



Effect of Acetylation of Bean Starch Zaragoza (*Phaseolus lunatus*) Red Variety on its Functional Properties

**Marrugo-Ligardo Y.*¹, Blanco-Santander C.²,
Severiche-Sierra C.^{1,2}, Jaimes-Morales J¹**

¹University of Cartagena, Department of Environment, Food and Health, Campus Zaragocilla-Area of Health Sciences. Cartagena de indias, Colombia.

²University Foundation Technological Comfenalco, Department of Occupational Health and Safety, Engineering Campus. Cartagena de indias, Colombia.

Abstract: The effect of acetylation of Zaragoza bean starch (*Phaseolus lunatus*), a red variety on its functional properties was evaluated to determine its possible use as a food ingredient. It was obtained and characterized the bean starch Zaragoza red variety, in its native form, undergoing a process of chemical modification by the acetylation method. The functionality was tested by formulating with each, a pastry cream, evaluating the viscosity, syneresis and freeze-thaw resistance. In the results an 18% yield of native starch was obtained, low yield compared to other vegetables; The gelatinization temperature for the starches was in the range of 77-83 ° C; The water retention capacity and the swelling power were higher than 95 ° C, which could be due to the crystallization of the amylose, the reduction of the stirring speed or variations in temperature. Comparing the preparations in water and cream with 4% of starch base, it was higher in the pastry cream at 80 ° C with 1,946 Cps, being a new alternative in food that requires viscosity. It is concluded that the chemical modification of starches, generates changes of vital importance on their chemical, physical and functional properties, compared to their native forms. According to the functional evaluation of native and acetylated starches in a food, the viscosities tend to be higher, as the degree of acetylation increases; Showing that they can be an alternative to be used as an ingredient in the manufacture of creams.

Keywords: Acetylation, Chemical Modification, Food, Functionality, Starches.

Introduction

Starch is the main storage polysaccharide synthesized by higher plants, usually isolated from various sources such as cereals, legumes, tubers and fruits¹. Its functionality depends on the average molecular weight of amylose and amylopectin, as well as the molecular organization of these glucans within the granule². Due to its high molecular weight, starch has been used as: fat substitute, thickeners, texturizing, adhesive, binder, film former, foaming stabilizer, bread anti-aging agent, gelling agent, glazing, humectant and stabilizer, mainly in the Food, textile and paper industry³.

This polysaccharide constitutes an excellent raw material to modify the texture and consistency of food⁴. In addition to being used as an additive in food systems due to its nutritional and functional characteristics, and its properties as a viscosifying and stabilizing agent for suspensions and dispersions⁵.

Native starches have certain limitations for industrial use. Therefore, they are modified to improve their functional properties and have a wide range of industrial applications. The resulting products or modified starches are, consequently, higher value-added products⁶. They are called native starches because they have not undergone any process of chemical modification during their production⁷.

Starch granules are chemically, physically and biochemically treated to cause the breakdown of some or all of the molecules. Starch modification allows to enhance or inhibit properties such as consistency, binder power, stability, changes in pH and temperature and improve gelation, dispersion Or fluency⁸.

Within the modifications made to the native starches we find the acetylation. Acetylation is a chemical modification of the starch by esterification, in which its hydroxyl groups (OH) are substituted by acetyl groups (CH₃-C = O), which give it greater stability, varying its physicochemical and functional properties, even having a low GS ; The introduction of acetyl groups, provides starch stability and retrogradation resistance, increases its hydrophobicity depending on chain length and starch GS⁹. Acetylated starches have different characteristics to the native ones, present approximately 6 ° C less at the gelatinization temperature (Tg) and the peak of maximum viscosity is higher with respect to the native, which indicates that the acetylated starches are dispersed more easily than the native people. Acetylation also increases the clarity and stability of the gels that are formed with this starch and reduces retrogradation. Acetylated starches with low GS are commonly used in the food industry as it imparts consistency, texture and stability¹⁰.

Experimental

Extraction of starch

For this determination, the protocol, established by Marrugo and collaborators (2012)¹¹, was followed, which consists of the following: 100 grams of Phaseolus lunatus seed were used, which were soaked for 12 hours; The seeds were husked and ground in a Corona grain mill, the dough obtained was allowed to soak in a 1: 3 (v / v) ratio for 2 hours. In a fabric strainer of 80 the suspension was filtered to remove the fibrous solid which was washed with 200 ml of distilled water; The filtrate was allowed to settle for 4 hours. After this time the supernatant liquid was removed and a dark layer with a high protein content. The starch (resulting solid) was washed 3 times with 300 ml distilled water for each wash, centrifuging at 2500 rpm for 10 minutes in the last wash in order to recover the starch, a Jouan B3.11 centrifuge was used. The obtained starch was artificially dried in aluminum trays in a dryer for 8 hours at 45 °C¹².

Determination of pH

A suspension was prepared by weighing 10 g of starch in a 100 ml beaker and adding 50 ml of water, continuously stirred at a moderate rate of 5 min. The pH was determined potentiometrically with a previously calibrated Mettler Toledo AG SG2 peachment¹³.

Determination of moisture

The moisture content of the starches was determined by weight loss, for which approximately 2.5 grams of the material to be analyzed was weighed into a pre-weighed Petri dish, which was dried for a period of 12 hours at 50 ° C In a MEMMERT oven, type UL 50¹⁴. The results were expressed in% moisture by the following formula.

$$\% \text{ Humidity} = \frac{P_1 - P_2}{P_1} \times 100$$

P1: Weight in g of the box with the sample

P2: Weight in g of the sample after drying

Determination of ashes

By the incineration method, 2 g of sample was accurately weighed in an analytical balance in a pre-weighed porcelain dish, which was brought to a muffle at about 300 ° C, the sample was completely calcined by raising the temperature to 600 ° C for 6 hours. After this time, the muffle was extinguished and the temperature was expected to drop to about 120 ° C, then the crucible was removed with the residue (ash) and placed in a

desiccator. Finally, after at least 40 minutes the crucible reached room temperature, and the weight of the ash was determined on an analytical balance¹⁵. The results were expressed as% ash by the following formula.

$$\% \text{ Ashes} = \frac{P_1 - P_2}{P_m} \times 100$$

P1 = weight in grams of the crucible with ash.

P2 = Weight in grams of the empty crucible.

Pm = weight in grams of the sample

Determination of protein content

This test was performed by the Kjeldhal method, in which 0.5 g of starch were weighed and placed in a digester bottle, then 8 ml of concentrated sulfuric acid and then 0.20 g of catalyst (K₂SO₄ + CuSO₄ 1:1). The sample was placed in an extraction cabinet for digestion, this process lasted until the solution took a clear green apple color, was then allowed to cool, and 150 mL of water was added, 14 mL of sodium hydroxide 50% and distilled for 40 minutes. The distillate was collected in 6 mL of 4% boric acid which had a mixed indicator solution (methyl red-methylene blue). After the distillation was completed, the solution was titrated with 0.02 N sulfuric acid¹⁶. The results were expressed as% Nitrogen by the following formula:

$$\% \text{ Protein} = \frac{V \times N \times 1,4}{\text{g sample}}$$

V = Volume of Sulfuric Acid Spent in titration.

N = Acid Normality.

% Protein =% N x Factor.

Factor: Flour: 5.7

Determination of lipid content

100 g of starch were weighed into a filter paper cartridge and transferred to a soxhletkit, enough petroleum ether was added to extract the fats in a pre-weighed balloon. This process was carried out for one hour, then the solvent of the balloon was evaporated and weighed with the extracted fat content¹⁷. The results were expressed as% fat by the following formula:

$$\% \text{ Greases} = \frac{P_2 - P_1}{P_m} \times 100$$

P1 = Weight in grams of the empty collector ball that is part of the extractor equipment.

P2 = Weight in grams of the ball containing the extracted fat.

Pm = Weight in grams of the sample.

Determination of crude fiber content

2 g of starch was weighed into a beaker and degreased with ether. 200 mL of hot 1.25% sulfuric acid was added and refluxed for 30 minutes. It was filtered hot through a cloth into a buchner, washed with hot water until removal of the acid reaction. The material was returned to the beaker, where 200 mL of 1.25% Sodium Hydroxide (NaOH) was added. It was heated for another 30 minutes, after which it was filtered again through the fabric. It was washed with hot water until the alkaline reaction was removed. The residue is then filtered through a Gooch crucible, previously prepared with asbestos. The crucible and its contents were brought to a closed oven and allowed to dry to constant weight at a temperature not exceeding 110 ° C, cooled and weight. Finally the crucible and its contents were calcined at a muffle at (550 ° C) for one to two hours¹⁸. The difference in weight before and after the calcination represents the raw fiber of the sample. The results were expressed as% crude fiber by the following formula:

$$\% \text{ FC} = \frac{P_1 - P_2}{P_m} \times 10$$

P1 = Weight in grams of calcined crucible.

P2 = Weight in grams of the empty crucible.

Pm = Weight in grams of the sample

Acetylation of starch

To a suspension of native starch (100g dry and 500ml distilled water) at pH 8.0 with constant stirring for 30 min, 10.2 g of acetic anhydride was slowly added maintaining the pH between 8.0-8.5; The pH was then adjusted to 4.5 with 0.5 M HCl, then filtered and the residue washed 4 times with distilled water, dried at 30 ± 2 ° C for 48 h and the starch obtained was ground and sieved (60 Mesh)¹⁹.

Determination of the percentage of acetyl and GS groups of the reaction by acetylation

For these determinations the method of Wuzburg (1964) was used²⁰. Measurement of acetyl content is a primary method for the characterization of acetate starches based on the titration of the acetyl residues after the sample has been subjected to an alkaline treatment. 1 g of starch acetate sample was weighed and placed in a 250 ml conical flask and 50 ml of 75% ethanol. The flask was then capped slightly and placed in a 50 ° C water bath with shaking for 30 min. Cool and add 40 mL of 0.5 N potassium hydroxide while stirring. The bottle was then capped and left for 72 hours with occasional stirring. After 72 hours the excess alkali was titrated with 0.5 N hydrochloric acid, using phenolphthalein as indicator. It was left for two hours, after which some extra alkali is titrated which may leach from the sample. Finally a white was titrated, replacing the acetate starch with the original starch.

Determination of swelling power and water absorption

The swellability is defined as the times it is able to absorb a material its own weight in water. The method used by Araujo (2004)²¹ with some modifications was used to determine the swelling power and water absorption. The determination was made in a temperature range between 55 and 95 ° C. Four grams of native starch were quantitatively transferred with 200mL of water to a 300mL three-necked balloon, into which a magnetic stirrer was introduced. In the central mouth of the balloon was connected a refrigerant and in one of the other two a thermometer, to the third mouth was placed a glass stopper to avoid the loss of water that is removed during the analysis. The balloon was placed on a heating plate with stirring. Temperature control was done and aliquots of 10mL were taken every 5 degrees between 55 and 95 ° C, this was done with agitation at a constant rate allowing the starch to be kept in suspension during the heating. These aliquots were placed in previously weighed centrifuge tubes. The centrifuge tubes with the room temperature aliquot were again weighed and centrifuged at 2200 rpm for 15 minutes. The supernatant liquid was decanted in previously tared Petri dishes, the boxes were dried in an oven at 60 to constant weight, placed in a desiccator and weighed. The centrifuge tubes were also weighed. The following calculations were performed.

$$W_1 = \frac{\text{Starchweightondrybasis (g)}}{\text{Starchweightondrybasis(g) + Dissolutionvolume (200 g)}} \times 100$$

$$W_2 = A \times \frac{W_1}{100}$$

$$W_3 = W_2 - b$$

$$\%SS = \frac{b}{W_2} \times 100$$

$$AA = \frac{a - W_3}{W_3}$$

Where:

W1 =% starch on dry basis of suspension

W2 = starch in each aliquot

W3 = residual starch in the sediment of each aliquot

A = aliquot weight (g)

% SS (% Soluble solids (g / g starch)

AA = water absorbed (g / g starch)

PH = Swelling Power

A = weight of the sediment in the tube

B = weight of the residue in the capsule

Determination of gelatinization temperature

100 mL of suspension was prepared with 7.5 g of starch on dry basis, which was heated with constant stirring (hot plate with magnetic stirring). A thermometer was inserted into the suspension. Heating was continued until the solution began to form gel²².

Determination of viscosity

7.5% (w / v) starch pastes were prepared in water, placing them in a boiling water bath for 15 min and then cooling them to room temperature. The apparent viscosity of the cold pastes was measured at 25 ° C in a Brookfield viscometer (RVF model, Stoughton, MA) at four deformation rates (2, 4, 10 and 20 min⁻¹) using needle No. 3. Finally The stability of the pulp was observed at a rate of 20 min⁻¹ in minutes 1, 2, 3, 4, 5, 10, 15, 20 and 30²³.

Stability to freeze-thaw

Suspensions of 5% (w / v) starch were subjected to a freeze cycle (-20 ° C for 18 h) and subsequent thawing (room temperature, 6 h). At the end, the samples were centrifuged at 3000g for 10 min. The percentage of water separated after subjecting the starch to this cycle was measured²³.

Determination of the percentage of syneresis

Syneresis is the tendency for a gel to contract and exude liquids, because the effect of water binding is not completely obtained. To determine syneresis, a suspension of gelled starch was taken at 90 ° C for 30 minutes (7.5% w / w), cooled in an ice bath to room temperature (25 °C). Samples were stored for 48, 72, 96 and 168 hours at 4 ° C. The syneresis was measured as the amount of water (%) released after centrifugation at 2300g for 15 minutes^{24,25}.

Functional tests in food

The milk was mixed with the starch, adding sugar and cinnamon, this mixture was deposited in a three-necked balloon which was connected to a condenser, and to a thermometer. It was then heated on a magnetic plate to a temperature of + 80 ° C, with the aid of a magnetic stirrer the homogeneous mixture was maintained.

Statistical treatment

In this research a totally random experimental design was handled. The determinations were performed in triplicate and the results are expressed as the mean ± standard deviation. For analysis of the data, an analysis of variance (ANOVA) and the analysis of means were applied, using a Tukey-Kramer test. Values of p <0.05 were considered significant.

Results and discussion

The starch used in this research project was obtained from the grinding of Zaragoza bean (*Phaseolus lunatus*) seeds extracted from previously selected legumes. The dry yield of starch of the seed could be by the method of extraction, since the residue was washed many times which could have caused losses, in addition by the Transfer of the samples into the centrifuge tubes. When performing the chemical modification by the acetylation method there was an increase in yield of 11.25%, this may be due to an increase in molecular weight due to the chemical groups that are added in the acetylation.

Table 1 presents the results corresponding to the physicochemical and bromatological composition of the native starch of Zaragoza bean (*Phaseolus lunatus*). Finding values between 0.542% moisture, 2.093% protein, 5.984% fat, 0.219% ash, 0.194% crude fiber and a pH of 7.553, the variability in the composition of the starch granule may be caused by differences In conditions of cultivation, climate and variety.

Table 1. Physicochemical and bromatological analyzes of the native starch of Zaragoza bean (*Phaseolus lunatus*) (The values correspond to mean of three replicates +/- standard deviation)

pH	7,553±0,133
Humidity %	0,542±0,021
Ashes%	0,219±0,005
Proteins%	2,093±0,061
Lipids%	5,984±4,153
Rawfiber%	0,194±0,002

It can be seen from Table 2, in general, that in all acetylated starches the degree of substitution was increased as the acetic anhydride volume and the reaction time were increased, however. The GS data reported in this study are low, with a minimum of 0.047 for acetylated starch with 2 mL of. The US FDA accepts a GS between 0.01 - 0.2 for use in food.

Table 2. Percentage of acetyl groups and degree of substitution of starch under study

Starch	Starch Amount of Acetic Anhydride	Percentage of Groups Acetils	G.S
Bean Zaragoza	2 mL	1,249 ± 0,002	0,047 ± 0,000
	5 mL	2,495 ± 0,005	0,095 ± 0,000

The functional properties of native starch, 2% acetylated starch and 5% acetylated starch of Zaragoza bean (*Phaseolus lunatus*). These values are shown in Table 3, as expected the swelling power increases with increasing temperature, since at high temperatures a progressive relaxation of the bonding forces within the granule occurs²⁶. This is evidenced from 75 ° C in acetylated starches, in the case of the native, this is shown from 80 ° C. In the measure that the acetylation is increased there is an evident tendency to increase in the value of the swelling capacity.

Table 3. Power of swelling and water absorption of vean starch Zaragoza

T	A.A			P.H		
	Zaragoza Native	Acetylated 2.5%	Acetylated 5%	Zaragoza Native	Acetylated 2.5%	Acetylated 5%
55	1,666 ± 0,574	1,209 ± 0,295	1,664 ± 0,862	2,666 ± 0,574	2,209 ± 0,295	2,664 ± 0,862
60	1,677 ± 0,263	1,390 ± 0,171	1,516 ± 0,346	2,677 ± 0,263	2,390 ± 0,171	2,516 ± 0,346
65	1,810 ± 0,513	1,6594 ± 0,247	1,800 ± 0,074	2,810 ± 0,513	2,6594 ± 0,247	2,800 ± 0,074
70	2,002 ± 0,268	1,906 ± 0,132	2,771 ± 0,170	3,002 ± 0,268	2,906 ± 0,132	3,771 ± 0,170
75	2,116 ± 0,480	6,427 ± 0,753	8,189 ± 0,321	3,116 ± 0,480	7,427 ± 0,753	9,189 ± 0,321
80	5,670 ± 0,633	15,923 ± 0,894	20,500 ± 0,827	6,670 ± 0,633	16,923 ± 0,894	21,500 ± 0,827
85	11,256 ± 2,386	25,890 ± 1,653	31,804 ± 0,590	12,256 ± 2,386	26,890 ± 1,653	32,804 ± 0,590
90	16,389 ± 2,816	29,252 ± 0,672	35,980 ± 1,170	17,389 ± 2,816	30,252 ± 0,672	36,980 ± 1,170
95	19,557 ± 4,205	32,398 ± 6,176	40,051 ± 3,276	20,557 ± 4,205	33,398 ± 6,176	41,051 ± 3,276

Table 4 shows the gelation temperature reached by native starch, 2% acetylated starch and 5% acetylated starch. The gelatinization temperature in the native starch of *Phaseolus lunatus* was 83 ° C, the latter being the highest among the starches in studies. This shows that when the granules were heated in water at high temperatures, the point where the granules swelled and lost their structural order was reached, which is due to the fusion of the crystals (birefringence) and its consequent expansion (gelatinization), leading to an increase in the viscosity of the sample. Phenomenon that was not notorious in 2% and 5% acetylated starches, which presented a more fluid consistency. The 5% acetylated starch had the lowest gelatinization temperature, with a value of 77 ± 0.563 ° C, which may be of

great interest to the food industry, in processes using viscous systems (creams, soups, sauces, Etc.), at a low temperature.

Table 4. Gelatinization temperature of starches (°C) (Values represent the mean of two trials +/- standard deviation)

Sample	Native	Acetylated 2%	Acetylated 5%
Bein Zaragoza	83 ± 0,707	80 ± 0,645	77 ± 0,563

Viscosity tests performed on native starches are presented in Table 5a, the viscosity was evaluated at 55-80 ° C, with a higher viscosity having 2% acetylated starch at 859,336 Cp. Deducting that in the chemical modification to this degree of acetylation, the starch an improvement of this property. Being of great utility for the viscous systems in the food industry. Viscosities were evaluated with a 4% starch concentration for both water and cream preparations. In the results a lot of variability was found, mainly the fact that the viscosities were significantly lower in preparations in water than in the starches in the cream preparation.

Table 5a. Viscosity in water of Zaragoza bean starch (Cps) (Values represent the mean of two trials +/- standard deviation)

Sample	Native	Acetylated 2%	Acetylated 5%
Frijol Zaragoza	434,67 ± 23,094	859,33 ± 19,425	667,33 ± 12,220

With the same starches, a functional food technology test was carried out. In Table 5b, this test consisted in developing a formulation of a custard, to evaluate the behavior of starches in terms of viscosity. With each starch this cream was prepared in triplicate.

Table 5b. Viscosity of cakes based on vean starches Zaragoza (Values represent the mean of two trials +/- standard deviation)

Sample	Cream with A. Native	Cream with A. Acetylated 2%	Cream with A. Acetylated 5%
Frijol Zaragoza	690,667 ± 4,619	1512 ± 36,661	1946,667 ± 16,653

In Tables 6 and 7 are shown the stability of the gels of the different native and acetylated starches was evaluated qualitatively after being subjected to cooling at 4 ° C and freezing at -18 ° C for 144 hours. According to the statistical analysis, the percentage of syneresis and the stability of the native starch presented significant differences with their acetylated counterparts, and also among the acetylates. In general, the starches under study present high percentages of syneresis and little stability to freezing - thawing, and there seems to be no correlation between the properties of the starches (amylose content, gel point, particle size, etc.) and resistance to syneresis²⁶.

Table 6. Stability to freezing - Defrost of Zaragoza vean starch (Cps) (Values represent the mean of two trials +/- standard deviation)

Sample	Native	Acetylated 2%	Acetylated 5%
Frijol Zaragoza	91,73 ± 3,4	45,527 ± 7,477	57,003 ± 19,116

Table 7. Saris vean starch syneresis (Cps) (Values represent the mean of two trials +/- standard deviation)

Sample	Native	Acetylated 2%	Acetylated 5%
Frijol Zaragoza	55,283 ± 15,924	60,593 ± 1,678	52,897 ± 2,722

Conclusions

The degree of substitution obtained in this study was low, however, when modified the starches showed

improvement in some of its properties, but in general it can be concluded that these starches presented different behaviors under the same conditions of acetylation, confirming that These behaviors depend on the intrinsic properties of each starch such as the amylose / amylopectin ratio, size and shape of the granule, botanical species and the physicochemical conditions of the modification. According to the results obtained in this research it can be said that native starch and its different acetylations, when used as an ingredient in food preparations, as in the case of creams, have higher levels of viscosity. This result is of vital importance to the food industry.

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