

## Research Article

### Formulation of Frankfurter-type Sausages with Yacon Peel Flour as Non-conventional Linker

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**Abstract:** The aim of study was to evaluate the replacement effect of wheat (*Triticum aestivum* L.) flour by Yacon (*Smallanthus sonchifolius* (Poepp) peel flour in a Frankfurter-type sausages formulation. In order to obtain an optimal incorporation level, five formulations from which Wheat Flour (WF) replacement with YPF were carried out as follows: 25, 50, 75 and 100%, respectively. Therefore, emulsifying stability, pH, water retention capacity, color, proximal composition and texture profile in Frankfurter-type sausages, were evaluated. Results showed that water retention capacity in sausage increased upon 6% when is replaced with up to 50% WF. pH decreased upon replacement with 75% WF. Conversely, Frankfurter-type sausages with added YPF had a higher protein content and hardness at different levels. In addition, the most similar formulation to the control sample was 25% YPF. Results demonstrated that wheat flour replacement (replaced with 25% of YPF) by yacon peel flour, which was beneficial for frankfurter-type sausages quality.

**Keywords:** Low-fat meat product, protein content, proximate composition, quality properties, textural attributes, texture profile analysis

## INTRODUCTION

Frankfurter type sausages are pork sausage meat consumed in many world cultures due to its high quality protein, safety and process profitability. An accurately amount of ingredients in its formulation is necessary to obtain nutritional, sensory and functional properties (Savadkoohi *et al.*, 2014). Among the ingredients used in the formulation of sausages are cereals and legumes, which are used as binders or extenders due to their ability to retain water and fat (Kurt and Kiliççeker, 2009; Ponsingh *et al.*, 2010). In cereals, the most used is wheat flour for their ability to produce gluten, a cohesive viscoelastic and proteinaceous material (Bernardino-Nicanor *et al.*, 2006). However, the intake of gluten-containing products causes allergic reactions and gastric intolerance, by causing a disorder called celiac disease (Iglesias-Puig *et al.*, 2015). The only effective treatment for this type of patients is permanently consuming a gluten-free diet.

Moreover, growing global demand for food requires food industries to seek new ingredients which used to be found in food and agricultural residues. Yacon peel, for example, is characterized by its high content of dietary fiber, protein and antioxidant substances. These components could provide functional and technological properties to various food formulations (Kim *et al.*, 2015).

Yacon (*Smallanthus sonchifolius* (Poepp.) H. Rob.), is a tuberous root known since the time of pre-Inca culture and is native from the Andes, where is commonly grown. Peel of this tuber has benefits for human health due to its antioxidant activity, reduction in glucose levels, triglycerides and blood cholesterol and prebiotic potential. Antioxidant activity is associated with phenolic compounds, among which may be mentioned as follows: chlorogenic acid, caffeic acid and ferulic acid. Prebiotic capacity is due to the presence of fructooligosaccharides (Franco *et al.*, 2016; Pereira *et al.*, 2013). However, currently, scientific

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literature regarding yacon peel, only physicochemical characteristics and production of a powder obtained through convective drying. Therefore, the aim of this study was to evaluate the replacement effect of wheat flour by yacon peel flour in a Frankfurter-type sausages formulation, which includes emulsion stability, pH, water retention capacity, color, proximal composition and sausages texture profile.

## MATERIALS AND METHODS

**Yacon Peel Flour (YPF) processing:** Yacon (*Smallanthus sonchifolius* (Poepp.) H. Rob.) was obtained from the local market in Palmira-Valle, Colombia. The fruits were washed and disinfected with hypochlorite at 100 ppm for 10 min (Djioua *et al.*, 2009). Fruits were peeled and shells miced into approximately 2\*2-cm cubes. 2\*2-cm<sup>3</sup> Therefore, peels were frozen at -30°C and dried through vacuum pressure lyophilization at 0.120 mBar and condenser temperature -80°C for 24 h. Dried material was ground through a 3-mm-diameter orifice using a mincer (IMA® M.V-MOM-10, Colombia). Ground material was sieved to 250 µm in a Rotap for 15 min (M® PS-35, Series 1329, Colombia). YPF was packed in 4 sauge bag, subsequently, vacuum sealed at -0.8 psi and stored at 5°C for later use.

**Sausage formulation and processing:** Cuts of pork lean meat with little connective tissue, were prepared and used. Meat was minced, with stainless steel knife, in small portions and introduced into a mill grinder (Torrey® M-12-FS, Mexico). In order to obtain a meat emulsion, ground beef was passed through cured process (SHARFEN® TC-1, Germany) in this stage, ground meat, fat and other ingredients, which are described in Table 1, were added. The emulsion temperature in the cutter was maintained below 10°C. Conversely, the cured process, Wheat Flour (WF) and/or YPF, were added. WF used in the standard formulation (control formulation), was replaced by YPF as follows: 25, 50, 75 and 100%, respectively. A meat emulsion was subjected to an inlaying hydraulic equipment (Javar® EM 30, Colombia), 23 mm diameter synthetic gut was used for inlaying (Amicel). Sausages were manually tied into a length of 7 cm

using thread. Cooking loss sausages measurement was conducted at core temperature of 75°C. Alternatively, control temperature was performed with a punch thermometer Checktemp® (HI 9850, England). Since the indicated temperature was reached, sausages were dipped into a cold-water bath with crushed ice at 4°C for 10 min. Therefore, sausages were processed in 4 gauge bags and vacuum-sealed at -0.8 psi. The sausages were held in a storage of 4°C for 48 h for further analysis and have allowed to equilibrate the ingredients.

**Emulsifying stability:** Emulsifying stability was determined in the meat mass, for this, approximately 30 g sample were extracted from the cutter and subsequently, placed in Petri dishes for further analysis. A slurry was made using 15 mL falcon tubes previously weighted with 6 g of meat mass, which were deposited and placed on their corresponding supports. Although, tubes were heated for 30 min in a water bath at 75±1°C followed by centrifugation at 3600 rpm for 5 min at 25°C, this provides more accurate and reliable estimates of detached lipid layer of the mass, which was drained (Choe *et al.*, 2013; Yasumatsu *et al.*, 1972). Emulsion stability was calculated following Eq. (1):

$$\%Ee = \frac{\text{meat mass weight in the tube after lipid layer draining}}{\text{meat mass weight in the tube before heating}} \times 100 \quad (1)$$

**Water retention capacity:** Water retention capacity was determined using the methodology proposed by Dzudie *et al.* (2002) with some modifications. A sample of 0.5 g was placed on a filter paper grade 1 and pressed among two plexiglass plates for 20 min under 1 kg of weight pressure. In addition, pressed meat area and liquid released, were determined following the ImageJ software (Image j® 1.40 g, Wayne Rasband, National Institutes of Health, USA). It is important to note that WRC was calculated following Eq. (2) and (3):

$$\text{water released} = \frac{(\text{total surface area} - \text{meat layer area})\text{cm} \times 61.1 \times 100}{\text{Total moisture of meat sample (mg)}} \quad (2)$$

Table 1: Ingredients in the sausages formulation  
Formulation for sausages

Ingredient (g)	YPF <sup>a</sup> (%)				
	0.0	25	50	75	100
Pork shoulder	1000	1000	1000	1000	1000
Nitro <sup>b</sup> salt	6.8	6.8	6.8	6.8	6.8
Fat	338	338	338	338	338
Water	250	250	250	250	250
Spices	71	71	71	71	71
Wheat flour	100	75	50	25	-
Yacon flour peel	-	25	50	75	100
Total per lot	1766 g	1766 g	1766 g	1766 g	1766 g

<sup>a</sup>: Yacon flour peel (% in wet weight of wheat flour); <sup>b</sup>: Nitrite salt = 6 g of sodium nitrite

$$WRC(\%) = 100 - (\%) \text{ free water} \quad (3)$$

**Proximate composition measurement:** The proximate composition analysis of the frankfurter type sausage, including dry matter, was determined (AOAC (1990), method 934.06), ash content AOAC (1990), method 942.05) in muffle (Fischer Scientific 550-58, USA), protein percentage (Kejhdal Method, AOAC (1990) method 32.1.22) and carbohydrates by difference of proximal analysis.

**pH measurement:** pH was measured using a digital pH meter (MP 230, Mettler Toledo, Switzerland) (Dzudie *et al.*, 2002) with a 10% buffer solution (w/v) of distilled water. In addition, solution was homogenised in a blender (1 min at 1900 rpm) using a polytron homogenizer (T25-B, IKA, Malaysia).

**Color measurement:** Color was assessed on the cut surface of the cooked sausages. Conversely, three replicate measurements were taken and results were expressed as CIE (Commission Internationalde l'Eclairage) L\*, a\* and b\* using colorimeter (Konica Minolta Model: CR-400, Osaka, Japan). Illuminant D65 (8 mm diameter measurement area) and 10° observer was used as reference. Therefore, the instrument was calibrated with a white ceramic plate ( $Y = 89.5$ ;  $x = 0.3176$ ;  $y = 0.3347$ ) (Fiorda *et al.*, 2015). For each treatment, three cylindrical samples, 5 cm long, were analyzed where a longitudinal miced meat was performed in order to analyze internal sausage color. In addition, tone angle ( $h^\circ$ ) and chroma using a Minolta Chromameter CR-300 (Minolta Co., Japan) or saturation index ( $C^*$ ), was determined following Eq. (4) and (5):

$$h^\circ = \text{Tan}^{-1} \left( \frac{b^*}{a^*} \right) \quad (4)$$

$$C^* = (a^{*2} + b^{*2})^{0.5} \quad (5)$$

**Texture Profile Analysis (TPA):** TPA was performed using an Universal Shimadzu texturometer (Tester EZTest EZ-S) for which, miced sausage of 15 mm thick were properly sliced. Samples were placed among two axial parallel plates, which were axially compressed to 50% of their original height (50% deformation) at a speed of 60 mm/min with a cylindrical aluminum flat compression probe, no downtime among two compressions. Results were used to analyze the hardness effort (Newton: maximum force required for the first compression), cohesiveness (dimensionless: the reason for the positive area under the curve during the second compression and area under the curve of the first compression) elasticity (mm distance traveled from the sample after the first compression), chewiness (N mm: hardness \* cohesiveness \* elasticity), stickiness (N mm: it is the area of the negative force and represents the work necessary to pull or take off from piston

compressor away from the sample) and gumminess (N: hardness \* cohesiveness) (Savadkoochi *et al.*, 2014).

**Statistical analysis:** The experiment had achieved random mixtures design with two replicates. Response variables were evaluated in triplicate. Data was analyzed by ANOVA procedures. To identify significant difference among treatments and statistical significance for all comparisons was made at  $p < 0.05$ . Tukey's multiple range test was used to compare the mean values of treatments. In addition, MINITAB software version 17TM was used.

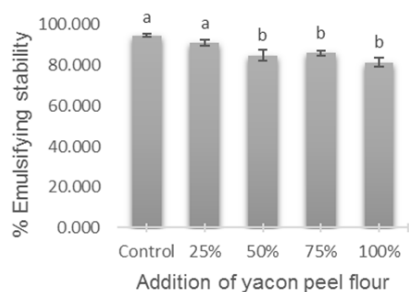
## RESULTS AND DISCUSSION

**Emulsion stability:** Figure 1a, shows emulsion stability of the meat masses corresponding to different treatments. In the meat mass, statistical differences were not presented when replacing 25% of WF by YPF, compared to control sample, however, other formulations showed minimum significant reduction ( $p < 0.05$ ).

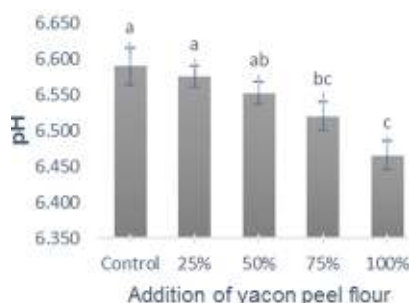
A decreasing in the emulsifying stability is explained by the difference in protein content between flours (6.13% protein on dry base for YPF compared to WF 13.74% protein on dry base). In addition, even though dietary fiber of YPF (53.77% dry base) far exceeds the WF (3.91%), the fiber exerted no positive effect on the emulsifying stability (Chinma *et al.*, 2016; Pereira *et al.*, 2013; Almendras and Kirigin, 2010).

It was concluded that is feasible to replace 25% of WF by YPF, without affecting significantly emulsifying stability of the meat mass of Frankfurter type sausages. As indicated above, the scientific literature reports no investigations where yacon peels are used, therefore, our results cannot be compared. However, as an illustration (Choe *et al.*, 2013), are highlighted due to had achieved an increasing in emulsifying stability when replacing fat by a mixture of pork skin and wheat fiber in Frankfurter type sausages.

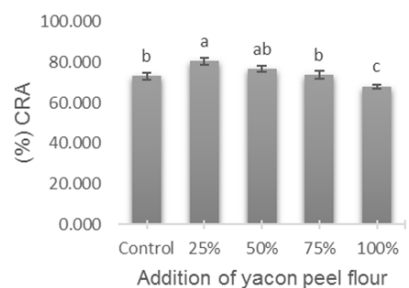
**pH:** Figure 1b, shows the addition effect of YPF on the sausages pH. pH values decreased significantly ( $p < 0.05$ ) compared to control sample, when overcoming the concentration of 50% in YPF incorporation. This result is because YPF presented lower pH values (6.04) compared to WF (6.56 - 6.83). Therefore, by increasing the amount of YPF in the formulation, final product showed lower pH values, lower than the control sample (Pereira *et al.*, 2013; Almendras and Kirigin, 2010). pH values in meat products are regarded as an intrinsic factor that directly affects the growth of microorganisms. Therefore, it could be said that pH for both the control sample and for other treatments represents a high risk in the growth of bacteria, yeasts and molds (Arief *et al.*, 2014). Dzudie *et al.* (2002) reported an increasing in pH in beef sausages with the addition of bean flour as extensor.



(a)



(b)



(c)

Fig. 1: Effect YPF added on (a) Emulsion stability, (b) pH, (c) Water Retention Capacity (WRC) in meat mass for elaboration of Frankfurter type sausages  
Data with different letters for each column represent significant difference at  $p < 0.05$

**Water retention capacity:** In meat products the ability to retain water before and after cooking is an important factor since this variable depends on the product juiciness and usefulness of the process (López-Vargas *et al.*, 2013; Savadkoochi *et al.*, 2014). Water retention capacity is defined as the rate of moisture retained in the sample compared to the initial moisture content, therefore, a higher percentage of water released, a lower moisture content will have the sample. Water retention is composed of the sum of bound water, hydrodynamic water and trapped physically water,

Figure 1c, shows the results of partial effect addition of YPF on water retention capacity of the sausages. It was evident that when increasing the concentration from 25 to 50% of YPF, there was

significant increasing ( $p < 0.05$ ) of 6% in water retention capacity, compared to control treatment. Treatments that used 75% of YPF showed no significant effect ( $p > 0.05$ ) in water retention capacity, compared to control treatment. This is explained by proximal composition of flours, YPF showed a higher dietary fiber content (53.77%) compared to WF (3.91%). It is well known that dietary fiber has the ability to bind water and for that feature, YPF could be used as a food ingredient, with the potential of increasing yields and minimize manufacturing costs (Pietrasik and Janz, 2010).

In relation to water retention capacity, results indicated that in the formulation of Frankfurter type sausages, it is feasible to replace even a 75% of WF by YPF, without presenting a significant effect with control treatment. Dzudie *et al.* (2002), reported a significant increasing in water retention capacity for different concentrations of bean flour in sausage formulation. Likewise, Ayadi *et al.* (2009) reported an increasing in water retention capacity by introducing carrageenan in the manufacture of turkey sausage and these phenomena are mainly due to the presence of macromolecules such as polysaccharides and fibers.

**Color:** Table 2, shows the color values in sausage with and without YPF addition. Coordinates of brightness ( $L^*$ ) and red-green ( $a^*$ ) are the most informative parameters for color change in sausages (Savadkoochi *et al.*, 2014).

Treatments with YPF presented brightness values ( $L^*$ ) ( $p < 0.05$ ) significantly lower compared to control sample and this is because flour has a brown hue when diluted with water. Savadkoochi *et al.* (2014), when incorporating tomato bagasse in 7% (p/p) in the formulation of frankfurter type sausage, showed  $L^*$  values lower than control samples.

Sausages with YPF showed significant reduction in the coordinate  $a^*$ , when exceeding 50% concentration and this is due to tonality of YPF, which was brown. Sanjeeva *et al.* (2010), reported reduction in the coordinate  $a^*$  by adding 5% of chickpea seed flour variety Desi as linker in bologna type mortadella. Values of the coordinate  $b^*$  showed no significant effect ( $p > 0.05$ ) at all concentrations.

Sausages with YPF showed a significant increasing in hue angle compared to control sample, showing a variation from 41.11 to 52.96 indicating a red color in control sample and yellow ochre color in all samples, which were evaluated with YPF replacement. This coloration phenomenon in sausages is due to the values of the coordinate  $b^*$  (yellow) remained stable and a decreasing in values of coordinate  $a^*$  (red). Similar considerations apply to the saturation index.

In sausages production, YPF addition significantly affected all parameters of color which plays a key role in this type of products, which have allowed a quality

Table 2: Color values in sausage with or without YPF added (Mean±S.D.)

Addition YPF (%)	Illuminate A				
	L*	a*	b*	Tone angle	Saturation index
Control, 0	62.69±0.49 <sup>a</sup>	10.60±0.07 <sup>a</sup>	10.66±0.31 <sup>a</sup>	44.11±2.20 <sup>c</sup>	15.03±0.27 <sup>ab</sup>
25	58.35±0.98 <sup>b</sup>	10.25±0.76 <sup>ab</sup>	11.56±0.13 <sup>a</sup>	48.50±1.95 <sup>bc</sup>	15.46±0.56 <sup>a</sup>
50	54.40±0.08 <sup>c</sup>	9.87±0.20 <sup>ab</sup>	11.33±0.35 <sup>a</sup>	51.00±2.29 <sup>ab</sup>	14.58±0.09 <sup>ab</sup>
75	55.04±0.75 <sup>c</sup>	9.17±0.46 <sup>bc</sup>	11.23±0.40 <sup>a</sup>	52.96±0.66 <sup>a</sup>	14.07±0.45 <sup>b</sup>
100	54.56±1.13 <sup>c</sup>	8.47±0.26 <sup>c</sup>	10.56±0.80 <sup>a</sup>	46.81±1.79 <sup>bc</sup>	14.48±0.69 <sup>ab</sup>

L\*: 0 = black y 100 = white; a\*: -60 = green y + 60 = red; b\*: -60 = blue y + 60 = yellow; Tone angle: 90° = yellow, 180° = green y 0° = red; Saturation index: distance from the coordinates in the origin to the determined color point; <sup>a-c</sup>: Averages in the same column with different superscripts are significantly different (p<0.05)

Table 3: Effect on the addition of YPF in sausages proximal composition

Sausages (%)	Mean±D.S (%)			
	Dry matter	Protein	Carbohydrates	Ashes
Control, 0	36.81±0.49 <sup>c</sup>	29.71±0.05 <sup>a</sup>	0.20±0.01 <sup>c</sup>	8.41±0.10 <sup>a</sup>
25	43.62±0.13 <sup>a</sup>	24.26±0.23 <sup>bc</sup>	0.36±0.01 <sup>bc</sup>	7.92±0.07 <sup>a</sup>
50	43.02±0.30 <sup>a</sup>	25.69±0.31 <sup>b</sup>	0.63±0.02 <sup>b</sup>	8.50±0.12 <sup>a</sup>
75	41.50±0.08 <sup>b</sup>	23.00±0.42 <sup>c</sup>	0.69±0.02 <sup>b</sup>	8.45±0.29 <sup>a</sup>
100	42.61±0.04 <sup>a</sup>	24.28±0.79 <sup>bc</sup>	1.30±0.23 <sup>a</sup>	7.94±0.01 <sup>a</sup>

YPF: Yacon peel flour; <sup>a-d</sup>: Averages in the same column with different superscripts are significantly different (p<0.05)

Table 4: Texture analysis in the sausages with the addition of YPF

Sausages (%)	Media±D.S					
	Hardness (N)	Elasticity	Cohesiveness	Adhesiveness (N.mm)	Chewiness (N.mm)	Stickness (N)
Control, 0	37.60±1.15 <sup>a</sup>	0.24±0.02 <sup>d</sup>	0.34±0.06 <sup>a</sup>	-0.66±0.08 <sup>a</sup>	3.25±0.62 <sup>ab</sup>	13.08±0.27 <sup>a</sup>
25	28.32±2.08 <sup>b</sup>	0.41±0.04 <sup>bc</sup>	0.30±0.02 <sup>a</sup>	-0.52±0.09 <sup>a</sup>	3.58±0.17 <sup>ab</sup>	8.77±1.32 <sup>b</sup>
50	24.90±0.80 <sup>c</sup>	0.52±0.02 <sup>a</sup>	0.31±0.03 <sup>a</sup>	-0.49±0.04 <sup>a</sup>	4.32±0.78 <sup>a</sup>	8.17±1.10 <sup>b</sup>
75	22.54±0.66 <sup>c</sup>	0.42±0.02 <sup>b</sup>	0.31±0.02 <sup>a</sup>	-0.44±0.12 <sup>a</sup>	2.88±0.27 <sup>b</sup>	6.71±0.32 <sup>b</sup>
100	22.76±0.97 <sup>c</sup>	0.33±0.01 <sup>c</sup>	0.30±0.03 <sup>a</sup>	-0.45±0.02 <sup>a</sup>	2.42±0.39 <sup>b</sup>	7.14±1.12 <sup>b</sup>

YPF: Yacon peeling flour; <sup>a-b</sup>: Averages in the same column with different superscripts are significantly different (p<0.05)

parameter associated with the final product acceptance by consumers (Afoakwah *et al.*, 2015).

**Proximal composition:** Mean values of the sausages proximal composition with YPF addition are observed in Table 3.

When Including 25% of YPF in the formulation, a significant increasing (p<0.05) approximated to 7% was observed in dry matter. Results are in concordance to those reported by Choe *et al.* (2013), where is reported that an increasing concentration of oat bran in chicken Frankfurter type-sausages, performed an increasing in dry matter content.

Protein content in sausages, performed a significantly decreasing (p<0.05) compared to control sample in 6.7%, when replacing the entire WF. As a result, it has long been thought that YPF provides lower protein percentage compared to WF. These results are comparable in variability to the report by Dzudie *et al.* (2002), where an introduction of bean flour as extensor in the sausages processing have allowed a decreasing in protein content for the final product. Moreover, Savadkoohi *et al.* (2014), reported a significant increasing in the protein content of beef sausages by inclusion of tomato bagasse in sausage formulation.

Nevertheless, the carbohydrate content in sausages with YPF increased significantly (p<0.05) on average 6.5 times when 100% WF replacement had achieved

with respect to control sample. An increased carbohydrate content is explained by fructo-oligosaccharides content in yacon peel (Santana and Cardoso, 2008). It is important to note that a meat sausage, Premium category, must contain at least 3% of carbohydrates. Therefore, sausages made with YPF are in this category. Savadkoohi *et al.* (2014) reported a 56.5% increasing in carbohydrate content by following an introduction of 7% in weight of tomato peel bagasse, in beef Frankfurter type-sausages.

However, the ash content showed no significant effect (p>0.05) in YPF increased concentration in the formulation. Choe *et al.* (2013) and Yetim *et al.* (2006), reported no significant effect on ash content by whey incorporation and wheat fiber in Frankfurter type-sausage processing.

**Texture profile:** Results of hardness, cohesiveness, elasticity, chewiness, adhesiveness and gumminess of the cooked sausages with addition of WF and YPF are shown in Table 4.

No significant effect (p>0.05) was observed in the parameters of cohesion and adhesiveness when have allowed a WF replacement by YPF. However, YPF addition significantly affected other texture parameters. Regarding the hardness, control sausage showed the highest value and as YPF amount was increased, hardness of the product decreased significantly

( $p < 0.05$ ). Das *et al.* (2008), reported an increasing in the sausages hardness when moisture content of the final product is decreased.

Calvo *et al.* (2008) and Savadkoobi *et al.* (2014), reported an increasing in hardness by increasing the concentration of tomato peel and bagasse in fermented Frankfurter type sausages. This phenomenon was mainly attributed to the presence of acid detergent fiber (30/100 g of dry matter) and protein (24.67% p/p) in these sub-products. Therefore, an increasing in fiber content and protein in sausages can modify the texture properties profile generating a harder and less moisture content product. Indeed, is considered that reducing the hardness is a negative aspect as this parameter, which is decisive in the final product acceptance by consumers.

Elasticity in the sausage showed a significant increasing ( $p < 0.05$ ) in YPF concentration compared to control sample, this increasing is mainly due to an increasing of the sausages dry matter (Savadkoobi *et al.*, 2014). Calvo *et al.* (2008) reported no significant changes in the elasticity of fermented sausages enriched with lycopene from tomato peel. Similarly, Savadkoobi *et al.* (2014), observed the same behavior in elasticity values in beef sausages when introducing tomato bagasse.

Despite having a significant effect ( $p < 0.05$ ) of sausages chewiness when is performed YPF, had achieved an increasing concentration in relation to control sample. The results had achieved an introduction of 6% flour in total sausages formulation. Choe *et al.* (2013) reported in their study a fat replacement by wheat fiber with chewiness parameter, which increased through a concentration of 15% of such mixture.

## CONCLUSION

This study evaluated the effects of WF replacement with YPF. Results demonstrated that it is feasible to replace 25% of wheat flour by yacon peel flour, without significantly affecting emulsion stability of the sausages meat mass or water retention capacity. Given these concerns, sausages color and hardness are affected by the inclusion of this agroindustrial residue in the sausage formulation. In relation to water retention capacity, results indicated that is possible a 75% WF replacement by sausages with added YPF, which had achieved a higher water retention capacity (6%). In addition, color is affected by yacon peel flour incorporation. Frankfurter type-sausages cohesiveness and adhesiveness did not present significant difference among them (YPF and WF).

## ACKNOWLEDGMENT

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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