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ULTRAVIOLET IRRADIATION AS AN ALTERNATIVE TO PURIFY THE SUGARCANE JUICE FOR SUGAR AND ETHANOL PRODUCTION

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ABSTRACT

The use of non-thermal radiation technology such as ultraviolet can simplify the process of clarification of sugarcane juice. Thus, the objective of this study was to purify the sugarcane juice by ultraviolet irradiation to produce sugar and ethanol. In the laboratory experiment physicochemical analyzes were performed – turbidity, color, pH and °Brix plus microbiological tests. For data analysis was used analysis of variance – ANOVA – followed by Tukey test and the nonparametric test of Kruskall-Wallis, all with significance level 0.05. The power of 250 W and an exposure time of 3 min. are more appropriate, where variations in the °Brix and pH were not significant, color and turbidity were reduced and no fungal contamination accompanied with the reduction of the number of colonies of aerobic and facultative bacteria. With this, it proposes changes to the production of sugar and ethanol production process by eliminating steps of sulfitation, liming, heating and decanting by ultraviolet irradiation.

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INTRODUCTION

Sugar and ethanol industry: The sugar and ethanol industry is one of the national industry sectors that may promote a significant technological development due to major importance their products provide both to the energetic source – ethanol production and surplus of energy through bagasse burn – and great aggregate value the sugar represents in the international market, in addition to ethanol sustainability as fuel (Macedo, 2007). In this context, the global interest on production and consume of biofuels – especially ethanol and biodiesel – has been increasing since the end of the last century (Walter et al., 2011). Partly, this interest was caused by environmental concerns due to needs to reduce greenhouse gases (Balat et al., 2008; Furtado et al., 2011; Liu et al., 2020). In addition to the carbon emissions mitigation, energetic safety and agriculture development are the main responsible for this bioenergetic project (Liu et al., 2020; Ramírez Triana, 2011).

Ethanol may be obtained through fermentation processes of any material that contains sugars or sugar indicative. Saccharomyces cerevisiae is the commonly used yeast for ethanolic fermentation and may ferment large amounts of sugar in the medium, when all nutrients and process conditions are provided in suitable quantities. Other microorganisms, as Leuconostocoenos and Zymomonasmobilis, are also viable for ethanol production (Han et al., 2019; Singh & Bishnoi, 2012; Wheals et al., 1999; Ghorbani et al., 2011). The sugar cane juice is a low-cost source of sugar, and in contrast to other agricultural sub products, do not require hydrolyses (Ghorbani et al., 2011). However, attention should be paid to the delay in juice extraction after sugarcane harvesting, which has been reported as one of the causes of quality changes (Yusof et al., 2000). The just must be clarified after the extraction due to oxidation of some components that may cause negative effects on consumption (Prati& Moretti, 2010). One of these effects is its darkening, this is related with the formation of melanoidins, from the Maillard reaction between reducing sugars and amino acids that are in the sugarcane, which contributes to the formation of a brown color (Oliveira et al., 2007; Thammasittirong et al., 2017).

The sugar cane extraction consists in the material process of the fiber separation (bagasse) and it is mainly performed through two processes: grinding and diffusion. In the grinding extraction the separation is made by mechanical pressure of grind rolls on shred sugar cane bed and water addition. In diffusion the separation is made by decomposed sugar cane movement through a water countercurrent flow (Oliveira et al., 2007; Prati& Moretti, 2010; Thammasittirong et al., 2017). The juice extracted in the grinding is a complex mixture containing a large number of suspended particles that are integral components of the sugar cane with some foreign matter incorporated to the juice by accident, through the sugar cane cut, harvest, transportation and operations in grinding. The soluble components of the juice are sucrose, glucose, fructose, proteins, oligosaccharides, polysaccharides, organic acids, amino acids and salts. The suspension material is widely constituted by little bagasse (lignocelulose bioproduct), land, sand, clay, waxes, lipids, gums and microorganisms. The relative quantity of components in both phases depends on variety, maturity and sugar cane conditions such as soil, in addition to the means and conditions of harvest (Doherty, 2011; Oliveira et al., 2007; Prati& Moretti, 2010; Yusof et al., 2000). Because of this, the juice must go through a simple clarification process that consists in a heat and lime treatment before the evaporation phase. In order to remove the thick impurities, the juice is first sieved and then treated with chemical agents, to coagulate part of the colloidal material (waxes, grease, proteins, gums, pectin, colorants), to precipitate certain impurities (silicate, organic acids, Ca, Mg, K, Na) and to modify the pH (Oliveira et al., 2007). In few words, to clarify the juice is to obtain the maximum removal of suspension particles, turbidity, soluble impurities – proteins, polysaccharides and inorganic materials – in order to produce clarified juice with low turbidity (Eggleston et al., 2010; Thai et al., 2012). Currently, there are five methods used in the sugar cane juice clarification process, which are the liming (the use of raw lime – CaO), used to clean and clear the juice, the sulfitation (the use of sulphur dioxide - SO_2) which helps to reduce the pH level, viscosity reduction, sugar reductors complex formation, juice preservation against some microorganisms and sugar yellow prevention. The next is the phosphatation (the use of phosphoric acid – P_2O_5) which helps to remove colorant materials and part of the juice colloids, the carbonation (the use of carbon dioxide - CO_2) which complements the clarification and lastly the use of magnesium oxide - MgO used to remove impurities without affect the sucrose contents (Doherty, 2011; Oliveira et al., 2007; Prati& Moretti, 2010; Thammasittirong et al., 2017; Yusof et al., 2000).

Ultraviolet radiation: In industrial processes, it has been widely studied on non-thermal methods to reduce pathogenic contents and to hold the product quality at the same time (Artés-Hernández et al., 2010). Thermal technologies are the most applied to food processing, despite these treatments negatively affect certain food components by reducing vitamins levels, modifying texture and coloration (Falguera et al., 2011a). Non-thermal technologies such as ozone, ultrasound and ultraviolet radiation (UV) have been applied to the food industry along with the benefits of pathogenic microorganisms' reduction and by preserving flavor and color features minimally (Artés-Hernández et al., 2010; Falguera et al., 2011b). The UV radiation covers a part of the electromagnetic spectrum. The other areas of this spectrum are: radio waves, micro waves, infra-red radiation, visible light, X-rays and gamma (γ) radiation. The aspect that features the spectrum each area properties is the radiation wave length. The UV radiation covers from 400 to 100 nm wave length (Diffey, 2002; Li et al., 2014). The UV thus covers the non-ionizing area of electromagnetic spectrum between X-rays and visible lights (Bintsis et al., 2000; Falguera et al., 2011a). The UV radiation changes with wave length and therefore the UV spectrum is divided by three regions: short waves called Ultraviolet C (UVC) which covers wave lengths from 200 to 280 nm, medium waves called Ultraviolet B (UVB) covering wave lengths from 280 to 320 nm and long waves called Ultraviolet A (UVA) with wavelengths ranging from 320 to 400 nm (Diffey, 2002; Falguera et al., 2011a; Graça et al., 2020). The UV radiation source may be either natural or artificial. In natural way it is produced both by heating a body to an incandescent temperature, and by solar radiation having the sun a distribution spectrum at wave length from 250 to 1200 nm (Bintsis et al., 2000; Diffey, 2002; Falguera et al., 2011a). The artificial sources are UV lamp bulbs that are grouped according to wave length that they emit. In mercury steam bulbs, mercury atoms get excited by the electron collision flowing between the lamp electrodes. The return of the electrons to your particular electronic state in the mercury atom releases energy in optical radiation form, that are ultraviolet, visible, and infrared radiation. Mercury bulbs are identical to fluorescent lamp bulbs, but removing the phosphorus protection cover, it emits mainly the UVC at 254 nm which has germicidal property (Bintsis et al., 2000; Diffey, 2002). The UV inactivation mechanism is the photo products formation of DNA. Among them the most important one is pyrimidin dimer that is formed between contiguous pyrimidin molecules in the same DNA chain and may interrupt either transcribing or translation (Franz et al., 2009). Precisely by direct altering the microbial DNA, the damage cause by UVC radiation leads to lethality (Bintsis et al., 2000). Sugars such as fructose, glucose and sucrose absorb little UV radiation at range from 240 – 360 nm despite fructose having larger absorption, 260-280 nm, than glucose and sucrose. These three kinds of sugar have high absorption at 200 nm (Falguera et al., 2011b). Noted that the application of the UVC is effective when applied to products surface, can thus be used as sanitizing agent (Artés-Hernández et al., 2010). The UV radiation use has high efficiency, little instrumentation, process reduction time, besides being economic possible (Birmpa et al., 2013). The UV radiation with 250 to 260 nm amplitude is lethal to the most part of the microorganism, including bacteria, virus, protozoa, mycelia fungi, yeast and algae (Bintsis et al., 2000).

The UV treatment is a disinfection method that may be used to inactivate microorganisms in food. The treatment can be applied at low temperature and may be led along with other non-thermal methods such as electric field pulse, high pressure and irradiation (Bermúdez-Aguirre & Barbosa-Cánovas, 2013; Tran & Farid, 2004). Germicidal treatment by UV radiation present the advantage of non-use residues (Yaun et al., 2004). The UV application as germicide are sub-grouped in three categories: (I) inhibition of microorganisms on surface; (ii) destruction of microorganisms on air; (iii) liquid sterilization (Bintsis et al., 2000). Recently, the demand for fresh food minimally processed has increased significantly and the inactivation processes of microorganism have the difficult task of not promote nutritional changes. Thereby the non-thermal technologies such as electric field and UV in batch or continuous processes at high or low pressure, have received special attention against pathogens (Bermúdez-Aguirre & Barbosa-Cánovas, 2013; Noci et al., 2008). The UV treatment features advantages such as being an effective method and economic viable. Currently, the pharmaceutics industry uses centrifugal UV irradiator to inactivate viruses in liquids, as serum plasma, without heat (Geveke& Torres, 2012). The biggest disadvantage of UV technology is your limited penetration in certain liquids (Falguera et al., 2011b; Geveke& Torres, 2012). The advantages associated to used UVC as non-thermal method are the ones who does not form known toxics bio products during the treatment, certain organic contaminants can be removed, there is no loss of flavor or odor when water is treated, and the energetic demand is very little when compare to thermal methods of pasteurization (Keyser et al., 2008). UVC is lethal to most microorganisms and can be applied with safety in industries. Currently, this technology is not in widely used in industries, nevertheless has potential to be working with solids and liquids, whereas in each case, a certain type of lamp is used to optimize the processes (Falguera et al., 2011b). On this subject, the innovation of sugar cane juice treatment methods is substantial to reduce costs, optimize the production and not encourage the generation of products that are harmful to the environment. In the meantime, it is justified the juice purification through non thermal irradiation methods as UV. This is an alternative that are residue free, having high efficiency in reducing the microorganisms amount and show potential to clarify the juice by reducing the turbidity and color, conserving the sucrose proportion as main objective of treatment. So, this study aims to purify the sugar cane juice by using ultraviolet irradiation to produce sugar and ethanol and to identify the best

operation conditions – for scale widening – by monitoring power parameters and exposure time.

METHODOLOGY

Raw material: Sugar cane juice in nature was obtained in market in the city of Uberaba/MG from the 2013 harvest. After extraction, the juice was filtered and poured in previously sterilized and identified glass jugs and then stored in refrigerator at 15°C for conservation. The used volume in each test was 50 mL.

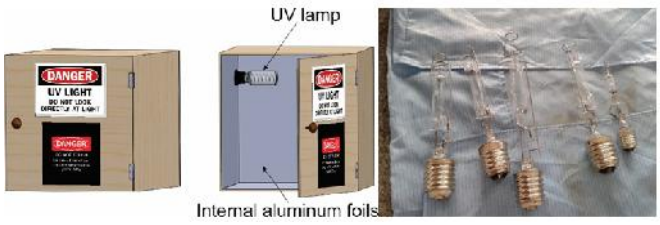
Experimental design: It has been used the full case with 4 repetitions in the factorial schedule 3X3 for this study experimental delimitation. Two factors have been analyzed – power and exposure time. There are three variation levels for each one and thus it has been set (-1) for the lowest level and (+1) for the highest level shown in Table 1. The data have been submitted to variance analysis by using the F test that satisfies the assumptions of independence, homoscedasticity and normality, followed by the Turkey´s test. The software used to data analysis and to elaborate the factorial experimental planning matrix with central settings was STATISTICA 8.0 (Statistica, 2007).

Table 1. Experimental lining level factors

VARIABLE	-1	0	+1
Power (W)	125	250	375
Exposure time (min.)	1	3	5

Homoscedascidity has been checked by Levene´s test as the normality has been checked by Kolmogorov-Smirnov´s test. It has been used non parametric variance analysis by using the Kruskall-Wallis test for the data that indicated violation of these criteria for ANOVA. And for the cases in which the F test showed significance, it has been verified the interaction occurrence between factors. The significance level used in all tests was 5% ($p < 0.05$). The independent variables were total soluble solids - °Brix, pH, color, turbidity, microbiological – fungi, aerobic and facultative bacteria.

Equipment and analysis methods: The UV chamber unit is built in wood. The interior is covered with aluminum sheets to promote the UV radiation reflection in order not to damage the unit. It has two exhaust fans in the back part to avoid overheating. It has mercury steam UV lamp bulbs with their rated power. The experimental unit and the UV lamps are shown in Figure 1.



Source: From authors.

Figure 1. Experimental unit and UV lamps

The °Brix analysis have been achieved using a fixed refractomery made by INSTRUTHERM model RTA - 100. pH readings have been achieved in a digital pHmeter brand HANNA Instruments Brasil model pH 21. Turbidity reading for the sugar cane juice has been achieved through turbidity meter HANNA Instruments Brasil model HI - 93703. The turbidity unit is in Nepholometric Turbidity Unit (NTU). The ICUMSA color index expresses the absorption index of a sugar solution. The sugar cane juice first is dissolved at 1°Brix. The absorption reading has been achieved in a spectrum photo meter BIOSPECTRO model SP-220 UV-Vis. with light ranging from 200 to 1000 nm in a 1cm at 420 nm quartz cuvette, where water was the blank sample. The color calculation is set according to Equation 1.

$$\text{ICUMSA (IU)} = \frac{\text{Abs}}{b \times c} \times 1000 \quad (1)$$

Where:

Abs = juice absorption at 420 nm;
b = sucrose concentration (solution °Brix);
c = cuvette length.

For fungi and bacteria amount determination, the methods of pour plate (dissemination or depth method) and spread plate (counting method) were used. The pour plate method is the one in which the nutritious mean (agar) is kept in liquid condition at 50 °C, and then poured on Petri´s dish being after slowly mixed with the mean. Therefore, the microorganisms develop all around the culture mean (Tortora et al., 2010). This technique may be used to obtain isolated colonies or to count colonies on dish by determining the UFC amount (colony forming units) (Okura &Rende, 2008). The original sample is submitted to a series of dilutions in order to sufficiently reduce the microbial population for both separated and plated colonies (Willey et al., 2008).
The achieved experiments have been sent to fungi and bacteria analysis. For the fungi counting the spread plate method has been used in the PDA (potato dextrose agar) culture means and for the bacteria counting both methods (spread and pour plate) have been used by PCA (plate count agar) culture means.

Experimental proceeding: In laboratory experiment physical, chemical and microbiological analysis of the clarified juice has been achieved after ultraviolet irradiation. The used methodologies for the physical and chemical analysis (turbidity, soluble solids (°Brix), pH and color) in sugar cane juice have been based on methods recommended by ICUMSA - International Commission for Uniform Methods of Sugar Analysis (CONSECANA-SP, 2006). For the microbiological analysis the colony number account by pour plate and spread plate methods have been used. The sugar cane juice in natura has been analyzed according to the same methodologies for the clarified juice.

RESULTS

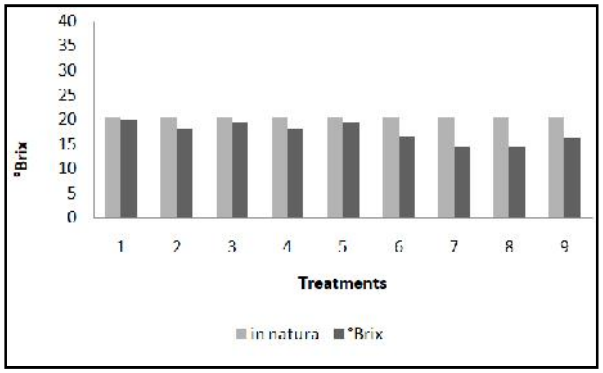
According to purposes to variance analysis used, the Kolmogorov-Smirnov normality test has been first used followed by Levene´s test for variance homogeneity. Levene´s test applied to variables has shown that only °Brix possesses variance homogeneity. Therefore, the non-parametric Kruskal-Wallis test for analysis of all variable results is applied for the Ultraviolet irradiation treatment method. The factorial planning resulted in 9 treatments, where their matrix with the temperature variation presented in Table 2.

Table 2. Matrix of factorial planning and treatment temperature variation

Treatment	Power (W)	Time (min.)	ΔTemperature (°C)
1	125	1	0.3
2	125	3	0.4
3	125	5	0.7
4	250	1	0.6
5	250	3	0.9
6	250	5	1.2
7	375	1	0.7
8	375	3	1.3
9	375	5	2.2

Brix: In natura juice, °Brix before ultraviolet treatment showed a value of 20.5. Thus, it is noticed that °Brix suffered a decrease after treatment. As ºBrix must stay still or as it is possible, treatments 7 and 8 must be discharged because they have the highest decrease in ºBrix value according to Figure 2. These treatments are related to the maximum power of 375 W respectively with times of 1 and 3 minutes. According to Table 2, the temperature is almost unalterable

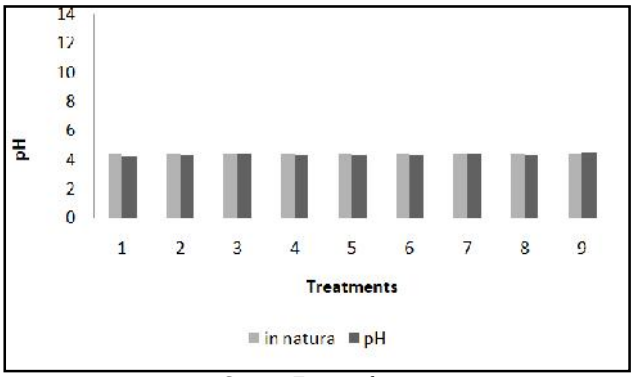
to the ultraviolet treatment, hence no alteration is attributed to ºBrix at those temperatures.



Source: From authors.

Figure 2. Brix average *versus* treatment

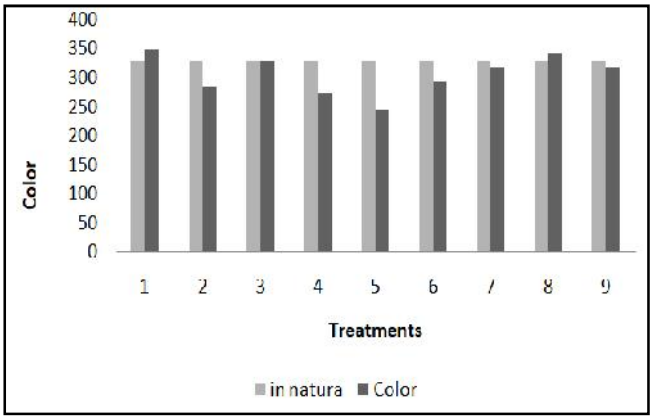
pH: According to Figure 3, pH on treatments submitted to ultraviolet irradiation have not shown significant alteration as all show values lower than the in natura juice (pH of 4.4).



Source: From authors.

Figure 3. pH average *versus* treatment

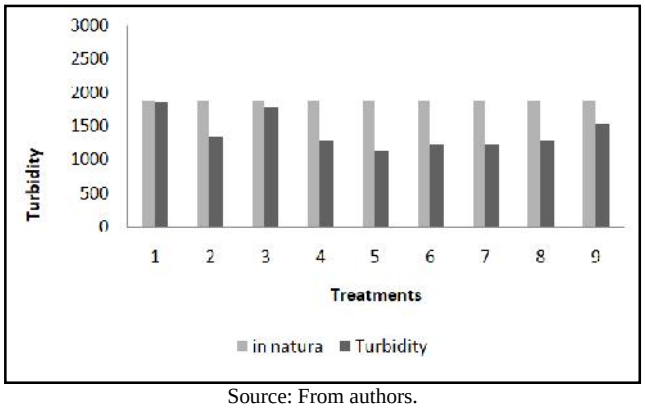
Color: The parameter color has shown variations for treatments submitted to ultraviolet though there was no relation between power and exposure time. As one of the clarifications aims to reduce color and in the in natura juice color was 328.8, treatments 2, 4 and 5 have shown major reduction in color according to Figure 4, thus treatment 2 relates to 125 W power and 3 min., and treatments 4 and 5 respectively have 250 W power and 1 and 3 min.



Source: From authors.

Figure 4. Color average*versus* treatment

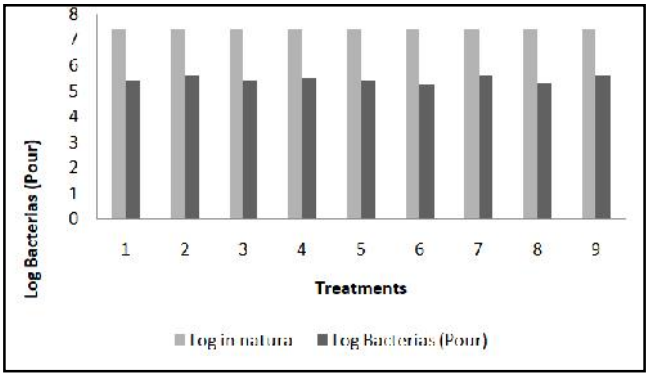
Turbidity: The parameter turbidity as well as color must have lower values after treatments. Turbidity in in natura juice was 1880 NTU. According to Figure 5, all treatments reduced the juice turbidity, pointing out treatment 5 with 250 W and 3 min. exposure time.



Source: From authors.

Figure 5. Turbidity average *versus* treatment

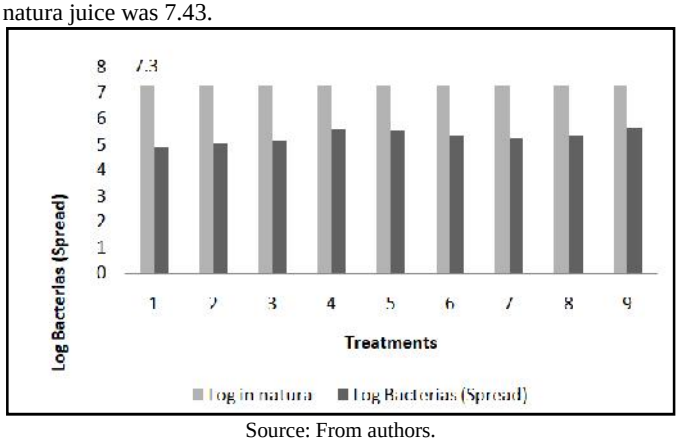
Fungi: Treatments submitted to ultraviolet irradiation have shown total fungi sterilization in sugar cane juice. Thus, aiming to cost reducing, it is recommended to use the lowest power treatment (125 W) and the fast exposure time (1 minute), which is the treatment 1. As clarification include other variables in analysis, it cannot be decided which is the best treatment only on sterilization to fungi.



Source: From authors.

Figure 6. Log bacteria average (pour) *versus* treatment

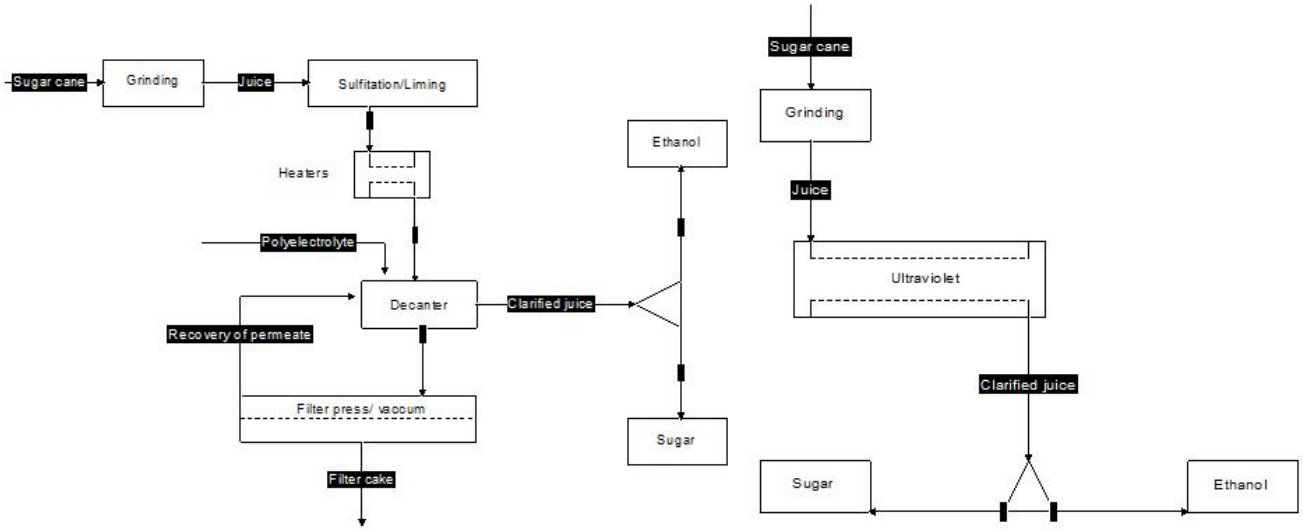
Bacteria-Pour plate and spread plate: The parameter facultative bacteria have not shown significant differences between treatments according to Figure 6. Therefore, it is not possible to state which treatment is the best one with ultraviolet method by considering this variable only. However, it is possible to observe, despite not showing total sterilization, the ultraviolet irradiation method can reduce the facultative bacteria present in the juice as Log Bacteria (Pour) in natura juice was 7.43.



Source: From authors.

Figure 7. Log bacteria average (spread) *versus* treatment

The aerobic bacteria variable either has not shown significant differences between treatments according to Figure 7. And as well as for facultative bacteria, it is possible to observe despite not showing total sterilization the ultraviolet irradiation method can reduce the aerobic bacteria amount present in juice as the initial Log Bacteria (Spread) was 7.3.



Source: From authors.

Figure 8. Conventional treatment and proposed route for ethanol and sugar production through UV irradiation treatment

The efficiency in reducing microorganisms on ultraviolet treatment has not shown satisfactory numbers as shown in Table 3.

Table 2. Microorganism reduction efficiency

Treatment	Efficiency	
	Bacteria (Pour)	Bacteria (Spread)
1	27%	32%
2	24%	31%
3	27%	29%
4	26%	23%
5	27%	24%
6	29%	27%
7	24%	28%
8	28%	27%
9	24%	22%

Table 3. In nature sugar cane juice composition and treatment 5

Variables	Values	Treatment 5
°Brix	20.5	19.5
pH	4.4	4.3
Turbidity	1880	1133.5
Color	328.83	244.9
Log Fungi	0	0
Log Bacteria (Pour)	7.43	5.42
Log Bacteria (Spread)	7.30	5.56

Source: From authors.

DISCUSSION

After observing each variable, it has been found that treatment 5 has shown better conditions to juice treatment by UV. Such treatment corresponds to 250 W power and 3 min. of exposure time where the relation to in natura juice the ºBrix and pH variations have not been significant, color and turbidity suffer significant reduction, there is no fungi contamination and the treatment has reduced the aerobic and facultative bacteria colony amount as relevant to juice sterilization.

Ultraviolet irradiation for described conditions have not provide temperature variation above 2 ºC. Treatment 5 and in nature juice values are shown in Table 4. In this work, the °Brix and pH have not suffered significant variations as results also found in studies with apple juice under ultraviolet irradiation, pre-heating and electrical field (Falguera et al., 2011a; Noci et al., 2008; Walkling-Ribeiro et al., 2008). The same behavior with the °Brix were found according to studies on peach nectar, (Flores-Cervantes et al., 2013; Gayán et al., 2012; Tran & Farid, 2004) orange juice, (Gayán et al., 2012; Tran & Farid, 2004) and starfruit juice (Bhat et al., 2011).

When it comes to the color, there has been significant reduction as observed in studies with different kinds of apple juice, (Falguera et al., 2011a) and starfruit juice (Bhat et al., 2011). On the other hand, color has been kept in another studies with apple juice, (Noci et al., 2008; Walkling-Ribeiro et al., 2008) orange juice (Gayán et al., 2012), peach nectar (Flores-Cervantes et al., 2013), strawberry and lettuce (Birmpa et al., 2013) apple, guava, pineapple, mango, strawberry and orange (Keyser et al., 2008). In contrast, UV treatment in watermelon juice with high pressure, significant increase in color was observed (Zhang et al., 2011) as well as in the sliced apple study that counts that increase to enzymatic activity caused by cellular membrane rupture with a consequent loss of compartmentalization (Gómez et al., 2010). Regarding to microbiological analysis, a reduction in microorganisms' amount was observed which has also been found in studies with apple juice contaminated with *Staphylococcus aureus* (Noci et al., 2008; Walkling-Ribeiro et al., 2008), *Brettanomyces*, *Saccharomyces*, *Acetobacter*, *Lactobacillus*, *Pediococcus*, *Oenococcus*, *Escherichia coli* and *Saccharomyces cerevisae* in grape juice (Fredericks et al., 2011; Geveke& Torres, 2012). In orange juice it also was found a reduction of aerobic bacteria, *Escherichia coli* and leaven (Gayán et al., 2012; Tran & Farid, 2004). A decrease of *Salmonella* spp. and Escherichia coli was also observed in fresh food such as strawberry, lettuce, apple, tomatoes, guava, pineapple, mango, orange and carrots (Bermúdez-Aguirre & Barbosa-Cánovas, 2013; Birmpa et al., 2013; Keyser et al., 2008; Yaun et al., 2004). The constant search for optimization in productive processes is based on technological innovation that not only creates new routes and equipment as well as it improves the existing ones. The sugar and ethanol industry which has a long way in this area has benefited greatly from innovation in sugar cane genetic field improvement, and foresees advance in industrial process. In this context, sugar cane juice treatment also called clarification is a phase of the process that is susceptible of innovation. As in this phase the expenditure of chemical supplies and energy are intense, it is very important to have proposals to reduce them. Therewith, treatment 5 for ultraviolet reduces color and turbidity by not changing the ºBrix and pH, and can be used to sugar cane juice clarification to produce sugar and ethanol. Therefore, an alteration in the productive process is proposed which the flowchart is shown in Figure 8.

CONCLUSION

Sugar cane juice purification treatment by ultraviolet irradiation has shown the best conditions related to 250 W of power and 3 min. of exposure time, in which the ºBrix and pH variations of in natura juice have not been significant and color and turbidity have suffered significant reduction.

At the same conditions no fungi contamination was observed but despite the presence of aerobic and facultative bacteria, the treatment reduced the colonies amount being relevant for juice sterilization. Ultraviolet being a suitable method for juice clarification not needing a pre-treat step. It is also possible to improve the technique with stirring the juice in the UV chamber, increasing the exposition of all volume, decreasing one of its disadvantages that is the little penetration into liquids. The use of non-thermal methods such as UVC irradiation may simplify the sugar cane juice clarification process once it does not use supplies, and may be automatized by rationalizing labor and decreasing production cost.

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