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RESEARCH ARTICLE

Evaluation of coffee pulp as substrate for polygalacturonase production in solid state fermentation

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ABSTRACT

The purpose of this research is to evaluate the coffee pulp, a by-product of coffee processing, as substrate for polygalacturonase production by solid state fermentation. In addition, it is a way to take advantage of the coffee pulp. Characterization of the coffee pulp revealed a high content of nutrients for fungi growth, such as reducing sugars (5.4% of dry pulp), proteins (9.4% of dry pulp), pectins (20.5% of dry pulp), which are inducers of pectic hydrolases production and source of carbon after degradation, and caffeine (1.4% of dry pulp), among others. The characterization of the cell-wall of coffee pulp revealed, after polysaccharides fractionation, the content in cell-wall pectins (25.5%), hemicelluloses (11.5%) and cellulosic residue (44%). A strain of *Aspergillus niger*, called van Thiegem, has been selected as a good producer of polygalacturonases (60 U/ml) using the coffee pulp as the growth substrate. During fermentation, reducing sugars, caffeine and phenolic compounds were consumed till almost exhaustion. A partial characterization of the polygalacturonase using high methoxyl pectin as substrate indicates an optimal pH of 4.0 and 45°C as optimal temperature, which are good values for the use of the enzyme in vegetable processing, including coffee processing.

Keywords: Coffee; Pulp; *Aspergillus*; Fermentation; Polygalacturonase

INTRODUCTION

The coffee industry occupies a predominant place in the economy of many countries in Latin America, Africa and Asia. This industrial activity also generates abundant by-products, such as pulp, mucilage, wash water and parchment (Alves et al., 2017). The availability of these by-products in large quantities leads to a problem of environmental contamination (Echeverria and Nuti, 2017). The world production of coffee was 125 million 60 kg bags (International Coffee Organization, 2020) in 2019, which indicates that these by-products are in a very important amount, being a problem their elimination.

Coffee pulp is one of the main by-products of coffee humid processing, because of the amount that it is generated (approximately 40% f.w.), and because of its high content of bio-compounds (Padmapriya et al., 2013). Coffee pulp has a high humidity (80-82%) and is rich in carbohydrates, proteins, minerals, tannins, pectins, caffeine and phenols (Echeverria and Nuti, 2017). Caffeine, tannins and some phenolics such as chlorogenic acid are of ecotoxicological concern and can limit their value-adding applications (Janissen and Huynh, 2018).

Coffee pulp has been used as a supplement in animal feed (Pedraza-Beltrán et al., 2012; Padmapriya et al., 2013), to produce pellets for heat generation (Cubero-Abarca et al., 2014), to obtain organic fertilizers and composts (Woldesenbet et al., 2015), bioethanol (Gurram et al., 2016), biogas (Juliastuti et al., 2018) and phenolic compounds (Geremu et al., 2016; Serna-Jiménez et al., 2018; Santos da Silveira et al., 2019). It is an interesting point of research the degradation of coffee pulp by microorganisms (Parani and Eyini, 2012; Kandasamy et al., 2016; Mata et al., 2016) for production of proteins and hydrolytic enzymes, particularly pectic enzymes and related (Venugopal et al., 2007; Murthy et al., 2009; Nava et al., 2011; Murthy and Naidu, 2012a; Oumer and Abate, 2018). Pectic enzymes are applied in the process of coffee

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benefit, specifically in the stage of fermentation of the grain (Murthy and Naidu, 2011). Pectic enzymes are also applied in the food industry for processes related to the clarification and extraction of fruit juices and oenological treatments (Kashyap et al., 2001; Tapre and Jain, 2014; Apolinar-Valiente et al., 2017). At the same time that pectic enzymes are produced by fermentation, the coffee pulp is transformed and detoxified (Mazzafera, 2002; Londoño-Hernandez et al., 2020), being also able for some of the uses mentioned above (Murthy and Naidu, 2012b). There is still need for research in alternative and profitable uses of coffee pulp (Oliveira and Franca, 2015) and, for that reason, the use of the pulp for the production of pectic enzymes by solid state fermentation requires further research. In this sense, the objective of this work was the evaluation of the coffee pulp as substrate for polygalacturonase production in solid state fermentation through a study of the physicochemical characteristics of the coffee pulp and its structural cell wall polysaccharides, together with the establishment of the biochemical characteristics of the produced polygalacturonase by an *Aspergillus niger* strain isolated from the same coffee pulp and coffee bean.

MATERIALS AND METHODS

Coffee pulp

The fresh pulp of coffee (*Coffea arabica*) was from the humid benefit of coffee, from a pulper in the eastern zone of the III Front, Santiago de Cuba (Latitude: 20°1' N, Longitude: 75°49' W), Cuba, dehydrated to the Sun for three days (Fig. 1). The mature coffee beans come from La Mandarina coffee plantation, also from Santiago de Cuba.

Fig 1. Sun dry coffee pulp from Santiago de Cuba, Cuba (20°1' N, 75°49' W).

Physicochemical analysis of the fresh and fermented coffee pulp

Humidity. One gram of sample is placed in an oven for 24 h at 105ºC. The % moisture being calculated by the weight difference before and after drying.

pH. To 5 g of the solid sample add 30 ml of distilled water and stir for 30 min. The suspension is then filtered through Whatman # 1 paper, the pH being measured in the filtrate.

Total nitrogen and crude protein. The total nitrogen and the crude protein were analyzed according to the Kjeldahl method (A.O.A.C., 1995) in a Büchi digester. The result is expressed as % nitrogen which, multiplied by 6.25, gives the crude protein % of dry pulp.

Total fat. The extraction of total fat is done using the Shoxlet method (A.O.A.C., 1995). The result is expressed as total fat % of dry pulp.

Reducing sugars. Reducing sugars were analyzed using the spectrophotometric method of Nelson (1944). The result is expressed as sugars % of dry pulp.

Caffeine. Caffeine was analysed according to Schufen et al. (1990). The result is expressed as caffeine % of dry pulp.

Total phenols. The total phenolic compounds were analysed by the Folin-Ciocalteu method (AOAC, 1995). The result is expressed as total phenols % of dry pulp.

Isolation and fractionation of the Alcohol Insoluble Solids

The dry coffee pulp was used to prepare the alcohol-insoluble solids (AIS; Apolinar-Valiente et al., 2010). Then, the AIS were sequentially extracted (Coll-Almela et al., 2015) yielding the coffee pulp cell wall polysaccharides: pectin soluble in chelating agents (ChSS), pectin soluble in dilute alkali (DASS), hemicellulose soluble in one molar sodium hydroxide (1MASS), hemicellulose soluble in four molar sodium hydroxide (4MASS), and a cellulose-rich residue (the Residue fraction).

Analysis of carbohydrate content in the polysaccharide fractions

Neutral sugars were determined by GLC after pre-treatment (30°C, 1 hr) with aq 72% sulphuric acid followed by hydrolysis with 1 M sulphuric acid (100°C, 3 hrs) and conversion of the products into alditol acetates (Ros et al., 1996). Uronic acids were determined in the sulphuric acid hydrolysate by the colorimetric 3,5-dimethylphenol assay (Scott, 1979). Results were expressed in mol % and % w/w. The degree of methylation (DM %) and acetylation (DA %) were determined by HPLC (Ros et al., 1996).

Average molecular weight determination of the polysaccharides

The average molecular weight determination was established by high performance size exclusión chromatography (HPSEC) using Ultrahydrogel columns (Waters) and refractive index detector (Ros et al., 1996). The calibration of the HPSEC system was performed according to Schols et al. (1990), using pectins as molecular weight standards.

Isolation of the strain of Aspergillus

Isolation of Aspergillus strains. It is made from mature coffee beans and fresh coffee pulp. The medium used was described by Gaime Perraud et al. (1993): pectin 2 g l^{-1}, ammonium sulphate 0.15 g l^{-1}, urea 0.05 g l^{-1}, chloramphenicol 0.25 g l^{-1}, agar 20 g l^{-1}, pH 5.5. The isolation was carried out by the direct seeding method and by the dilution method (Casadesús and Rojas, 1981).

Purification of strains. It is carried out by means of the method of dilutions in Agar and sowing by exhaustion, using the medium Agar-Sabouraud-Dextrose and direct sowing.

Taxonomic identification. Cultures are used on the media Agar-Czapek and Agar-Extract of Malta, making macroscopic and microscopic observations of the morphology of the crops.

Solid state fermentation

The coffee pulp is mixed with a solution of urea, ammonium sulphate and potassium phosphate, having a final humidity of 60% and pH 5.5. Then, three columns of 20 x 3 cm were loaded with the prepared pulp and placed in vertical position (Raimbault and Alazard, 1980; Loera et al., 1999; Brand et al., 2001). A stream of 100% humid air feeds continuously the column from the bottom to the top. At time intervals a sample of fermented pulp was taken and analyses were carried out as described previously and also as follows for the enzymes.

Extraction of the enzymes

For the extraction of the enzymes, an aqueous extraction was carried out. To one part of the fermented coffee pulp, six parts of water were added. Then the suspension was stirred for one hour and pressed in a laboratory press. The extract was filtered using a Whatman # 1 filter and then centrifuged at 6000 *g* x 10 min. The enzyme activity was measured in the supernatant, which was kept at 4°C.

Polygalacturonase activity determination

Polygalacturonase refers to the total enzymatic activity in the extract that contributes to the release of reducing sugars from polygalacturonic acid or high methoxyl pectin as the enzyme substrate (Ros et al., 1991). The polygalacturonase was assayed by measuring the increase in reducing end groups (Nelson, 1944). The polygalacturonase activity was expressed in enzyme units per ml of extract.

Experimental design and data processing

The extraction of the cell-wall material and its fractionation into polysaccharide fractions was carried out once. The fermentation was carried out three times in the same conditions (three replicates). All the other analyses were done at least three times each. Then, average values and coefficient of variation were calculated using Statistix 8 for Windows from Analytical Software (Tallahassee, FL, USA).

RESULTS AND DISCUSSION

Characteristics of the dry coffee pulp

The main characteristics of the coffee pulp are shown in Table 1. Being a pulp dried under the Sun, still contains 11% of humidity, which is in agreement to the 11.6% of humidity reported by Londoño-Hernandez et al. (2020) in an also three days Sun dried coffee pulp from Mexico. It has been reported values of crude protein of 8% (Zuluaga-Vasco, 1989), 12% (De Acosta et al., 1997), 13.2% (Munguía Ameca et al., 2018) and 13.4% (Londoño-Hernandez et al., 2020), being our value (9.4%) in agreement with them and close to the 10% reported by Woldesenbet et al. (2015). The same for the value of caffeine (1.4%), since Porres et al. (1993) reported a content of caffeine in coffee pulp of 1.3%, Zuluaga-Vasco (1989) 0.8%, Juliastuti et al. (2018) 0.9%, while Woldesenbet et al. (2015) reported 2.3% and Munguía Ameca et al. (2018) the highest (3.0%). The content of pectins in the coffee pulp used was 20.5% (after AIS fractionation, see below), being higher than the 6.5% reported by Pulgarin et al. (1991) and also by Woldesenbet et al. (2015), and the 2.3% reported by Juliastuti et al. (2018). Hemicelluloses and cellulosic

Table 1: Physicochemical characteristics of dry coffee pulp

Parameter	% Of dry pulp (Average value*)
pH	5.5
Dry matter	89
Humidity	11
Total nitrogen	1.5
Proteins (N x 6.25)	9.4
Caffeine	1.4
Total phenols	0.3
Reducing sugars	5.4
Fats	0.8
***Average of three**	**Analysis (cv < 5%)**
Pectin**	20.5
Hemicellulose**	9.2
Cellulose**	35.6
****From AIS**	**fractionation**
Total analysed dry matter	84.1

residue were 9.2 and 35.6%, respectively, also after AIS fractionation. Juliastuti et al. (2018) reported a different content in hemicelluloses (21.6%) and cellulose (57.9%). Differences in polysaccharides are due to the different origin and treatments of the coffee pulp (Juliastuti et al., 2018). The amount of reducing sugars (5.4%) is very low compared to the 50% reported by Pandey et al. (2000), 44% by Porres et al. (1993) and 51% by Braham and Bresani (1979). The reason could be that our pulp was three days drying under the Sun and microorganisms degraded the reducing sugars, resulting in a lower amount, and that these authors included the monomers of the structural polysaccharides in the reducing sugars, resulting in a higher amount. Others authors (Woldesenbet et al., 2015; Gurram et al., 2016) reported a lower content of reducing sugars (12.4% of fresh mass and 40% of dry mass). The content of total phenolic compounds (0.3%) was lower than the amount reported by Woldesenbet et al. (2015) of caffeic acid (1.6%) and chlorogenic acid (2.6%), lower than the amount reported by Munguía Ameca et al. (2018) of ferulic acid (0.4%), caffeic acid (0.5%) and chlorogenic acid (0.5%), and also lower than the total phenolic compounds content (3.5%) reported by Juliastuti et al. (2018). The content in fats (0.8%) is a bit higher to the 0.5% reported by Woldesenbet et al. (2015), and lower than the 1.6% of Londoño-Hernandez et al. (2020) and the 1.7% of Munguía Ameca et al. (2018). Total analysed dry matter was 84.1% (Table 1), being the constituents not analysed lower than 5%, on the basis of a dry matter content of 89%.

Extraction and characterisation of the dry coffee pulp cell wall as alcohol insoluble solids

The analysis of neutral sugars and uronic acids in the alcohol insoluble solids extracted from the coffee pulp, allows us to know in a preliminary way the fractionation of the polysaccharides of the cell wall of the coffee pulp, the constituent sugars of the alcohol insoluble solids. This allows to obtain a previous information about the content of the polysaccharidic sugars of the coffee pulp and, in this way, to evaluate the possibility of its use as substrate-support in a solid state fermentation. From 100 g of dry coffee pulp, 81 g of alcohol insoluble solids were obtained.

The main carbohydrate components of the dry coffee pulp AIS were glucose (33 mol%), galacturonic acid (28 mol%) and arabinose (16 mol%), accompanied by smaller amounts of galactose, xylose, mannose and rhamnose (Table 2). The carbohydrates accounted for 60.4% of the AIS, while the remainder probably included protein, lignin, phenolic compounds and some moisture. In this research, only the carbohydrate part was studied. On average, the galacturonic acids present in the AIS were not much methylesterified (DM 23%, Table 3), while, based on the galacturonic acid content, a low degree of acetyl substitution existed (DA 12%). To the best of our knowledge, the sugar composition of the AIS (cell-wall) from coffee pulp has not been reported before. Thus, it can no be compared with the composition of the AIS from other coffee pulps. The sugar composition of the AIS from coffee pulp suggests the presence of pectins, hemicelluloses and cellulose as polysaccharides constituents of the AIS. These are the normal structural polysaccharides found in plant cell-walls (Ros et al., 1996), which was confirmed and quantified by the following AIS fractionation into structural polysaccharides.

The sugar contents of coffee pulp reported by Gurram et al. (2016), expressed as percentages of dry mass, were as follows: arabinose 5.8%, galactose 5.2%, glucose 20.2%, xylose 4.2%, and mannose 4.7 %. In our coffee pulp AIS, the same sugars were as follows: arabinose 8.0%, galactose 3.5%, glucose 20.5%, xylose 5.6%, and mannose 2.6 %. There is some similarity, although differences are due to the different origin and treatments of the coffee pulp (Juliastuti et al., 2018).

Isolation and characterisation of pectin fractions

Fractionation of the dry coffee pulp AIS was carried out using non-destructive extractants. All buffer-soluble pectins and Ca^{2+}-complexed pectins were isolated in one single fraction (ChSS), representing 18.5% of the AIS (14.9% of the coffee pulp). Extraction with 50 mM sodium hydroxide at 0 °C, which

Table 2: Carbohydrate composition (mol% (% w/w)) of Alcohol Insoluble Solids (AIS) and polysaccharides from AIS fractionation isolated from dry coffee pulp

Carbohydrate*	AIS	ChSS	DASS	1MASS	4MASS	Residue
Rhamnose	3 (1.4)	7 (1.5)	4 (0.3)	2 (0.8)	1 (0.6)	3 (1.3)
Fucose	nd	nd	nd	nd	nd	nd
Arabinose	16 (8.0)	20 (4.0)	15 (1.1)	12 (5.2)	23 (14.8)	19 (8.3)
Xylose	11 (5.6)	2 (0.5)	3 (0.2)	21 (9.2)	12 (7.7)	9 (4.1)
Mannose	4 (2.6)	8 (2.1)	5 (0.4)	10 (5.1)	14 (11.3)	1 (0.6)
Galactose	6 (3.5)	14 (3.5)	8 (0.7)	9 (5.0)	11 (8.5)	4 (2.1)
Glucose	33 (20.5)	9 (2.2)	6 (0.6)	20 (10.5)	24 (19.1)	46 (25)
Galacturonic acid	28 (18.8)	39 (10.5)	60 (5.9)	27 (15.9)	16 (14.3)	18 (10.9)
TOTAL	100 (60.4)	100 (24.4)	100 (9.1)	100 (51.6)	100 (76.3)	100 (52.3)

AIS: Alcohol Insoluble Solids, ChSS: Pectin extracted using chelating agents, DASS: pectin extracted using diluted sodium hydroxide, 1MASS: Hemicellulose extracted using 1M sodium hydroxide, 4MASS: Hemicellulose extracted using 4M sodium hydroxide, *Average of three analysis (cv < 5%). nd: not detected

Table 3: Degree of methylation, degree of acethylation and average molecular weight distribution, when analysable, of Alcohol Insoluble Solids (AIS) and polysaccharides from AIS fractionation isolated from dry coffee pulp

Parameter*	AIS	ChSS	DASS	1MASS	4MASS	Residue
Degree of methylation (%)	23	35	32	nd	nd	nd
Degree of acethylation (%)	12	9	7	nd	nd	nd
Average molecular weight (kda)	-	60	80	80	70	-

AIS: Alcohol Insoluble Solids, ChSS: Pectin extracted using chelating agents, DASS: pectin extracted using diluted sodium hydroxide, 1MASS: Hemicellulose extracted using 1M sodium hydroxide, 4MASS: Hemicellulose extracted using 4M sodium hydroxide, *Average of three analysis (cv < 5%). nd: not detected

triggered ester removal and detachment of hydrogen bonds, resulted in the solubilisation of 6.9 % of the AIS (5.6% of the dry coffee pulp). Pectin represented 25.4% w/w of the dry coffee pulp AIS and 20.5% w/w of the dry coffee pulp.

Table 2 shows the sugar composition of the pectin fractions. The potential polysaccharides in each fraction based on the sugar composition, considering also the type of fractionation carried out, are the non-covalently linked to the cell-wall pectins (ChSS) and the covalently linked to the cell-wall pectins (DASS). Since the sugar content of these fractions was 24-60% (w/w), some non-carbohydrate material, such as salts and proteins, were obviously present, probably due to the resistance of some salts to removal by dialysis. Methyl-esterified galacturonic acid was the predominant sugar residue in the ChSS fraction (39 mol% GalA, DM 35%). The composition of the ChSS fraction suggests that it consists mainly of not much methyl-esterified homogalacturonan regions. The most abundant neutral sugar was arabinose (20 mol%). Other sugars such as glucose and galactose were present only in small amounts. The DASS fractions contained lower neutral sugars than the ChSS fraction. HPSEC analysis (Table 3) showed that the average molecular weight for the ChSS and DASS fraction were 60 and 80 kDa, respectively. With quantitative differences in amounts and composition, a similar result was found for the same pectin fractions in the cell-wall (AIS) of lemon albedo (Ros et al., 1996) and in the cell-wall (AIS) of mandarin segment membrane (Coll et al., 2015).

Isolation and characterisation of the hemicellulose fractions

The residual material after pectin extraction was sequentially extracted using 1 M sodium hydroxide and 4 M sodium hydroxide. The 1MASS fraction represented 2.2% of the dry coffee pulp, while the 4MASS fraction represented 6.9% of the dry coffee pulp. Hemicellulose represented 11.5% w/w of the coffee pulp AIS and 9.2% w/w of the coffee pulp. According with the carbohydrate composition, being the fraction 1MASS extracted as a hemicellulose, it seems to be an intermediate fraction between a pectin very linked to the cell-wall-core and a hemicellulose, since in 1MASS galacturonic acid is still the main carbohydrate (27 mol%), but closely followed by xylose (21 mol%) and glucose (20 mol%). Hemicellulose 4MASS has as main sugars glucose (24 mol%) and arabinose (23 mol%), and important amounts of xylose (12 mol%), mannose (14 mol%), galactose (11 mol%) and still galacturonic acid (16 mol%). HPSEC analysis (Table 3) showed that the average molecular weight for the 1MASS and 4MASS were 80 and 70 kDa, respectively.

Isolation and characterisation of the residue fraction

After extraction with alkalis, an insoluble residue was obtained, which represented 44% of the dry coffee pulp AIS (35.6% of the dry coffee pulp). The main sugar in the residue fraction (Table 2) was glucose (46 mol%) followed by arabinose (19 mol%), galacturonic acid (18 mol%) and xylose (9 mol%). The sugar composition confirms that the residue is a cellulose-rich material. Although the percentage of cellulosic glucose in the residue was not determined, an analogy with other residue fractions from fruit origin (Ros et al., 1996; Coll et al., 2015) would suggest that most of it is cellulosic glucose. However, uronic acids present in the residue are 18 mol%. These uronic acids have survived the alkaline extraction conditions, which suggests that they might originate from a polymer of galacturonic acid and/or glucuronic acid strongly linked to cellulose.

The results on carbohydrates composition of the dry coffee pulp indicate that it could be a good substrate for pectic enzymes (polygalacturonase) production. For this reason next point is to carry-out assays of polygalacturonase production by solid state fermentation.

Production of polygalacturonase by solid state fermentation

The course of the solid substrate fermentation of the coffee pulp for polygalacturonase production is shown in the Fig. 2. The pH, with an initial value of 5.5 (Fig. 2A), changed to 4.5 at the end of the fermentation (days 7-10). There was a continuous increasing in the total proteins (Fig. 2B), which is, indirectly, a measure of the growth of *Aspergillus niger* van Thiegem. Then started a continuous production of the enzyme polygalacturonase (Fig. 2C), with a maximun of activity (60 U/ml) at day 7, and then decreased. Three different compounds were consumed by the fungi for its growth during the solid state fermentation: caffeine, phenolic compounds and reducing sugars. Caffeine decreased during fermentation from initial values of 0.28 (mg/ml) (Fig. 2D) to 0.06 mg/ml at the end of the fermentation (day 9). It is a 79% reduction of caffeine, higher than the 52% reduction of caffeine reported by

Londoño-Hernandez et al. (2020) in fungal detoxification of coffee pulp by solid state fermentation. Phenolic compounds decreased during fermentation from initial values of 1.6 mg/l (Fig. 2E) to 0.7 mg/l at the end of the fermentation (day 10). Reducing sugars decreased during fermentation from initial values of 16 mg/ml (Fig. 2F) to 1 mg/ml at the end of the fermentation (day 10). The general trend of the different fermentation parameters, as shown in the Fig. 2, is in agreement with the fermentation behaviour reported by several authors (Ros et al., 1991; Rodríguez-Fernández et al., 2011; Ruiz et al., 2012). The activity (60 U/ml) produced by the isolated strain of *Aspergillus niger* van Thiegem (measured by the reducing-ends method of Nelson) was of the same order of magnitude to the 28 U/ml (measured by the viscosimetric method) from a pectinase hyperproducing mutant of *Aspergillus niger* in solid state fermentation of coffee pulp (Antier et al., 1993). One viscosimetric unit corresponds, approximately, to two reducing-ends units (Ros et al., 1991).

Partial characterization of the *Aspergillus niger* van Thiegem polygalacturonase

In order to evaluate the applicability of the *Aspergillus niger* van Thiegem polygalacturonase for vegetable processing and other uses, a partial characterization of the enzyme was carried-out. Although polygalacturonase is more active, at the same pH and substrate concentration, on polygalacturonic acid than on pectin, it was decided to use pectin as substrate, since polygalacturonic acid become unsoluble at pH 3.0 and lower, while at pH 2.0, pectin is still soluble. The Figure 3 shows the polygalacturonase activity produced by *Aspergillus niger* van Thiegem on dry coffee pulp vs pH, in the range pH 2.0 – pH 9.0, being the maximun of activity using high methoxyl pectin as the substrate at pH 4.0. Finally, the Figure 4 shows the polygalacturonase activity produced by *Aspergillus niger* van Thiegem on dry coffee pulp vs temperature, being

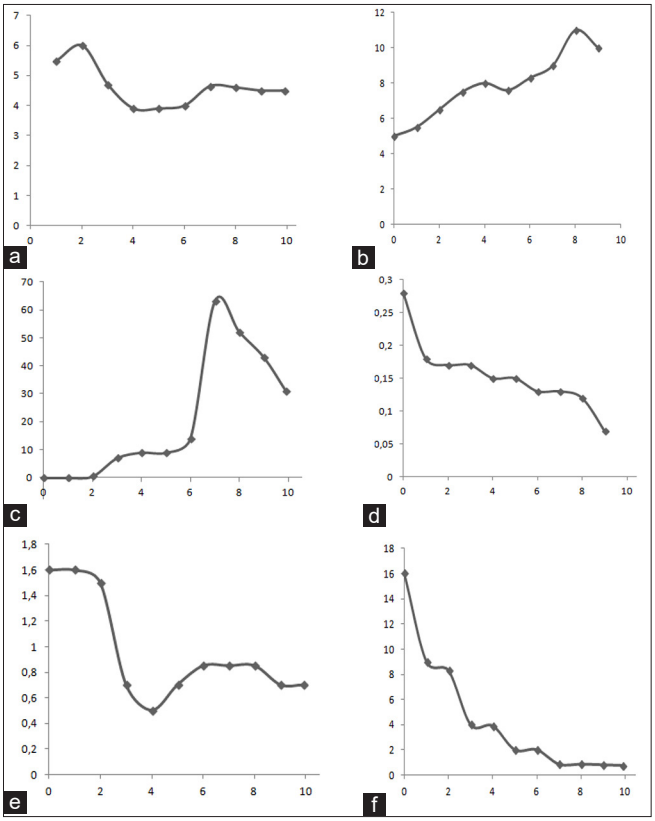

Fig 2. Evolution of pH (A), % total proteins (B), polygalacturonase (U/ml) (C), caffeine (mg/ml) (D), total phenols (mg/l) (E) and reducing sugars (mg/ml) (F) during fermentation of the dry coffee pulp by *Aspergillus niger* van Thiegem. Error bars are not shown in each point of the figure, since for all points (average of three analyses) cv < 5%.

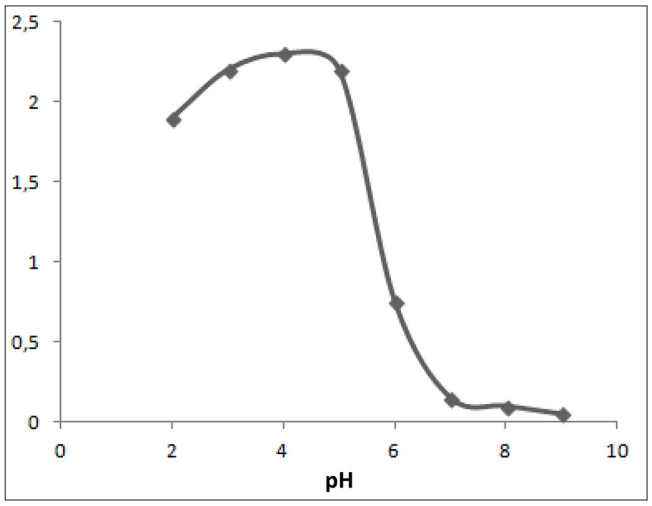

Fig 3. Polygalacturonase (U/ml) activity produced by *Aspergillus niger* van Thiegem on dry coffee pulp vs pH. The maximun of activity using high methoxyl pectin as the substrate is at pH 4.0. Error bars are not shown in each point of the figure, since for all points (average of three analyses) cv < 5%.

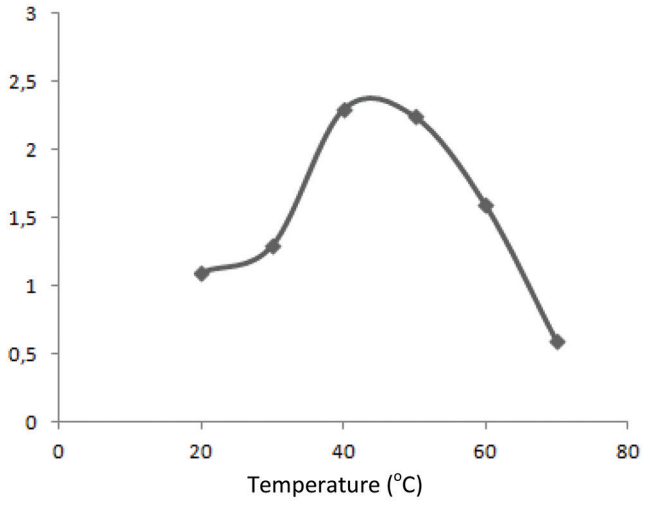

Fig. 4. Polygalacturonase (U/ml) activity produced by *Aspergillus niger* van Thiegem on dry coffee pulp vs temperature. The maximum of activity using high methoxyl pectin as the substrate is at 45oC. Error bars are not shown in each point of the figure, since for all points (average of three analyses) cv < 5%.

the maximun of activity using high methoxyl pectin as the substrate at 45ºC. These results are similar to those found in polygalacturonases from *Aspergillus sp.* (pH 4.5, 40ºC, Buga et al., 2010; pH 4.8, 45ºC, Kant et al., 2013). Other interesting enzymatic characteristics require further research.

CONCLUSION

Due to the chemical composition of dry coffee pulp, which is especially rich in reducing sugars and polysaccharides such as pectins and cellulose, it is a good substrate for pectic enzymes (polygalacturonase) production by solid state cultivation of *Aspergillus niger* van Thiegem. At the same time, a detoxification (caffeine consumption) of the pulp occurs, which converted the fermented pulp in a good material for animal feed, organic fertilizers and compost ingredient. The characteristics of the polygalacturonase indicate the use of the enzyme in vegetable processing, including coffee processing.

Declaration of conflicting interests

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Authors' contributions

This manuscript contains the main results of Rosa Amarilis Rodríguez Frómeta's doctoral thesis, from the University of Havana (Cuba). This doctoral thesis was carried out by Rosa Amarilis at the University of Murcia (Spain), under the direction of Dr. José Laencina and Dr. José María Ros. The manuscript has been prepared by Dr. Amarilis and Dr. José María Ros. Currently Dr. Laencina is retired.

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