RESEARCH ARTICLE

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Biotechnological potential of apple pomace for value-added products

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Abstract
Agri-food processing waste and by-products are important to be valued in an integral unit to the main process. This study focused on showing the potential valorization of apple pomace as substrate towards valuable products by a biotechnological mean. Apple pomace was fermented by *B.subtilis* at 37 °C, 150 rpm, and 72 h. Reducing sugars, total phenol content and α-amylase activity were followed throughout the fermentation. The results showed that *B.subtilis* assimilated apple pomace sugars and stimulated the release of sugars into the medium during fermentation. α-amylase activity detected in the medium also indicated the degradation of pomace by *B. subtilis*. However, the total phenol content was found to be low. The α-amylase activity at 24th h was 29.6% higher when the fermentation initiated with a former fermentation medium than that of started with the inoculum based on agar and liquid incubation media. Overall results showed –for the first time- that apple pomace can be valued towards α-amylase activity, reducing sugar and total phenol content by the activity of *B.subtilis* cells.

Keywords: Apple pomace valorization, Fermentation, *Bacillus subtilis*, α-amylase enzyme

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INTRODUCTION

Apple is among the most consumed fruits and the annual world production of apples was reported as 83.1 million metric tons in 2017. About one third of the world total production of apple is used in the production of juice, wine, jams and dried products**;** and 65% of the total amount of processed apple is juiced (Lyu et al., 2020) Apple pomace is the waste product of the juice processing plants and huge amounts of apple pomace are generated worldwide every year. Most part of the apple pomace is disposed of through storage and incineration processes whereas a fraction of it is used as livestock feed. On the other hand, its composition rich in carbohydrates with some minerals, proteins, and vitamins provides it to be used as a low-cost substrate in microbial conversions. The simple sugars in apple pomace are mainly glucose (22.7%), fructose (23.6%) and galactose (6% to 15%) (Lyu et al., 2020). Several bacteria, yeasts, and fungi have been used for the production of enzymes, ethanol, biopolymers, fatty acids, polysaccharides, and organic acids from apple pomace. β-Glucosidase by *Aspergillus foetidus*, lignocellulolytic enzymes by *Candida utilis*, pectin methylesterase by *Aspergillus niger*, pectolytic enzymes by *A. niger*, polygalacturonase by *Lentinus edodes* and pectinase by *Polyporus squamosus* are some examples for fermentation enzymes of apple pomace (Kosseva, 2011). Cellulase production (Sun et al., 2010), extraction of phenolic antioxidants (Ajila et al., 2011), production of lignocellulosic enzymes

(Gassara et al., 2012), feed additive studies such using fermented apple pulp on animal development (Ajila et al., 2015) are other examples for the fermentation studies for microbial evaluation of apple pomace.

The genus *Bacillus* is among the most important microorganisms used in industrial processes due to its high capacity of secreting enzymes such as protease, α-amylase, β-glucanase and lipolytic enzymes (Su et al., 2020). The literature reports some fermentation studies by *Bacillus* strains that use apple pomace as an agro-waste for value-added products. A fibrinolytic protease enzyme was produced by solid phase fermentation on different vegetable solid substrates including apple pulp by using a *Bacillus cereus* strain that they isolated and mutated (Venkata et al., 2014). Alkaliphilic *Bacillus subtilis* with genetic modifications was used in the fermentation of apple pomace hydrolysate for 2,3-butanediol production (Bialkowska et al., 2016). The production of polyhydroxyalkanate (PHA) was studied with different *Bacillus* species using various herbal wastes including apple pulp (Kumar et al., 2016). An amylase production by solid phase fermentation was studied with a *Bacillus thuringiensis* strain isolated in environments containing three different herbal solid substrates including apple pulp (Rana et al., 2017). Pectinase production was optimized by a co-culture of *B. subtilis* and *B. pumilus* in a submerged fermentation using apple pomace as the carbon source (Kuvvet et al., 2017).

The forementioned studies have reported the production of some value-added products from apple pomace. In the present study, different from the existing literature, we aimed to explore and suggest how apple pomace can be valued in a biotechnological process rather than focus on a specific compound. In the study, only liquid phase of fermentation medium was considered. The course of two main components in apple pomace, that is reducing sugar and total phenolic compounds, during fermentation of apple pomace by a strain of *Bacillus subtilis* was monitored. The activity of α-amylase enzyme was also measured to investigate the microbial production of a starch degrading enzyme in a fermentation process of apple pomace.

MATERIALS AND METHODS

Materials and Microorganism

Apple pomace was supplied from a commercial apple juice factory and dried in a forced-air drying oven (Zhicheng ZRD-5110) at 60 ^{0}C for 16-17 h. Then, the pomace was ground and kept in a refrigerator at +4 ^{0}C. The starch content of the pomace was found approximately 16.30 % (Sulewska et al., 2014). No method was used to hydrolyze starch content of apple pomace before fermentation. The chemicals were of among the commercial brands as Merck, Sigma-Aldrich and Applichem. *Bacillus subtilis* strain was purchased from the Public Health Agency of Turkey. Gene sequence analysis of the strain was provided by Ankara University Biotechnology Institute (Sanger et al., 1977)F. & Coulson, A. R. (1975.

Culture Media and Conditions

The culture medium used for *B. subtilis* was based on tryptic soy medium (TS Broth composition: 1.7% (w/v) peptone from casein, 0.3% (w/v) peptone from soy hydrolyzate, 0.5% (w/v) NaCl, 0.25% (w/v) glucose, 0.25% (w/v) K_2HPO_4 (pH 7)). In some experiments, tryptic soy agar (TSA) and broth (TSB) were used by introducing starch or apple pomace into the medium instead of glucose and named as induced-TSA/TSB medium. All media were sterilized at 121 °C for 20 minutes in an autoclave (ALP) before fermentation. The microorganism was incubated on induced TSA at 30 °C for 16-18 h and then inoculated into 10 ml induced TSB pre-culture medium. After 24 h incubation at 30 °C and 150 rpm in an orbital shaker (Edmund Bühler SM-30), the preculture medium was transferred into 100 ml fermentation medium that contained 2.5% (w/v) dried apple pomace, 1.7% (w/v) peptone from casein, 0.3% (w/v) peptone from soy hydrolysate and 0.5% (w/v) NaCl. Fermentation conditions were 37 °C, 150 rpm, and 72 h. The samples taken off from the medium were centrifuged at 10000 rpm (Hottich Mikro 22) and the liquid phase was analyzed for reducing sugar, total phenol content (TPC) and α-amylase enzyme activity. The cell growth could not be followed as the pomace particles were present in the medium together with the cells. Instead, the decrease in reducing sugars content and an increase in α-amylase activity -that provides also released reducing sugars- were considered as the indicator of the cell growth.

Reducing Sugar Assay

The reducing sugar analysis was carried out spectrophotometrically at 575 nm (Shimadzu 1601) according to the DNS method (Miller, 1959; Sadasivam S., 2008).

Total Phenol Content

Total phenol content of fermentation medium was measured spectrophotometrically at 760 nm (Shimadzu 1601) by using the Folin-Ciocalteu method (Keskin-Šašić et al., 2012; Škerget et al., 2005). Briefly, 1000 µL of 10% Folin reagent (v/v) was added to 200 µL of sample and mixed. After the mixture was waited for 4-5 minutes at room temperature, 800 µL of 7.5% sodium carbonate solution was added. The sample was then waited in a dark place for 30 minutes. Absorbance values of samples were read against blank at 760 nm. The total phenol concentration of samples was calculated as gallic acid equivalent (GAE).

α-Amylase Enzyme Assay

Alpha-amylase enzyme activity was measured spectrophotometrically (Shimadzu 1601A) by the Sigma

Aldrich method. One unit of enzyme activity was defined as 1 mg maltose released for 3 min under pH 6.9 and 20 °C conditions ("Enzymatic Assay of α-Amylase (EC 3.2.1.1)," n.d.). Briefly, a- 500 µL / b- 700 µL / c- 1000 µL enzyme samples mixed with 1% starch solution at 20 °C. The mixtures were incubated in a water-bath and stirred at 20 °C for 3 min. 1000 µL of colour reagent was added to each mixture and mixed. After the mixtures were waited for 15 minutes in a boiling water-bath, a- 500 µL / b- 300 µL / c- 0 µL enzyme samples were added into the mixtures. The samples were waited on ice until they reached room temperature. Then, 1000 µL distilled water was added for dilution. Absorbance values of samples were read against blank at 540 nm wavelength.

Total Antioxidant Activity

Total antioxidant activity of samples was determined in terms of free radical scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Savatovic et al., 2009; Yen and Chen, 1995).1 mL of sample and 3 mL methanol was mixed with 1 mL of 0.3 mM DPPH solution in methanol and after 10 min in the dark at room temperature, the absorbance at 517 nm was measured (A_{sample}). The similar procedure was repeated without sample ($A_{control}$). Percent inhibition of DPPH radicals (%inh.) was then calculated according to Eq. (1):

$$\%inh. = \frac{A_{control} - A_{sample}}{A_{control}} x100 \qquad (1)$$

In this study, all experiments and analyses were carried out at least in dublicate. The standard deviations were within ±10%.

RESULTS AND DISCUSSION

Identification of the Strain

The strain used in the study was analyzed by 16S rDNA sequences method. The 16S rDNA sequencing of the isolate showed high homology (98.76%) with *B. subtilis,* thus it is classified as a variant of *B. subtilis.* Agarose gel image and DNA sequence analysis of PCR product are given in Picture 1.

Effect of Nitrogen Source on Fermentation Products

The effect of nitrogen source on the fermentation of apple pomace by *B.subtilis* was investigated in the medium containing 2.5% (w/v) dried apple pomace, 0.5% (w/v) NaCl and ammonium sulphate or urea. The cultivation medium used in these experiments was apple pomace-induced TSA/TSB medium. The fermentation carried out in TSB involving apple pomace instead of glucose was referred to the control medium. The concentration of nitrogen was adjusted to 1.63 g/L referring TSB medium in all fermentation media. (Also, it was estimated that apple pomace had no nitrogen.) Initial pH values of fermentation media were adjusted to 5.00 ± 0.30 by K_2HPO_4.

The changes in the concentrations of reducing sugar and total phenol throughout the fermentations carried out with different nitrogen sources are shown in Figures 1 and 2, respectively. The profiles of reducing sugar concentration were similar to each other in all media where there was a decrease at the beginning of the fermentation indicating the utilization of the sugars in apple pomace as substrate by the cells (Figure 1). However, the reducing sugar concentration remained constant or slightly increased with time. This shows that the releasing rate of sugars from apple pomace by the action of enzymes was higher than the rate of consuming of them by the cells after a certain period of fermentation. The variations in total phenol concentration with time were also similar in all fermentation media (Figure 2). There were no considerable changes in phenolic content of the medium with time and nitrogen source indicating that the phenolic compounds released from apple pomace at the beginning of (or before) the fermentation were not used by the cells and remained constant throughout the fermentation. This variation also showed that the cells did not contribute the release of phenolic compounds from apple pomace. The α-amylase activity of the cells in the fermentation media was measured at 24 h and 48 h and the results are given in Table 1. The enzyme activity was higher at the 24 h of fermentation. This variation was in coherent with the periods of consuming of reducing sugar by the cells and then the increase in the concentration of reducing sugar, in order. Organic nitrogen source appeared to be more effective for α-amylase secretion by *B. subtilis* in the fermentation of apple pomace. The literature also reported that the combination of organic nitrogen sources like peptone, tryptophan or yeast extract had more stimulating effect than inorganic nitrogen sources likely ammonium salts on α-amylase production by *Bacillus subtilis* (Dash et al., 2015).

Effect of Cell Inoculum Size and Type on Fermentation Products

Fermentation of apple pomace cultured with different amounts of *B.subtilis* strains -grown on starch-induced TSA/TSB medium- was carried out to investigate the effect of inoculum size on the fermentation course. The OD values of the pre-culture media were 0.32 and 1.0. In the same experimental set, the medium in which the cells previously cultured in the starch-induced preculture medum (OD value=1) then fermentation medium containing 2.5% (w/v) dried apple pomace, 0.5% (w/v) NaCl, %0.3 peptone from soy hydrolysate, 1.7% peptone from casein and then stored at +4°C for 3 months were also tested as the preculture of the fermentation (which was called former fermentation medium thereafter).

The changes in the concentrations of reducing sugar and total phenolics as well as the pH of the medium

Table 1. α-Amylase activity the 24. and 48. hours of fermentations carried out with different nitrogen sources.

Nitrogen source	α-amylase activity U/mL	
	t=24 h	t=48 h
TSB (control)	1.920 ± 0.162	1.467 ± 0.159
Urea	1.802 ± 0.083	1.787± 0.107
Ammonium sulphate	1.526 ± 0.104	1.438 ± 0.198

Table 2. α-Amylase activity at the 24. and 48. hours of fermentations carried out with different cell inoculum sizes and types.

Samples	α-amylase activity U/mL	
	t=24 h	t=48 h
Inoculum with the culture of OD=1.0	2.311 ± 0.187	2.068 ± 0.076
Inoculum with the culture of OD=0.32	2.060 ± 0.106	1.968 ± 0.076
Inoculum with former fermentation medium	2.996 ± 0.275	2.349 ± 0.148

Table 3. α-Amylase activity at the 24. and 48. hours of fermentations carried out in the presence and absence of metal ions

Samples	α-amylase activity U/mL	
	t=24 h	t=48 h
Inoculum with the culture of OD=1.0 (without metal ions-control)	2.311 ± 0.1875	2.068 ± 0.0768
Inoculum with the culture of OD=1.0 (in the presence of metal ions)	2.483 ± 0.0128	1.153 ± 0.0514

Table 4. α-Amylase activity and total antioxidant activity of the medium for the fermentation carried out under optimal conditions (ND: not determined)

Samples	α-amylase activity U/mL		Total antioxidant activity (inh.%)			
	t=24 h	t=48 h	t=0	t=24 h	t=48 h	t=72 h
average of two runs	3.022	2.075	22.72	18.88	13.21	24.64
control	ND	ND	17.31	14.51	14.96	16.06

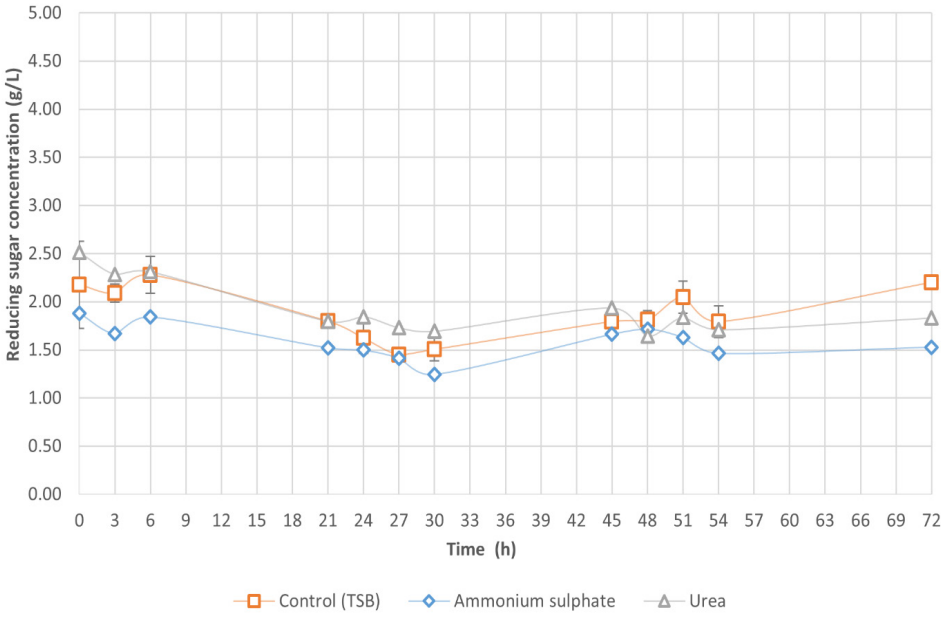


Figure 1. Effect of nitrogen source on reducing sugar concentration throughout the fermentations (T=37°C, N=150 rpm)

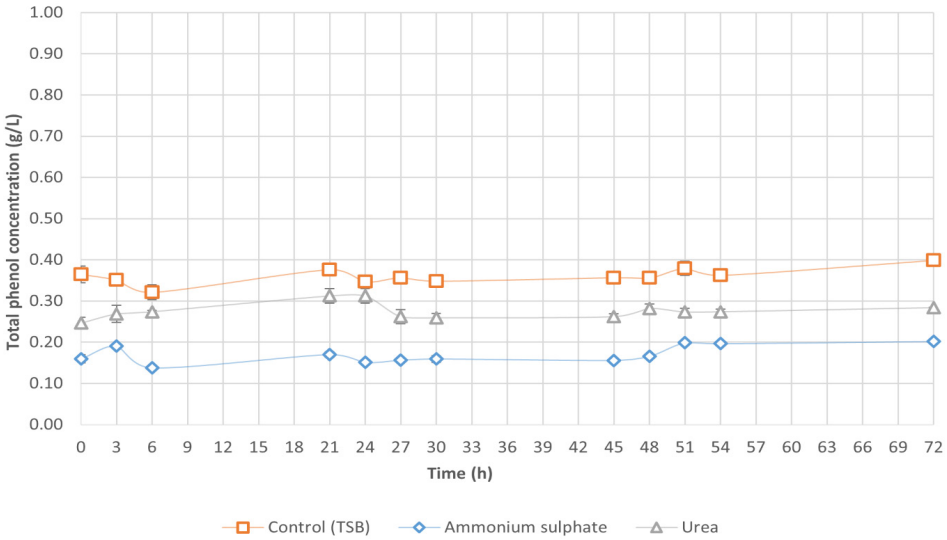


Figure 2. Effect of nitrogen source on total phenol concentration throughout the fermentations (T=37^{0}C, N=150 rpm)

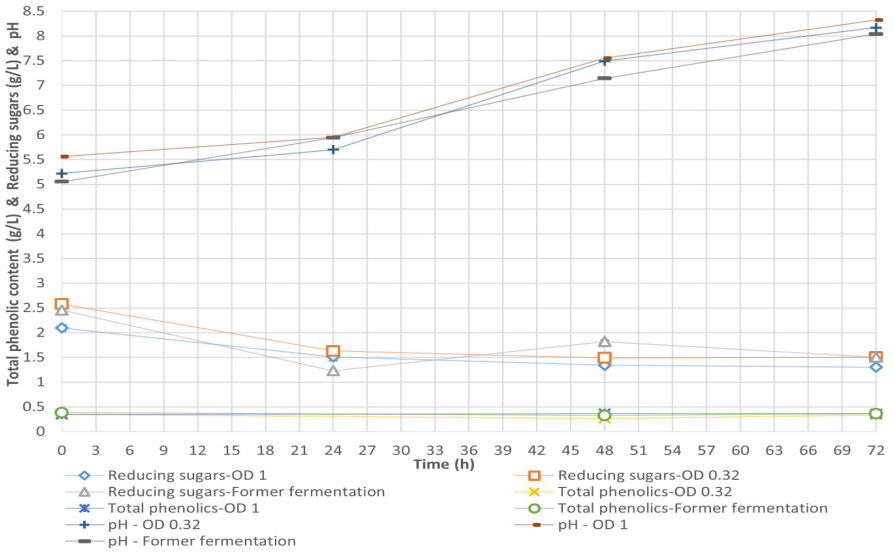


Figure 3. Effect of cell inoculum size and type on pH, concentrations of reducing sugars and total phenolics throughout the fermentations (T=37^{0}C, N=150 rpm)

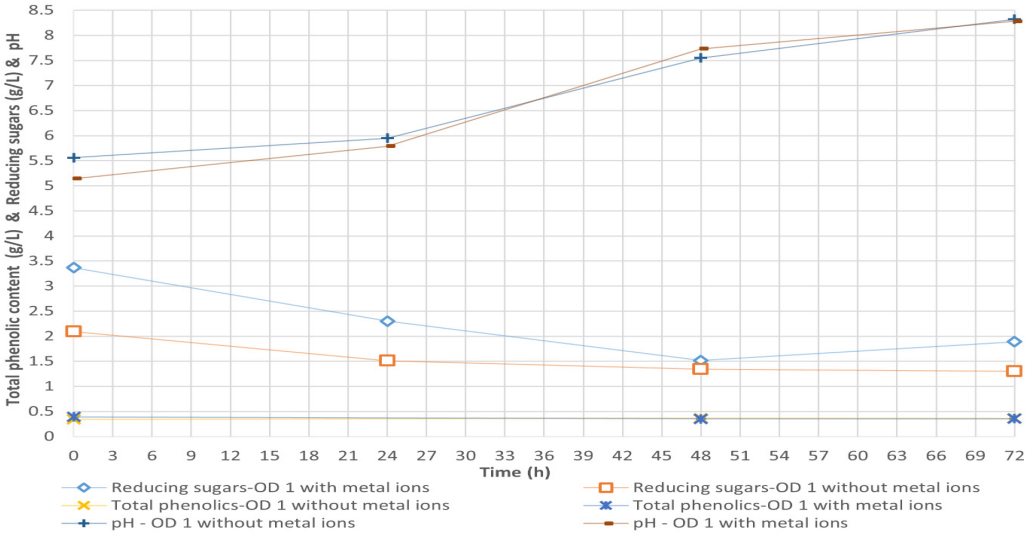


Figure 4. Effect of metal ions on pH, concentrations of reducing sugars and total phenolics throughout the fermentations (T=37^{0}C, N=150 rpm)

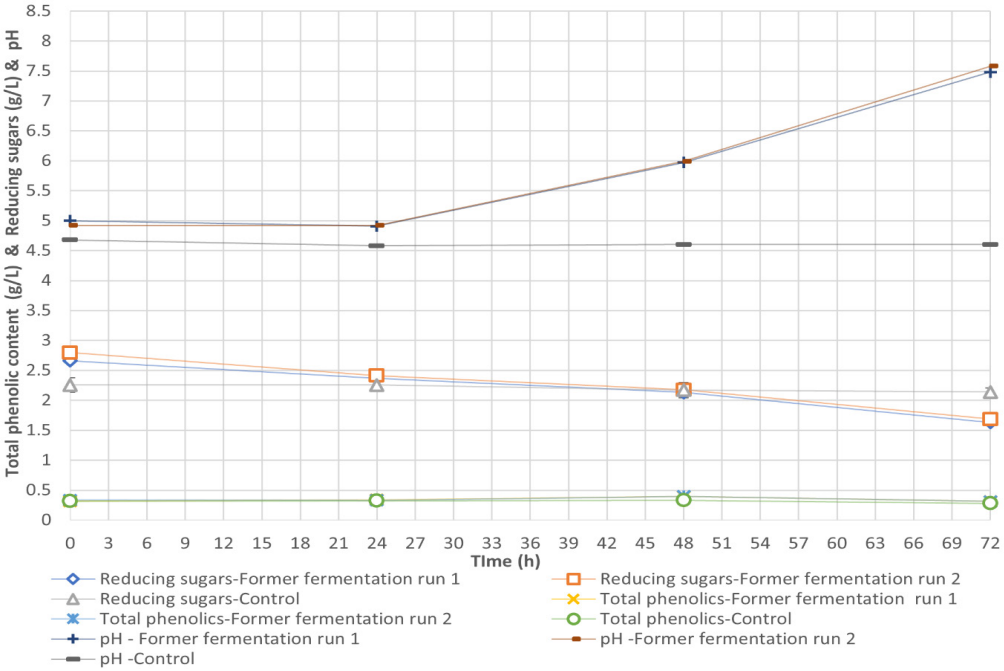
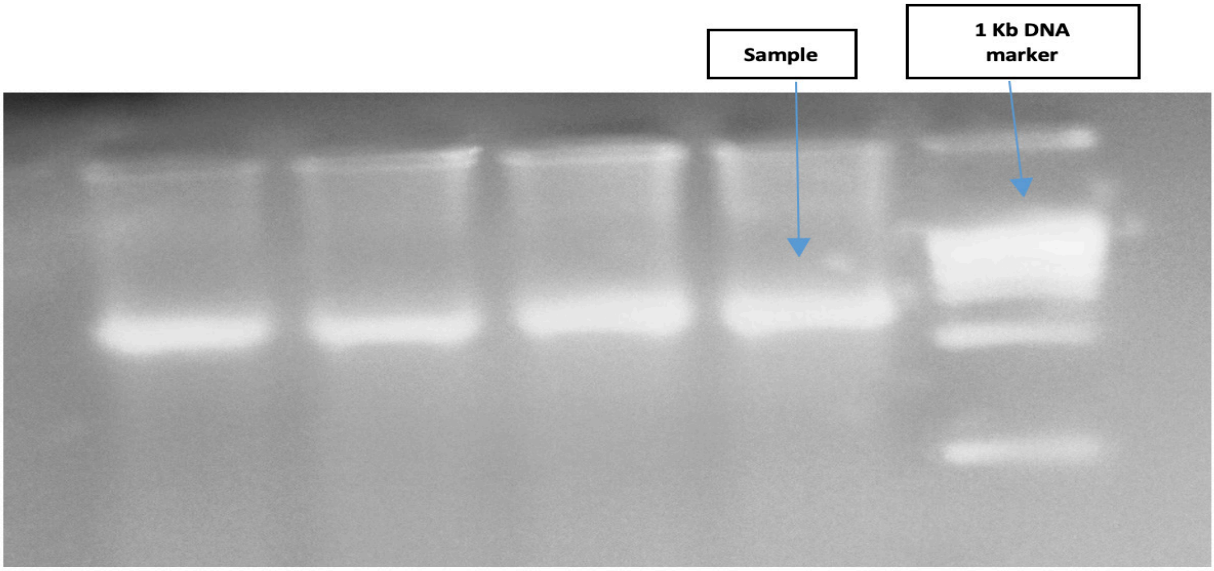


Figure 5. Comparison of fermentation course under optimal conditions with control (T=37^{0}C, N=150 rpm)



DNA sequence analysis

NNNNNGGCGCTGCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTA
ACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAACCGCATG
GTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCAC
CAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG
GCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTA
AAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAAC
TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCT
TAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAAGAGAG
GGAATTCNNCGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGNNTGCGACTCTCTGGTCTGTAGCTAC
CGCTGAGAAGNNAAACGTNGG

Picture 1. Agarose gel image of 16S PCR product (The sample was loaded into the gel in 4 replicates and an example of DNA sequence analysis for the product

throughout the fermentations are shown in Figure 3. The time variations of reducing sugar concentration were similar to each other in all media where there was a decrease at the very beginning of the fermentation and then remained almost constant with time. However, the rate of decrease and the following increase in reducing sugar concentration inoculated with a former fermentation medium were higher than others. The time variation of total phenol concentration during all fermentations were also similar to each other. The phenolic compounds released from apple pomace at the beginning of the fermentation were very low and did not change with time considerably. The pH of all fermentation media increased with time possibly referring the release of nitrogen metabolites to the medium.

The α-amylase activity of the cells in the fermentation media at 24 h and 48 h is given in Table 2. The enzyme activities were higher at the 24 h of the fermentations where the inoculation with a former fermentation medium was more effective for α-amylase secretion by *B. subtilis* in the fermentation of apple pomace.

Effect of Metal Ions on Fermentation Products

The effect of metal ions on the fermentation of apple pomace by *B.subtilis* strain was investigated by introducing $CaCl_2$. $2H_2O$ (0.1 g/L), $MgSO_4$. $7H_2O$ (0.1 g/L), $MnSO_4$. $4H_2O$ (0.01 g/L) and $FeSO_4$. $7H_2O$ (0.01 g/L) in the medium composed of 2.5% (w/v) dried apple pomace, 0.5% (w/v) NaCl, %0.3 peptone from soy hydrolyzate and 1.7% peptone from casein. The agar medium used in these experiments was starch-induced-TSA medium.

The changes in pH, concentrations of reducing sugar and total phenolics with fermentation time are shown in Figure 4. The fermentation course without metal ions was also shown in Figure 4 for comparison. Although there were no differences between the total phenols and pH courses with metals introduction into the medium, the rate of sugar release from the apple pomace and of consumption by the cells appeared to increase with the presence of metals.

The α-amylase activity of the cells in the fermentation media was measured at 24 h and 48 h and the results are given in Table 3. The enzyme activities of samples taken from the medium containing metal ions were slightly higher when compared to those without metal ions.

Fermentation Course Under Optimal Conditions

The abovementioned results obtained in the fermentation of apple pomace by *B. subtilis* showed the advantage of inoculation of the fermentation medium with a former fermentation medium (stored at +4 ^{0}C for 3 months). In this part of the study, the optimal apple pomace fermentation medium was used to culture *B.subtilis*. To show the repeatability of the fermentations, two runs were conducted in parallel and compared with the run without any inoculation. The fermentation medium composition was as follow: apple pomace (2.5%), peptone from casein (1.7%), peptone from soy hydrolyzate (0.3%), NaCl (0.5%).

The changes in the concentrations of reducing sugar and total phenols as well as the pH of the media throughout the fermentations are shown in Figure 5. A continuous decrease in the reducing sugar concentration with time was observed in both fermentation runs where the concentration remained constant in the control run. The decrease in sugar concentration indicated that sugars of apple pomace were utilized as substrate by the cells and that the releasing rate of sugars from pomace was lower than the rate of consumption of them. The variations in total phenol concentration with time are also similar for both fermentations where it increased up to 48 h and then decreased. The initial increase showed that the cells may contribute the release of phenolics by their enzymes. However, the decrease after a certain time indicated the loose of enzyme activities and domination of free release of phenolics from pomace over the enzymes action. The oxidation of phenolics might have also occurred. The profile of control run also showed the free release of phenolics from the apple pomace. The pH of the fermentation media increased after 24 h due to the changes in medium composition by the action of enzyme activities on the cells. The formation of alkaline compounds based ammonia might have increased the medium pH.

The α-amylase activities at the 24 h and 48 h of the fermentations are given in Table 4. Parallel fermentation runs resulted in the similar activities. The α-amylase activities were higher at the 24 h than those at 48 h. This was compatible with the change in reducing sugar concentration where it decreased at 48 h by the action of α-amylase activity to degrade starch.

Total antioxidant activity of the fermentation medium was also followed (Table 4). The control medium possessed almost the constant antioxidant activity throughout 72 h arising from the phenolic compounds present in the medium. The fermentation medium possessed more antioxidant activity compared to that of control indicating the release of phenolics by the cells at the 72 th h of fermentation. The antioxidant activity increased after 48 h where the concentration of total phenolic compounds was at its highest value.

Research Needs and Perspectives

Apple pomace has a low economical value, it is difficult to disposal; also causes pollution when it is discarded into the environment. However, valuable products can be produced in a simple fermentation medium by using this food processing chain product as shown in the present study. Phenolic compounds are useful for human and animal health, sugars are the main substrates for fermentations and enzymes produced using pomaces are cheaper than produced using synthetic medium.

To be made the fermentation medium more cheaper, urea should be preferred as the nitrogen source instead of peptone since there wasn't a significant difference in yields when peptone or urea were used. In further studies, solid biomass of fermentation medium, that is the mixture of microbial biomass and remaing pomace, can be evaluated as a feed additive or fertilizer. This study supports circular bioeconomy and sustainability by using renewable resources and eco-friendly processes for value-added products for the market.

CONCLUSIONS

The overall results of the present study showed that apple pomace can be valorized in a simple biotechnological process. It is considered that all of the outcomes have a potential for industrial usage. In the sterilized fermentation medium, the amount of sugars decreased or increased in all the experiments. Also, α-amylase activities were detected in all the experiments. These results showed that the sugars were consumed by the cells and α-amylase enzyme was started to secrete when the sugars became insufficient for the growth of the cells. *Bacillus subtilis* assimilated reducing sugars of apple pomace and secreted α-amylase enzyme, which has an important role in chemical and food industries. Phenolic compounds of apple pomace released in the first period of fermentation probably before the enzymes of *B.subtilis* started to degrade them. Therefore, by finalizing the fermentation where phenolics concentration was still high would provide the process to obtain phenolic compounds. The α-amylase activity at 24 th h was found to be 29.6% higher when the fermentation initaited with a former fermentation medium than that of started with the inoculum based on starch induced agar (Inoculum with the culture of OD=1.0 both Table 2 and Table 3) and liquid incubation media. The activity of the enzyme can be enhanced by further formulation of fermentation medium.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

This study is a part of the Master's Thesis of Sıla Sözgen. The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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REFERENCES

Ajila, C.M., Brar, S.K., Verma, M., Tyagi, R.D., Valéro, J.R., 2011. Solid-state fermentation of apple pomace using Phanerocheate chrysosporium – Liberation and extraction of phenolic antioxidants. Food Chem. 126, 1071–1080. https://doi.org/10.1016/j.foodchem.2010.11.129

Ajila, C.M., Sarma, S.J., Brar, S.K., Godbout, S., Cote, M., Guay, F., Verma, M., Valéro, J.R., 2015. Fermented Apple Pomace as a Feed Additive to Enhance Growth Performance of Growing Pigs and Its Effects on Emissions. Agriculture 5, 313–329. https://doi.org/10.3390/agriculture5020313

Bialkowska, A.M., Jędrzejczak-Krzepkowska, M., Gromek, E., Krysiak, J., Sikora, B., Kalinowska, H., Kubik, C., Schütt, F., Turkiewicz, M., 2016. Effects of genetic modifications and fermentation conditions on 2 , 3-butanediol production by alkaliphilic Bacillus subtilis. Appl Microbiol Biotechnol 2663–2676. https://doi.org/10.1007/s00253-015-7164-2

Dash, B.K., Rahman, M.M., Sarker, P.K., 2015. Molecular identification of a newly isolated bacillus subtilis BI19 and optimization of production conditions for enhanced production of extracellular amylase. Biomed Res. Int. 2015, 9 pages. https://doi.org/10.1155/2015/859805

Enzymatic Assay of α-Amylase (EC 3.2.1.1). Retrieved from https://www.s_gmaaldr_ch.com/techn_cal-documents/protocols/b_ology/enzymat_c-assay-of-α-amylase.html

Gassara, F., Ajila, C.M., Brar, S.K., Verma, M., Tyagi, R.D., Valero, J.R., 2012. Liquid state fermentation of apple pomace sludge for the production of ligninolytic enzymes and liberation of polyphenolic compounds. Process Biochem. 47, 999–1004. https://doi.org/https://doi.org/10.1016/j.procbio.2012.03.001

Keskin-Šašić, I., Tahirović, I., Topčagić, A., Klepo, L., Salihović, M., Ibragić, S., Toromanović, J., Ajanović, A., Velispahić, E., 2012. Total Phenolic Content and Antioxidant Capacity of Fruit Juices. Bull. Chem. Technol. Bosnia Herzegovina 39, 25–28.

Kosseva, M., 2011. Management and Processing of Food Wastes, Second edi. ed, Comprehensive Biotechnology. Reference Module in Earth Systems and Environmental Sciences. https://doi.org/https://doi.org/10.1016/B978-0-08-088504-9.00393-7

Kumar, P., Ray, S., Kalia, V.C., 2016. Production of co-polymers of polyhydroxyalkanoates by regulating the hydrolysis of biowastes. Bioresour. Technol. 200, 413–419. https://doi.org/http://dx.doi.org/10.1016/j.biortech.2015.10.045

Kuvvet, C., Uzuner, S., Cekmecelioglu, D., 2017. Improvement of Pectinase Production by Co-culture of Bacillus spp. Using Apple Pomace as a Carbon Source. Waste and Biomass Valorization 0, 1–9. https://doi.org/10.1007/s12649-017-0142-4

Lyu, F., Luiz, S.F., Azeredo, D.R.P., Cruz, A.G., Ajlouni, S., Ranadheera, C.S., 2020. Apple pomace as a functional and

healthy ingredient in food products: A review. Processes 8, 1–15. https://doi.org/10.3390/pr8030319

Miller, G.L., 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Anal. Chem. 31, 426–428. https://doi.org/10.1021/ac60147a030

Rana, N., Verma, N., Vaidya, D., Dipta, B., 2017. Production of Amylase from Bacillus thuringiensis J2 Using Apple Pomace as Substrate in Solid State Fermentation. Int. J. Curr. Microbiol. Appl. Sci. 6, 3465–3474. https://doi.org/10.20546/ijcmas.2017.608.415

Sadasivam S., M.A., 2008. Biochemical Methods, Third Edit. ed. New Age International Publishers, New Delhi.

Sanger, F., Nicklen, S., Coulson, A.., 1977. DNA sequencing with chain-terminating. Proc Natl Acad Sci USA 74, 5463–5467.

Savatovic, S., Tepic, A., Sumic, Z., Nikolic, M., 2009. Antioxidant activity of polyphenol-enriched apple juice. Acta Period. Technol. 220, 95–102. https://doi.org/10.2298/apt0940095s

Škerget, M., Kotnik, P., Hadolin, M., Hraš, A.R., Simonič, M., Knez, Ž., 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chem. 89, 191–198. https://doi.org/10.1016/j.foodchem.2004.02.025

Su, Y., Liu, C., Fang, H., Zhang, D., 2020. Bacillus subtilis: A universal cell factory for industry, agriculture, biomaterials and medicine. Microb. Cell Fact. 19, 1–12. https://doi.org/10.1186/s12934-020-01436-8

Sulewska, H., Adamczyk, J., Gygert, H., Rogacki, J., Szymanska, G., Smiatacz, K., Panasiewicz, K., Tomaszyk, K., 2014. A comparison of controlled self-pollination and open pollination results based on maize grain quality. Spanish J. Agric. Res. 12, 492–500. https://doi.org/10.5424/sjar/2014122-4970

Sun, H., Ge, X., Hao, Z., Peng, M., 2010. Cellulase production by Trichoderma sp . on apple pomace under solid state fermentation. African J. Biotechnol. 9, 163–166.

Venkata, E., Raju, N., Goli, D., 2014. Effect Of Physiochemical Parameters On Fibrinolytic Protease Production By Solid State Fermentation. Mater. Sci. 3, 1937–1954.

Yen, G., Chen, H., 1995. Antioxidant Activity of Various Tea Extracts in Relation to Their Antimut agenicity. J. Agric. Food Chem. 27–32.