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Development and characterization of a dehydrated mixture based on pumpkin flour (*Cucurbita maxima*) incorporating modified starch of yam (*D. alata* cv. Diamante 22) with potential application for instantaneous soups

Desarrollo y caracterización de una mezcla deshidratada a base de harina de ahuyama (*Cucurbita maxima*) incoporando almidón modificado de ñame (*D. alata* cv. Diamante22) con potencial aplicación para sopas instantáneas

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Abstract

In Colombia, food industry seeks the use of raw materials of high nutritional value such as pumpkin (*Cucurbita maxima*) and yam (*Dioscorea alata cv.* Diamante 22) for the development of processed foods and diversification of agricultural production. The purpose of this work was to develop a dehydrated mixture using pumpkin flour and modified yam starch. The study included the formulation of dehydrated mixture based on the starch/flour ratio and the type of starch modification. With analysis by infrared spectroscopy (FT-IR) peaks were identified in 1366 - 1367 cm^{-1} corresponding to beta-carotene in the flour. With starch modifications gelatinization temperature increased reaching values of 83.6 and 86.3ºC. All the rehydrated formulations showed pseudoplastic rheological behavior where the viscosity decreased with the shear rate. The mixture most preferred by consumers and that showed the highest overall desirability (0.47) was the formulation with 60% flour and 10% enzymatically modified starch. Finally, the pumpkin flour conferred favorable sensory characteristics and the starch improved the stability and consistency properties in the prepared product.

Keywords: Instant soup, acetylated starch, enzymatically modified starch, rheological behavior, sensory evaluation.

Resumen

En Colombia, la industria alimentaria busca el aprovechamiento de materias primas de alto valor nutricional como ahuyama (*Cucurbita maxima*) y ñame (*Dioscorea alata cv.* Diamante 22) para el desarrollo de alimentos procesados y diversificación de la producción agrícola. El objetivo de este trabajo fue desarrollar una mezcla deshidratada usando harina de ahuyama y almidón modificado de ñame. El estudio comprendió la formulación de la mezcla en función de la relación almidón/harina y el tipo de modificación de almidón. Con el análisis por espectroscopia de infrarrojo (FT-IR) se identificaron picos en 1366 - 1367 cm^{-1} correspondientes a betacarotenos en la harina. Con las modificaciones del almidón, la temperatura de gelatinización aumentó alcanzando valores de 86.3 y 83.6ºC. Todas las formulaciones rehidratadas evidenciaron un comportamiento pseudoplástico, donde la viscosidad disminuyó con la velocidad de corte. La mezcla de mayor preferencia por los consumidores y que mostró la mayor deseabilidad general (0.47) fue la formulación con 60% de harina y 10% de almidón modificado enzimáticamente. Finalmente, la harina de ahuyama confirió características sensoriales favorables y el almidón mejoró las propiedades de estabilidad y consistencia en el producto preparado.

Palabras clave: Sopa instantánea, almidón acetilado, almidón modificado enzimáticamente, comportamiento reológico, evaluación sensorial.

1 Introduction

Pumpkin (*Cucurbita maxima*) and yam (*Dioscorea alata cv.* Diamante 22) are transitory crops of great economic interest in Colombia. These vegetable products as raw materials have been used mainly for fresh consumption, and the lack of transformation and agro-industrialization processes favors an increase in post-harvest losses, making difficult their use as viable agro-industrial products of second or third quality (Plan estratégico de ciencia, tecnología e innovación del sector agropecuario, 2016).

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Flours and various starches have nutritional and techno-functional properties that allow the development of a variety of attractive food products for the consumer. These include instant soups that, due to its simple preparation, are part of the most common powder mixtures on the market meeting modern people requirements (Pacheco, 2001). In the formulation of these products, flours can be used as the main ingredient or to be combined with other flours or starches for giving adequate sensory characteristics as taste, consistency and stability (Wicks *et al*., 2006).

The flour is a considered as techno-functional ingredient and generally provides macronutrients in the mixture, so it is possible to consider that its ingestion may play an important role in the diet either by its content of carbohydrates, fiber, proteins or minerals. Several studies have been conducted for the formulation of these products with flour sources such as *Arracacia xanthorriza* (García *et al*., 2007), *Oryza sativa* and *Phaseolus vulgaris* L. (Carvalho *et al*., 2013), *Cucurbita maxima L.* and *Cajanus cajan L.* (Praderes *et al*., 2010), *Kappaphycus alvarezzi*, *Ulva lactuca* and *Gracilaria verrucose* (Jayasinghe *et al*., 2016), *Bananas* "Matooke" (Muranga *et al*., 2011), and some legumes (Haleem and Omran, 2014). In addition, the quantity of starch used in dehydrated mixtures for instant soups has a decisive influence on thickening and acceptability properties that condition the final quality of the product (Pacheco *et al*., 2008). Some rheological studies have shown that starches can give the prepared mix a stable consistency due to the gel swelling capacity produced when temperature increases during cooking process (Qian *et al*., 2019; Ulfa *et al*., 2019; Lawal *et al*., 2015; Sahnoun *et al*., 2015). This phenomenon is caused by the increase in the solubility of leached amylose molecules after starch is subjected to modification processes, either chemically (Moin *et al*., 2019; Siroha *et al*., 2019, Shah *et al*., 2017) or enzymatically (Asiri *et al*., 2018; Figueroa *et al*., 2019).

The main purpose of this study was to develop a pumpkin flour dehydrated mixture incorporating yam starch modified by acetylation and enzymatic process, with potential application for instant soup. The study included the formulation and evaluation of stability during storage by characterization at proximal, physicochemical, techno-functional and sensory level.

2 Materials and methods

The study was carried out in the Laboratory of Agricultural Processes of Universidad Nacional de Colombia - Sede Medellín and the laboratories of the Unitary Operations Plant of Universidad de Sucre, "Los Pericos" Farm. Vegetable raw materials used correspond to pumpkin and yam, which were acquired from local suppliers in the city of Sincelejo, Sucre. Both pumpkin and yam crops were located on a farm at 200 meters above sea level, temperature and relative humidity of 28 ± 2ºC and 82 ± 5%, respectively. Pumpkin was harvested after 120 days, once it reached its post-harvest maturity marked by the intense orange of its pulp, and yam after 240 days when the tuber reached its full brown color.

2.1 *Obtaining flour and modified starches*

The flour used came from pumpkins (*Cucurbita maxima*) meeting the quality requirements defined in the Colombian Technical Standard - NTC 1291 (ICONTEC, 1977) and was processed following the methodology proposed by Pérez and Pacheco (2005), with some modifications. Pumpkins were disinfected and then the material was peeled, chopped, and dried by forced convection at 50ºC until reaching a final moisture content of 10% d.b. Subsequently, the dry product was subjected to grinding using a hammer micro-grinder (Tecnal WILLYE TE-650) and sieved up to particle size of 180 μm.

The obtaining of native yam starch (*Dioscorea alata cv.* Diamante 22) was carried out using a bubbling equipment. Acetylation process was performed by preparing an aqueous starch suspension at 43% (p/V) adding to 8.0 mL of acetic anhydride (Salcedo *et al*., 2016). For enzymatic modification was handled 100 mL of 0.1 M buffer solution and 20 g of starch in which 10 μL of enzyme α-amylase (*Liquozyme extra*) were dissolved, followed the methodology proposed by Salcedo *et al*. (2018).

2.2 *Formulation of dehydrated mixture*

The development of powdered mixture was carried out by the procedure described by Praderes *et al*. (2010), supported in tests preliminary that allowed to determine the ranges of variation of the flour/starch ratio.

Table 1. Formulations for the development of the dehydrated mixture.

Composition (%)	F 1	F 2	F 3	F 4
Flour	40	60	40	60
Acetylated starch	30	10	-	-
Enzymatically modified starch	-	-	30	10
Milk	9	9	9	9
Salt	6	6	6	6
Garlic	6	6	6	6
Onion	6	6	6	6
Pepper	0.5	0.5	0.5	0.5
Cumin	2	2	2	2
Gum	0.5	0.5	0.5	0.5

Four formulations were established (Table 1), in which 40 to 60% pumpkin flour and 10 to 30% enzymatically and chemically (acetylation) modified starch were incorporated. In addition, lactose-free milk powder, xanthan gum, salt, vegetables and spices powder (garlic, onion, cumin, and pepper) were added in fixed proportions according to the provisions established in the Colombian Technical Standard - NTC 4482 (ICONTEC, 1998). The amount of water for sample rehydration was defined by dissolving 20 g of powder mixture in 200 mL of water, in a standardized cooking time of 10 minutes. A pumpkin dehydrated mixture existing in the Colombian market was chosen as a benchmark (commercial sample).

2.3 *Fourier transform Infrared spectroscopy (FT-IR)*

Infrared spectra of flour and modified starches were obtained using a Fourier Transform Infrared Spectrometer (Thermo Scientific Nicolet iS5 iD1 Transmission) in the region of 4000 to 500 cm^{-1}. The formation of crystals was done by mixing 20 mg starch with KBr in a ratio of 1:5 (starch: KBr) using a pressure of 220.63 bar. Thirty-two readings were made at a resolution of 4 cm^{-1}. The results were processed with OmnicTM SpectaTM software version 9.0 (Figueroa *et al*., 2016).

2.4 *Viscoamylogram*

The viscosity profile (pasting properties) of starch dispersions was determined by the technique proposed by the AACC (2000) using a rheometer (Anton Paar, MCR 302, Austria). Two grams of starch in dry base, dissolved in 25 mL of distilled water, were deposited in an aluminized sample holder. Temperature was maintained at 50ºC for one minute, then raised to 95ºC in 7.5 minutes, kept at 95ºC for 5.0 minutes, immediately cooled down to 50ºC in 7.5 minutes, and finally at 50ºC for two minutes (Delgado *et al*., 2018). Spindle rotational speed (Anton Paar, ST24-2D/2V, Austria) was 960 rpm during the first 10 seconds, allowing the starch suspension to be dispersed uniformly, and then reduced to 160 rpm for the rest of the experiment.

2.5 *Proximal, physicochemical and techno-functional characterization*

The content of crude protein, crude fiber, crude fat, moisture, ash, and pH of mixtures were established according to AOAC protocols (2012). Soluble solids (ºBrix) were measured by a refractometer (HI 96861) and water activity (a_w) determined with a dew point hygrometer Aqualab 4TE (Decagon Devices, Inc., Washington, USA) at 25ºC (Fitch *et al*., 2019; Cortés *et al*., 2007). The color quantification was done with a Konica Minolta Chroma meter CR-400 colorimeter considering D65 illuminant and observer of 2º, where color levels were expressed as $L*$ (luminosity), $a*$(green-red chromaticity) y $b*$(blue-yellow chromaticity). Total color difference ($\Delta E*$) was calculated using Eq. (1), where the value referenced with a subindex zero is the reference color or standard sample (Pérez *et al*., 2018).

$$\Delta E* = \sqrt{(L*-L_0^*)^2 + (a*-a_0^*)^2 + (b^* - b_0*)^2} \quad (1)$$

For techno-functional evaluation, the water absorption capacity (WAC) was determined using the method described in the AACC (2012) and cold water solubility (CWS) as stated by Salcedo *et al*. (2018).

The flow curves of the formulations were obtained with a viscometer (Brookfield DV-III Ultra, USA) with coaxial cylinder geometry, performing a shear rate sweep (γ) in the range from 0 to 100 s^{-1} (Contreras *et al*., 2018; Pereyra *et al*., 2019). The experimental values of flow curves were adjusted to Power Law model (Equation 2), where n is the flow index (dimensionless) and K is the coefficient of consistency ($Pa.s^n$) (Sánchez *et al*., 2018).

$$\sigma = K(\gamma)^n \quad (2)$$

2.6 *Sensory and stability evaluation*

A sensory evaluation was performed using a hedonic acceptance test, with a five-point scale, where (1 = dislike very much, 2 = dislike lightly, 3 = neither like nor dislike, 4 = like lightly, 5 = like very much), based on the Colombian Technical Standard - NTC 3884 (ICONTEC, 1996) and as proposed by Ramirez (2012). The valuation was made according to the possible changes in smell, color, flavor, and consistency of the prepared mixture. The soups were given to taste to 50 untrained panelists (25 men and 25 women), who performed their university studies and that his/her age ≥ 20 years old (Delgado *et al*., 2018).

The stability of the dehydrated product was determined for a period of two months under controlled conditions (temperature of 25 ± 2ºC and 75 ± 5% relative humidity). The product was stored in a climate chamber (Memmert, ICH 260L) in aluminized bags, evaluating protein, fat, and crude fiber in the initial and final stage of storage and analyzing the content of moisture, ash, soluble solids (°Brix), water activity (a_w), CIELAB color space (ΔE*), WAC and CWS every 15 days. Analyses were performed in triplicate.

2.7 *Statistical analysis*

For the statistical analysis of the results, a multi-factor categorical design was applied with factors: type of modified starch and starch/flour ratio, performing analysis of variance (ANOVA) and comparison of means test determined by Tukey test at 5% significance level using Statgraphics Centurion XVI software (Version 16.1.18).

The simultaneous optimization by multiple responses was performed based on the overall desirability function (D), using the R-Studio 6.18 statistical software. The approach is to first convert each response (y) into a dimensionless function, known as individual desirability function (d), calculated by Eq. 3. In turn, it was assigned for each of the d a weight factor (w) in the range 1-5, being 1: very unimportant, 2: unimportant, 3: moderately important, 4: important, 5: very important, and k is the number of responses (Silva *et al*., 2013). Since the desirability approach requires that each response be transformed into a utility without units whole limits are $0 < d < 1$, the higher values of d indicate more desirable response values ($d1$), otherwise they indicate less desirable response values ($d0$).

$$D = (d_1 \times d_2 \times \cdots \times d_k)^{\frac{1}{k}} \tag{3}$$

Individual desirability functions were calculated based on the optimization criteria: maximization, minimization or proximity to a reference according to eqs. (4)-(6).

$$d = \begin{cases} 0 & , \quad y_i < d0 \\ \left(\frac{y_i - d0}{d1 - d0}\right)^w & , \quad d0 \le y_i \le d1 \\ 1 & , \quad y_i > d1 \end{cases} \tag{4}$$

$$d = \begin{cases} 1 & , \quad y_i < d1 \\ \left(\frac{y_i - d0}{d1 - d0}\right)^w & , \quad d1 \le y_i \le d0 \\ 0 & , \quad y_i > d0 \end{cases} \tag{5}$$

$$d = \begin{cases} 0 & , \quad y < Li \\ \left(\frac{y - Li}{ref - Li}\right)^w & , \quad Li \le y \le ref \\ \left(\frac{y - Ui}{ref - Ui}\right)^w & , \quad ref \le y \le Ui \\ 0 & , \quad y > Ui \end{cases} \tag{6}$$

where $i = 1, 2, \ldots, k$ and ref is a reference values that lies between the lower (Li) and upper (Ui) as described by Derringer and Suich (1980).

3 Results and discussion

3.1 *Fourier transform Infrared spectroscopy (FT-IR)*

Fig. 1 shows the spectral profiles of pumpkin flour and modified yam starches versus their native reference. In Fig. 1(a), the signals identified between 1650 and 1550 cm^{-1} allow to infer the presence of proteins in pumpkin flour related to amines I and II, respectively (Borges *et al*., 2014) and the peak in 1366 - 1367 cm^{-1} corresponds to the standard region of beta-carotene (Kushwaha *et al*., 2014). In Fig. 1(b), the signals in 1160, 1080, 1020 and 930 cm^{-1} indication typical C−O stretching vibration peaks un the fingerprint region the available carbohydrates in the samples studied. With the modifications starch the stretching mode H−O−H in the region from 3000 to 3600 cm^{-1} was evidenced and an increase in the absorption band in 2930 cm^{-1}, probably due to the vibration of asymmetrical stretching of C−H in $-CH_2-$ (Demiate *et al*., 2000). The absorption peaks in modified starches increased their intensity compared to native sample due to the possible incorporation or substitution of OH groups that influence water retention and absorption properties (Mu *et al*., 2015).

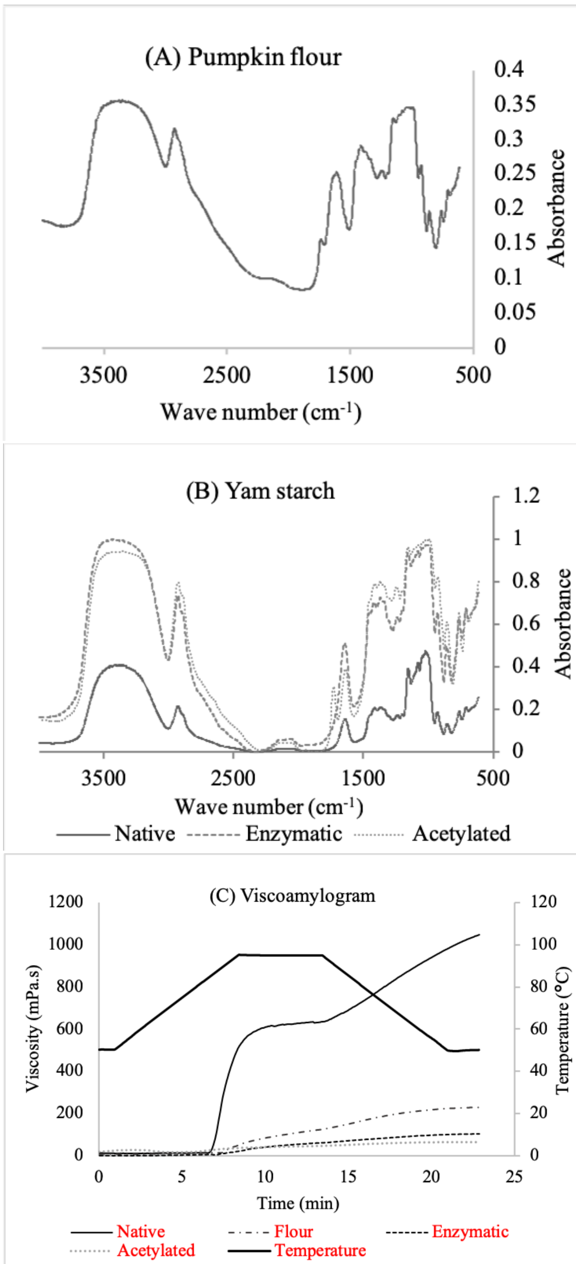

Fig. 1. Fourier transform infrared spectroscopy (FT-IR) of pumpkin flour (A); FT-IR of native starch, acetylated and enzymatic of yam (B); Viscoamylogram of pumpkin flour, native starch and modified of yam (C).

After the acetylation reaction an increase in the signal of 1740 cm^{-1}, characteristic of vibrational modes in the carbonyl groups present starch was observed showing a high degree of modification in the granule and corroborating the effect of acetic anhydride by esterification similar to reports in corn, cassava, sweet potato and oat starches (Lawal *et al*., 2015; Figueroa *et al*., 2016; Shan *et al*., 2017). Also, spectra of the acetylated starch revealed the characteristic band of the acetate group in 1260 cm^{-1} and 1230 cm^{-1} (Qian *et al*., 2019). For the enzymatic starch, an increase in absorbance in peaks located at 1010 and 1160 cm^{-1} was evidenced caused by the vibrations of C-O, C-C or O-H functional groups, whose chemical bonds are present in amylose and amylopectin molecules (Salcedo *et al*., 2018). These bands suffer tension or bending vibrations due to the breaking of α-D- (1,4) glycosidic in polymeric chains product of the biocatalytic action of some hydrolases such as α-amylase or amyloglucosidase (Mu *et al*., 2015).

3.2 Pasting properties (viscoamylogram)

Pumpkin flour provided higher viscosity than modified starches, as shown in Fig. 1(c). However, the absence of a viscosity peak in flour is attributed to the restriction of granules swelling caused by helicoidal complexes developed between starch chains and lipids (Wongsagonsup *et al*., 2015). Starch modifications increased the initial gelatinization temperature reaching values of 86.3 and 83.6ºC, with respect to the native (Table 2) possibly because the modification processes reduced the order of double helices present in amorphous region, altering the diffusion of water molecules, as well as the endothermic process during gelatinization (Chen *et al*., 2011; Salcedo *et al*., 2018). In fact, an decrease of the peak of maximum viscosity was observed after the chemical and enzymatic attack due to the depolymerization of the amylose and amylopectin chains, causing a weakening at the molecular level and affecting interaction of the granules for the formation of the network at the beginning of the gelatinization process (Sun *et al*., 2016). The viscosity of the suspension remained slightly constant during the isothermal stage, indicating that the starch granules achieve structural stability (breakdown). On the other hand, modified starches reached viscosities higher than the maximum viscosity of 257.4 and 579.8 mPa.s during the cooling stage; however, retrogradation decreased (setback), an effect that could be favored by to the weak rearrangement of bonds in linear amylose chains solubilized during heating, being resistant to thermal and mechanical stress (Zhu *et al*., 2015; Salcedo *et al*., 2016).

Table 2. Pasting properties in native and modified yam starches. Arithmetic mean ± standard error.

Pasting properties	Native	Acetylated	Enzymatically modified
Gelatinization Temperature (°C)	80.5 ± 1.4	86.3 ± 0.7	83.6 ± 1.3
Peak viscosity (mPa.s)	1320.8 ± 0.6	54.6 ± 3.2	130 ± 1.1
Viscosity at 95°C, 5 min (mPa.s)	1651 ± 0.9	156 ± 1.2	325 ± 0.5
Viscosity at 50°C (mPa.s)	2542.8 ± 1.1	257.4 ± 0.9	579.8 ± 2.3
Breakdown	-330.2 ± 0.3	-101.4 ± 2.6	-195 ± 0.4
Setback	891.8 ± 2.2	101.4 ± 0.5	254.8 ± 0.8

3.3 Proximal, physicochemical and techno-functional characterization

All established formulations had a protein contribution (4.9 g/100 g to 6.35 g/100 g) lower than the commercial reference (8.6 g/100 g). However, the values obtained can be considered to depend on the incorporation of pumpkin flour (F2 and F4). For fiber and fat content, the same tendency was presented, reaching values of (3.8 g/100 g to 2.8 g/100 g) and (1.89 g/100 g to 1.36 g/100 g), respectively. For fiber and fat content, the same behavior was presented, reaching values of (3.8 g/100 g to 2.8 g/100 g) and (1.89 g/100 g to 1.36 g/100 g), respectively. Similar results have been reported in the development of instant soup mixtures using pumpkin flour as a base, which vary according to the proximal composition thereof. This may depend on various factors such as soil fertility, the variety, stage of maturity and climate (Praderes *et al*., 2010; Dhiman *et al*., 2017; Indrianingsih *et al*., 2019). Regarding ash content, the different formulations presented values from 10.87 g/100 g to 13.07 g/100 g, so that this powder mixtures could contribute with some essential elements required in the diet, given that ash content in an indicator of mineral availability in foods which are important for the proper physiological functioning of the human body (Jayasinghe *et al*., 2016).

When analyzing moisture content, for both formulated and commercial samples, data obtained were lower than the maximum limit (11% d.b) specified in the Colombian Technical Standard - 4482. Similarly, it was found that water activity (a_w) content was below the 0.6 where almost all microbial action is inhibited (García *et al*., 2005; Dhiman *et al*., 2017). The mentioned above, minimizes the possibilities of deterioration by reactions of chemical or microbial origin that affect the physical and sensory quality of the dehydrated mixture, as suggested by Jayasinghe *et al*. (2016) who developed a soup mix powder using algae as a nutritional component, presenting water activity levels between 0.50 and 0.56.

Regarding soluble solids content, Dinu *et al*. (2016) have reported values from 5.5 to 12.6 °Brix in different genotypes of pumpkin and these values are higher than those found in the mixtures evaluated. It is worth mentioning that the variety of pumpkin used is characterized by a high sugar content, which could favor the sensory attributes of the product in terms of flavor. For pH, values close to the neutral range were obtained, suggesting that the mix is suitable for human consumption (Praderes *et al*., 2010). According to the CIELAB color space, for F4 and F2, reddish and yellow shades were shown according to the green-red (a*) and blue-yellow (b*) chromaticity, associated to the dark color produced by the caramelization and Maillard reactions that occur during the drying process and natural pigmentation of pumpkin due to the presence of carotenoids (Wongsagonsup *et al*., 2015). The inclusion of 30% of modified starch in F1 and F3 decreased the intensity in the powdered mixture coloration, making yellow predominate between yellow-orange, reflected in the increase of luminosity (Table 3). In addition, the value of total color difference (ΔE^*) which correlates the human visual judgment show values greater than 6, hence a very obviously difference with the reference product (commercial product) is given.

The different formulations reached higher WAC and CWS values than the commercial sample, results possibly associated to the high hygroscopic condition of the pumpkin flour related to proteins hydrophilic capacity (Table 3).

Table 3. Proximal, physicochemical, techno-functional and sensory characterization of dehydrated mixture of pumpkin flour with modified yam starch. Arithmetic mean ± standard error. Means in the same row with different letters are significantly different ($p < 0.05$), Tukey HSD tests. First letter (type of modification), second letter (starch/flour ratio).

Characteristics	Formulation				
	Commercial	F1	F2	F3	F4
Proximal (%)					
Protein	8.6 ± 0.28	5.6 ± 0.21ac	6.4 ± 0.07ad	4.9 ± 0.09bc	6.2 ± 0.14bd
Fiber	1.7 ± 0.07	3.1 ± 0.02ac	4.0 ± 0.56ad	2.8 ± 0.14bc	3.8 ± 0.42bd
Fat	0.8 ± 0.07	1.4 ± 0.04ac	1.8 ± 0.06ad	1.3 ± 0.02bc	1.8 ± 0.02bd
Moisture	5.0 ± 0.65	4.8 ± 0.03ac	4.7 ± 0.06ad	5.4 ± 0.22bc	4.3 ± 0.09bd
Ash	16.0 ± 0.34	10.8 ± 0.09ac	12.1 ± 0.09ad	12.7 ± 0.34bc	13.0 ± 0.33bd
Physicochemical					
pH	6.2 ± 0.01	6.3 ± 0.01ac	6.3 ± 0.01ac	6.3 ± 0.05ac	6.3 ± 0.01ac
a_w	0.3 ± 0.02	0.2 ± 0.02ac	0.2 ± 0.01ac	0.2 ± 0.01ac	0.2 ± 0.02ac
Soluble solids (°Brix)	4.2 ± 0.05	4.0 ± 0.10ac	4.7 ± 0.15ad	4.0 ± 0.11bc	4.9 ± 0.05bd
Color					
L	50.1 ± 0.29	45.6 ± 0.64ac	42.2 ± 0.35ad	45.8 ± 0.49ac	42.0 ± 0.25ad
a*	1.6 ± 0.08	3.1 ± 0.03ac	3.9 ± 0.04ad	3.3 ± 0.14bc	4.1 ± 0.03bd
b*	19.4 ± 0.62	25.1 ± 0.73ac	27.0 ± 0.32ad	26.2 ± 0.59bc	27.5 ± 0.30bd
Techno-functionals (%)					
CWS	3.96 ± 0.13	4.3 ± 0.17ac	5.6 ± 0.11ac	4.5 ± 0.10bd	5.9 ± 0.10bd
WAC	113.0 ± 0.55	169.0 ± 0.72ac	197.0 ± 0.45ac	162.0 ± 0.25bd	192.7 ± 0.53bd
Sensory score (numerical value)	374	404	414	372	431

Note: a_w (Water activity), L (CIE colour space co-ordinate: degree of luminosity), a* (CIE colour space co-ordinate: degree of green-red), b* (CIE colour space co-ordinate: degree blue-yellow).

In turn, the substitution of hydroxyl groups (−OH) in the starch molecules with acetyl groups of acetic anhydride ($CH_3C{=}O$), induces a steric hindrance that interferes with the structural order of the native starch, as well as in the re-association of amylose and amylopectin after gelatinization (Das *et al*., 2010). Similar results have been reported in rice starch and corn starch (Moin *et al*., 2019; Sahnoun *et al*., 2015). Since the substituent group is bulky, it improves the access of water to the amorphous area and thus the leaching of the amylose, contributing to the increased hydration of the F1 and F2 mixtures (Ulfa *et al*., 2019). Similarly, the biocatalytic activity of α-amylase affected the hydrophilic properties of starch, causing an increase in micellar force to bind water that, when incorporated into the mixture (F3 and F4), favors its CWS (Praderes *et al*., 2010). This indicates that the enzyme attacked glycosidic bonds, facilitating the release or availability of soluble polymer components of starch as mentioned by Figueroa *et al*. (2019).

Rheological parameters analysis for the different formulations after rehydration was established through Power Law model, taking into account the rheological behavior obtained in preliminary tests ($R^2 > 0.99$), similar to that evaluated in fishmeal mixture by Barragán *et al*. (2016). Coefficient of consistency K values increased from 0.068 to 0.424 Pa.s^n as the concentration of flour in the formulation increased. Regarding the power law index (n) all formulations including the commercial sample showed values of $n < 1$ which oscillated between 0.593 and 0.883, associated with the pseudoplastic nature of rehydrated mixtures and subjected to cooking process (Wongsagonsup *et al*., 2014). As show in Fig. 2 (a), for F1 and F3 the shear stress appeared to be slightly proportional to the shear rate, which favors suspensions stability, whereas a nonlinear curve was observed for F2 and F4. This behavior is typical of non-newtonian fluids, probably due to the incorporation of modified starches, given their orderly linear conformation during heating (Song *et al*., 2006). Analogously, with the results established in Fig. 2(b), it is observed that the apparent viscosity decreases as deformation rate increases.

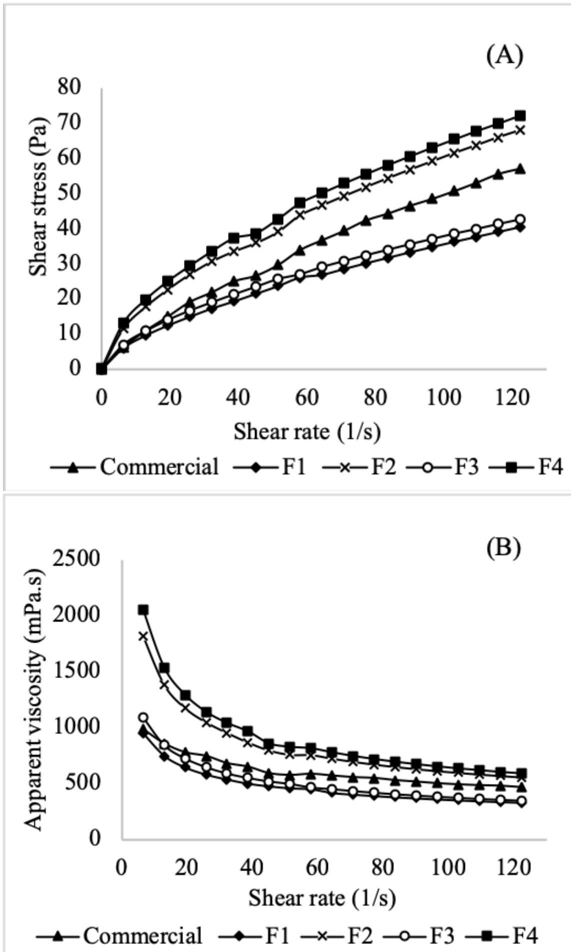

Fig. 2. Rheological behavior of pumpkin flour with modified yam starch: Flow curve (A); Rheogram (B).

The apparent viscosity patterns showed that mixtures with the addition of acetylated starch (F1 and F2) resulted in a weak gel structure with higher ability to flow, in contrast to those handled with enzymatically modified starch (F3 and F4), coinciding with the viscosity values reporteds in the Table 2 (Rivera *et al*., 2013; Shah *et al*., 2017).

3.4 Sensory and stability evaluation

Sensory quality attributes of the different formulations and the commercial sample are presented in Fig. 3. F2 and F4 samples showed greater acceptance in terms of consistency, coinciding with the apparent viscosity patterns according to Fig. 2(b). It is also evident that when the formulations have 60% flour, yellow-orange color predominates in the mixture (Table 3), favoring the level of consumer preference.

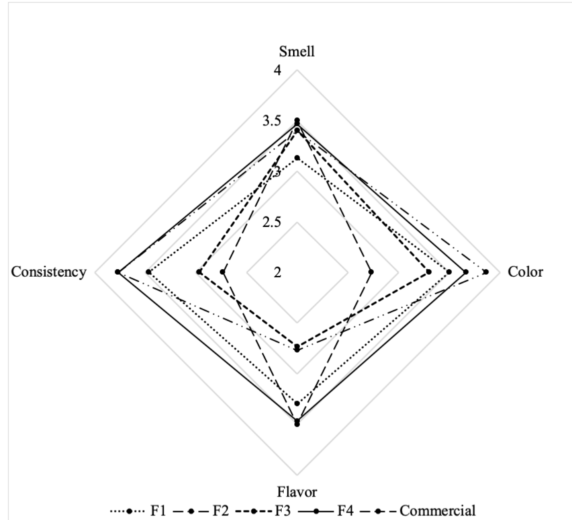

Fig. 3. Qualification of sensory attributes of the formulations established for the development of pumpkin flour with modified yam starch using hedonic scale.

Regarding of smell, the different formulations presented similarities in the qualification, probably attributed on the ionodora condition starch. The opposite happened for the flavor, since the scores did not follow a defined pattern. This could indicate that the incorporation of other ingredients inferred in the results of the panelists, as mentioned by Muranga *et al*. (2011), who evaluated soups bases on banana flour. It is necessary to mention that in dehydrated mixture the dominant smell and flavor of the main ingredient should be highlighted (Abeysinghe and Illepruma, 2006).

In the stability analysis of powder mixtures, the values of water activity (a_w) and moisture content showed a slight increase at the end of storage (Fig. 4), however, it can be considered that samples reached thermodynamic equilibrium. This increase could be due to the absorption of small amounts water vapor from the atmosphere by the samples stored through diffusion of vapours by the microscopic spores of the packing material (Sharma *et al*., 2013). A similar situation occurred with the pH of the mixtures which remains close to neutrality as evidenced in Fig. 4(c). As for the color, the powder mixtures presented changes without tendency in the first thirty days (Fig. 4(e)), possibly attributed to the oxidation of β-caroteno, so it is necessary to leave at rest and perform maturation processes as they suggest by Dhiman *et al*. (2017) and Mala *et al*. (2016).

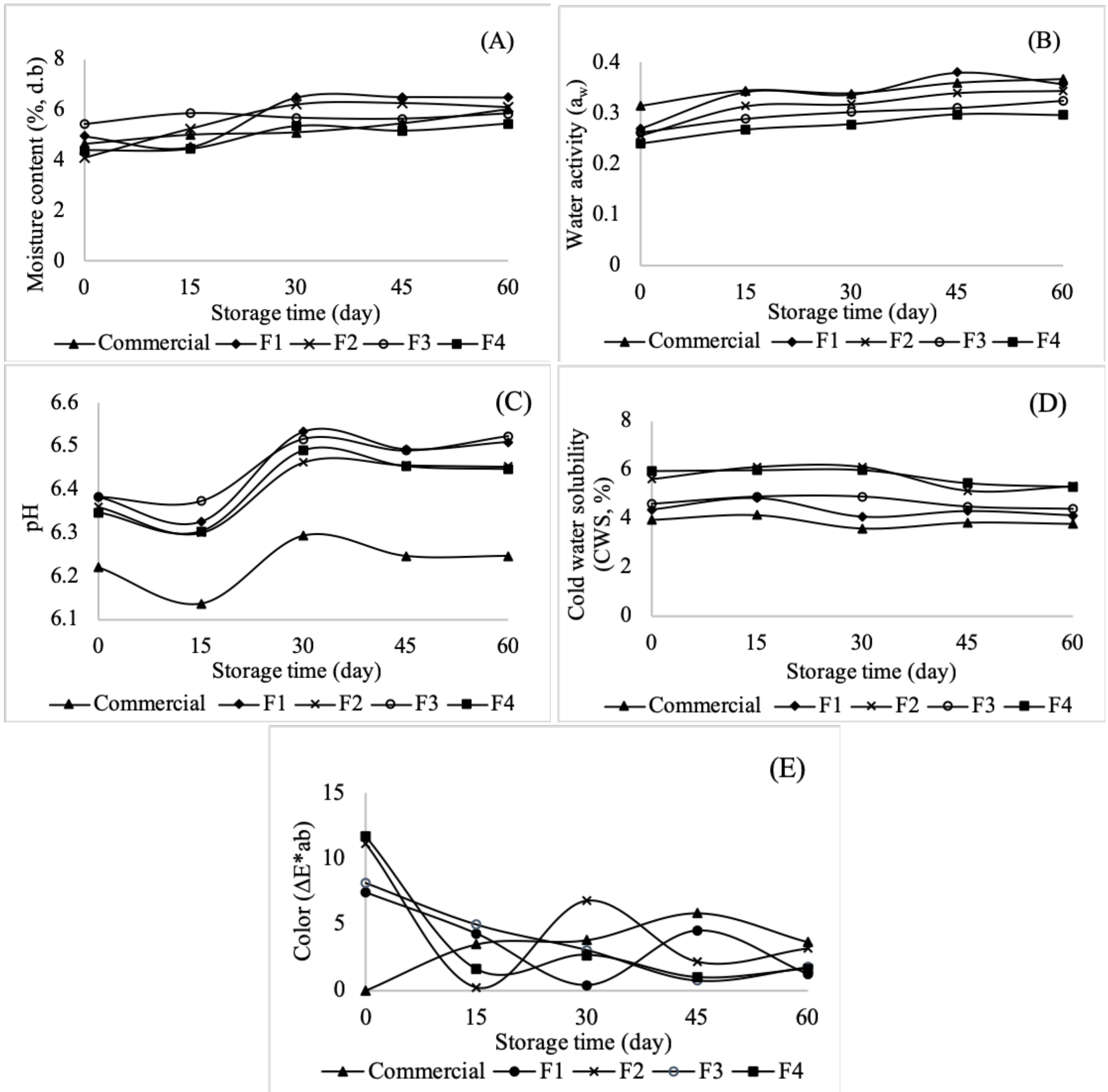

Fig. 4. Stability evaluation of the dehydrated mixture formulated with pumpkin flour incorporating modified yam starch (storage for 60 days at room temperature): moisture content (%) (A); Water activity (a_w) (B); pH (C); Cold water solubility (CWS, %) (D); Color (ΔE*) (E). Arithmetic mean ± standard error.

For content of protein (5.7% ± 0.07), fiber (3.42% ± 0.21), ash (12.27% ± 0.07) and WAC (185.32% ± 3.01) there were no statistically significant differences in time ($P > 0.05$). It allows inferring that it is possible to extend the shelf life of the product under the conditions of temperature and relative humidity handled, similar to that reported by García *et al.* (2007), who obtained analogs results during a storage period of 90 days when they evaluated a powder mixture using white and yellow arracacha flour.

3.5 *Simultaneous optimization*

The objective of the optimization study was to identify the formulation that exhibited high nutritional value and greater sensory acceptability. In addition, the mixture should contain low moisture content and low water activity (a_w) to facilitate its preservation. In relation to the above, protein, ash, soluble solids and sensory evaluation responses were maximized and moisture content and water activity (a_w) were minimized subject to what is described in section 2.7.

Table 4. Optimization of the combined responses in the powder mixture formulation of using general desirability method.

Responses (y)					Individual desirability (d)				
	Criterion	w	$d0$	$d1$	Commercial	F1	F2	F3	F4
Protein	max	5	5	10	0.72	0.12	0.28	0.05	0.24
Fiber	8.5	4	-	-	0.5	0.16	0.3	0.12	0.27
Fat	10	3	-	-	0.08	0.14	0.18	0.13	0.18
Moisture	min	5	11	5	1	1	1	0.92	1
Ash	max	4	16	10	0.11	1	0.71	0.59	0.6
pH	6.25	3	-	-	0.96	0.82	0.86	0.82	0.87
a_w	min	4	0.5	0.2	0.61	0.77	0.81	0.79	0.87
Soluble solids	max	2	3.5	6	0.29	0.2	0.62	0.21	0.58
CWS	6.5	4	-	-	0.25	0.39	0.74	0.45	0.83
WAC	150	4	-	-	0.26	0.52	0.05	0.75	0.26
Sensory	max	5	300	600	0.24	0.34	0.38	0.24	0.43
	Overall desirability (D)				0.31	0.36	0.4	0.31	0.47

Note: the original values for the control factors are shown in table 3. w: weight factor, $d0$: desirability 0, $d1$: desirability 1.

The responses were optimized simultaneously (Table 4) and the mixture that obtained the highest desirability (0.47) was when 60% pumpkin flour and 10% enzymatically modified starch (coded as F4) were used.

Conclusions

The highest overall desirability was obtained when 10% of enzymatically modified yam starch and 60% of pumpkin flour were incorporated, formulation identified as F4. Further, the product was stable for two months under environmental storage conditions.

Pumpkin flour provided the mix favorable sensory and techno-functional characteristics, constituting a good alternative to be used as the main ingredient in the formulation of dehydrated products to human consumption. However, it is suggested to enhance its nutritional contribution by combining it with flours from other sources of vegetable origin containing a higher protein value. The modifications favored the properties of yam starch. Acetylation modification resulted in a weak gel structure with greater resistance to thermal and mechanical stress, whereas enzymatic hydrolysis conferred greater viscosity, consequently granting stability and consistency in the prepared product.

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