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Potential of an ultra-high pressure-treated gluten-free mix as an improving ingredient in gluten-free breadmaking

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Abstract

In the search for ingredients to improve the quality of gluten-free breads, a suspension of rice flour, cassava starch, and egg albumin (GF-Mix) was subjected to high pressure processing (HPP), to develop an ingredient capable of providing gluten-free breads of desirable quality. A central composite rotational design was followed, varying pressure (300–600 MPa), exposure time (5–10 min), and processing temperature (30–50°C). The samples were evaluated regarding pasting properties, instrumental color, and optical microscopy. The combination of high pressure and high temperature altered the pasting curves and reduced the parameters such as trough, final viscosity, and setback viscosity of the samples. An increase in the pasting temperatures was observed with the higher pressure and temperature conditions (600 MPa and 50°C) and intermediate processing times (4–8 min). Optical microscopy showed a loss of birefringence with increased pressure and temperature. The technological characteristics of the GF-Mix, processed through HPP at 600 MPa, 50°C, for 7.5 min, indicate a potential ingredient for gluten-free breadmaking, due to changes occurring in the starch leading to an ingredient with characteristics similar to a viscoelastic network.

Keywords: pasting properties; optical microscopy; birefringence; gelatinization.

Practical Application: HPP-modified GF-Mix mimics gluten, enhancing GF bread.

1 INTRODUCTION

Many strategies have been used in gluten-free baking in an attempt to improve final product quality, especially in relation to sensory characteristics. The total replacement of wheat flour with starches or alternative gluten-free flours, such as rice and cassava, requires the use of ingredients, additives, and processing aids to mimic gluten functionality (Capriles & Arêas, 2014).

Due to the high concentration of starch, gluten-free bread formulations result in breads with a faster aging rate during storage than breads made from conventional formulations (Gray & Bemiller, 2003). These transformations are responsible for huge economic losses, both for the bakery industry and for the consumer. In this context, novel technological methods and packaging stand out among the main new strategies to be studied.

High pressure processing (HPP) has proven to be an interesting technique in bakeries, as it can modify the structure of starch granules and contribute to the better quality of gluten-free products (Vallons et al., 2011). The mechanism of HPP-induced gelatinization is different from that of gelatinization by heat (Yamamoto & Buckow, 2016), high pressure significantly affected the amorphous and ordered structure of starch (Błaszczak et al., 2005), that is, there was a hydration of the amorphous phase, and fusion with the crystalline structure (Pei-Ling et al., 2012), inducing the partial gelatinization of starch (Bauer & Knorr, 2005). In addition to starch modifications, the protein denaturation mechanism is also affected by HPP, inducing changes in the conformational structure of proteins, with the formation or rearrangement of hydrogen bonds, disulfide bonds, electrostatic and hydrophobic interactions, and the HPP-modified protein can positively or negatively affect the functional and sensory properties of food products (Yang and Powers, 2016).

Of the existing techniques to evaluate the modification of starches, the analysis of pasting properties in the Rapid Visco Analyser (RVA) is one of the most used. The RVA rapidly heats and cools a starch-in-water suspension while stirring the sample at a constant rate. During this process, the viscosity of the sample is continuously measured, allowing the construction of a graph of viscosity over time (Bauer & Knorr, 2005). The information provided on the gelatinization and functional properties of starch can be used to infer the contribution of starch to the technological properties of bread dough (Balet et al., 2019).

Other techniques that can contribute to evaluating changes in starches and proteins are optical microscopy and instrumental color analysis. Optical microscopy allows evaluating the structure of starch at a microscopic level, verifying starch

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gelatinization through the Maltese cross, while instrumental color analysis using the CIE*Lab* system allows evaluating the appearance of the gluten-free mix in terms of color, indicating if other reactions may have occurred. Together, these techniques can provide valuable information about the physicochemical characteristics of a gluten-free mix treated by HPP.

In view of the modifications caused by HPP on starch and proteins, the present study aimed to evaluate the technological properties of a gluten-free mix (GF-Mix) made from rice flour, cassava starch, and modified egg albumin, treated by HPP using different pressure, time, and temperature conditions, with the aim of obtaining an ingredient to improve gluten-free breadmaking.

2 MATERIALS AND METHODS

2.1 Material

A gluten-free mix (GF-Mix) composed of 49% cassava starch (Amafil, Cianorte-PR), 41% rice flour (FA-1001—SL Alimentos, Mauá da Serra-PR), and 10% pasteurized acidified egg albumin (Sohovos®— AB Brasil Indústria e Comércio de Alimentos Ltda., Sorocaba-SP) was prepared according to Almeida (2011). The resulting GF-Mix presented 10.22% moisture, 11.40% proteins, 0.67% ash, 0.85% lipids, and 76.85% carbohydrates.

The rice flour acts as a bulking agent and contributes partially to the protein content of the formulation; cassava starch has the function of providing consistency and cohesiveness; and the acidified and dehydrated egg albumin has the function of replacing gluten, being responsible for the incorporation of air and consistency (Almeida, 2011).

2.2 Methods

2.2.1 Sample preparation and HPP treatment

A suspension of GF-Mix (25 g/100 g) was kept under agitation on an LGI-MSH-5 magnetic stirrer (LGI Scientific, São Paulo-SP, Brazil) for 18 h, for hydration of starch. Subsequently, the samples were packed in polyethylene bags to avoid contact with the pressure-transmitting fluid (water). The samples were subjected to the HPP process in a random manner, in an Avure QFP 2L-700 high isostatic pressure equipment (Avure Technologies®, Livonia-MI, USA), with a 2 L stainless steel pressurization chamber.

Based on preliminary studies and a literature review (Almeida, 2011; Bauer & Knorr, 2005; Capriles & Arêas, 2014), the pressure conditions (300–600 MPa), chamber temperature (30–50°C), and treatment time (5–10 min) were chosen and varied according to the central composite rotational design (CCRD) described in Table 1. In total, 18 experiments were performed with 8 factorial points (23), 6 axial points (2 x 3), and 4 repetitions of the central point.

After the HPP treatment, the samples were frozen at -40 ± 2°C in a UK05B ultra freezer (KLIMAQUIP, Pouso Alegre, Brazil) for 1 h, and then freeze-dried in a L108 freeze-dryer (Liobras Ind. Com. e Serv., São Carlos, Brazil). Finally, they were milled until obtaining powders with a particle size ≤ 0.250 mm to perform the analyses. The results were compared to the control sample, which consisted of GF-Mix subjected to all processing stages, except the HPP treatment. The effect of the combination of pressure, temperature, and exposure time on the instrumental color, pasting properties, and microscopic structure of the mix was evaluated.

Table 1. Ultra-high pressure (HPP) treatment conditions (real values) of GF-Mix and instrumental color parameters.

	Independent variables			Instrumental color parameters		
Treatment	**Pressure (MPa)**	**Time (min)**	**Temperature (°C)**	**L***	**a***	**b***
T1	360.81	6.00	30.07	94.51 ± 2.04	0.45 ± 0.05	6.53 ± 0.11
T2	539.19	6.00	30.07	95.86 ± 1.59	0.46 ± 0.03	6.93 ± 0.17
T3	360.81	9.00	30.07	95.68 ± 1.33	0.43 ± 0.04	6.85 ± 0.37
T4	539.19	9.00	30.07	96.81 ± 0.20	0.52 ± 0.04	7.22 ± 0.06
T5	360.81	6.00	44.93	95.21 ± 1.29	0.66 ± 0.01	7.58 ± 0.19
T6	539.19	6.00	44.93	97.14 ± 0.04	0.55 ± 0.02	7.10 ± 0.03
T7	360.81	9.00	44.93	94.54 ± 0.31	0.82 ± 0.04	9.48 ± 0.04
T8	539.19	9.00	44.93	97.95 ± 0.09	0.45 ± 0.00	5.93 ± 0.06
T9	300.00	7.50	37.50	96.49 ± 1.10	0.66 ± 0.07	6.76 ± 0.50
T10	600.00	7.50	37.50	96.91 ± 0.58	0.64 ± 0.10	6.25 ± 0.64
T11	450.00	5.00	37.50	96.42 ± 0.56	0.77 ± 0.07	8.60 ± 0.54
T12	450.00	10.00	37.50	97.67 ± 0.47	0.87 ± 0.04	6.45 ± 0.51
T13	450.00	7.50	25.00	97.05 ± 0.3	0.54 ± 0.08	7.91 ± 0.56
T14	450.00	7.50	50.00	95.79 ± 0.52	0.45 ± 0.05	7.64 ± 0.67
T15*	450.00	7.50	37.50	94.86 ± 0.55	0.99 ± 0.08	9.41 ± 0.47
T16*	450.00	7.50	37.50	96.02 ± 0.13	0.82 ± 0.01	8.36 ± 0.09
T17*	450.00	7.50	37.50	97.58 ± 0.03	0.44 ± 0.01	6.37 ± 0.03
T18*	450.00	7.50	37.50	95.61 ± 0.15	0.78 ± 0.04	8.72 ± 0.16
Control	-	-	-	98.85 ± 0.29	0.39 ± 0.06	6.58 ± 0.26

Means ± standard deviations of three repetitions; Control: without HPP treatment; *Central point.

2.2.2 Instrumental color

The instrumental color of the HPP-treated and control samples was evaluated, in triplicate, using a MiniScan HUNTERLAB (Reston, USA) spectrophotometer, following the *CIELab* system, determining L*, a*, and b* color parameters (Minolta, 1994).

2.2.3 Pasting properties

The pasting properties of the HPP-treated and control samples were determined using 3 g of sample, in a RVA 4500 Rapid Visco Analyser (Perten Instruments, Melbourne, Australia), using the software Thermocline for Windows, version 2.3 (Jessup, USA), and the profile Standard 1, according to AACCI method 76-21.02 (AACCI, 2010), in triplicate.

2.2.4 Optical microscopy

The raw materials (rice flour, cassava starch, and modified egg albumin), the HPP-treated samples, and the control were visualized using a BX51/BX52 microscope (Olympus Corporation, Tokyo, Japan), coupled with an Evolt E-330 camera (Olympus Imaging Corporation, Tokyo, Japan), according to the methodology of Vigneau et al. (2000), with 100x magnification, to verify the presence of native and modified starch granules, as well as the structure of albumin.

2.2.5 Statistical analysis

The response surface methodology was used to analyze the effect of the independent variables pressure, temperature, and exposure time (P, T, and t) using the software Protimiza Experimental Design (Campinas-SP, Brazil). The dependent variables were the instrumental color (L*, a*, and b*) and the pasting properties (hot peak viscosity, trough, final viscosity, breakdown, setback, pasting temperature, and time to reach peak viscosity).

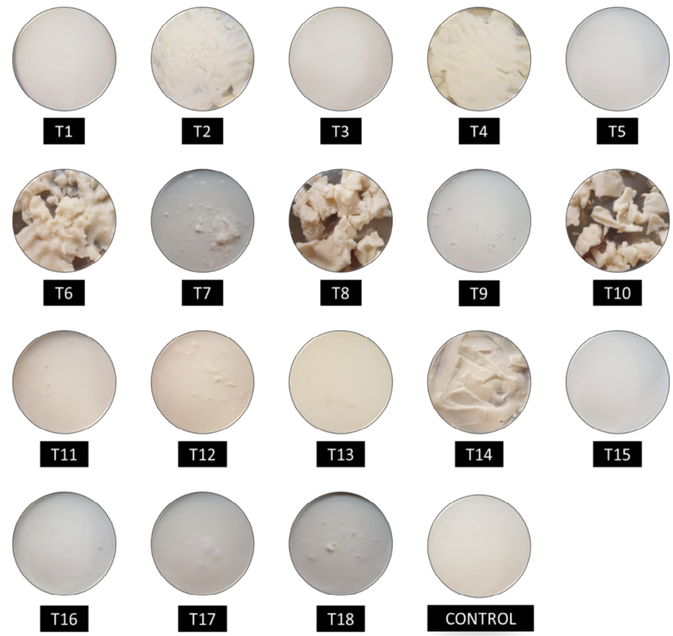
3 RESULTS AND DISCUSSION

3.1 Characterization of the HHP-treated samples

The HHP-treated and the control samples were characterized regarding visual aspect, instrumental color, pasting properties, and microscopic structure, and the results are described and discussed in Sections 3.1.1 to 3.1.4.

3.1.1 Visual aspect

Figure 1 shows the visual aspect of the 18 samples of the gluten-free mix (GF-Mix) treated by ultra-high pressure (HPP) and the control (untreated sample). Little or no significant differences were observed for the consistency of the treatments T1, T3, T5, T7, T9, T11, T12, T13, T15, T16, T17, and T18 of GF-Mix in water (25 g/100 g), subjected to 300–450 MPa, which remained in the liquid form. Minimal changes in the structure of starch and protein may have occurred, with no effects on consistency, with treated samples remaining very similar to the control (untreated sample). In contrast, changes in the consistency of T2, T4, and T14 were observed after the HPP treatment,



T1: pressure 360.7 MPa, time 6 min, temperature 30.1°C; T2: pressure 539.3 MPa, time 6 min, temperature 30.1°C; T3: pressure 360.7 MPa, time 9 min, temperature 30.1°C; T4: pressure 539.3 MPa, time 9 min, temperature 30.1°C; T5: pressure 360.7 MPa, time 6 min, temperature 44.9°C; T6: pressure 539.3 MPa, time 6 min, temperature 44.9°C; T7: pressure 360.7 MPa, time 9 min, temperature 44.9°C; T8: pressure 539.3 MPa, time 9 min, temperature 44.9°C; T9: pressure 300.0 MPa, time 7.5 min, temperature 37.5°C; T10: pressure 600.0 MPa, time 7.5 min, temperature 37.5°C; T11: pressure 450 MPa, time 5 min, temperature 37.5°C; T12: pressure 450 MPa, time 10 min, temperature 37.5°C; T13: pressure 450 MPa, time 7.5 min, temperature 25°C; T14: pressure 450 MPa, time 7.5 min, temperature 50°C; T15: pressure 450 MPa, time 7.5 min, temperature 37.5°C; T16: pressure 450 MPa, time 7.5 min, temperature 37.5°C; T17: pressure 450 MPa, time 7.5 min, temperature 37.5°C; T18: pressure 450 MPa, time 7.5 min, temperature 37.5°C; Control: without HPP treatment.

Figure 1. Visual aspect of the gluten-free mix (GF-Mix) suspensions treated by ultra-high pressure (HPP) and the control.

and were similar for the samples treated at 450 MPa and 50°C (T14), and 539.6 MPa and 30°C (T2 and T4), all exhibiting a pasty consistency. The greatest changes in consistency were observed for the samples T6, T8, and T10, which became rigid after the HPP treatment, probably due to the application of higher pressures (539.6–600 MPa), leading to greater changes in the structure of the starch and proteins present in GF-Mix.

According to Pei-Ling et al. (2010), the pressure level at which the starch suspension is gelatinized after the HPP treatment depends strongly on the pressure, starch concentration, process temperature, and exposure time used in the treatment. The final quality of a bakery product is directly linked to the viscosity of the dough.

For a bread to be considered of good quality, it needs to be evenly baked and have an airy texture. These characteristics are related to the ability of the dough to incorporate air, retain air bubbles, and maintain its stability, which are influenced by the initial viscosity of the dough (Patil et al., 2020). Bhaduri (2013) reported that the low viscosity of a gluten-free formulation decreased hardness, gumminess, and chewiness and provided better consumer acceptance. Thus, we can verify that the HPP treatment at higher pressures (> 539.6 MPa) can contribute to viscosity reduction.

3.1.2 Instrumental color

As can be seen in Table 1, very similar L*, a*, and b* values were observed for all treatments. The L* values (lightness) ranged from 94.51 to 97.95, a* values (green–red) from 0.43 to 0.99, and b* values (blue–yellow) from 5.93 to 9.48, indicating a color very close to white, with a slight yellow tone for all samples. Thus, it can be stated that the majority of the flours were similar to white wheat flour, once Ortolan et al. (2010) stated that a white wheat flour is characterized by L* value ≥ 93, a* close to zero (≤ 0.5 or negative), and b* value ≤ 8. According to the CCRD, the independent variables had no significant effect ($p \geq 0.05$) on the instrumental color parameters, thus mathematical models and response surfaces were not generated.

3.1.3 Pasting properties

The effects of the independent variables (P, T, and t) of the HPP treatment were significant ($p \leq 0.05$) on the responses to pasting temperature (°C), trough (cP), final viscosity (cP), and setback (cP). No significant effect ($p \geq 0.05$) was observed for hot peak viscosity (cP), breakdown (cP), and time to reach maximum viscosity (min), with values ranging from 1,159.3 to 4,595.7 cP, 390.0 to 1882.3 cP, and 5.02 to 6.31 min for these parameters, respectively, while the control presented 4,439.0 cP, 1,800.7 cP, and 5.27 min, respectively (Table 2).

The pasting temperature indicates the temperature of the initial increase in viscosity during heating, that is, when the starch starts to gelatinize (Singh et al., 2007). In the present study, these values ranged from 71.73 to 77.45°C and were affected by all independent variables (pressure, time, and temperature), and the response surfaces are shown in Figures 2D, 2E, and 2F. High pressure combined with mild temperatures (Figure 2E) increased the pasting temperature of the starch suspensions throughout the whole process time (Figure 2D), probably due to the change in the granular structure and crystallinity of the starch granules.

Regarding the trough (cP), final viscosity (cP), setback (cP), and pasting temperature (°C), the effects of the independent variables were statistically significant ($p \leq 0.05$), thus mathematical models and response surfaces were built to represent such effects, as shown in Table 3 and Figure 2.

The values of trough, final viscosity, and setback ranged from 570.0 to 3,168.3 cP, 931.0 cPa to 4,546.3 cP, and 361.0 to 1,421.0 cP, respectively. The response surfaces in Figures 2A–2C demonstrate that these parameters were affected by the independent variables' pressure (MPa) and temperature (°C), with no significant effect on the exposure time.

The results of the trough (Figure 2A) show the lowest viscosity reached during the heating cycle, due to the breaking down of the gelatinized starch granules under agitation, which leads to a lower viscosity of the suspension.

The final viscosity (Figure 2B) represents the viscosity after cooling the system and resuming the initial temperature (50°C) and depends on the ability of the starch molecules (amylose and

Table 2. Pasting properties of GF-Mix treated by ultra-high pressure (HPP) and control.

	Hot Peak viscosity (cP)	Trough (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)	Peak time (min)	Pasting temperature (°C)
T1	4,746.3 ± 20.1	2,864.0 ± 135.1	1,882.3 ± 155.1	4,208.7 ± 121.6	1,344.7 ± 17.7	5.40 ± 0.11	72.62 ± 0.70
T2	3,558.3 ± 15.1	3,168.3 ± 50.3	390.0 ± 37.5	4,546.7 ± 38.9	1,378.3 ± 32.2	6.29 ± 0.08	74.57 ± 0.39
T3	4,718.7 ± 67.0	3,014.3 ± 68.2	1,704.3 ± 108.9	4,435.3 ± 48.7	1,421.0 ± 19.5	5.51 ± 0.11	72.93 ± 0.33
T4	3,194.0 ± 30.7	2,866.0 ± 39.6	328.0 ± 52.7	4,001.3 ± 54.8	1,135.3 ± 91.0	6.31 ± 0.17	75.12 ± 0.55
T5	4,120.3 ± 43.1	2,654.7 ± 139.7	1,465.7 ± 96.6	3,873.7 ± 147.5	1,219.0 ± 13.1	5.53 ± 0.11	72.60 ± 0.76
T6	1,159.3 ± 7.5	570.0 ± 9.3	589.3 ± 16.8	931.0 ± 8.6	361.0 ± 2.9	5.02 ± 0.03	77.45 ± 0.04
T7	4,692.3 ± 113.2	3,063.7 ± 150.5	1,628.7 ± 111.5	4,338.7 ± 127.0	1,275.0 ± 26.5	5.27 ± 0.20	72.68 ± 0.05
T8	2,077.3 ± 20.3	1,423.7 ± 19.7	653.7 ± 26.5	1,938.3 ± 19.2	514.7 ± 16.4	5.69 ± 0.08	76.90 ± 0.43
T9	4,230.7 ± 102.1	2,686.7 ± 46.6	1,544.0 ± 60.8	4,058.7 ± 59.1	1,372.0 ± 13.1	5.49 ± 0.03	72.60 ± 0.04
T10	2,075.7 ± 32.3	1,430.3 ± 44.1	645.3 ± 22.1	1,943.0 ± 43.8	512.7 ± 4.0	5.71 ± 0.06	75.55 ± 0.39
T11	3,034.3 ± 14.1	2,000.3 ± 16.0	1,034.0 ± 27.5	3,097.3 ± 3.1	1,097.0 ± 13.5	5.47 ± 0.00	72.98 ± 0.37
T12	2,377.7 ± 81.6	1,815.7 ± 64.9	562.0 ± 29.7	2,779.3 ± 76.4	963.7 ± 11.8	5.60 ± 0.00	73.70 ± 0.39
T13	4,250.7 ± 61.3	2,601.7 ± 55.7	1,649.0 ± 53.8	3,881.7 ± 74.0	1,280.0 ± 26.2	5.36 ± 0.03	72.63 ± 0.02
T14	1,932.0 ± 26.9	1,461.0 ± 12.8	471.0 ± 23.3	2,281.3 ± 31.8	820.3 ± 25.4	5.40 ± 0.05	74.27 ± 0.02
T15*	3,743.7 ± 40.7	2,642.3 ± 42.7	1,101.3 ± 45.6	3,841.3 ± 45.1	1,199.0 ± 26.7	5.78 ± 0.11	73.78 ± 0.37
T16*	4,212.0 ± 72.2	2,985.7 ± 64.9	1,226.3 ± 93.0	4,114.7 ± 56.3	1,129.0 ± 55.9	5.69 ± 0.03	73.22 ± 0.33
T17*	4,442.0 ± 87.5	2,821.3 ± 29.5	1,620.7 ± 89.4	4,011.0 ± 15.5	1,189.7 ± 15.0	5.11 ± 0.06	72.32 ± 0.44
T18*	4,595.7 ± 36.6	2,885.3 ± 105.7	1,710.3 ± 77.0	4,055.0 ± 42.4	1,170.0 ± 70.9	5.16 ± 0.11	71.73 ± 0.65
Control	4,439.0 ± 99.0	2,638.3 ± 62.5	1,800.7 ± 38.4	3,973.3 ± 69.8	1,335.0 ± 9.0	5.27 ± 0.05	72.68 ± 0.02

Means ± standard deviations of three repetitions. T1: pressure 360.7 MPa, time 6 min, temperature 30.1°C; T2: pressure 539.3 MPa, time 6 min, temperature 30.1°C; T3: pressure 360.7 MPa, time 9 min, temperature 30.1°C; T4: pressure 539.3 MPa, time 9 min, temperature 30.1°C; T5: pressure 360.7 MPa, time 6 min, temperature 44.9°C; T6: pressure 539.3 MPa, time 6 min, temperature 44.9°C; T7: pressure 360.7 MPa, time 9 min, temperature 44.9°C; T8: pressure 539.3 MPa, time 9 min, temperature 44.9°C; T9: pressure 300.0 MPa, time 7.5 min, temperature 37.5°C; T10: pressure 600.0 MPa, time 7.5 min, temperature 37.5°C; T11: pressure 450 MPa, time 5 min, temperature 37.5°C; T12: pressure 450 MPa, time 10 min, temperature 37.5°C; T13: pressure 450 MPa, time 7.5 min, temperature 25°C; T14: pressure 450 MPa, time 7.5 min, temperature 50°C; T15, T16, T17, and T18: pressure 450 MPa, time 7.5 min, temperature 37.5°C; Control: without HPP treatment; *Central points.

amylopectin) to reassociate, and can be related to the amylose/amylopectin ratio.

The tendency to retrograde or setback (Figure 2C) is based on the difference between the final viscosity and the trough, indicating how much viscosity increases with cooling. The transformations that the starch granules and molecules undergo during gelatinization and retrogradation are the main determinants of the pasting behavior (Thomas & Atwell, 1999).

The results showed that the increase in pressure combined with high temperature favored a reduction in viscosity of the GF-Mix suspension. Most HPP-gelatinized starches develop a lower viscosity at normal paste concentrations, forming smooth-textured gels, while excessive pressurization likely weakens the structure of starch gels (Pei-Ling et al., 2010).

Therefore, the combination of pressure and temperature in the HPP process may have induced the gelatinization of the starch present in the GF-Mix suspension, which reduced the paste viscosity in the RVA curve, when using the Standard 1 method. van Rooyen et al. (2023) proposed that a three-dimensional starchy network, created under suitable conditions, can retain the gases from fermentation, similar to the protein network called gluten. This three-dimensional network would have its base of support in the hydrogen bonds created between the amylose and amylopectin molecules.

Starch retrogradation decreases the sensory quality of foods rich in starch, increasing firmness, which is the main cause of the aging of breads. In this study, the decrease in setback may lead to a slower retrogradation of gluten-free bread made with GF-Mix subjected to higher pressure and temperature conditions (600 MPa and50 °C, respectively), contributing to better quality during shelf life.

All these results indicate that HPP changed the behavior of the paste viscosity curve of GF-Mix, especially when combining high pressures with high temperatures.

3.1.4 Optical microscopy

The raw materials (rice flour, cassava starch, and egg albumin) were visualized individually in the optical microscope to assess the structure of cassava and rice starch granules, and egg albumin (Figure 3), aiming to elucidate the structural changes after the HPP treatment at different pressure, temperature, and process time conditions. Cassava starch granules mostly have an irregular polyhedron form, while some are spherical, elliptical, with flat surfaces, and lengths varying from 5.01 to 34.67 μm (Ren, 2017). Rice starch granules are pentagonal and angular, with lengths ranging from 3 to 5 μm (Singh et al., 2007). In the present study, albumin showed an irregular and flat structure, similar to broken glass. Figures 3A and 3B show the images captured with polarized light, which exhibited the Maltese cross, thus indicating the presence of non-gelatinized starch. The images of egg albumin (Figure 3C) were obtained without polarized light, as the structure of albumin cannot be observed when using this tool.

The effect of the combination of high pressure, temperature, and exposure time on the molecular structure of starches (rice

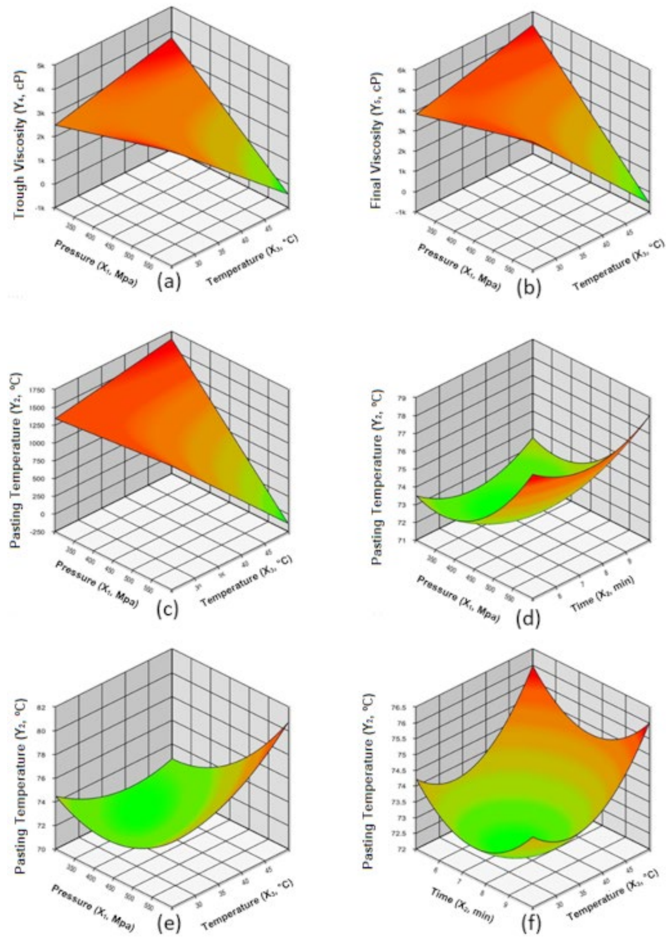


Figure 2. Response surface. (A) Response surface for trough viscosity (cP) as a function of pressure (MPa) and temperature (°C); (B) response surface for final viscosity (cP) as a function of pressure (MPa) and temperature (°C); (C) response surface for setback (cP) as a function of pressure (MPa) and temperature (°C); (D) response surfaces for pasting temperature (°C) as a function of pressure (MPa) and time (min), € as a function of pressure (MPa) and temperature (°C), and (F) as a function of time (min) and temperature (°C).

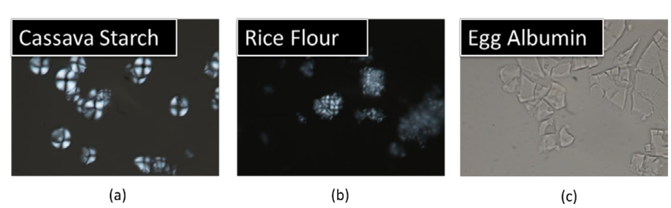


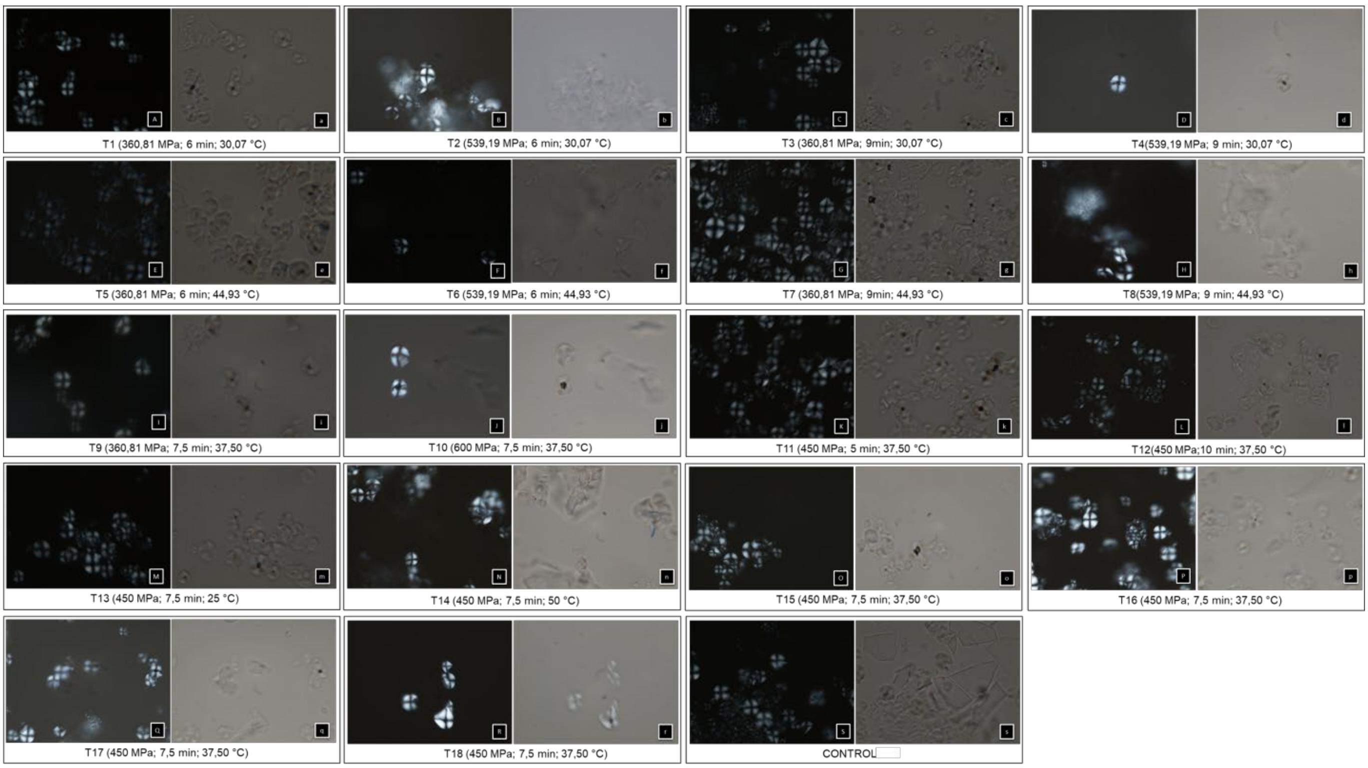
Figure 3. Microscopic structures of cassava and rice starches and egg albumin. (A) Cassava starch granules and (B) rice starch granules, under polarized light, and (C) egg albumin structure, using optical microscopy with 100x magnification.

and cassava) and proteins (egg albumin) was observed with the aid of optical microscopy (Figure 4). Loss of birefringence (which indicates a crystalline organization) was observed for some starch granules after the HPP treatment at 450 MPa and 50°C (T14—Figure 4N) and at 539.19 MPa and ~30°C (T2 and T4—Figures 4B and 4D), indicating partial gelatinization of starch. Although more extreme treatments, mainly T6, T8, and

Table 3. Coded models generated by the regression, % explained variation (R^2), and calculated F for the responses trough (cP), final viscosity (cP), setback (cP), and pasting temperature (min).

Responses	Coded regression model	R^2 (%)	F calc	F Lack of fit
Trough (Y_1)	$Y_1 = 2386.39 + 416.03\ X_1 - 448.05\ X_3 - 485.09\ X_1X_3$	73.84	13.2	10.3
Final viscosity (Y_2)	$Y_2 = 3470.50 + 658.81\ X_1 - 664.50\ X_3 - 655.89\ X_1X_3$	79.97	18.6	92.4
Setback (Y_3)	$Y_3 = 1084.36 - 242.78\ X_1 - 196.44\ X_3 - 170.77\ X_1X_3$	87.55	32.8	2.2
Pasting temperature (Y_4)	$Y_4 = 72.46 + 1.33\ X_1 + 0.71\ X_1^2 + 0.45X_2^2 + 0.52\ X_3 + 0.48\ X_3^2 + 0.62\ X_1X_3$	88.36	13.9	1.3

X_1 = Pressure (MPa); X_2 = Time (min); X_3 = Temperature (°C). For use with the coded values of the independent variables, in the range of -1.68 to +1.68.



T1: pressure 360.7 MPa, time 6 min, temperature 30.1°C; T2: pressure 539.3 MPa, time 6 min, temperature 30.1°C; T3: pressure 360.7 MPa, time 9 min, temperature 30.1°C; T4: pressure 539.3 MPa, time 9 min, temperature 30.1°C; T5: pressure 360.7 MPa, time 6 min, temperature 44.9°C; T6: pressure 539.3 MPa, time 6 min, temperature 44.9°C; T7: pressure 360.7 MPa, time 9 min, temperature 44.9°C; T8: pressure 539.3 MPa, time 9 min, temperature 44.9°C; T9: pressure 300.0 MPa, time 7.5 min, temperature 37.5°C; T10: pressure 600.0 MPa, time 7.5 min, temperature 37.5°C; T11: pressure 450 MPa, time 5 min, temperature 37.5°C; T12: pressure 450 MPa, time 10 min, temperature 37.5°C; T13: pressure 450 MPa, time 7.5 min, temperature 25°C; T14: pressure 450 MPa, time 7.5 min, temperature 50 °C; T15: pressure 450 MPa, time 7.5 min, temperature 37.5°C; T16: pressure 450 MPa, time 7.5 min, temperature 37.5°C; T17: pressure 450 MPa, time 7.5 min, temperature 37.5°C; T18: pressure 450 MPa, time 7.5 min, temperature 37.5°C; Control: without HPP treatment.

Figure 4. Optical microscopy under bright field or light (lower case letters) and under polarized light (uppercase letters), with 100x magnification, of all treatments by HPP and control.

T10, which used pressures above 539 MPa in combination with temperatures above 44°C, showed greater loss of birefringence, the granules showed some structural integrity.

The results corroborate the findings of Oh et al. (2008), who reported that rice and potato starches did not completely lose birefringence, even after HPP treatment at 600 MPa. In contrast, Pei-Ling et al. (2012) found that cassava starch granules completely lost their birefringence at pressures above 450 MPa. Concerning egg albumin, its original structure was observed only in the control, thus suggesting a structural reorganization.

Pasqualone et al. (2010) reported that the technological increase in the participation of cassava starch in the formulation of gluten-free breads was to provide cohesiveness, aiming at gas retention. The modification induced by HHP (> 450 MPa) on the GSF-Mix may suggest that samples treated under these conditions can help in structure and expansion, as well as in texture.

4 CONCLUSIONS

The effect of different HPP process conditions on suspensions prepared with rice flour, cassava starch, and egg albumin was investigated. The pasting properties were affected by the HPP treatment, mainly the trough, final viscosity, setback, and pasting temperature. The combination of high pressure (600 MPa) and high temperature (50°C) favored the reduction of viscosity, especially a lower setback, which indicates a lower tendency to retrograde in products high in starch. The parameter pasting temperature was affected by all independent variables, increasing with the increase in pressure and temperature for the intermediate exposure times (4–8 min).

Optical microscopy showed modification of starch granules (cassava and rice) with the increase in pressure, especially when combined with higher temperatures, with a partial loss of birefringence. Concerning egg albumin, there was evidence of a loss of structure for all treatments studied.

Therefore, the use of HPP under the conditions of this study allowed the modification of the pasting properties of the GF-Mix, as well as the partial loss of birefringence of the starch granules.

The HPP process at 600 MPa, 50°C, and 7.5 min appears to be the most promising condition for the treatment of GF-Mix for application in gluten-free breads, mainly due to the setback results, which showed a lower retrogradation tendency, possibly contributing to a better sensory quality of breads during shelf life.

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