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Research Article

Application of Modified Cassava Starch as a Fat Substitute in Cracker Production

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Crackers are a popular food with an appreciable share of the consumer market. Fat is essential for the sensory properties of this product; however, a high fat content is associated with health disorders. For this reason, developing low-fat products with the same desirable attributes as the corresponding full-fat ones is a high priority for the food industry. The objective of the study was to evaluate the inclusion of modified cassava starch as a fat substitute in crackers by measuring their physical and sensory properties and behavior during storage. Fat reduction in crackers led to higher fracturability values for all treatment but the pregelatinized (PREGEL) one (11.73 ± 0.39 N). The expansion ratio decreased with fat reduction, and the treatments with the highest expansion ratio and specific volume were the control (CTRL) (10.51 ± 0.21 and 1.97 ± 0.09 mL/g), the one with amyloglucosidase (AMG) (10.24 ± 0.10 and 2.20 ± 0.023 mL/g), and the pregelatinized (PREGEL) one (10.40 ± 0.16 and 1.99 ± 0.15 mL/g). The samples with modified starch showed an average total fat reduction of 49.51% compared with the control treatment. The results of sensory analysis by the acceptability test showed a greater inclination towards the CTRL and AMG treatments, in both color (6.73 ± 1.81 and 6.45 ± 2.08, respectively) and texture (6.95 ± 1.72 and 6.67 ± 1.69, respectively) parameters. During storage, the fracture strength decreased from 13.66 ± 0.32 N to 11.39 ± 0.57 N for AMG and from 12.5 ± 0.42 N to 10.77 ± 0.61 N for CTRL treatment, while the moisture content increased for both AMG (from $4.09 \pm 0.85\%$ to $4.72 \pm 0.13\%$) and CTRL (from $3.87 \pm 0.14\%$ to $4.31 \pm 0.43\%$) treatments between 0 and 30 days of storage. According to these results, it can be concluded that physically and enzymatically modified cassava starches can work as fat replacers in crackers.

1. Introduction

Crackers are popular products with an appreciable share of consumer demand. Their consumption is growing significantly because they have a long shelf life, they can be consumed easily and quickly, and they are economically accessible to the general population [1]. These foods are generally defined as dry, thin, and crispy bakery products usually made from wheat flour, fat (or vegetable butter), salt, and leavening agents (yeast, chemical leaveners, or a combination) [2]. In this type of product, fat is an essential ingredient that, in addition to being responsible for the soft and crispy texture of the crackers, gives flavor and aeration [3]. However, excess calorie intake and high fat content (especially saturated fat) are associated with health disorders such as obesity, cancer, high blood cholesterol, and coronary heart disease [3]. Therefore, several strategies to replace saturated and trans fats in food products without adversely affecting sensory properties have been an increasingly pressing concern for the food industry [4]. In this context, carbohydrate-based fat replacements such as processed starch may mimic fat by binding water and provide lubricity, body, and a pleasant mouthfeel [5, 6].

Starch has potential utility because it is an innocuous polysaccharide derived from renewable sources, and it is a product of relatively low cost for the food industry compared with others used for similar purposes. It yields good results by modifying the texture and consistency of food due to its gelling and thickening properties [7].

However, in its native state, starch tends to present limitations in industrial applications due to its mechanical resistance, thermal decomposition, high retrogradation, and syneresis. These deficiencies can be overcome by modifying starch through physical, chemical, and enzymatic methods [8]. Currently, under the concept of clean and green labels, the physical and enzymatic methods emerge as environmentally friendly, safe, and nontoxic technologies [9].

The aim of this study was to evaluate the inclusion of physically, chemically, and enzymatically modified cassava starch in reduced fat crackers, considering their physical properties, rheological behavior, effect at the sensory level, and behavior during storage.

2. Materials and Methods

2.1. Materials Used for Starch Modification. Native cassava starch and oxidized cassava starch were provided by Poltec S.A.S. (Baking XP, Poltec S.A.S., La Estrella, Antioquia, Colombia), and pregelatinized starch was obtained from Podium (100 starch, Podium Alimentos, Zona Rural, Tamboara, PR, Brazil). Enzymatically modified starches were obtained by enzymatic hydrolysis of native cassava starch with amyloglucosidase from *Aspergillus niger* (Dextrozyme® GA, Novozymes, Krogshoejvej, Bagsvaerd, Denmark), pullulanase from *Bacillus licheniformis* (OPTIMAX® L-1000, DuPont™ Genencor® Science, Wuxi, China), α-amylase from *B. licheniformis* (Liquozyme® Supra 2.2X, Krogshoejvej, Bagsvaerd, Denmark), and β-amylase from *Hordeum vulgare* (OPTIMALT® BBA, DuPont, Genencor Science, Wuxi, China).

2.2. Enzymatic Hydrolysis of Starch. Native cassava starch (25 g) was suspended in 200 mL of sodium citrate/citric acid buffer solution (pH 4.5 and 5) at 60°C and 250 rpm (Shaker, MaxQ 4450, Germany) for 30 min. Then, the indicated amount of enzyme was added for each treatment (400 μL of pullulanase, 400 μL of β-amylase, 50 μL of α-amylase, and 50 μL of amyloglucosidase) keeping the suspension under stirring at 60°C for 6 h. After hydrolysis and a 30 min rest, two washes were carried out with distilled water at room temperature. The suspensions were centrifuged (Hettich Universal 320R, Sigma-Aldrich, USA) at $5478 \times g$ for 7 min, and the supernatant was removed. The enzymatically modified starch was oven dried (UFB500, Memmert, Germany) at 38°C for 12 h, and it was ground, sieved, and stored for further analysis and application.

2.3. Determination of the Degree of Hydrolysis. The degree of hydrolysis was determined as a function of the production of reducing sugars using the 3.5-dinitrosalicylic acid method. The absorbance was detected at 540 nm with a UV-Vis spectrophotometer (UV-2550, Shimadzu, Japan). The degree of starch hydrolysis was expressed in dextrose equivalents [10].

2.4. Pasting Properties of Modified Starches. The pasting properties of the modified starches were measured using a microviscoamylograph (Brabender® GmbH & Co. KG, Duisburg, Germany). Six grams of sample with a 12% moisture content was dissolved in 110 mL of distilled water. The slurry was heated from 30 to 92°C at a rate of 7.5°C/min, held at 92°C for 5 min, and cooled at the same rate from 92 to 46°C. The evaluated parameters were pasting temperature (PT), peak viscosity (PV), breakdown (BD), setback (SB), and final viscosity (FV) [10].

2.5. Ingredients Used for Cracker Production. Wheat flour (Tres Castillos, Cartagena, Colombia); baking powder (Carolesen, Colorisa S.A., Sabaneta, Colombia); salt, water, and yeast (Fermipan Brown, Mexico City); and margarine (Astra, SIGRA, Bogotá, Colombia) were used for the basic formulation of the crackers. DATEM (Panodan M2020, Danisco Ltd., Braband, Denmark) was used as emulsifier.

2.6. Preparation of Crackers. Crackers were made according to the method proposed by Li et al. [11]. The formulation was slightly modified according to preliminary tests where the proportion of each ingredient, kneading time, mixing, and baking conditions was evaluated. Initially, the sponge method was used by mixing water (30 g), yeast (0.4 g), and wheat flour (65 g) with a fermentation time of 20 h. The fermented sponge was then mixed and kneaded with the following additional ingredients: 35 g of wheat flour, 0.4 g of baking powder, 2.5 g of salt, 10 g of water, 7 g of margarine, 0.5 g of emulsifier, and 2 g of modified cassava starch, using a mixer (KSM150PSER Kitchen aid Artisan, USA). After the crackers were sheeted (Skyfood CLM-400 16″ tabletop dough roller and sheeter) and molded, they were baked in an electric oven (Tecnoeka KL 884-HT Padova, Italy) in two stages: 180°C for 4 min and 160°C for 6 min. Finally, the crackers were allowed to cool to room temperature and packed into polythene bags for further analysis.

2.7. Characterization of Crackers. Weight loss was calculated as the ratio between weight before and after baking expressed as a percentage [12]. The width was calculated as the average value of six crackers from edge to edge, and the thickness average was measured using a digital Vernier gauge (0.01 mm resolution) by stacking six crackers one on top of the other. The expansion ratio was calculated by dividing the cracker's average width value by the average thickness value [13]. The AACC method 10-05.01 millet seed displacement was used to measure the specific volume [14]. The moisture percentage was determined by the AACC 44-19 approved drying method [14]. The water holding capacity (WHC) was determined according to the methodology reported by Santiago-García et al. [15]: adding 10 mL of distilled water to 0.5 g of macerated sample, stirring for 10 min, and leaving at rest for 24 h at room temperature, finally centrifuging at $2264 \times g$ for 10 min (Hettich Universal 320R, Sigma-Aldrich, USA), removing the supernatant immediately, and weighing the precipitate. The macerated sample was placed in a dew point hygrometer to determine water activity (aw) (Aqualab Series 3TE, Decagon Devices, Pullman, WA, USA) [13].

Cracker fracturability was measured by a 3-point bending test at room temperature using a reversible blade at the sharp end and a texture analyzer (TA-XT2i, Stable Micro

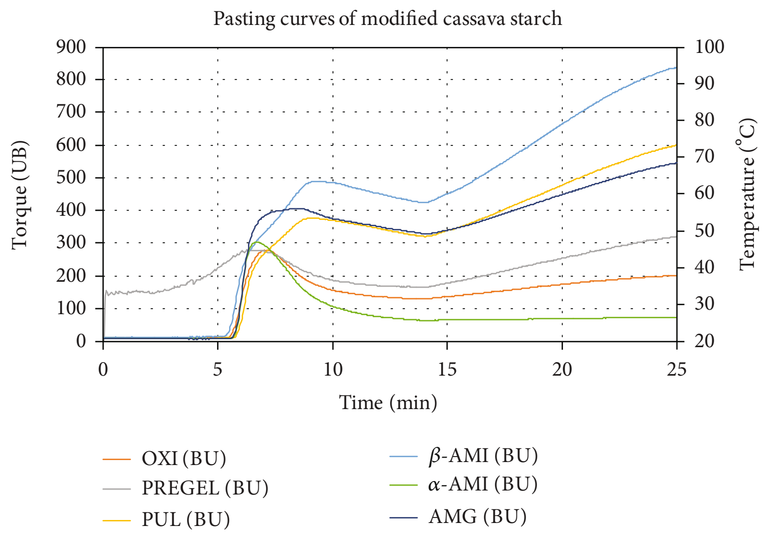

Figure 1: Pasting profiles (viscosity curves) of the investigated native and modified cassava starches: oxidation treatment (OXI), pregelatinization treatment (PREGEL), enzymatic alpha amylase treatment (α-AMI), beta amylase treatment (β-AMI), amyloglucosidase treatment (AMG), and pullulanase treatment (PUL).

Systems, Godalming, UK) equipped with a 50 kg load cell. The test parameters were pretest speed, 5.0 mm/s; test speed, 1.0 mm/s; and posttest speed, 10.0 mm/s [16, 17]. The surface color of the crackers was analyzed in the CIELAB space with a 10° D65 illuminant using a sphere spectrocolorimeter (SP64, X-Rite Inc., MI, USA). The values of L^* (luminosity), a^* (green/red), b^* (blue/yellow), h (tone), and c^* (saturation) were determined, and the ΔE (total color difference) was calculated with respect to the control treatment [18].

For further analysis, one of the enzymatic treatments was selected according to the type of modification and based on the behaviors observed in physicochemical analyses in relation to the treatment without fat reduction (CTRL). The crude protein content was determined by the Kjeldahl method (AOAC 928.08), fat content by the Soxhlet extraction (AOAC 920.39), and the ash content by incineration at 525°C for 24 h (AOAC 942.05). Similarly, to assess the effect of these starches in crackers at the sensory level, a consumer acceptability test was conducted, and samples were evaluated by scoring the acceptability of the product on an unstructured scale of 10 cm, being 0 = I dislike it very much and 10 = I like it very much [19]. The acceptability test was conducted with a panel of 100 people—51 men and 49 women between 16 and 36 years of age—who qualified texture, color, and general acceptability.

2.8. Storage Behavior. For the storage tests, we evaluated the selected control treatment (CTRL) and enzymatic treatment according to the results of the physicochemical and sensory analyses. Samples were stored in polyethylene bags at an average temperature of 25 ± 3°C and relative humidity of 64 ± 4% for 30 days. Fracturability and moisture content were evaluated on days 0, 10, 15, 20, and 30.

2.9. Morphological Characterization: Scanning Electron Microscopy (SEM). Samples were degreased by the Soxhlet extraction and freeze dried (Labconco Freezone 12, Labconco Corporation, Kansas City, USA). Freeze drying was performed with two heating ramps from −40°C to 0°C at 0.03°C/min for 1 h and then brought to 30°C at a rate of 0.03°C/minute. The morphological identification of the crackers was carried out with a scanning electron microscope (EVO MA10, Carl Zeiss, Oberkochen, Germany) on day 0 of storage. The samples were spray coated with a 5 nm gold layer (Quorum, Q150R ES, UK) with an acceleration voltage of 10 kV [20].

2.10. Statistical Analysis. The data were reported as a mean ± standard deviation of at least two independent repetitions. Statistical analyses were performed in Statgraphics 18 statistical software (Manugistics Inc., Maryland, USA). Variance analysis was performed by one-way ANOVA followed by Tukey's post hoc test. Differences between means with probability $p < 0.05$ were considered statistically significant. Principal component analysis (PCA) was used to visually display relationships among the cracker's main quality properties. For the sensory analysis, the nonparametric Friedman test was used in the R statistical package (RS Team Inc., Boston, MA, 2015). The treatments evaluated were oxidation treatment (OXI), pregelatinization treatment (PREGEL), enzymatic alpha amylase treatment (α-AMI), beta amylase treatment (β-AMI), amyloglucosidase treatment (AMG), pullulanase treatment (PUL), treatment without fat reduction and without addition of starch (CTRL), and fat reduction without addition of starch (NEG).

3. Results and Discussion

3.1. Degree of Hydrolysis. The reducing sugar content released into the solution system was related to the degree of starch degradation. The final products of the hydrolysis depend on the type of enzyme, its specificity, the granular structure of the starch, the amylose/amylopectin ratio, and others [10]. α-Amylase and amyloglucosidase showed the

Table 1: Physical properties of crackers with modified cassava starch as a fat substitute[†].

Treatment	aw	Moisture (%)	Weight losses (%)	WHC (gH_2O/g)	Fracturability (N)	Width (cm)	Height (cm)	Expansion ratio	Specific volume (mL/g)
CTRL	0.14 ± 0.01^{cd}	3.04 ± 0.12^{b}	34.65 ± 1.3^{ab}	3.80 ± 0.37^{bc}	12.8 ± 0.33^{cd}	7.49 ± 0.02^{ab}	0.71 ± 0.01^{d}	10.51 ± 0.21^{a}	1.97 ± 0.09^{ab}
NEG	0.14 ± 0.01^{d}	4.24 ± 0.41^{a}	35.88 ± 0.90^{a}	3.58 ± 0.23^{c}	14.86 ± 0.20^{ab}	7.39 ± 0.21^{b}	0.73 ± 0.02^{cd}	10.05 ± 0.09^{ab}	1.67 ± 0.19^{b}
OXI	0.16 ± 0.02^{bcd}	3.69 ± 0.13^{ab}	34.66 ± 1.29^{ab}	4.06 ± 0.42^{abc}	15.24 ± 0.17^{a}	7.33 ± 0.09^{b}	0.78 ± 0.02^{ab}	9.41 ± 0.20^{c}	1.85 ± 0.01^{ab}
PREGEL	0.23 ± 0.01^{a}	3.25 ± 0.22^{b}	31.92 ± 1.17^{bc}	4.81 ± 0.09^{a}	11.73 ± 0.39^{d}	7.69 ± 0.05^{a}	0.74 ± 0.01^{cd}	10.40 ± 0.16^{a}	1.99 ± 0.15^{ab}
PUL	0.15 ± 0.02^{cd}	3.63 ± 0.21^{ab}	33.39 ± 0.96^{abc}	4.11 ± 0.06^{abc}	15.27 ± 0.5^{a}	7.50 ± 0.07^{ab}	0.79 ± 0.07^{a}	9.54 ± 0.18^{c}	1.58 ± 0.05^{b}
β-AMI	0.19 ± 0.05^{abc}	4.27 ± 0.51^{a}	30.58 ± 1.98^{c}	3.97 ± 0.12^{bc}	12.73 ± 1.15^{cd}	7.38 ± 0.02^{b}	0.75 ± 0.01^{bc}	9.86 ± 0.11^{bc}	1.98 ± 0.06^{ab}
$\propto$-AMI	0.17 ± 0.03^{bcd}	3.68 ± 0.45^{ab}	35.33 ± 1.18^{ab}	4.17 ± 0.21^{abc}	13.61 ± 0.16^{bc}	7.52 ± 0.12^{ab}	0.79 ± 0.02^{a}	9.55 ± 0.23^{c}	1.98 ± 0.22^{ab}
AMG	0.21 ± 0.03^{ab}	3.54 ± 0.25^{ab}	36 ± 0.84^{a}	4.54 ± 0.45^{ab}	13.53 ± 0.40^{bc}	7.57 ± 0.02^{ab}	0.74 ± 0.01^{cd}	10.24 ± 0.10^{ab}	2.20 ± 0.023^{a}

[†]Water activity (aw), water holding capacity (WHC), treatment without fat reduction, treatment without addition of starch (CTRL), treatment with fat reduction without addition of starch (NEG), oxidation treatment (OXI), pregelatinization treatment (PREGEL), enzymatic alpha amylase treatment (α-AMI), beta amylase treatment (β-AMI), amyloglucosidase treatment (AMG), and pullulanase treatment (PUL).

highest degrees of hydrolysis: 39.01 ± 0.89% and 36.13 ± 0.3%, respectively. α-Amylase is an endoamylase that hydrolyzes α-1.4 bonds at random locations [21], and amyloglucosidase or glucoamylases cleave both α-1.4 and α-1.6 bonds in external glucose residues. Pullulanase and β-amylase were less efficient in degrading starch granules and had less effect on the hydrolysis degree with results of 11.12 ± 0.99% and 10.5 ± 0.71%, respectively. The action of these enzymes was more limited. β-Amylase is an exo-hydrolase that specifically cleaves α-1.4 bonds from the nonreducing ends of the glucan chain, stopping by the presence of breakpoints, outermost branching (α-1.6) linkages [22]. Debranching enzymes such as pullulanase catalyze the hydrolysis of α-1.6 glycosidic bonds in amylopectin [21]. Huang et al. [23] made similar observations when comparing α-amylase and β-amylase in cassava starch.

3.2. Pasting Properties of Modified Starches. Changes in starch granules during gelatinization and retrogradation are the main determinants of pasting properties and are mainly assessed through changes in viscosity of starch suspensions during heating and cooling. In the food industry, this characteristic is essential due to its multiple applications [24]. Figure 1 shows the pasting properties of the evaluated starches. Native cassava starch had the lower values for PV and BD, whereas SB and PT tended to be higher when compared with those reported by Asaoka et al. [25]. The increase in PT in enzymatically hydrolyzed cassava starches may be related to the amylose content decrease due to the reduction of the granule amorphous region [10]. Pregelatinized starch presented a significant decrease in PT, which implies an earlier swelling state of the granules during heating [26]. Whereas the maximum viscosity of that starch was one of the lowest in this study, it indicates that the granules have swelling and hydration capacity in water under heating and represents the equilibrium point between swelling and breakage. In pregelatinization, the higher the degree of gelatinization of the starch, the lower the presence of residual granular starch and therefore the lower the degree of swelling, which leads to a lower PV. These observations coincide with those reported by Liu et al. [27] for pregelatinized starch by extrusion and by Rodrigues dos Santos et al. [24], for starch treated by spray drying. In starches obtained by hydrolysis with α-amylase, this parameter showed an increase with respect to native starch, thus suggesting that—in addition to amylose molecules—some amylopectin molecules were degraded during hydrolysis [28]. Salcedo-Mendoza et al. [29] observed an increase in BD in starches modified by enzymatic hydrolysis with α-amylase, indicating a greater instability of the paste. Moreover, the SB tended to be higher and coincided with those observed by those authors, who pointed out the susceptibility to retrogradation or loss of water (syneresis) in starches modified enzymatically with α-amylase. Higher PV and FV were observed in corn starches modified with amyloglucosidase with respect to those modified by α-amylase [30]. They also observed a reduction in viscosity caused by α-amylase that did not change after heating and cooling, which coincides with what was observed in this study.

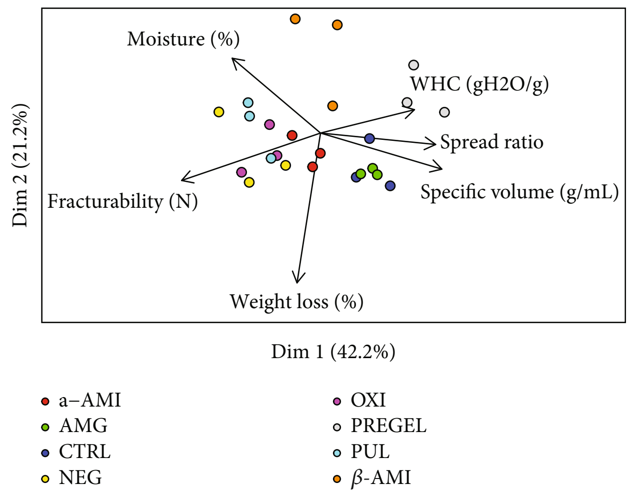

Figure 2: Principle component analysis (PCA) describing the interrelationship between the type of starch in the cracker's physical properties: oxidation treatment (OXI), pregelatinization treatment (PREGEL), enzymatic alpha amylase treatment (α-AMI), beta amylase treatment (β-AMI), amyloglucosidase treatment (AMG), pullulanase treatment (PUL), treatment without fat reduction and without addition of starch (CTRL), and treatment with fat reduction without addition of starch (NEG).

3.3. Cracker Characterization. Hadnađev et al. [31] define crackers as baked small products, usually flat, with a moisture content of less than 5%—ranging from 3.05% to 4.47% (w/w) and aw values between 0.12 and 0.24 [3], which agrees with the results presented in Table 1. The replacement of fat by carbohydrate-based mimetics tends to decrease water loss during baking; thus, it is associated with lower weight loss and higher moisture and water activity (aw) in the product [3, 13, 32]. Treatments including starch (except for α-AMI and AMG) tended to decrease or maintain weight loss; however, the differences in moisture content were not significant ($p > 0.05$) between these treatments and the CTRL. The water holding capacity (WHC) of the final product showed a higher value in treatments including starch—the PREGEL and AMG treatments stand out. This may be due to the structure of these molecules, which have a greater contact area that facilitate hydration [15].

Texture properties, in addition to taste and appearance, are one of the most important quality parameters in crackers [31]. While mixing the dough, fat acts as a lubricant and competes with the aqueous phase, thus avoiding the formation of the gluten network. Therefore, the fat reduction phase results in the hydration of the flour and the formation of a stronger gluten network during the mixing process, thus obtaining viscoelastic doughs [33]. In the fracturability measurements (Table 1), the enzymatic treatments (except for PUL) and the PREGEL treatment showed no significant differences ($p > 0.05$) with respect to the CTRL treatment. Sudha et al. [34] evaluated the inclusion of gels of two carbohydrate derivatives as fat replacements in crackers, reducing the fat content in proportions of 50%, 60%, and 70%; they found an increase in the fracture force applied when the fat reduction was higher.

Table 2: Color characteristics of cracker formulations including modified cassava starch as a fat substitute[†].

Treatment	Color L^*	a^*	b^*	c^*	h	ΔE
CTRL	80.18 ± 0.76^{a}	1.19 ± 0.12^{bc}	21.46 ± 1.64^{b}	21.49 ± 1.64^{b}	86.82 ± 0.35^{bcd}	
NEG	80.81 ± 1.22^{a}	0.61 ± 0.07^{d}	17.9 ± 0.69^{d}	17.91 ± 0.69^{d}	88.06 ± 0.2^{a}	3.66
OXI	81.74 ± 0.92^{a}	1.18 ± 0.19^{bc}	19.7 ± 1.19^{bcd}	19.73 ± 1.18^{bcd}	86.56 ± 0.72^{cd}	2.35
PREGEL	79.63 ± 1.27^{a}	0.72 ± 0.23^{d}	20.33 ± 0.63^{bc}	20.34 ± 0.64^{bc}	87.98 ± 0.60^{ab}	1.35
PUL	79.4 ± 1.14^{a}	1.19 ± 0.19^{bc}	22.02 ± 0.82^{b}	22.05 ± 0.83^{b}	86.91 ± 0.38^{abc}	0.97
β-AMI	80.52 ± 0.38^{a}	0.98 ± 0.14^{cd}	18.86 ± 0.21^{cd}	18.89 ± 0.21^{cd}	87.02 ± 0.39^{abc}	2.63
α-AMI	79.79 ± 1.56^{a}	2.05 ± 0.16^{a}	24.54 ± 0.36^{a}	24.63 ± 0.36^{a}	85.23 ± 0.41^{e}	3.22
AMG	79.91 ± 0.53^{a}	1.52 ± 0.04^{b}	20.17 ± 0.09^{bcd}	20.23 ± 0.09^{bcd}	85.68 ± 0.11^{de}	1.36

[†]Color difference (ΔE). Treatment without fat reduction, treatment without addition of starch (CTRL), treatment with fat reduction without addition of starch (NEG), oxidation treatment (OXI), pregelatinization treatment (PREGEL), enzymatic alpha amylase treatment (α-AMI), beta amylase treatment (β-AMI), amyloglucosidase treatment (AMG), and pullulanase treatment (PUL).

Table 3: Cracker acceptability test[†].

Treatment	Color	Texture	Acceptability
CTRL	6.73 ± 1.81^{a}	7.19 ± 2.25^{a}	6.95 ± 1.72^{a}
OXI	6.13 ± 1.94^{b}	6.35 ± 2.63^{b}	6.37 ± 1.65^{b}
PREGEL	5.79 ± 1.75^{b}	7.17 ± 2.26^{ab}	6.11 ± 1.91^{b}
AMG	6.45 ± 2.08^{ab}	7.22 ± 2.05^{a}	6.67 ± 1.69^{ab}

[†]Treatment without fat reduction and without addition of starch (CTRL), oxidation treatment (OXI), pregelatinization treatment (PREGEL), and enzymatic amyloglucosidase treatment (AMG).

However, with the inclusion of the substitute and when adding an emulsifier, a considerable reduction in the fracture force was observed, obtaining softer crackers but not yet at the level of treatment without fat reduction. Similar results were reported by Zoulias et al. [6] using carbohydrate or protein-based fat mimetics to replace up to 50% of the fat in crackers. According to the authors, the baking process can be divided into two stages, identified as "heating" and "crust and crumb development," where the greatest changes occur since the dough forms an open porous structure and a dry surface layer, which significantly alter the color and texture [35]. During this process, the crackers propagate in all directions until they stop by their own weight, which allows them to expand. It is apparently due to the structure property of gluten in the flour, suitable to form a two-dimensional folding film instead of a three-dimensional elastic network, as in the case of bread [13].

The results of the width, height, and expansion ratio of the crackers are given in Table 1. The values decreased with fat reduction as reported by Min et al. [36] when they incorporated water-soluble pectin-enriched material (PEM) as a fat substitute into cracker formulations, and it affected the dimensions of the crackers after baking. While the treatment without fat reduction had the largest diameter and lowest height (i.e., the largest extension), the crackers in which the fat was replaced with PEM presented a reduced diameter and increased height. Other authors observed this behavior when using carbohydrate derivatives as fat substitutes [13, 34, 37]. A higher value of specific volume is associated with better development of the protein-starch network, which gives the product a greater capacity to retain the released gas and, therefore, increases its volume. In this study, the treatments with the highest specific volume were CTRL, AMG, and PREGEL.

Figure 2 shows the PCA of the physical properties evaluated in the crackers, with a representation quality of 63.4%. It showed a positive correlation between the expansion ratio and the specific volume, which was also observed by Falsafi et al. [38] when evaluating the application of resistant starch in cookies; less expansion was also reflected in less volume. Moreover, this parameter was negatively correlated with the cracker's fracturability, with a higher expansion ratio resulting in lower fracturability as reported by Mudgil et al. [39]. Similarly, moisture and weight loss in baking showed an indirect relationship; weight loss during baking is due to water evaporation as reported before [37, 40].

There are also browning reactions between amino acids and carbohydrates during the baking process (Maillard reaction). Caramelization of sugars, reactions between proteins and lipid oxidation products, and complex pigment reactions may occur [35]. Regarding the color parameters (Table 2), no significant differences ($p > 0.05$) were observed in the luminosity values (L^*). Lee and Puligundla [41] found that the partial substitution of fat by native and modified (acetylated and hydroxypropylated) starches of rice in the production of muffins was slightly increased L^* , although not significantly. Meanwhile, the CTRL treatment and those modified by oxidation and enzymatic hydrolysis (except β-AMI) presented higher values for a^* and b^*, that is, a greater tendency towards yellow and red colors, as reflected by the tone (h) with values close to 90 indicating yellow tones. In addition, these treatments had the highest saturation values (c *). Colla and Gamlath [42] explain that fats and oils increase the internal temperature of baked goods, which can accelerate the rate of browning reactions, so that higher fat contents tend to present higher browning and higher values of the aforementioned parameters. When all treatments were compared with the CTRL without fat reduction through the total color difference (ΔE), it was observed that the PUL, AMG, and PREGEL treatments were more similar, with values indicating color differences (ΔE) between the samples not easily discernible by the human eye [18].

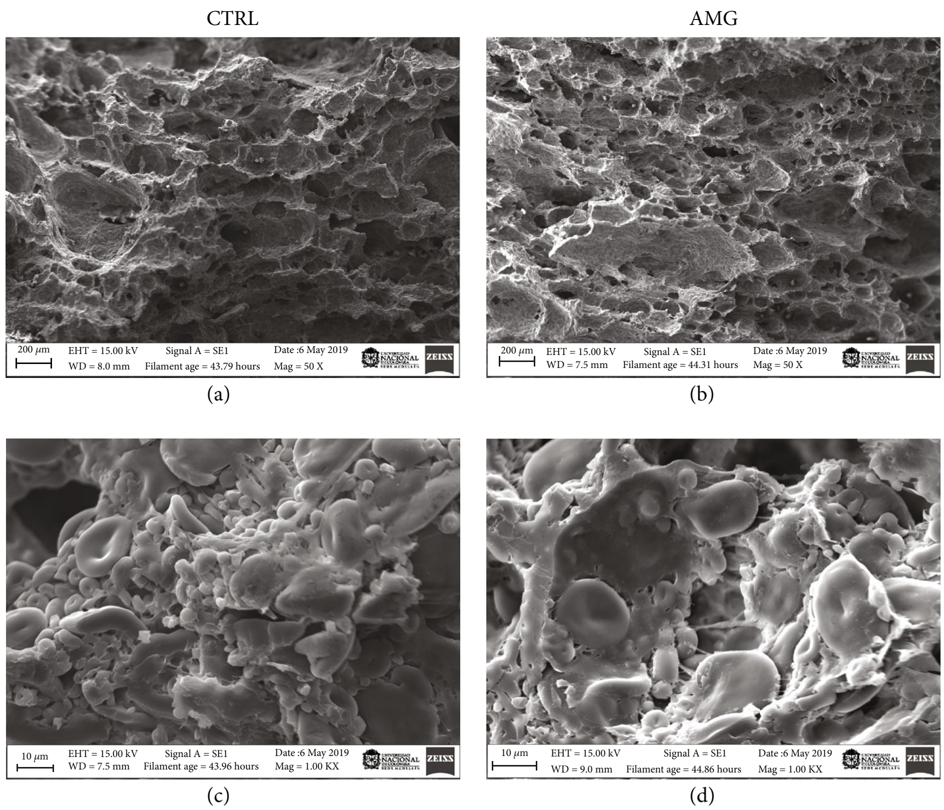

Figure 3: Scanning electron microscopy of cracker cross-section: control (CTRL) with 50x (a) and 1 Kx (c) magnifications and with amyloglucosidase (AMG) modified cassava starch with 50x (b) and 1 Kx (d) magnifications.

The sensory analysis test was performed for each type of modification: chemical treatment (oxidation (OXI)), physical treatment (pregelatinization (PREGEL)), the treatment without fat reduction and without addition of starch (CTRL), and the amyloglucosidase treatment (AMG) was selected according to the behaviors observed in physical analyses in relation to the treatment without fat reduction (CTRL) based on parameters such as color, texture, specific volume, and the expansion ratio. The acceptability test results (Table 3) showed a greater inclination towards the CTRL and AMG treatments, both in the color and texture parameters, which was also observed in the general acceptability assessment of the products. The PREGEL treatment was also rated with a crispy texture. Among the observations made by the panelists, the OXI treatment showed a tendency to be rated as the hardest when biting the cracker. However, although these results coincide with the behavior observed by instrumental analyses, the distribution of the response (standard deviations) suggested that crackers with fat reduction expressed a wider range of scores than the control. This could be related to the different sensory preferences of the evaluators regarding this type of product [31]. In addition, the content of protein and ash in the different treatments was not affected by fat substitution, with average values of $11.05 \pm 0.06\%$ and $3.34 \pm 0.04\%$, respectively. By contrast, there was an average reduction of total fat of $49.51 \pm 3.32\%$ in the final product.

3.4. Storage Behavior and Morphological Characterization. According to the results obtained for specific volume, water holding capacity, expansion ratio, texture, and color properties, the AMG enzymatic treatment was selected to evaluate its stability in storage with respect to the CTRL treatment without fat reduction. Figure 3 presents SEM micrographs of the internal cross-section of CTRL crackers without fat reduction and AMG with fat reduction and inclusion of modified cassava starch. The treatments showed a porous and cracked structure as a result of the expansion of gas bubbles during baking. However, the AMG treatment (Figure 3(b)) presented a rougher and more porous structure than the CTRL treatment (Figure 3(a)) with deeper and larger pores. Nandeesh et al. [43] explained this open structure as a product of the expansion of gas bubbles because of the temperature increase, also increasing the steam pressure inside them and the tensile stress in the membrane and leading to the rupture and formation of holes and tunnels through which the gas finds its way out. These results coincide with those reported by Ahmed and Abozed [44], who evaluated the incorporation of a residual Jamaican flower fiber (*Hibiscus sabdariffa*) into the microstructural properties of crackers with reduced fat content and observed a rougher and cracked surface when applying the fiber. Figures 3(c) and 3(d) show an interconnected starch and protein matrix that forms the structure; fat melts during baking and covers the surface, thus giving it a softer appearance [45]. Figure 3(d) shows a more compact structure in

Table 4: Cracker fracturability and moisture behavior during 30 days of storage†.

Treatment	Time (days)	Fracturability (N)	Moisture content (%)
AMG	0	13.66 ± 0.32[a]	4.09 ± 0.85[a]
	10	11.34 ± 0.75[bc]	4.18 ± 0.38[a]
	15	12.31 ± 0.8[ab]	4.44 ± 0.58[a]
	20	11.37 ± 0.64[bc]	4.75 ± 0.53[a]
	30	11.39 ± 0.57[c]	4.72 ± 0.13[a]
CTRL	0	12.5 ± 0.42[ab]	3.87 ± 0.14[a]
	10	11.4 ± 0.66[bc]	3.42 ± 0.07[a]
	15	11.8 ± 0.63[bc]	3.79 ± 0.42[a]
	20	11.72 ± 0.22[bc]	4.22 ± 0.96[a]
	30	10.77 ± 0.61[c]	4.31 ± 0.43[a]

†Treatment without fat reduction and without addition of starch (CTRL), and enzymatic amyloglucosidase treatment (AMG).

which the starch granules are more immersed in the matrix, and fibrous patterns interconnect the granules with the rest of the structure, while the CTRL treatment in Figure 3(c) shows more intact granules. According to Nandeesh et al. [43], the protein component of the dough has been described as a network covering the starch granules that can control the structure of the product through its interaction with the protein matrix during heating.

Table 4 shows the treatment's values of fracturability force and moisture content during storage. The CTRL treatment showed lower fracture strength compared with AMG treatment over time. There was also a slight decrease in fracture force for each treatment between zero and thirty days of storage. Similarly, the moisture content of the crackers exhibits a tendency to increase in the two treatments due to the associated effect of mass transfer to achieve hygroscopic balance. These results agree with those reported by Chugh et al. [32] when evaluating the effect of storage time on crackers made with carbohydrate derivatives (combination of polydextrose and guar gum) as fat replacers.

4. Conclusions

According to the results obtained in this study, the application of modified cassava starch as a fat substitute in salt crackers caused a decrease in weight loss during baking and increased the aw, water holding capacity (WHC), and fracture strength. Among the modified starches used in the formulation of the crackers, the treatment with the enzymatic starch AMG and the physical one (PREGEL) produced a product with a texture, specific volume, weight loss, WHC, and sensory characteristics closer to that without fat reduction (CTRL), and a total fat reduction in the final product of 49.51% was achieved. The storage of crackers affected their textural properties, showing a slight decrease in the fracture force and an increase in the hygroscopic behavior of the product. From the sustainability and food security point of view, it is also considered a good achievement once physical and enzymatically methods are considered clean label processes. This study indicates that enzymatic and physically modified starch can be favorable as a fat replacer for the production of different bakery products. The application of starches obtained through sequential physical and enzymatic modifications as fat replacers in this type of product could be evaluated as a future approach.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Additional Points

Novelty Impact Statement. Research on strategies to replace saturated and trans fats in food products without negatively affecting sensory properties has been an increasingly pressing concern for the food industry. Among the alternatives studied, carbohydrate-based fat replacers, such as modified starches, have been postulated to mimic fat. Therefore, in the present study, it was observed that fat reduction and application of modified starch influenced the quality properties of crackers such as texture, specific volume, weight loss, water holding capacity, and morphological properties. A total fat reduction in the final product of 49.51% was achieved.

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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