA and amyloglucosidase-AMG were tested individually and in combination at three concentrations. Chickpea suspensions with 3% protein, equivalent to milk contribution, were produced at laboratory scale in RVA while simulating PP. Highest viscosity reductions was obtained with the highest enzymatic activity (TA.3 = 1.21 CU/g flour; MA.3 = 0.15 CU/g flour; AMG.3 = 0.69 mg of glucose released/ g flour). TA was the most effective in reducing final viscosity and increasing free sugars, particularly combined with TA+MA+AMG. RVA findings were validated in PP-production using TA.3+MA.3+AMG.3. Viscosity decreased from 50697 ± 8907 cP observed in the control to 4505 ± 171 cP when using TA.3+MA.3+AMG.3. Due to homogenization, the whiteness index was higher for PP beverages (67-71) than for RVA suspensions (65-67). This study demonstrates the potential of using RVA as a tool to optimize enzyme concentrations for chickpea beverage production and the successful scale-up of the process to PP-level.

Keywords: Plant-based beverages; chickpea; amylases; viscosity; homogenization.

Abbreviations: AMG, amyloglucosidase; CBB, chickpea-based beverages; Ctrl, control; CU, Ceralpha units; MA, maltogenase; PBB, plant-based beverages; PP, pilot plant; RVA, Rapid Visco Analyzer; TA, thermostable α-amylase; TSS, total soluble solids; TTA, total titrable acidity; WI, whiteness index.

1. Introduction

Plant-based beverages (PBB) are colloidal suspensions or emulsions of disintegrated plant material, being a viable option for those looking for dairy-free alternatives. Although a large assortment of PBB are available in the market, they exhibit significant variations in their nutritional profiles, including low protein content, in most cases below the whole milk protein contribution (3.18%) (Grau-Fuentes et al., 2023). Therefore, PBB still require the improvement of nutritional properties to be considered as milk nutritionally (Singhal et al., 2017). The nutritional profile of PBB is greatly dependent on the main plant source used and the processing conditions applied during their production (Grau-Fuentes et al., 2023; Jeske et al., 2017). Pulses are promising for producing PBB due to their balanced nutritional composition, although some technological challenges related to processing and preservation are still prevalent (Lopes et al., 2020; Mäkinen et al., 2016; Xiong et al., 2024). Soybeans have been widely used in PBB production due to their nutritional attributes, but their beany flavor and allergenicity have prompted looking for alternatives (Vanga and Raghavan, 2018).

Chickpeas are a good source of proteins, ranging from 17 to 22%, with adequate levels of essential amino acids and good digestibility (Kaur & Prasad, 2021). Furthermore, chickpea protein has a more neutral taste and light color, allowing for increased consumer acceptability, which makes it a good option for product development (Boukid, 2021). In fact, several researches have been conducted using chickpeas or chickpeas flour to prepare beverages through microwave processing (Sharma et al., 2024a), fermentation (Mariscal et al., 2023; Wang et al., 2018), extrusion and enzymatic hydrolysis (Silvestre-De-León, 2020; Silvestre-De-León et al., 2021), ultrasound (Vallath and Shanmugam, 2022) and conventional cooking (Sharma et al., 2024b). Those studies assessed the characteristics of the resultant beverages in terms of nutritional composition, physicochemical properties, protein quality,

functionality, and sensory attributes. However, chickpea starch content spans from 41% to 51%, which can pose a challenge during thermal processing owing to starch gelatinization (Mäkinen et al., 2016), particularly when scaling up production. In fact, Lopes et al., 2020 reported that gelatinization of chickpea preparations occurred at lower (5-6%) and high (10%) chickpea concentrations, which were prevented by controlling the time and temperature of the thermal treatment. Nonetheless, thermal treatments are necessary at the end of the PBB manufacturing process to guarantee consumption safety and extend the shelf life of PBB, although the high-temperature conditions may induce an increase in viscosity in starchy PBB (Xie et al., 2023). To prevent this issue, enzymatic treatments using amylases have been utilized to liquify the starch present in the initial powder or flour. Deswal et al. (2013) optimized the conditions for enzymatic treatment to produce a PBB from oats, another starchy source, using a central composite rotatable design. According to their study, all parameters evaluated (initial solids and α-amylase concentrations and liquefaction time) markedly impacted the beverage yield, total solids, and rheological properties. On the other hand, Silvestre-De-León (2020) reports that sequential hydrolysis of chickpea flour slurry (12% w/v) with alcalase followed by α-amylase increased the solubilization of compounds from 10.08 to 29.70%. Furthermore, adding starch-degrading enzymes can enhance the palatability of the end product by releasing sugars during hydrolysis, which relates to sweetness, along with improving its fluidity and consistency (Mariscal, 2021; Patra et al., 2023). Therefore, enzymatic treatments might be an elective alternative to produce PBB.

To effectively monitor these enzymatic processes and the associated viscous properties of starchy materials, the Rapid Visco Analyzer (RVA) has proven to be an invaluable tool (Santamaria et al., 2023). Its capacity to require minimal sample amounts, coupled with the ability to customize profiles for evaluating various conditions, makes it particularly

advantageous in this context. Additionally, the RVA provides results comparable to those from other viscometers, facilitating reliable assessments of starch behavior during processing (Balet et al., 2019). In fact, Yılmaz Tuncel et al. (2021) successfully monitored starch hydrolysis in chickpea based beverage production by using the Micro Visco Amylo-Graph. Authors highlighted the effectiveness of this analytical tool in understanding the intricate processes involved in its formulation, although only α-amylase was employed, and the process was not scaled up for validation.

Research on chickpea beverages has primarily concentrated on formulation and processing conditions at the laboratory scale to address various physicochemical challenges and the functional characterization of the formulations. However, it is essential to investigate the scalability of these processes for larger production volumes to ensure the potential commercial viability and feasibility of large-scale manufacturing. It is hypothesized that integrating enzymatic treatments with accurate monitoring techniques, such as the RVA, can optimize the production of chickpea-based beverage (CBB). Therefore, the primary objective of this study was to evaluate the potential of RVA to simulate pilot plant conditions and secondly, to determine the optimal type and concentration of amylase(s) for producing CBB with a 3% protein content, comparable to that of milk, at the laboratory scale. Additionally, findings were validated by scaling up the production of CBB at the pilot plant level and assessing their physicochemical properties.

2. Materials and methods

2.1. Raw materials

Chickpea flour was procured from *Productora de Alimentos Especializados de Monterrey S.A. de C.V*. (Monterrey, Mexico). Flour had a particle size of 806 ± 133.75 nm (diameter) (Ruiz-Zambrano et al., 2024). All the enzymes used for starch hydrolysis, including a thermostable α-amylase (BAN 480 LS, 1.25 g/ml), maltogenic amylase (Maltogenase 2X L, 1.26 g/ml) and amyloglucosidase (Amylase AG 300 L, 1.17 g/ml), were obtained from Novozymes (Bagsvaerd, Denmark). Standards for starch and free sugars determination were prepared with Fleischmann's corn starch (Mississauga, Ontario, Canada, 0-15 mg/ml) and D-glucose (Sigma-Aldrich, St. Louis, MO, USA, 0-10 mg/ml), respectively. Salt (Sifto® Hy-Grade® Food Grade Salt) and calcium lactate (Dr. Paul Lohmann, product code 512083003) were also used in the formulation of the PPB.

2.2. Chemical composition of the chickpea flour

Moisture content was measured using a moisture analyzer (Denver IR 35 moisture analyzer; Denver, CO, USA). Protein, crude fat, and ash contents were determined according to the AOAC official methods 978.02, 920.39, and 923.03, respectively (AOAC International, 2000). A 6.25 conversion factor for N, was used for protein determination. The non-starch carbohydrate content was estimated by difference.

Starch and free sugars were determined based on the procedure described by Jeong *et al*. (2010) with some modifications. Chickpea flour (10 mg) or chickpea suspensions (50 mg) were suspended in 1 ml of 50 mM sodium acetate buffer at pH 5, containing 5 mM $CaCl_2$. After adding 5 µl of Thermamyl 2X (240 KNU-T/g; Novozymes, Bagsvaerd, Denmark), tubes were incubated in a boiling water bath for 30 minutes, and then centrifuged (16200 x*g*, 4°C, 5 min). Reducing sugars in the supernatant were quantified with 3,4-dinitrosalicylic acid and the absorbance read at 535 nm using a microplate reader (Epoch 2 Biotek Instruments, Winooski,

VT, USA). Free-reducing sugars content was determined under the same conditions, but the enzyme volume was replaced with sodium acetate buffer. All analyses were performed in triplicates.

2.3. Enzymatic activity

Enzymatic activities in the commercial preparations were quantified. Thermostable α-amylase (TA) and maltogenic amylase (MA) was measured according to the Ceralpha method assay kit (K-CERA, Neogen, Lansing, Michigan, USA). Amyloglucosidase (AMG) was measured according to the procedure described by Aleixandre et al. (2022), using maltose (10 mg/ml) as substrate. Briefly, AMG solutions in sodium phosphate buffer (0.1 M, pH 6.9) and maltose substrate were preincubated at 37°C for 10 min and for ten more minutes after mixing them. The enzymatic reaction was stopped by submerging the samples in a boiling water bath for 10 minutes. Sample absorbance was measured using a D-glucose assay kit (GOD/POD) (K-GLUC 08/18, Neogen, Lansing, Michigan, USA) at 510 nm, using a microplate reader (Epoch 2 Biotek Instruments, Winooski, VT, USA). AMG enzyme activity was measured as mg of glucose released in 10 minutes at 37°C. All analyses were performed in triplicates.

2.4. Suspension preparation

The suspensions were prepared using a Rapid Visco-Analyser (RVA-4 Series, Newport Scientific Pty., Ltd., Warriewood, NSW, Australia). Based on preliminary trials conducted in a pilot plant setting, the testing profile and sample processing were specifically designed to replicate the established production conditions for PBB at a pilot scale. The RVA profile conditions are described in Table 1. From those initial tests, the level of the enzymatic treatments was selected. Three different levels of enzymatic activities were evaluated using the RVA. For TA, 0.12, 0.24 and 1.21 CU/g flour were used, corresponding to level 1, 2 and 3,

respectively. For MA, 0.01, 0.03, and 0.15 CU/g flour corresponded to level 1, 2 and 3, respectively. In the case of AMG, levels 1, 2 and 3 were 0.07, 0.14, and 0.69 mg of glucose release, respectively. Samples were prepared by suspending chickpea flour (15.8% w/w), calcium lactate (0.1% w/w), enzyme preparations (0.49% w/w), and water (83.61% w/w) to obtain 28.5 g of suspension. This recipe was designed to achieve suspension with a protein content of 3%, considering the protein concentration determined in the chickpea flour. Suspensions were initially prepared using each enzyme individually at three different enzymatic levels. TA, MA, and AMG were tested combined. A control sample (Ctrl) was prepared without adding enzymes. These preparations are referred to as *suspensions*.

2.5. Physical attributes determination

Color, pH, total titratable acidity and total soluble solids were determined in the suspensions and CBB. Color attributes were determined using a Konica Minolta CM-3500d benchtop spectrophotometer (Osaka, Japan) with SpectraMagic NX software using illuminant D65 per the CIE *L*a*b** color system. The whiteness index (WI) is calculated as follows:

$$WI = 100 - \sqrt{\left(100 - L^{*}\right)^{2} + a^{*2} + b^{*2}}$$

pH was measured directly using a digital pH meter (Fisherbrand Accumet AB315 pH/mV, Pittsburgh, PA, USA). Total titratable acidity (TTA) of CBB was measured using 0.01 M NaOH and three drops of phenolphthalein 1% (w/v) indicator were used for titration. TTA was expressed as ml milliequivalents (mEq) of acid/L (Rincon et al., 2020).

To determine the total soluble solids (TSS), the sample (1 g) was centrifuged at 16200 x*g* for 10 minutes at 4°C. TSS of the supernatant were measured using two methods: a) using a compact digital refractometer (Abbemat 3100, Anton Paar Germany GmbH, Ostfildern,

Germany) and expressed as a percentage on a °Brix scale. b) Gravimetrically after drying at 105°C overnight.

2.6. Beverage preparation

Enzyme concentrations were selected based on the results obtained in the RVA suspension preparations. Beverages were produced by mixing chickpea flour (15.8% w/w), calcium lactate (0.1% w/w), salt (0.1% w/w), water, and enzyme preparations considering 3 kg as total solids. The beverage formulation was designed for beverages with 3% protein. Four different beverages were produced: 1) Control: no enzymes added; 2) TA.3 = Only TA at 1.21 CU/g flour; 3) TA.3 + AMG.3 containing TA and AMG at 1.21 CU/g flour and 0.69 glucose released/g flour, respectively; 4) TA.3+MA.3+AMG.3 containing TA = 1.21 CU/g flour, MA = 0.15 CU/g flour and AMG = 0.69 mg glucose released/g flour. The procedure followed herein was adopted from the one used at the pilot plant for PBB from different sources.

Calcium lactate was added while agitating water at 3000 rpm at room temperature. Then, the temperature increased to 60°C and the enzyme preparation was added, followed by chickpea flour addition. Salt was added after 30 minutes, and the mixture was stirred under the same conditions for 10 minutes. Then, the mixture was screened through a kitchen mesh strainer. An aliquot of 350 ml was taken, and the rest was homogenized in a two-phase homogenizer (APV Gaulin laboratory homogenizer, model 15MR-8TA, 316 stainless steel 2 Stage valve. Wilmington, Massachusetts, USA). The second-stage pressure was set to 4,136.85 kPa, and the total pressure was 20,684.27 kPa. Once homogenized, the mixture was pasteurized (3000 rpm, 90°C, 30s) and then cooled in a cold-water bath with manual agitation until the temperature reached 25°C. The non-homogenized beverage was pasteurized under the same conditions using a hot plate and a magnetic stirrer for agitation. The beverages were

packed in plastic bottles and stored at 4°C for further physical and chemical analyses. These preparations are referred to as *beverages*.

2.7. Viscosity and sedimentation

The final viscosity of the suspensions prepared in the RVA was considered as the apparent viscosity of the suspensions. For the beverages, viscosity measurements were done at room temperature (25°C), right after transferring the beverage, using a viscometer (Brookfield, LVDV-E, Massachusetts, USA) with appropriate cylindrical spindles (LV 60-64). Rotational speed and spindle were selected so that the torque readings were within the 40-60% range of the equipment scale, after 3 minutes of revolution.

For sedimentation analysis throughout storage, the volume of the upper phase relative to the total volume was assessed following the method outlined by Rincon et al., (2020). In this process, 15 ml of the sample was transferred into 15 ml plastic tubes in triplicate and stored at 4 °C in a tube rack. The volume of the upper phase was recorded every 24 hours for a duration of 168 hours (7 days), with sedimentation expressed as a percentage of the upper phase relative to the total volume (15 ml).

2.8. Statistical analysis.

All experimental data are expressed as mean ± standard deviation of three replicates. Data were subjected to one-way ANOVA followed by mean comparison for significant differences using Tukey's HSD test at $p \leq 0.05$ by the Minitab 21 statistical software (version 21.4.3, 64-bit, Pennsylvania, USA). Pearson's correlations were used to evaluate correlations between TSS measured gravimetrically and using a refractometer, starch and free sugars content and starch content and apparent viscosity.

3. Results and discussion

Chickpea flour and enzyme activities were evaluated before starting the study. The chickpea flour had 11.26% ± 0.92 moisture, 19.33% ± 0.54 protein, 8.17% ± 0.89 crude fat, 3.12% ± 0.01 ash, 50.70% ± 0.96 starch, and 7.42% ± 1.32 non-starch carbohydrate content. TA and MA activities were 1192.82 CU/ml and 146.52 CU/ml, respectively. AMG enzyme activity was 636.20 mg of glucose released/ml of AMG.

3.1. Chickpea flour suspensions

Preliminary tests on a pilot-plant scale were made to produce CBB. Similar conditions were recorded using a RVA to simulate pilot-scale production of PBB and produce chickpea suspensions.

The effects of the three different enzymes on the pasting properties were evaluated (Figure 1). Peaks observed at the onset of the pasting profile and upon enzyme addition were attributed to changes in paddle speed. The control suspension exhibited a pasting temperature of 71°C, a peak viscosity of 3020 cP, and a final viscosity of 4081 cP. Previous studies have reported pasting temperatures within the range of 68.60-73.66°C for chickpea flour slurries prepared with 10, 12.5 and 15% flour concentration, which is consistent with what was observed herein (Yılmaz Tuncel et al., 2021).

Prior investigations used enzyme addition to prevent starch gelatinization during heat processing to improve compound solubilization (Silvestre-De-León, 2020), and to reduce the viscosity of the final product (Tan et al., 2023; Yılmaz Tuncel et al., 2021). In the current study, enzymes (TA, MA, and AMG) reduced the apparent viscosities during heating and cooling. The extent of these reductions was enzyme- and enzyme-activity-dependent. TA led to the lowest apparent viscosities, followed by MA and then AMG. The suspension prepared

with TA at its highest activity level (TA.3 = 1.21 CU/g flour) reached a peak viscosity of 361 cP and a final viscosity of 69 cP, which is approximately 8 and 60 times lower than that of Ctrl (3020 cP and 4081 cP respectively). Suspensions TA.1 and MA.3 exhibited similar pasting profiles. The viscosity reduction promoted by TA was expected, considering that TA is an endoenzyme that randomly cleaves internal α-1,4-glycosidic linkages in starch molecules to produce low-molecular-weight products, such as maltose, maltotriose, and branched oligosaccharides of 6–8 glucose units. In contrast, MA also cleavages α,1-4 glycosidic bonds in starch, hydrolyzing them internally and externally from the non-reducing end of the chain. Nevertheless, MA displays preferences for straight-chain starch or cyclic dextrins for rapid hydrolysis and branched starch to a lesser extent, having moderate impact on the reduction of starch viscosity (Liu et al., 2023). AMG had low impact on the pasting properties of the suspensions, which might be explained because it has a preference for hydrolyzing oligosaccharides rather than polysaccharides to produce glucose, and when used alone, it does not hydrolyze starch completely (Farias et al., 2021).

After conducting tests using enzymes individually, combinations of TA, MA, and AMG (TA+MA+AMG) were prepared at the three different levels of enzymatic activity and their effect on the pasting profile of chickpea suspensions were evaluated (Figure 2). The combination of TA.3+MA.3+AMG.3 resulted in a peak viscosity 71% lower than that achieved using TA.3 individually (102 and 361 cP, respectively). Nevertheless, their final viscosities were similar (TA.3 = 63 cP, TA.3+ MA.3+AMG.3 = 69 cP). However, lower level in the enzymatic combination, TA.1+MA.1+AMG.1 and TA.2+MA.2+AMG.2, do not lead to lower viscosities than those of TA.3. Due to their mechanisms of action, it is imperative to use enzyme

combinations to complete starch hydrolysis. TA can catalyze starch liquefaction but not hydrolyze it entirely to glucose unless AMG is present. Similarly, in the absence of an α-amylase like TA, MA and AMG actions are not enough to achieve complete starch hydrolysis due to preferential selectivity and substrate specificity (Farias et al., 2021; Presečki et al., 2013). The higher impact of the enzyme combinations compared to the enzymes used individually could be explained by the increased enzyme activity and the synergistic effects of the enzymes when combined, resulting in more efficient and complete starch hydrolysis. During starch hydrolysis, α-amylase breaks down starch into oligosaccharide products that serve as substrates for MA and AMG. Then, MA and AMG hydrolyze the oligosaccharides into sugars preventing them from inhibiting the action of the α-amylase and maintaining the starch digestion rate. Additionally, AMG may expose catalytic sites for α-amylase by attacking the protruding amylopectin branches of amylopectin on the starch surface (Liu et al., 2023; Zhang et al., 2013). The changes induced by α-amylase on starch increase the gelatinization temperature thus reducing the pasting parameters like setback, breakdown and peak viscosities, in addition to shorten the amylose and amylopectin chains improving the starch solubilization and lowering the pasting viscosity (Saeid et al., 2024).

3.1.1. Characterization of chickpea suspensions.

The physicochemical properties of plant-based beverages directly affect the quality of the product, which in turn affect its overall acceptability (Ramesh et al., 2022). The results of the physicochemical parameters evaluated are presented in Table 2. Significant differences were observed in pH among the chickpea suspensions ($p<0.05$), but within a narrow range and had no practical implications. The control sample showed the lowest pH value (6.14), and AMG.2, the highest (6.25). Similar pH values have been reported for chickpea beverages in previous

works (Rincon et al., 2020; Sharma et al., 2024b). On the other hand, titratable acidity values were within 1.52 to 1.99 mEq of acid/L, and no significant differences were observed among them.

The color characteristics of chickpea suspensions were determined based on the CIE *L*a*b** color space and the Whiteness Index (WI) was calculated (Table 2). The *L**, corresponding to lightness, was between 74.54 and 76.22; *a** (redness) ranged between -0.15 and 1.74; whereas *b** (yellowness) varied from 20.99 to 23.96. No trend was identified regarding the levels of enzymes used. Furthermore, the WI of the suspensions was between 65.22 and 67.86. Since PBB intends to replicate some of the physical properties of milk, WI is an important parameter. WI for commercial PBB has been reported to be within 51.57 to 75.95 range (Jeske et al., 2017), thus the WI of chickpea suspensions was comparable to that of other PBB. Color attributes of PBB rely on the characteristics of the plant source used for preparation, the natural pigments present in it, and the properties of their colloidal particles, which influence the way light waves are absorbed and scattered in the PBB. Processing conditions like high-pressure homogenization and oil addition can influence this parameter by reducing the particle size and coating proteins, which leads to more light scattering (McClements, 2020).

Total soluble solids (TSS) were evaluated gravimetrically and assessing the °Brix, to determine the impact of the enzyme and enzyme activity levels on compound solubilization. TSS of the suspensions was within the 4.0 and 9.57 °Brix, as shown in Table 3. The control suspension had the lowest TSS content; thus, enzymatic treatment improved compound solubilization. TSS was also influenced by the enzyme level used for suspension preparation, as a higher TSS was obtained when using higher enzyme activity. Rincon et al. (2020) reported

a TSS of 4.04 °Brix when prepared a chickpea extract by using conventional cooking, which aligns with the TSS found in the control sample. The enzymatic treatment with TA led to the highest amount of TSS, compared to the individual addition of MA or AMG. However, suspensions prepared using TA+MA+AMG exhibited higher TSS contents than those prepared with TA individually. Silvestre-De-León et al. (2021) explained the increase in TSS after enzymatic treatments based on the modifications induced to starch during hydrolysis, as it was broken down to dextrins, maltose, and glucose, thus enhancing its solubility. Those authors obtained a TSS of 11.13 °Brix using an enzymatic treatment of chickpea flour, involving both amylase and protease, which is higher than that obtained in this study with only amylases (Silvestre-De-León, 2020). In contrast, when TSS were measured gravimetrically, TSS ranged from 7.34 to 34.73 g/100 g. When comparing the TSS values measured gravimetrically and with a refractometer, the gravimetric method showed a wider range of TSS. Despite these differences, the same trend was observed across the samples for both methods. The highest TSS was achieved using the TA+MA+AMG combination, followed by TA at the highest enzyme activity level (3 = 1.21 CU/g flour), while the control sample had the lowest TSS. These values were similar to those reported in a previous study using amylase and protease (Silvestre-De-León, 2020). A positive strong correlation ($r = 0.957$, p-value = 0.001) was found when comparing the data obtained using the two methods. The determination coefficient increased ($r = 0.988$, p-value <0.001) when TSS > 5.81 °Brix or TSS > 9.65 g/100 g (measured gravimetrically) but the correlation is not significant ($r = 0.681$, p-value = 0.137) at lower TSS. This suggests that the results obtained with the two methods exhibit more similar trends at higher TSS but differ at lower concentrations.

The effects of the enzymatic treatments on the concentration of starch and free sugar in the suspensions were assessed (Table 3). The starch content in chickpea suspensions varied

between 4.62 g/100 g and 8.58 g/100 g. The control sample displayed a starch content of 7.43 g/100 g, which was majorly decreased with the addition of increasing levels of TA. Likewise, suspensions prepared with enzymes combination, TA.3+MA.3+AMG.3, exhibited even lower starch contents of 4.62 g/100 g, confirming again the synergistic effect. The free sugar content ranged from 0.07 g/100 g to 3.29 g/100 g. The control sample exhibited the lowest free sugar content, indicating that chickpea flour naturally contains low levels of free sugars. The highest sugar concentrations were obtained at the highest level of TA or with the enzymes' combination TA.3+MA.3+AMG.3. Therefore, the free sugar content observed in the suspensions was directly related to the degree of starch hydrolysis, explaining the negative correlation between starch content and free sugar levels ($r = -0.953$, p-value <0.001).

Additionally, the pasting profile revealed a positive correlation between the starch content and the apparent final viscosity of the suspensions ($r = 0.867$, p-value <0.001); thus, the higher the starch content, the higher the apparent viscosity observed. The pasting characteristics are dependent on the starch performance, and its hydrolysis alters its ability to retain and link water molecules, leading to reduced viscosity and higher free sugar content. These changes improve the physical properties of chickpea-based beverages, besides providing sweet taste to the CBB. Taking this into account, TA.3 and TA.3+MA.3+AMG.3 were considered the suspensions with the most promising attributes to be scaled up. Additionally, AMG hydrolyzes dextrins into glucose, thus, combining them could increase the sweetness of the beverage and its palatability without adding sugar (Basinskiene and Cizeikiene, 2020).

3.2. Chickpea beverage scalability in a pilot plant.

Based on the results obtained with the RVA suspensions, TA.3, and TA.3+AMG.3, were applied to produce CBB at the pilot-plant scale. In addition, a control beverage (Crtl) and the TA.3+AMG.3 were prepared for comparison purposes. Unlike the laboratory-scale procedure, the pilot plant process included a homogenization step, which had not been applied in previous experiments. The impact of this homogenization was also evaluated. The resulting beverages are shown in Supplementary Figure ESM1. Beverages made with TA.3 (1.21 CU/g flour) and without enzymes (Ctrl) exhibited a dough-like texture rather than a liquid one. This behavior was expected for Ctrl but contradicted the RVA findings for TA.3, where the suspension took on a fluid form rather than a doughy one. On the other hand, beverages made with the enzyme preparations TA.3+AMG.3 and TA.3+MA.3+AMG.3 had a liquid-like consistency, as expected. Partial hydrolysis of starch could lead to starch gelatinization and retrogradation during and after pasteurization, which could explain this phenomenon (Sethi et al., 2022, 2016).

To validate the findings obtained at the laboratory scale, all beverages underwent evaluation for the same attributes as suspensions (Table 4). Additionally, the viscosity and phase separation were measured. Chickpea beverages had pH and TTA values between 6.04 - 6.11 and 1.99 - 2.12 mEq acid/L, respectively. These parameters were like those obtained with the RVA approach.

Color attributes of the beverages were consistent with those obtained with the RVA suspensions. However, the beverages had slightly higher values for lightness and whiteness index than their suspensions counterparts. Homogenization may induce changes in color

attributes, similar to those observed in the present work, as particle size and number are modified, affecting the light reflection (Bernat et al., 2015).

In the beverages, TSS were within the range of 5.26-12.13 °Brix and 4.61-49.31 g/ 100 g, which were slightly higher than the measurements obtained for the suspensions. Viscosity of the homogenized beverages ranged from 4050 cP to 50697 cP, with the Ctrl having the highest viscosity. The lowest viscosity was achieved using TA.3+MA.3+AMG.3 preparation. Viscosity measurements obtained using a viscosimeter were higher than the apparent viscosity measured with the RVA, however, similar trends were observed in both cases. Viscosity of the chickpea beverages was higher than that of commercial PBB (2.21 - 47.8 cP) (Jeske et al., 2017). The formulation used in this study contained 15.8% chickpea flour solids, which likely contributed to the increased viscosity of the beverages. Furthermore, conditions for measuring viscosity are not standard, thus leading to discrepancies within measurements that impair proper comparison as PBB exhibit shear thinning behavior (Yao et al., 2022).

The values for non-homogenized beverages were 1357 ± 11 cP for TA.3, 1396 ± 2 cP for TA.3+AMG.3, and 1259 ± 22 cP for TA.3+MA.3+AMG.3. The higher viscosity observed in the homogenized beverages compared to their non-homogenized counterparts can be attributed to the homogenization process, followed by pasteurization. These treatments may have induced the formation of a weak gel due to protein aggregation, which helped stabilize the beverage and contributed to the viscosity increase (Bernat et al., 2015; Mäkinen et al., 2016; McClements et al., 2019).

Starch and free sugar contents of the beverages were consistent with those of their suspensions' counterparts produced in the RVA. In both cases, the TA.3+MA.3+AMG.3 enzyme preparation led to the lowest starch and highest free sugar contents. Beverage

prepared with TA.3 (1.21 CU/g flour) had 7.71 g/100 g starch content, while its suspension exhibited 5.47 g/100 g, suggesting higher starch hydrolyzation when the beverage was prepared at the laboratory scale, possibly due to more controlled conditions that allowed higher hydrolysis compared to PP scale. Correlations observed in suspensions among starch and free sugar contents, as well as for free sugars and TSS-°Brix, were also identified in the beverages (R^2 = 0.941 and 0.927 respectively).

Sedimentation was evaluated due to its importance in the shelf life of the beverage. Chickpea beverages produced at the pilot plant scale using TA.3, TA+MA.3 and TA.3+MA.3+AMG.3 were assessed for phase separation, by measuring the upper phase volume that appeared during the 7-day evaluation. As depicted in Figure 3.A, the beverages exhibited up to 8% phase separation. However, the rate of separation differed throughout the evaluation period. TA.3 did not show any phase separation, as this was prevented due to its high viscosity. For the TA.3+AMG.3 beverage, most of the phase separation took place within the first 48 hours (Figure 3.B). On the other hand, for the TA.3+MA.3+AMG.3 beverage, a progressive increase was observed (Figure 3.C), which has been attributed to the presence of insoluble particles like proteins, starch, fibers, and cellular materials in the raw materials (Mäkinen et al., 2016).

The phase separation of non-homogenized beverages was also evaluated. The final phase separation for TA.3+AMG.3 was 30.22% ± 0.38, while for TA.3+MA.3+AMG.3, it was 26.22% ± 0.77. Homogenization significantly decreased the sedimentation, increasing the stability of the CBB by reducing its particle size.

4. Conclusions

RVA was used to simulate pilot plant process conditions for producing PBB with chickpea flour, and simultaneously monitor the apparent viscosity. The chickpea suspensions where prepared with 15.8% chickpea flour to yield a 3% protein content, similar to that of milk and soy beverages, applying different enzymatic treatments. RVA curves showed that enzymatic treatments reduced the peak and final viscosities, with a parallel reduction of the starch content and increase of the TSS and free sugar content. Based on RVA simulation, TA.3 and TA.3+MA.3+AMG.3 enzyme preparations were chosen to prepare a chickpea beverage and results validated at the pilot plant. These findings prove that RVA can be a useful tool for evaluating hydrolysis parameters and simulating pilot plant conditions, highlighting its potential to be used as a quick method for testing different parameters in beverage development. In this preliminary approach, a CBB formulation with 3% protein was produced. Future studies should be undertaken to perform the sensory evaluation and full rheological characterization of the beverages. PBB are becoming more popular, and validating laboratory findings on a pilot scale is important for their scalability.

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Declaration of interest: The authors declare no conflicts of interests

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Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used Paperpal to check grammar and spelling and improve text cohesiveness and readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Figure captions.

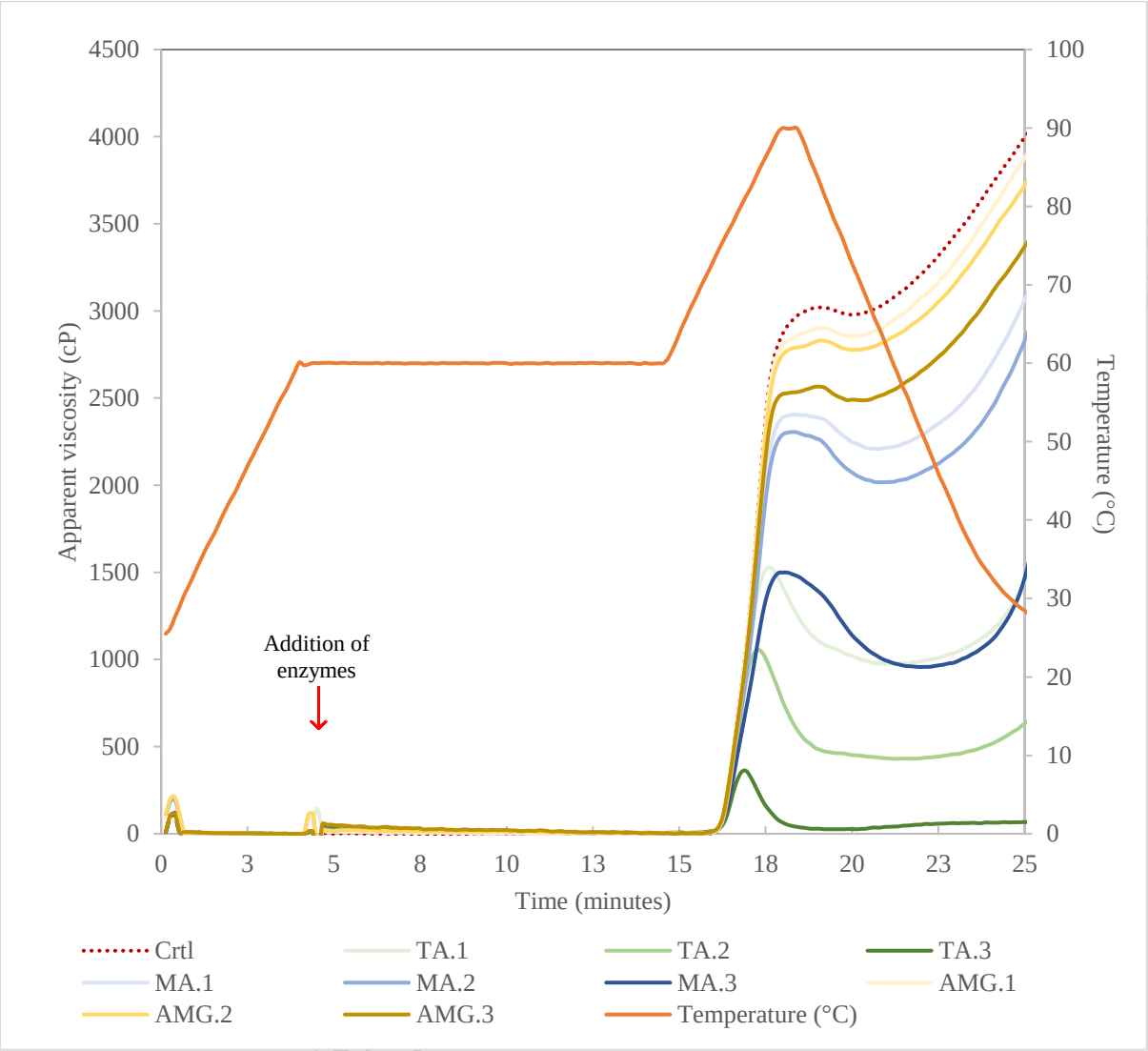

Figure 1. Pasting profile of native chickpea flour prepared using thermostable α-amylase (TA), maltogenase (MA) or amyloglucosidase (AMG) individually. The number next to the label in the legend corresponds to the enzymatic activity used to prepare gels. TA: 1 = 0.12, 2 = 0.24, 3 = 1.21 CU/g flour. MA: 1 = 0.01, 2 = 0.03, 3 = 0.15 CU/g flour. AMG: 1 = 0.07, 2 = 0.14, 3 = 0.69 mg of glucose released/ g flour. A control sample (Crtl) was prepared without enzyme addition. Conditions for the RVA profile are described in detail in Table 1.

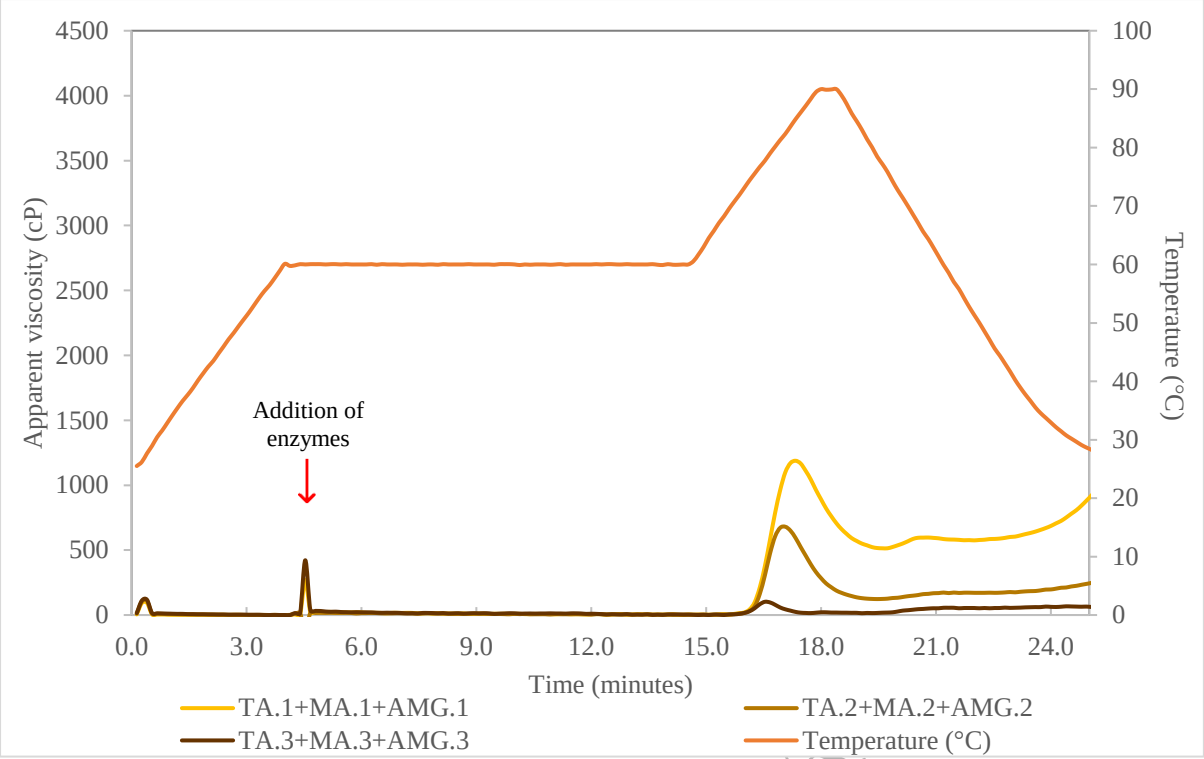

Figure 2. Pasting profiles of native chickpea flour prepared using thermostable α-amylase (TA), maltogenase (MA) and amyloglucosidase (AMG) mixed. The number next to the label in the legend corresponds to the enzymatic activity used to prepare gels. TA: 1 = 0.12, 2 = 0.24, 3 = 1.21 CU/g flour. MA: 1 = 0.01, 2 = 0.03, 3 = 0.15 CU/g flour. AMG: 1 = 0.07, 2 = 0.14, 3 = 0.69 mg of glucose released/g flour. Conditions for the RVA profile are described in detail in Table 1.

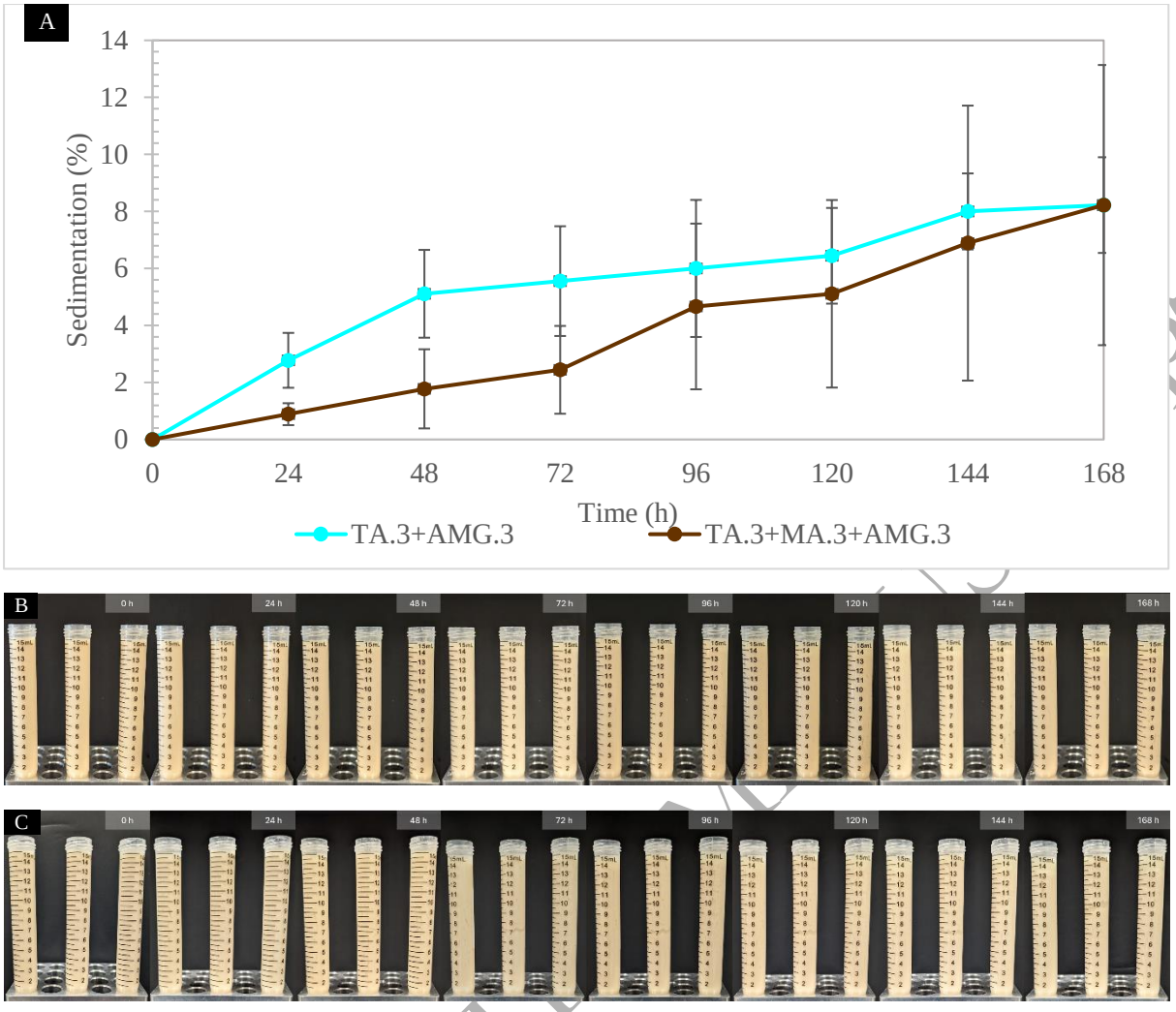

Figure 3. Sedimentation of chickpea beverages. A) Phase separation plot. B) TA.3+AMG.3 sample pictures during the evaluation course. C) TA.3+MA.3+AMG.3 sample pictures during the evaluation course. TA, thermostable α-amylase; MA, maltogenic amylase; AMG, amyloglucosidase. Numbers next to the sample identifier correspond to the enzymatic activity used for preparing the beverages. TA: 3 = 1.21 CU/g flour. AMG: 3 = 0.69 mg of glucose released/ g flour.

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Table 1. Rapid Visco Analyzer profile conditions.

Time (mm:ss)	RVA Function	Value	Description
00:00	Temperature (°C)	25	Starting conditions
00:00	Speed (rpm)	0	Starting conditions
00:10	Speed (rpm)	960	Speed increases to 960 rpm
00:10	Temperature (°C)	25	Temperature increases to 60°C in 3:50 min
04:00	Temperature (°C)	60	Temperature set to 60°C
04:00	Speed (rpm)	960	Speed decreases to 160 rpm in 10 s
04:10	Speed (rpm)	160	Speed set to 160 rpm
04:12	Speed (rpm)	0	Speed decreases to 0 rpm for enzyme addition
04:27	Speed (rpm)	0	Speed increases to 160 rpm
04:30	Speed (rpm)	160	Speed set to 160 rpm
04:30	Temperature (°C)	60	Temperature set to 60°C
14:30	Temperature (°C)	60	Temperature increases to 90°C 3:24 min
17:54	Temperature (°C)	90	Temperature set to 90°C
18:24	Temperature (°C)	90	Temperature decreases to 25°C in 6:00 min
24:24	Temperature (°C)	25	Temperature set to 25°C
25:24	End		

Table 2. Physical attributes of chickpea suspensions obtained with the Rapid Visco Analyzer.

Sample	pH [1]	TTA [2]	*L** (Lightness)	*a** (Redness)	*b** (Yellowness)	WI
Control	6.14 ± 0.05 c	1.72 ± 0.12 a	75.63 ± 0.03 bc	1.74 ± 0.06 a	23.95 ± 0.07 a	65.79 ± 0.06 f
TA.1	6.17 ± 0.02 abc	1.87 ± 0.12 a	76.22 ± 0.14 a	0.99 ± 0.23 d	21.59 ± 0.34 e	67.86 ± 0.13 a
TA.2	6.18 ± 0.03 abc	1.92 ± 0.12 a	75.42 ± 0.14 cd	1.11 ± 0.35 cd	22.16 ± 0.46 d	66.89 ± 0.22 b
TA.3	6.19 ± 0.00 abc	1.92 ± 0.23 a	75.61 ± 0.07 bc	1.09 ± 0.05 d	22.78 ± 0.03 bc	66.61 ± 0.04 b
MA.1	6.23 ± 0.01 ab	1.93 ± 0.30 a	75.63 ± 0.02 bc	1.47 ± 0.02 abc	22.96 ± 0.01 b	66.48 ± 0.01 d
MA.2	6.21 ± 0.04 abc	1.85 ± 0.23 a	75.75 ± 0.04 b	1.24 ± 0.09 cd	22.67 ± 0.05 bc	66.78 ± 0.03 bc
MA.3	6.23 ± 0.02 ab	1.99 ± 0.20 a	75.73 ± 0.02 b	1.30 ± 0.03 bcd	22.33 ± 0.06 cd	67.00 ± 0.03 b
AMG.1	6.23 ± 0.03 ab	1.52 ± 0.11 a	74.69 ± 0.00 ef	1.63 ± 0.04 ab	23.63 ± 0.07 a	65.33 ± 0.05 g
AMG.2	6.25 ± 0.01 a	1.66 ± 0.12 a	74.58 ± 0.02 f	1.01 ± 0.02 d	23.14 ± 0.05 b	65.61 ± 0.02 f
AMG.3	6.19 ± 0.05 abc	1.93 ± 0.11 a	74.83 ± 0.03 e	1.75 ± 0.01 a	23.94 ± 0.04 a	65.22 ± 0.03 g
TA.1+MA.1+AMG.1	6.15 ± 0.01 bc	1.73 ± 0.11 a	74.54 ± 0.01 f	-0.15 ± 0.08 f	20.99 ± 0.03 f	67.00 ± 0.01 b
TA.2+MA.2+AMG.2	6.15 ± 0.01 bc	1.86 ± 0.11 a	75.22 ± 0.05 d	0.58 ± 0.04 e	22.07 ± 0.09 de	66.81 ± 0.05 bc
TA.3+MA.3+AMG.3	6.17 ± 0.01 abc	1.80 ± 0.00 a	76.05 ± 0.11 a	1.46 ± 0.05 abc	23.96 ± 0.04 a	66.10 ± 0.11 e

[1] pH was measured at 25°C

[2] TTA: Total titratable acidity. TTA was measured as mEq of acid/L.

3 WI: Whiteness index.

Suspensions identifiers: Crtl, no enzymes added; TA, thermostable α-amylase; MA, maltogenic amylase; AMG, amyloglucosidase.

Numbers next to the sample identifier correspond to the enzymatic activity used for preparing the suspensions TA: 1 = 0.12, 2 = 0.24, 3 = 1.21 CU/g flour. MA: 1 = 0.01, 2 = 0.03, 3 = 0.15 CU/g flour. AMG: 1 = 0.07, 2 = 0.14, 3 = 0.69 mg of glucose released/g flour.

Values are expressed as the mean ± standard deviation of three replicates.

Table 3. Total soluble solids, starch, and free sugar content of the suspensions.

Sample	TSS (°Brix)	TSS (g/ 100 g)	Starch (g/ 100 g)	Free-sugar (g/ 100 g)
Control	4.05 ± 0.02 j	7.34 ± 1.30 f	7.43 ± 0.46 abc	0.07 ± 0.05 g
TA.1	6.93 ± 0.05 f	13.78 ± 0.58 e	7.37 ± 0.36 bc	0.85 ± 0.03 e
TA.2	7.78 ± 0.06 d	20.67 ± 0.26 cd	6.72 ± 0.09 cd	1.39 ± 0.10 d
TA.3	8.78 ± 0.02 b	29.36 ± 0.80 b	5.47 ± 0.16 ef	2.12 ± 0.13 b
MA.1	4.44 ± 0.05 i	9.24 ± 2.13 f	8.02 ± 0.19 ab	0.21 ± 0.01 g
MA.2	4.69 ± 0.03 h	9.69 ± 0.39 f	7.45 ± 0.32 abc	0.48 ± 0.03 f
MA.3	5.81 ± 0.05 g	9.65 ± 0.61 f	7.25 ± 0.49 bc	1.47 ± 0.06 d
AMG.1	4.09 ± 0.01 j	7.98 ± 0.74 f	8.05 ± 0.57 ab	0.13 ± 0.01 g
AMG.2	4.14 ± 0.03 j	9.05 ± 0.60 f	8.58 ± 0.62 a	0.13 ± 0.02 g
AMG.3	4.32 ± 0.09 i	8.57 ± 1.99 f	8.14 ± 0.45 ab	0.08 ± 0.00 g
TA.1+MA.1+AMG.1	7.29 ± 0.02 e	19.56 ± 0.55 d	6.64 ± 0.51 cd	1.29 ± 0.10 d
TA.2+MA.2+AMG.2	8.17 ± 0.03 c	24.11 ± 2.82 c	5.99 ± 0.10 de	1.88 ± 0.07 c
TA.3+MA.3+AMG.3	9.57 ± 0.07 a	34.73 ± 0.44 a	4.62 ± 0.28 f	3.29 ± 0.08 a

TSS: Total soluble solids.
Suspensions identifiers: Crtl, no enzymes added; TA, thermostable α-amylase; MA, maltogenic amylase; AMG, amyloglucosidase.
Numbers next to the sample identifier correspond to the enzymatic activity used for preparing the suspensions TA: 1 = 0.12, 2 = 0.24, 3 = 1.21 CU/g flour. MA: 1 = 0.01, 2 = 0.03, 3 = 0.15 CU/g flour. AMG: 1 = 0.07, 2 = 0.14, 3 = 0.69 mg of glucose released/g flour.
Values are expressed as the mean ± standard deviation of three replicates.

Table 4. Attributes of chickpea flour beverages produced at the pilot plant.

Parameter	Ctrl	TA.3	TA.3 + AMG.3	TA.3+MA.3+AMG.3
pH [1]	6.11 ± 0.03 a	6.12 ± 0.03 a	6.09 ± 0.01 ab	6.04 ± 0.02 b
TTA[2]	1.99 ± 0.01 b	2.43 ± 0.12 a	2.11 ± 0.12 ab	2.09 ± 0.23 ab
*L** (Lightness)	77.00 ± 0.09 c	77.91 ± 0.04 b	76.71 ± 0.03 d	78.57 ± 0.12 a
*a** (Redness)	2.44 ± 0.01 ab	2.23 ± 0.01 bc	2.55 ± 0.01 a	2.00 ± 0.20 c
*b** (Yellowness)	19.09 ± 0.17 a	17.36 ± 0.02 b	19.35 ± 0.03 a	19.14 ± 0.16 a
WI	70.01 ± 0.04 c	71.81 ± 0.02 a	69.62 ± 0.04 d	71.20 ± 0.05 b
TSS (°Brix)	5.26 ± 0.10 c	8.16 ± 0.03 c	11.74 ± 0.04 b	12.13 ± 0.06 a
TSS (g/ 100 g)	4.61 ± 0.15 c	20.61 ± 0.38 c	49.31 ± 1.51 a	39.49 ± 7.24 b
Viscosity (cP)	50697 ± 8907 a	26730 ± 240 b	7747 ± 391 c	4505 ± 171 c
Starch (g/ 100 g)	9.51 ± 0.40 a	7.71 ± 0.15 b	5.21 ± 0.45 c	4.86 ± 0.17 c
Free sugars (g/ 100 g)	0.13 ± 0.04 d	0.74 ± 0.04 c	4.65 ± 0.08 b	4.90 ± 0.12 a

[1] pH was measured at 25°C
[2] TTA: Total titratable acidity. TTA was measured as mEq of acid/L.
WI: Whiteness index.
TSS: Total soluble solids.
Beverages' identifiers: Crtl, no enzymes added; TA, thermostable α-amylase; MA, maltogenic amylase; AMG, amyloglucosidase.
Numbers next to the sample identifier correspond to the enzymatic activity used for preparing the beverages. TA: 3 = 1.21 CU/g flour. MA: 3 = 0.15 CU/g flour. AMG: 3 = 0.69 mg of glucose released/ g flour.

Values are expressed as the mean ± standard deviation of three replicates.