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Bioethanol production from potato peel waste using Saccharomyces cerevisiae

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A B S T R A C T

The present work consists of producing bioethanol from potato (*Solanum tuberosum* L.) peels using *Saccharomyces cerevisiae*. The different physicochemical and biochemical analyses showed that potato peels are rich in nutritional elements that make them favorable to alcohol fermentation. The total soluble sugars content, the pH value and the ethanol content were evaluated. The results indicated limited ethanol production. The addition of yeast extract, peptone and urea to the fermentation medium improved the ethanol yield produced by the yeast. The physicochemical characterization of purified bioethanol revealed that density, boiling temperature and refractive index are close to those of absolute ethanol. The in vitro antibacterial activity of bioethanol was tested on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Bacillus cereus*. Significant inhibition zones were observed. This study showed that potato peel waste can be a feedstock for bioethanol production.

Keywords: bioethanol, fermentation, potato peels, *Saccharomyces cerevisiae*, valorization.

И З В О Д

У овом раду представљено је добијање биоетанола из љуске кромпира (*Solanum tuberosum* L.) уз помоћ *Saccharomyces cerevisiae*. Примењеним физичко-хемијским и биохемијским анализама у љусци кромпира је утврђено обиље хранљивих елемената који је чине погодном за алкохолну ферментацију. Испитан је садржај укупних растворљивих шећера, pH вредност и садржај етанола. Добијени резултати су указали на ограничену продукцију етанола. Додавањем екстракта квасца, пептона и урее ферментационој подлози побољшан је принос етанола добијен посредством квасца. Физичко-хемијском карактеризацијом пречишћеног биоетанола утврђено је да су његова густина, температура кључања и индекс рефракције приближни вредностима код апсолутног етанола. Антибактеријска активност биоетанола у in vitro условима испитана је према *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* и *Bacillus cereus*. Примећене су значајне зоне инхибиције. Ово истраживање је показало да се љуска кромпира, која је отпадни материјал, може употребити као сировина за добијање биоетанола.

Кључне речи: биоетанол, ферментација, љуска кромпира, *Saccharomyces cerevisiae,* валоризација.

1. Introduction

Potatoes are among the most widely grown crops worldwide, and have been continuously expanding as a crop for basic human nutrition (FAO, 2018). In Algeria, potato production is a major agricultural activity, with more than 2 million tons produced in 2015, and approximately 60% of the potatoes are oriented for consumption (FAOSTA, 2015). Potato peels are the main by-product of processing, producing around 8% waste by weight and becoming a major environmental issue. The potato industry generates on average 100 kt of peels worldwide annually (Chang et al., 2011). Current research is focused on potato peel waste recycling pathways for the pharmaceutical and/or energy industry, resulting in an enhanced potato peel waste management (Wu et al., 2010). In agricultural activities, potato peel waste is used for the production of low-value animal feed or as fertilizer (Nelson et al., 2010). Potato peel has been previously characterized, and contains starch (15–25%), non-starch polysaccharides (25–30%), acid insoluble and acid soluble lignin (15–20%), proteins (18%), lipids (1%), and ash (6–10%) (Mader et al., 2009; Liang et al., 2014; Spyridon et al., 2019).

This diverse composition can facilitate (contribute to) the production of bio-based products and fuels (Bezirhan et al., 2019; Arapoglou et al., 2010). Peschel et al. (2006) found an industrial approach for polyphenols extraction from potato peel. Recent studies have also reported a promising method for lactic acid production with mixed microbial consortia

in batch fermentation mode (Chang et al., 2011; Liang et al., 2015).

Among the renewable resources, starch is a potentially useful material for energy production because it is inexpensive and easily available (Peschel et al., 2006; Chiumenti et al., 2018; Achinas et al., 2017). Starch has been used in many industrial areas such as paper production, corrugated boards, biofuels, and the pharmaceutical, textile and especially food industry (Chen et al., 2016). On the other hand, many companies have already begun to use starch for the production of pharmaceutical products. In spite of its abundance, low cost and natural origin, there is still a major concern about the use of this type of renewable resources for production. Also, the biofuels industry can decrease land that is available for food production or, in order to create more arable land, it can increase the incentives to cut down forested areas (Sahajwalla et al., 2018). In order to avoid potential competition with agricultural resources for foods and also to provide additional raw-material sources, the utilization of waste is the current trend (Lagaron et al., 2011).

The objectives of this study are: finding a new source of bioethanol production, enhancing energy security, and valorizing potato waste.

2. Materials and methods

2.1. Potato peels (Solanum tuberosum L.)

Potato peels were collected from a restaurant located in Sidi Hammou, Tighennif (Mascara, Algeria). The collected peels were cleaned and cut into small pieces to speed up drying at a temperature of 40°C for 24 h (Kacimi et al., 2008). Grinding and sieving were subsequently carried out. Then, the resulting powder was stored in sterile glass bottles in the dark and at room temperature (Figure 1).

Figure 1. Preparation of potato peel powder

2.2. Acid hydrolysis of potato peel starch

Hydrolysis is the necessary step before the fermentation of starchy substrates. It is a process of breaking down the bonds of amylopectin and amylose. It can be done using enzymes or acids. Based on the results of Ojewumi et al. (2018) investigation on sweet potato peel waste, the hydrolysis was carried out using a 0.5 M HCL solution with a ratio of 1:3 (w/v) compared to starch-based substrate. Peel powder (100g) was mixed with a volume of 300 ml of HCL and brought to a boil (100°C) in a reflux assembly for 1 hour. After this step, the solutions were filtered.

It should be noted that the duration of the hydrolysis was selected by carrying out several tests by taking a test volume at each time interval (0min, 15min, 30min, 45min, 60min). The presence of starch was tested by adding an iodine solution; the disappearance of the blue color indicated starch hydrolysis (Tasic et al., 2009).

2.3. Preparation of fermentation substrates

Two categories of substrates were prepared, one using peel powder and the other using the resulting hydrolyzate. Regarding the peel powder, the concentration chosen was 10 %(m/v).

For the optimization of bioethanol production, three compounds were added separately and together to the hydrolyzate and the peel powder solution. The compounds were yeast extract (1%: m/v) and peptone (1%: m/v), to approximate the composition of the standard medium for *Saccharomyces cerevisiae*: Yeast extract Peptone Glucose medium (YPG). The third compound was urea which was added in an amount of 0.4% (m/v).

The pH of prepared substrate fermentation was then adjusted to 5 and sterilization was avoided at 121 ° C for 15 minutes (Ojewumi et al., 2018).

2.4. Saccharomyces cerevisiae

The yeast used was commercialized organic yeast (LEVANOVA; levadura fresca; LO963). It is the fresh form of *Saccharomyces cerevisiae*. The choice of this strain was motivated by several advantages such as: rapid growth, easy cultivation, high fermentation power. As being in dough form, the yeast used was rehydrated at a rate of 6% (6 g of yeast per 100 mL of medium) in the base substrates and in the YPG reference medium. The strain was reactivated on the YPG medium (Guiraud, 1998).

2.5. Inoculation of substrates

The prepared mediums were inoculated with the yeast suspension so as to obtain an optical density of 0.1/620 nm. Then, they were incubated at 30°C with agitation at 150 rpm. Therefore, all the trials were

started under the same conditions: microbial load, volume, pH value, temperature and agitation rate.

2.6. Monitoring of fermentation

Incubation was maintained for 5 days. Samples were taken at different times and several assays were thus carried out.

2.7. Chemical analysis

Reducing sugars, sucrose and total sugars were determined by the Dubois method (Dubois, 1956) (A.O.A.C, 1970). The contents of water and dry matter were determined by drying 10 g of potato peels at 105°C for 18 h (Salgarolo, 2003). The ash content was determined by incinerating one gram of potato peels at a temperature of 600°C for 3 h (Audigie et al., 1983; Hamon et al., 1993) and mineral salts were determined according to the methods advocated by Godon (1997). The pH value was measured using a pH meter.

2.8. Ethanol extraction and purification

At the end of the process, the fermentation broth obtained mainly contained ethanol, water and other substances resulting from fermentation. The fermentation broth was placed in a balloon heated at a temperature of 78°C. According to Didderen et al. (2008), this separation, based on alcohol volatility, allows approximately 95% ethanol recovery.

For ethanol recovery, the distillate (ethanol, toluene) was placed in a separatory funnel. The separation was thus done according to the difference in the density of both solvents (ethanol 0.7 kg/L, toluene 0.8 kg/L). At the end, the upper phase was recovered. The confirmation of the presence of ethanol was done by a flame production test after contact of the liquid with the fire. The purified bioethanol was also tested by measuring its density, boiling temperature and refractive index.

2.9. Antibacterial effect of purified bioethanol

The purified bioethanol samples were screened for antibacterial activity using the agar diffusion technique against the strains selected (Table 1). Wells of 6 mm diameter were made on agar plates (containing Mueller Hinton agar) which were previously seeded by spreading 0.1 ml of culture at a concentration of 108 CFU/mL. Each well was then filled with 100 µL of sample. The plates were incubated at 37°C/18 h and the resulting inhibition zone was measured in mm (Stephen, 2012).

Table 1.
Selected strains for the antibacterial assay

Staphylococcus aureus	ATCC : 43300
Escherichia coli	ATCC : 25922
Pseudomonas aeruginosa	ATCC : S14C1B
Bacillus subtilis	ATCC : 168
Bacillus cereus	ATCC : 14579

3. Results and discussions

3.1. Physicochemical and chemical analyses of peel powder

Potato peel composition is given in Table 2. As shown, the tested sample had a pH value of 4.96, which was favorable for yeast growth. The contents of total sugars, dry matter and ash were 50 g/100g, 24% and 5.3%, respectively. These results show that peel powder is rich in nutritional elements that make it favorable to alcoholic fermentation.

Table 2.
Chemical composition of the potato peel powder

Parameter	Values
Dry matter content (%)	24 ± 0.668
Humidity (%)	76 ± 0.668
Ash content (%)	5.3 ± 1.45
Organic matter content (%)	94.6 ±1.98
pH value	4.96 ± 0.52
Total sugar content (g/100g)	50 ± 1.07

3.2. Fermentation course monitoring

During fermentations under the experimental conditions specified above, the concentrations of residual sugars, ethanol and optical density (OD) were quantified. As shown in Figure 2, at the start of fermentation, the initial OD was around 0.1. After 98 hours of incubation, the biomass content resulted in an absorbance of 2.003 for the YPG medium. For the prepared media, the maximum ODs were recorded for the powder and the hydrolyzate supplemented with both YE, PEP and UR. They were in the order of 1.46 and 1.16, respectively (Figure 2).

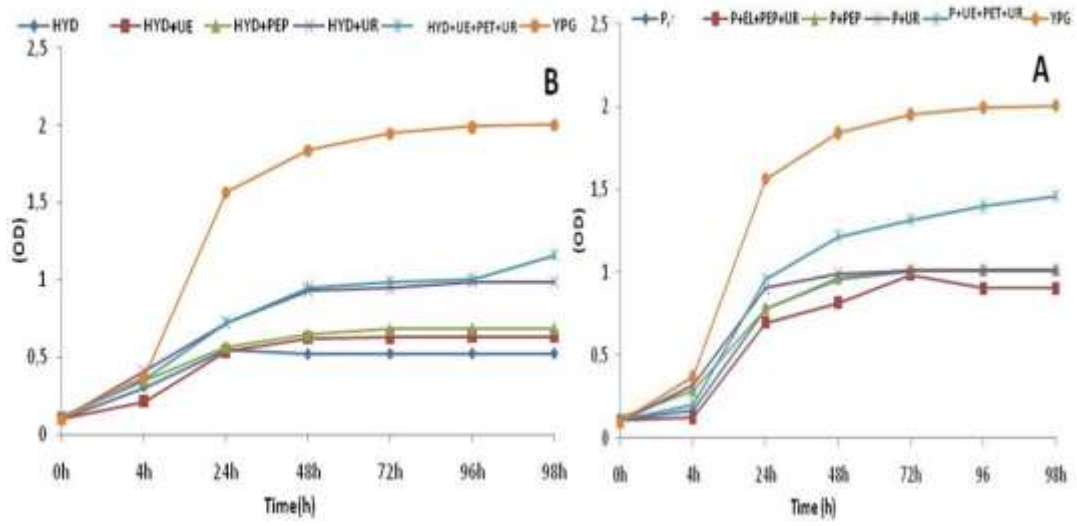

Figure 2. Evolution of OD during the cultivation of *S. cerevisiae* in the YPG and the media based on (A): peel powder (P), (B): hydrolyzate

The evolution of the microbial load for the tests where a single component was added was practically close to that where the basic substrates (powder alone and hydrolyzate alone) were used, with the exception of the case of urea when OD values were slightly higher. This is in agreement with the results of Gbohaida et al. (2016), who found that the growth of *Saccharomyces cerevisiae* was increased by adding urea to cashew apple juice. It should be noted that the OD increased remarkably to 48 h, which corresponds to the growth phase. Then, from 72 hours, its variation was almost stable, which is usually the case with the stationary phase of yeast growth. These results are similar to those recorded by Chibi et al. (2018). In the stationary phase, the yeast was no longer multiplying and its activity decreased regularly. This decrease in activity corresponded to a regular decrease in the speed of sugar consumption. During its growth, in alcoholic fermentation, S*accharomyces cerevisia*e produced ethanol, which, when accumulated, was the main cause of stress. It therefore slowed down the development of yeasts and possibly caused an irreversible damage (Wang et al., 2013). This was because yeast can resist a certain level of alcohol. In addition, the glycerol produced during fermentation (the major co-product after ethanol and CO_2) participated in maintaining the oxidation-reduction balance and in protecting the cell during osmotic and thermal stress (Fugelsang et al., 2007; Hohmann et al., 2002). From Figure 3, it is clear that there was a reduction in the level of sugar content over time. It was justified by their transformation into metabolites. In the first 24 hours, the consumption of sugars was significant in the case of fermentations using YPG and hydrolyzate-based medium. Then, it slowed down until the third day, when it stabilized. These results were in agreement with those of Abdelhafez et al. (2015) and those of Ojewumi et al. (2018), who carried out alcoholic fermentations on potato peel hydrolyzate medium. However, in the case of the powder-based substrates, the decrease in total sugars was moderate until day 4. Sugars were the main source of energy for the development and growth of microorganisms.

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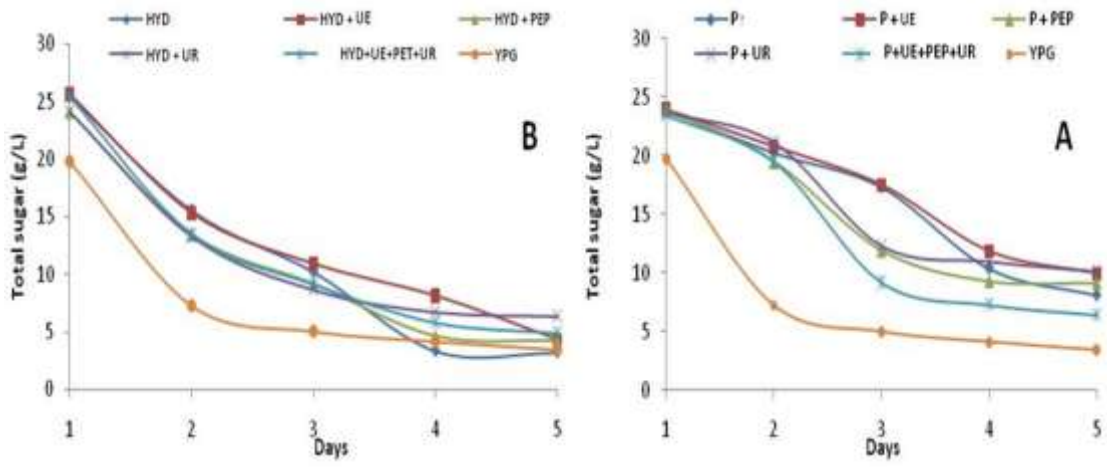

Figure 3. Residual sugar levels in g/L during the cultivation of S. cerevisiae in the YPG and the media based on (A): peel powder, (B): hydrolyzate

As shown in Figure 4, all of the tests looked the same, with the exception of substrates containing urea. Changes in pH depended on microbial metabolism. Its decrease can be explained, according to Akin (2008) by the assimilation of the nitrogen source by the yeasts. During fermentation, secondary metabolites other than ethanol were formed in small quantities, namely organic acids. It was in fact known that these substances caused an acidification of the medium, which decreased the pH value (Cooper et al., 1975). In addition, the carbon dioxide formed (CO_2) can influence it. This compound can be dissolved in the liquid medium in the form of carbonic acid (H_2CO_3), which was dissociated into bicarbonate (HCO_3^-), carbonate (CO_3^{-2}) and hydrogen (H^+) ions (Winter, 1989). Several studies have shown that at the end of fermentation, the pH values stabilized. On the other hand, the increase observed for the tests where urea was added was also noted by Gbohaida et al. (2016) by testing several yeast strains. This rise can be explained by the fact that the degradation of urea releases ammonium carbonate ($(NH_4)_2CO_3$). *Saccharomyces cerevisiae* can use this compound by degrading it in two steps to ammonia and CO_2. The enzymes responsible for this degradation are urea carboxylase and allophanate hydrolase (Cooper et al., 1975).

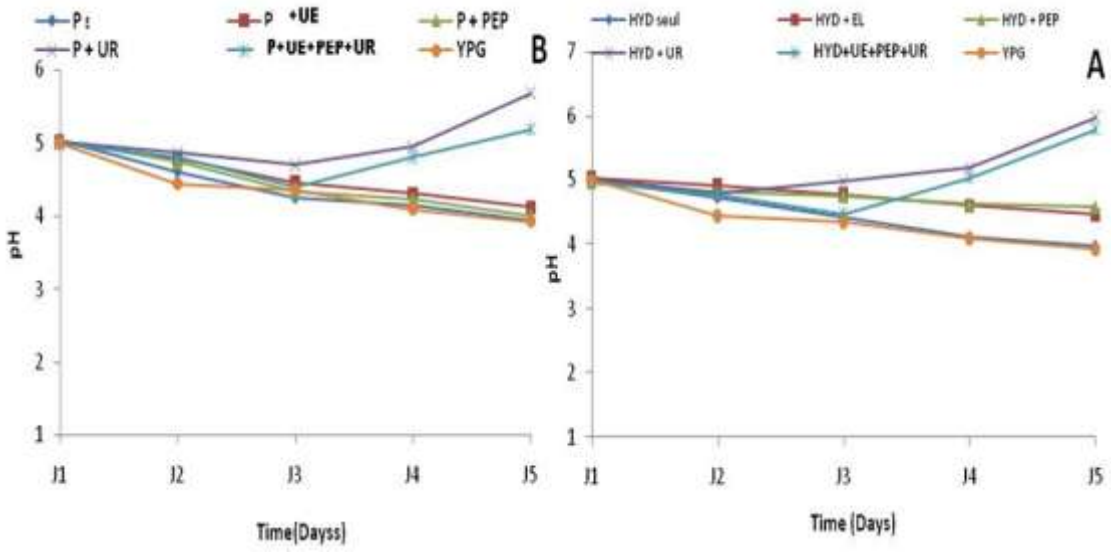

Figure 4. pH value during the cultivation of S. cerevisiae in the YPG and the media based on (A): peel powder, (B): hydrolyzate

As shown in previous (or these) results, *it is clear that the addition of peptone, yeast extract and urea to the peel powder and hydrolyzate was beneficial* and improved the growth of *Saccharomyces cerevisiae*. In fact, yeast extract was a valuable source of nutrients and a growth factor. It can contribute, through its amino acid content, to the stimulation of the growth of microorganisms (Sommer et al., 1998). Urea was a considerable growth factor for yeasts, especially in the biosynthesis of amino acids, proteins and nucleic acids and in other functions such as osmoregulation (Winter et al., 1989; Salma et al., 2013). Peptone, a digestible protein, served as the primary source of nitrogen in culture media (Russell, 2003). Thereafter, there was no difference in quantitative terms between the medium based on powder and that based on hydrolyzate. The rate of bioethanol was at its maximum at 48 hours. Then, it decreased at the end of fermentation. These results were close to those of Arapoglou et al. (1998), Sheikh et al. (2016) and Ojewumi et al. (2018), who also found similar results when working on sweet potato. According to Azad et al. (2014), the decrease in the level of ethanol may be due to its transformation by the yeast into other products.

3.3. Characterization of purified bio-ethanol

3.3.1. Flame test

According to this test, the presence of the flame indicated the presence of ethanol. Several studies carried out on alcoholic fermentation by *Saccharomyces cerevisiae* were based on the confirmation of the presence of ethanol by this test (Figure 5).

Figure 5. Results of the flame test.

3.3.2. Ethanol yields

The yield of this alcohol was interpreted in g/L, knowing that 1ml of pure ethanol was equal to 0.789 g/l. The results are illustrated in Figure 6. As shown, the highest yield was recorded for the medium containing peel powder, peptone, yeast extract and urea.

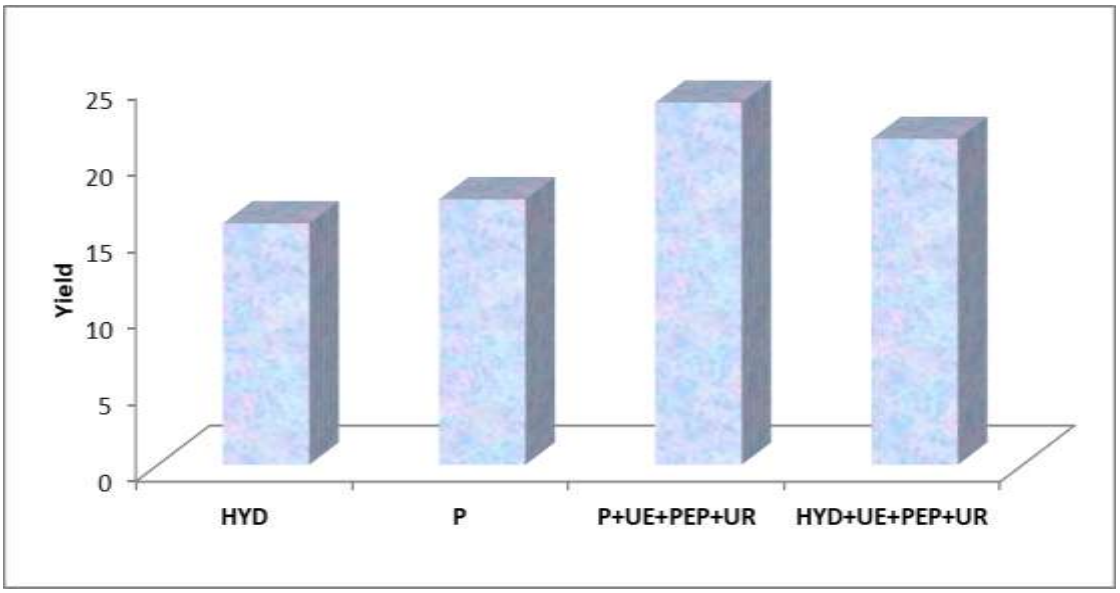

Figure 6. Ethanol yields from *S. cerevisiae* fermentations in selected mediums

3.3.3. Color and smell

The macroscopic observation of the samples obtained allowed us to determine their color and even the smell, which was characteristic of absolute ethanol. Regarding the color, it was variable. The color of purified ethanol varied depending on the fermentation medium (Figure 7).

Figure 7. Purified ethanol samples

According to Table 3, and by comparing with absolute ethanol, the closest density was that of the medium containing peel powder, peptone, yeast extract and urea with a value of 0.808 kg/L. By referring to the toxicological data for ethanol (2011 edition), the boiling temperature is 78.37°C, which was close to the results obtained. As shown in the table, the boiling point was the same for absolute ethanol and that obtained from P+YE+PEP+UR.

The refractive index was a good way to check the purity of alcohol. The results established in Table 3 showed that the refractive index values varied between 1.372 and 1.395. These values are close to the refractive index of absolute ethanol (1.364).

Table 3.
The physicochemical analysis of different samples of purified ethanol

	Density (kg/L)	Boiling Temperature (°C)	Refractive index
P	0.662	82	1.395
P+YE+PEP+UR	0.808	78	1.372
HYD	0.682	69	1.377
HYD+YE+PEP+UR	0.714	80	1.383
Absolute ethanol	0.799	78	1.364

3.4. Evaluation of the antibacterial potential of purified bioethanol

The evaluation of the antibacterial activity of purified ethanol was illustrated in Table 4. In general, the samples tested showed variable activity spectra. The whole samples tested were active on Gram negative and Gram positive bacteria. *B. cereus* was more resistant, which can be attributed to the sporulation of this species. According to Russell et al. (2003), ethanol was widely used clinically for its antisepsis action. It can readily diffuse into the cytoplasmic membrane.

Table 4.
Inhibition diameters (mm) obtained using purified and absolute ethanol samples against the strains tested

	P	P+YE+PEP+UR	HYD	HYD+YE+PEP+UR	Ethanol
E. coli	19	12	15	–	35
S. aureus	20	10	12	10	15
P. aeruginosa	22	11	35	13	20
B. cereus	20	–	–	15	28
B. subtilis	22	8	19	12	9
- : Lack of inhibition zones					

In addition, when present in large amounts, ethanol impaired membrane permeability, which reduced the activity of transport proteins. Therefore, proton penetration increased. When the balance between this passive penetration of protons promoted by alcohol and their active excretion by cell metabolism

was no longer maintained, the cell eventually died through intracellular acidification (Soubeyrand et al., 2005).

4. Conclusions

The present study was focused on the valorization of the potato peel by a biotechnological process: the production of a renewable ecological additive, which is more respectful of the environment, such as bioethanol. This study revealed that the peel powder potato medium was rich in sugars and mineral salts and constituted a favorable environment for the development of the species *Saccharomyces cerevisiae*, but its lack of nitrogen sources was a limiting factor. In this sense, yeast extract, peptone and urea were used as a nutrient source to enrich the culture medium. This addition generated a better production of biomass. The physicochemical properties of absolute ethanol and the produced bio-ethanol were similar. It can therefore be used for different applications. This study concluded that food wastes could be used for ethanol production. A new guide for ethanol should be developed for production, usage and waste management in Algeria as soon as possible.

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Declaration of competing interest

The authors have no conflict of interest to declare.

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