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Effect of Thermal treatment and acid modification for corn starch extracted from some Iraqi cultivated corn genotypes on their resistance to α- amylase activity and some physical properties

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ABSTRACT

The influence of temperature and citric acid treatments on increasing the resistance of starch's extracted from ten genotypes of corn by treatment with alpha-amylase activity was studied. The study also included the effect of these two treatments on increasing the percentage of amylose, in addition to some physiochemical behavior such as; water binding capability, swelling capacity, and solubility. The research findings revealed significant differences among the studied genotypes in response to the two treatments. In most cases, both treatments resulted in an increased in amylose content, typically within the range of 0-10%, with the acid treatment showing a more pronounced effect compared to the other treatment. The percentage of resistant starch varied significantly across the genotypes, ranging from 14.079% to 26.40%. Furthermore, the thermal and acid treatments had noticeable impacts on the physical characteristics of the starch samples. Overall, solubility and water binding values increased, while swelling values decreased due to these treatments.

Keywords: Cornstarch, Amylose Content, Enzymatic Hydrolysis, Physicochemical Properties, A-Amylase Activity.

1. Introduction

Corn holds a significant position as the third high production of cereal crop worldwide, serving as a considerable source of vitamins, minerals and energy. It dominates the starch market, accounting for over 80% of the global share[19]. The diverse range of corn genotypes has been noted to impact on various starch properties, as reported by multiple researchers[3]. This genetic diversity results in starches with distinct chemical and functional characteristics[33]. In the past two decades, extensive research has focused on studying thermal and chemical modifications of starch to achieve new properties and functionalities. One of the areas of interest has been converted some the starch to a material that can resist the action of α- amylase, aiming to reduce energy intake for individuals following diets to combat obesity. The research findings indicate that heat treatment promotes the formation of amylose crystals through retrogradation, rendering them resistant to amylase enzymes[26]. This resistance may have potential implications in the developing of new starch-based products with improved functionalities. Also, the use of treatment with organic acids may cause the breakage of some branches of amylopectin, which increases the straight unbranched chains, which helps to form crystals containing α- helix chains that are resistant to amylase enzymes[40]. Although these mechanisms have a mutual effect, the response of starch types is different depending on their crystalline structure, which is the aim of this current work.

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2. Material and Methods

2.1 Material

It was collected the following ten mazes (Zea mays) to isolate and study their starch which were; "Sara, Al-Maha, Fajar -1-, Bagdad -3-, ZP.434*A, ZP.434*B, MSI*B, Dhqan, Corpeto, Dracma)" and synthetic variety "Sara, Al-Maha, Fajar -1- and Bagdad -3". These genotypes were purchased from the Iraqi Ministry of Agriculture/ General Commission for Agricultural Research /Field Crops Research department, while Ministry of Agriculture in Kurdistan Region-Iraq supplied the following genotypes; (ZP.434*A, ZP.434*B and MSI*B). Also, the Iranian Dhqan type of maze obtained from Sianandaj, Agricultural college, the University of Kurdistan. Syngenta company Italia S.p.A company provided Corpeto genotype and Dracma genotype.

However, to produce seeds and in the year 2019, it was cultivated six genotypes "ZP.434*A, ZP.434*B, MSI*B, Dhqan, Corpeto and Dracma" in Qlyasan research station located in the Agriculture Engineering Science College / Sulaimani University.

2.2 Methods

2.2.1 Starch isolation

One kilogram of corn kernels was milled using laboratory mill to obtain on fine flour after sieved with sieve (100 μ). The produced flour was mixed with adequate water (about 50ml distil water for each 100g flour) permitting for protein to make network as a suitable way to remove it during purification starch. The produced ball dough was put in cheese cloth then it was immersed in adequate amount of water to release the starch granules from protein matrix, this process continues for about 1hour with change the immersed water several time to ensure that most of starch left the ball to the solution. The protein mass inner the cloth was diluted again with 1 liter of distilled water, and the resulting slurry was filtrated twice times with another clean cheesecloth. This step was repeated until no starch remained in the residue. Next, the colloidal solution of isolated starch was filtered to remove the course materials and fiber using a 75 μm mesh sieve. The slurry was then subjected to centrifugation at 4000 rpm for 20 minutes. The grey colored upper layer that rich in proteins, was carefully removed using a spatula. The sample was re-suspended with additional water, and another round of centrifugation for 15 minutes was performed. The previous steps were repeated several times until the purified starch becomes bright white, indicating the removal of all or most impurities. To further purify the starch, a proteinase-k solution (40 U/mg) was added to the starch suspension (final volume of starch solution 2 liters contained 20 mg of enzyme) utilized to remove as much protein as possible. The soluble protein was discard using the steps of centrifuge and washing as above. The purified starch was dried for 24 hours at 40 ˚C[16, 29].

2.2.2 Chemical composition

For each starch sample, moisture percentage was determined according to[1], while protein, lipid, and ash were determined according to[6]. The difference between the sum of all components percentage (100%) and the total of moisture, protein, lipids and ash percentage is total carbohydrate percentage[25].

2.2.3 Amylose content

The dried starch for each sample under research was used to determination their content of amylose according to[37]. Amylopectin also was determined by difference using the following equation;

Amylose content (%) = (85.24 x A) - 13.19
Amylopectin (%) = 100 - % Amylose
While A is a spectrophotometer's absorbance

2.2.4 Acid modification of natural and gelatinized starch

According to official method[20] , purified starch (15g) was suspended in forty milliliter of 0.1 M citric acid then it was heated to 120°C for 45 minutes. After that the slurry was cooled to room temperature using cold water. according to All samples were incubated at 35°C for 24 hours, after the end of this period, 30 ml of distilled water was added and mixed to reach to uniform matrix which was neutralized with 1M NaOH. The mixture was centrifuged at 4000 rpm for 15 minutes. The separated water was discarded but the precipitate was washed. This process was repeated several times to remove all soluble materials. To obtain on modified starch contains less than 12% moisture, it was dried at 40 ˚C. then it was milled and sieved through 100 mesh screens. The same procedure was repeated with gelatinized starch (prepared by drying the starch paste which obtained from the amylograph after the test finished).

2.2.5 Swelling power and solubility

This test carries out depending on the method of[34], which based on the ability of starch samples to swell and soluble The values of these parameters were calculated using the following equations:

$$\text{Swelling power } \left(\frac{g}{g}\right) = \frac{\text{weight of wet sediment}}{\text{Weight of sample} - \text{weight of dried supernatant}}$$

$$\text{Solubility } (\%) = \frac{\text{weight of dried supernatant}}{\text{weight of wet sediment}} \times 100$$

2.2.6 Water Binding Capacity (WBC)

To determine the ability of tested starches to capture the molecules of water, it was used the method described by[39], with modifications by[24]. The process involved make a suspension by suspending 5g of samples (dry weight) in 75 ml of distilled water for 1 hour. After agitation, the suspension was subjected to centrifuged at 4000 ×g for 20 minutes to discard the collected water over the starch. The obtained of wet starch was allowed to drain for 10 minutes which subsequently was weighed to calculate its water-binding capacity using the following equation.

$$\text{Water banding capacity } \% = \frac{\text{Weight of residue} - \text{Weight of sample}}{\text{Weight of sample}} \times 100$$

2.2.7 Determination the percentage of starch hydrolysis by alpha-amylase

The researchers employed the colorimetric method described by[15] with slight modifications to evaluate the reduced sugar content. For this analysis, the 3,5-Dinitrosalycylic acid as the color reagent was used. For this test, approximately 0.5 grams of starch was mixed with 15 ml of deionized water. Then, 35 ml of a buffer solution, prepared in advance and consisting of sodium phosphate and sodium chloride, was added to the mixture. This resulted in a prepared sample that was ready for the evaluation of reducing sugar content using the colorimetric method. First, prepare starch suspension with starch and water, then boil the buffer solution in the water bath and, add the starch solution to the buffer and let the solution for cool. After that, 0.5 ml of solution was taken and 1 ml of α-amylase (microbial α-amylase source, 0.5 mg/ml) was added, then left for 30 min at 37 ˚C; after this time, 1 ml of reagent was added (3, 5-Dinitrosalycylic acid)

then put into a water bath at boiling degree for 15 min then left to cooling and 9 ml of deionized water was added, the absorbance was measured at 540 nm using UV-light spectrophotometer against blank solution (without starch), the reducing sugar produced was calculated from a standard curve prepared by a different concentration of glucose, and following the equations;

Equation of regression of Glucose concentration:

Y (mg glucose/ ml solution) = 1.534 × absorbance + 0.04815

According to Megazyme of[23], and the percentage of analyzable starch was calculated with some modification.

Amylolytic analyzable starch= (concentration of glucose mg/ml * 0.9* 70)/ [weight of starch(mg)] ×100

whereas;

- 0.9 to convert factor for free glucose to starch
- 70 is (dilution factor)

Statistical Analysis

The obtained data underwent one-way analysis of variance (ANOVA) with a significance level of $P < 0.05$. The statistical analysis was performed, and the results were further separated using the multiple-range Duncan's test. XLSTAT, version (2016.02.28451), a statistical software, was utilized to analysis of the data. The reported results include mean values and standard deviations for the different experimental groups or treatments.

3. Results and Discussion

3.1 Chemical composition

The proximate analysis of cornstarch extracted from various genotypes (Table 1) revealed varying percentages of moisture, protein, oil, ash and carbohydrates. Moisture content is considered an essential factor as it provides insights into the drying process, resistance to microbial and enzyme action during storage, and physiochemical properties. The moisture percentage ranged from 9.253% to 12.245%. The relatively low moisture content is advantageous as it reduces the risk of microbial growth during starch storage, as mentioned by[4]. The content of cornstarch of protein varied between 0.770% and 1.540%, and statistical analysis demonstrated a significant difference among the five samples in their protein content. For acceptable study purposes, purified starches typically contain low levels of protein and oil, as indicated by[32]. The oil content in the ten cornstarch genotypes ranged from 0.309% to 0.664%, consistent with common starches. The ash content, ranging from 0.122% to 0.214%, aligns with values reported in previous studies[2, 5]. Consequently, these hybrid corn-derived starches can be classified and used as regular corn starches, as discussed in references[7, 17]. Differences in the chemical compositions of cornstarch may be attributed to the difference of corn genotypes of their content of chemical components in addition to the hardness or the strength of chemical materials to bind with starch granules which could influence their responses to extraction treatments

Table 1: Proximate analysis of cornstarch extracted from studied corn genotypes.

Genotypes	Moisture content %	Protein content %	Oil content %	Ash content %	Carbohydrate content %
Sara	9.812±0.001^{h}	1.433±0.058^{b}	0.453±0.001^{h}	0.126±0.001^{h}	88.176±0.055^{c}
Al Maha	9.253±0.001^{j}	1.210±0.010^{d}	0.491±0.001^{f}	0.194±0.001^{b}	88.852±0.009^{a}
Fajr-1	10.212±0.001^{d}	1.433±0.015^{b}	0.461±0.001^{g}	0.146±0.001^{e}	87.748±0.013^{f}
Bagdad-3	10.283±0.001^{c}	1.250±0.010^{c}	0.592±0.002^{c}	0.141±0.001^{f}	87.734±0.006^{f}
ZP.434*A	12.245±0.001^{a}	1.150±0.010^{e}	0.432±0.001^{i}	0.122±0.001^{i}	86.051±0.010^{h}
ZP.434*B	10.152±0.001^{e}	1.540±0.010^{a}	0.612±0.002^{b}	0.214±0.001^{a}	87.482±0.009^{g}
MSI*B	9.644±0.001^{i}	1.433±0.015^{b}	0.664±0.001^{a}	0.136±0.001^{g}	88.123±0.014^{d}
Dhqan	9.953±0.001^{g}	1.150±0.010^{e}	0.584±0.002^{d}	0.172±0.001^{d}	88.141±0.006^{d}
Corpeto	10.563±0.001^{b}	1.143±0.015^{e}	0.309±0.002^{j}	0.176±0.001^{c}	87.809±0.015^{e}
Dracma	10.143±0.001^{f}	0.770±0.010^{f}	0.507±0.002^{e}	0.136±0.001^{g}	88.444±0.011^{b}

3.2 Amylose and Amylopectin content

A significant difference in amylopectin and amylose percentages was found among cornstarch extracted from these genotypes under study (Table 2). The amylose percentage ranged between 19.935% for Sara and 31.095% for ZP.434*B, appearing the wide range of differences between the genotypes in this parameter[38] studied several genotypes of cornstarch to reach the final results and claimed that the range of amylose % was between 18-35% for typical starch. Because starch composites of amylose and amylopectin therefore these two components determine all physical, chemical and biological properties of starch depended on the ratio, molecular weight, crystal characteristics of these two components. However, resistance starch formation powerful depends on the ratio of unbranched chain of starch such as amylose.

Table 2: Amylose and Amylopectin content of cornstarch extracted from studied corn genotypes.

Genotypes	Sara	Al maha	Fajr-1	Bagdab-3	ZP.434*A	ZP.434*B	MSI*B	Dhqan	corpeto	dracma
Amylose %	19.935 ±0.010^{j}	24.113 ±0.012^{g}	20.140 ±0.002^{i}	20.271 ±0.005^{h}	26.669 ±0.005^{e}	31.095 ±0.003^{a}	29.531 ±0.025^{d}	30.192 ±0.002^{b}	29.944 ±0.003^{c}	26.319 ±0.002^{f}
Amylopectin %	80.07 ±0.004^{a}	75.897 ±0.001^{d}	79.864 ±0.002^{b}	79.735 ±0.001^{c}	73.344 ±0.004^{g}	68.910 ±0.002^{j}	70.486 ±0.001^{h}	73.811 ±0.002^{e}	70.061 ±0.002^{i}	73.681 ±0.001^{g}

3.3 Modifying starch by citric acid

3.3.1 Amylose content

Increasing of amylose percentage in the starch assists to formation a lot of resistant starch because unbranched chain of starch has a high ability to retrograde and form network or gel which is prime material for resistant starch formation. Citric acid is one of the best organic acid that can breakdown the branch chain (α-1,6 glycosidic bond) from amylopectin increasing amylose[8]. Significant increasing in amylose percentage was occurred in all samples of treated corn genotypes' starches (Figure 1). Sara genotype showed a considerable response toward this treatment. Amylose percentage increased in this type of starch from 19.935 to 31.22 and 30.069 of acid modification for both starch and gelatinized starch, respectively. Formation of a lot of amylose not enough to claim this genotype will produce a high amount of resistant starch. The result showed that there is other factor may be the nature of amylose crystal which is formed by cooling the starch slurry after cooking. It was noticed that the natural starch of these genotypes of corn had a high resistance to amylase activity. However, after acid treatment, it lost its resistance. Rebuilding a new network through inter- intra molecular links in starch matrix, especially in unbranched chains of amylose, is ruled to the presence of a considerable excess amount of amylose. Citric or other organic acids may assist in increasing unbranched chains without severely breaking down these chains to increase the long chain of amylose. Indeed, this behavior will be emphasized by increasing starch – iodine binding[31, 10]. Debranching of any starch branched chains, like amylopectin, also can be explained for amylose increasing. Spending a long time treating the starch with acid is considered a primary factor in increasing the unbranched chains (amylose)[11].

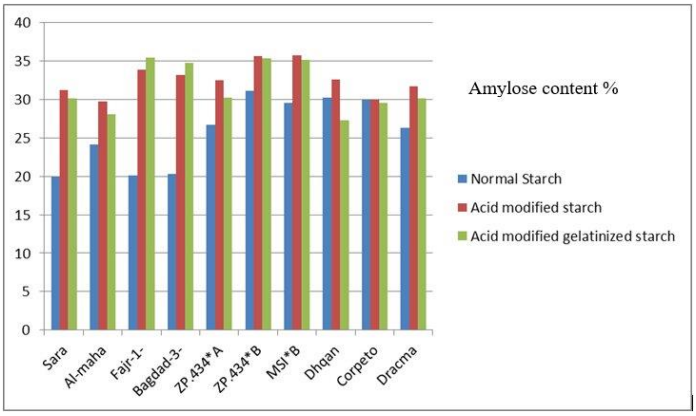

Figure 1: Effect of Citric acid treatment and acid modified gelatinized starch on Amylose content of studied cornstarch

3.3.2 Swelling Power g/g (SP)

The SP values shown in (Figure 2) exhibited significant variations among the different corn starch genotypes. Present starch granules in an adequate amount of water with thermal treatment and stirring or centrifuging for some time must let the granule take much of water, which will swell it. This phenomenon will permit the building of a new structure called gel, which will have new properties such as strength, resistance to α-amylase activity tension, or other physical characteristics depending on the ability of starch granules to absorb water and bind it with hydroxyl groups of starch chains[30]. In this study, except of two genotypes, the reduction of the SP value for acid-modified starches was observed compared with native uncooked starches which ranged from 15.232 to 11.906 g/g. The genotype that had a high swelling power was Dracma (15.232 g/g), while the lower value was observed in Bagdad-3 (11.906 g/g). Aqueous hydrochloric solution may alter the SP properties of wheat starch by decreasing its value from 15.4 to 5.0 g/g during a period of time[21]. Severely break down of starch chains by acid, which must role is only to remove most the amylopectin branched chains, was the primary factor in decreasing the SP value. Starch that had high amount of soluble dextrin or small and medium chains of carbohydrates will decrease the amount of bonded water, which is necessary for high SP value, while, the excess of free water may be predominant[18]. The chemical structure of starch especially, the amylopectin ratio has an impacted role on the SP values of starches from different sources. However, any factor, including citric, that can change the starch components structure, especially amylopectin indeed will strongly influence the SP value[38,29].

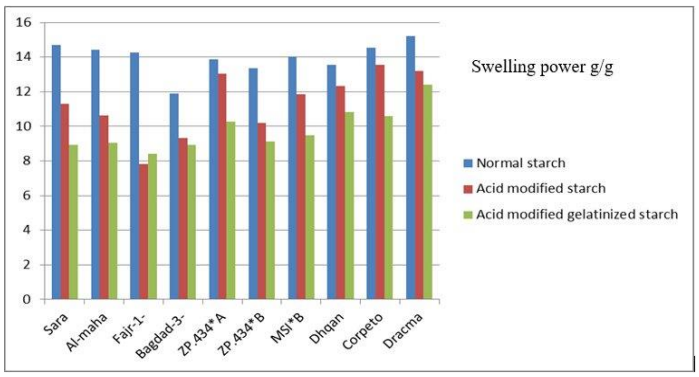

Figure 2: Effect of Citric acid treatment and acid modified gelatinized starch on swelling power of studied cornstarch genotypes.

3.3.3 Solubility %

Figure (3) shows the significant differences among the tested starches. Native starch had a low value of solubility index compared with acid modified starch ranged from 14.497 for Bagdad-3 to 6.274 % for Al Maha. Although, the Al Maha value is low, but its ratio is higher than its counterpart in native starch. Higher solubility properties have been noticed in starch modified by lactic or citric acid compering with natural untreated starch[33]. One of the considerable factors that assist starch granules swell and

soluble to form a gel is the presence of channels within it, which permit to water and other solutions to penetrate through the granules[14]. Also, the solubility of starch may increase in the autoclaved starch due to dextrin formation and debrancher chains of starch[28]. However, an increase in small molecular weight and debranched chains of starch, in addition to an increase of amylose, assists in increasing water absorption due to thermal treatment, especially in the autoclaving process[22, 27]. This process can change a lot of nature starch to resistant starch that is very preferable for high water-binding properties[12].

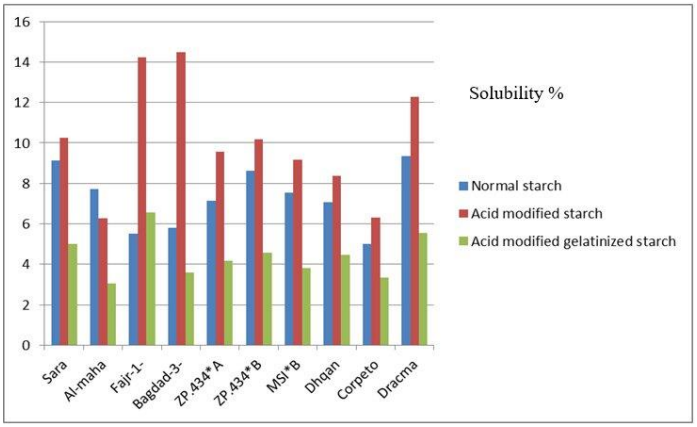

Figure 3: Effect of Citric acid treatment and acid modified gelatinized starch on solubility of studied cornstarch genotypes.

3.3.4 Water Binding Capacity % (WBC)

Capture of water in the starch gel is considered an acceptable characteristic, therefore the genotype that has a higher value of WBC may be more favorite than the lower value. Nature uncooked starch of Bagdad-3 showed a higher value 165.341 % compared with Dracma (109.896 %) which had the lower value as shown in figure (4). Treatment of samples of tested starch with acid increased the WBC for Dracma to be 243.400 % compared with MSI*B which was 141.114 %. Using gelatinized starch caused increasing of WBC for Sara and Al Maha which were 212.200 and 211.973 %, respectively, while it was reduced in ZP.434*B to 127.735 %. The findings presented in the study align with what[8, 36] found; they noticed that acid modification can potentially increase starch fractions, with small molecular weight. Water can be caught by hydroxyl units through hydrogen bonding, resulting in an increase in WHC. This characteristic led to the use of this type of starch which has high WHC as a result of acid modification in the fat replacer in the industries[9]. Therefore, it was suggested that citric acid treatment can be a promising approach to enhance the water-binding properties of starch, making it a viable option for reducing the need for fat in certain food products.

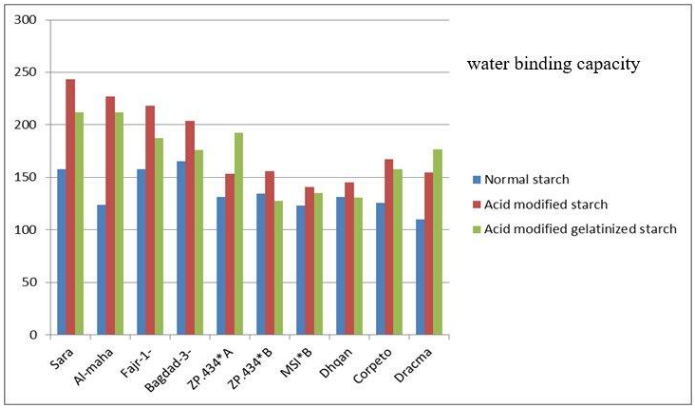

Figure 4: Effect of Citric acid treatment and acid modified gelatinized starch on water binding capacity of studied cornstarch genotypes.

3.3.5 Effect of Cornstarch Treatments by α- Amylase activity

-Ability of α- amylase to hydrolyze raw cornstarch;

In this an experiment, it was attempted to understand the responsibility of some extracted types of cornstarch of studied genotypes of corn. The importance of such experiment is to assist in selecting the more suitable cornstarch to prepare resistance starch, which is the more requested type of starch for its low energy release. The results of α- amylase activity on extracted untreated starch (Figure 5) showed significant differences among the samples in their ability to release reducing sugar due to the activity of α- amylase. The natural untreated starch of the sample appears as a highly resistant starch to α- amylase since only 14.079% of Sara of untreated starch was analyzed, in comparison, the Dhqan genotype was the most analyzable starch, which was 26.40% of untreated starch. Therefore, the resistance of studied untreated starch ranged from 14.079 to 26.40%. These results agreed with[13], who found that α- amylase can hydrolyze about 20% of raw cornstarch at optimal conditions. However,[35] found that the structure, especially the number of pores on starch granules, in addition to its content of amylose and the crystallinity of starch crystals can impact α- amylase activity.

-Ability of α- amylase to hydrolyze acid-modified corn starch;

The impact of citric acid treatment on α-amylase activity to hydrolyze acid-pretreated cornstarch is presented in (Figure 5). Citric acid treatment partially breaks down amylopectin branches, leading to the production of unbranched or low-branched chains, similar to amylose. This process enhances the starch's ability to undergo crystallization within the retrogradation mechanism. Crystallization facilitated by the unbranched chains increases the starch's resistance to α-amylase action, making it more difficult for α-amylase enzymes to break down the starch molecules. The experimental results indicated a significant difference

among the samples. Despite the Sara genotype initially having a higher ability to resist α-amylase activity compared to the other genotypes, this resistance decreased following the acid modification treatment. The acid treatment seems to have an impact on reducing the overall resistance of the starch to α-amylase activity, leading to increased susceptibility to enzymatic hydrolysis. The percentage of hydrolyzing raw starch was 14.079% to become 13.970%. In addition, Sara and MSI*B genotypes were not influenced by acid treatment, while, in general, this modification causes a significant decrease in the analysis of acid-modified starch by α amylase. However, this phenomenon must be applied to all types of cornstarch. The results of this experiment confirmed that some samples disobey the effect of citric acid modification, such as Corpeto and Dracma genotypes.

-Ability of α- amylase to hydrolyze thermally treated cornstarch;

The results of this experiment (Figure 5), clarified that although there was a clear difference among the cornstarch varieties in their α- amylase activity response. However, they did not have a high resistance against this activity. When the results were compared in column 1 (which was normal starch genotypes), with the results of column 3 (which was gelatinized starch), it was observed that the action of α- amylase was somewhat higher in both substrates than citric-modified starch. The response of some samples to α- amylase was higher than raw starch or acid-modified starch, while the others were more resistant to α- amylase activity. However, Corpeto genotype had the most resistance to α- amylase activity, which hydrolyzed only 19.98 % of gelatinized starch. Al Maha genotype had the least resistance toward α- amylase that hydrolyzed about 27.19% of starch. Other sample values were between these values.

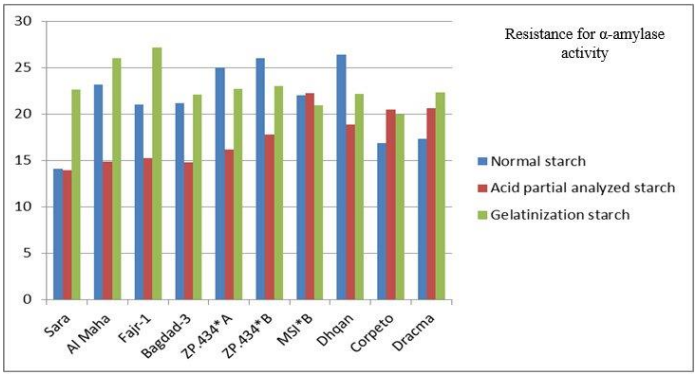

Figure 5: Resistance of normal starch, acid partial analyzed starch and gelatinization starch for α-amylase activity.

Conclusion

The application of citric acid and thermal treatments can lead to modifications in natural cornstarch, resulting in an increasing in amylose percentage. This phenomenon is attributed to the debranched chains of starch. Due to these treatments, various physical properties related to gel formation were influenced. Additionally, the resistance of cornstarch to the action of α-amylase increased, indicating improved stability under enzymatic conditions. However, it is crucial to note that not all types of cornstarch exhibited the same response to these treatments. The effects of citric acid and thermal treatments may vary depending on the specific characteristics of each cornstarch type, suggesting that factors like genotype, processing methods, and initial composition play a role in determining the outcomes of these modifications.

Conflict of interests

None

Author Contribution

The authors contributed equally to this work, from the implementation and design of the research, the analysis of the results and to the writing of the manuscript.

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