CHEMICAL ENGINEERING TRANSACTIONS
VOL. 37, 2014
A publication of AIDIC
The Italian Association of Chemical Engineering
www.aidic.it/cet
Guest Editors: Eliseo Ranzi, Katharina Kohse- Höinghaus
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ISBN 978-88-95608-28-0; **ISSN** 2283-9216
DOI: 10.3303/CET1437102

Vinegar Production from Pineapple Wastes –Preliminary Saccharification Trials

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This study is located in the within of a research devoted at processing wastes both in developing and in developed Countries, so reducing both environmental pollution and seasonal fruit losses. In particular, the full work intended to completely process pineapple wastes into vinegar which may be then used as dressing, food preservative, and disinfectant. The preliminary trials presented here deepened the first process step (i.e. the saccharification) and looked into the feasibility of producing the greatest yield of reducing sugars from peels and core of pineapples.
Wastes were cut into thin strips, chopped in a mixer, and divided into samples of peel and core to which distilled water was added. For enhancing reducing sugar yield, physical treatments were arranged to disaggregate the fibrous structure followed by enzyme treatments to breakdown cellulose polymers and to hydrolyse sucrose. The optimal time-temperature conditions of each process step were searched for gaining the highest reducing sugars yield at the end of the saccharification. Cellulolytic enzymes were tested for 4-8-18-24 h at 30-40-50 °C, invertase addition was arranged, and amylolytic enzymes were evaluated.
All determinations were done in duplicate and a factorial ANOVA with Tukey's test at $p \leq 0.05$ was used to measure the significance of the differences among treatments.
The conditions allowing the greatest reducing sugar yield were: the addition to 100 g of waste fresh weight (fw) of 0.025 mL of thermostable α-amylase before a 10 min treatment at 143.27 kPa followed by 24 h-50 °C incubation with 0.05 g pectinase/kg_{fw}, 6 g cellulase/kg_{fw}, 1 g hemicellulase/kg_{fw}, and 0.05 % glucoamylase and pullulanase (V_{enzyme}/kg_{fw}). Then, samples were incubated with 0.05 g invertase/kg_{fw} for 3 h at 50 °C. Under these conditions, more than 100 g of reducing sugars per kg of fresh peels and about 330 g of reducing sugars per kg of fresh core were obtained.

1. Introduction

Most nations, whether economically advanced or at different stages of development are faced with the issue of disposal and treatment of wastes (Itelima et al., 2013). Agro-industrial wastes are generated in large amounts every year and their reuse in processes is of particular interest due to their availability, low cost, and characteristics that allow at obtaining different value-added compounds (De Freitas Borghi et al., 2009). Tropical and subtropical fruits processing have considerably higher ratios of by-products than the temperate fruits (Schieber et al., 2001). Pineapple (*Ananas comosus*) by-products are not exceptions. Indeed, several efforts have been made in order to utilize pineapple wastes, which have already been used as the substrate for the production of bromelain and organic acids (Dacera et al., 2009), fibre and phenolic anti-oxidants (Larrauri et al., 1997), ethanol and biogas (Nigam, 1999). Since pineapple has the second highest production volume of all tropical fruits in the world (FAO, 2009), the production of processed items results in massive waste generation, estimated about 40-50 % from fresh fruit as peels and core (Buckle, 1989). Even if pineapple residues are rich in sugars, especially in sucrose, glucose and fructose and other components like minerals and vitamins (Abdullah and Hanafi, 2008), they cannot be used in full. The pineapple peel contained an appreciable amount of insoluble fibre-rich fraction which primarily consisted of cellulose, pectin substances, hemicellulose, and notable proportions of lignin (Huang

Please cite this article as: Roda A., De Faveri D.M., Dordoni R., Lambri M., 2014, Vinegar production from pineapple wastes –preliminary saccharification trials, Chemical Engineering Transactions, 37, 607-612 DOI: 10.3303/CET1437102

et al., 2011). A multitude of different pretreatment technologies have been suggested during the last decades (Alvira et al., 2010) in order to hydrolyze the cellulosic waste materials.
The present study, devoted to turn pineapple wastes into vinegar, investigated throughout preliminary trials the first step (i.e. saccharification) of the process with the purpose of obtaining the greatest yield of reducing sugars from peel and core of pineapples.

2. Materials and methods

2.1 Materials

Enzymes
Cellulolytic and pectinolytic enzymes were: cellulase (from *Aspergillus niger*, 0.8 enzyme units/mg solid, Sigma C1184-25KU, Sigma-Aldrich, USA), hemicellulase (from *Aspergillus niger*, 1.5 enzyme units/mg solid, using a β-galactose dehydrogenase system and locust bean gum as a substrate, Sigma H2125-150KU, Sigma-Aldrich, USA), and pectinase (>200 enzyme units PL/g, Enartis Zym Quick, Enartis, Novara, Italy). Amylolytic enzymes were: thermostable α-amylase (from *Bacillus licheniformis,* 135 KNU/g, Liquezyme-X), glucoamylase (from *Aspergillus niger,* 400 AGU/g, Dextrozyme GA), and pullulanase (from *Bacillus acidopullulyticus,* 400 AGU/g, Dextrozyme GX) and provided by M/s Novozymes A/S (Denmark). For sucrose hydrolysis, invertase (from bakery's yeast *Saccharomyces cerevisiae*, >300 enzyme units/mg, Sigma-Aldrich, USA) was employed.

Raw material
Pineapples (average fresh weight of 1.73±0.49 kg) were purchased from the supermarket in Italy. They were kept at 22 °C before undergoing the saccharification process. The pineapples were washed and the wastes were separated from the edible pulp and the crown. The pineapple wastes used in the study, peel and core, were separately processed. The peels were manually cut in small pieces using knife and then chopped in an electric blender (La Moulinette, Moulinex, Groupe SEB, France) to obtain a homogeneous mixture. Similarly, pineapple core mash was prepared. Samples of 50 g and 20 g of peel and core respectively, were stored in freezer (-18 °C) prior to use.

2.2 Saccharification procedure

Hydrolysis of cellulose polymers and of sucrose in peel and core samples (treatment 1 – T1)
Each sample of pineapple peel (50 g) and core (20 g) was added in duplicate at 1:2 ratio to distilled water and put into 100 mL Pyrex bottle sealed with screw cap. Then, hydrolysis was performed at pH 4.00 adding 6 g/kg_{fw} of cellulase, 1 g/kg_{fw} of hemicellulase, and 0.05 g/kg_{fw} of pectinase under different times and temperatures: 4-8-18-24 h and 30-40-50 °C, respectively. Then, the samples were brought down to 21±2 °C, filtrated using cheese cloth, and poured to the beaker. The filtered sample was added with 0.05 g/kg_{fw} of invertase and left for 3 h at 50 °C before being centrifuged at 3000 rpm for 15 min at 24 °C.

Physical pre-treatment and use of amylolytic enzymes on peel and core samples (treatment 2 – T2)
To enhance reducing sugar yield, pineapple peel and core samples were subjected to 10 and 30 min 143.27 kPa treatment before enzymatic hydrolysis with and without the addition of 0.025 % thermostable α-amylase (V_{enzyme}/kg_{fw}). Then, 0.05 % glucoamylase and pullulanase (V_{enzyme}/kg_{fw}) were mixed with cellulolytic enzymes and incubated under the conditions tested in T1 which allowed at gaining the greatest sugar yield. The samples were filtrated and incubated with invertase under the condition reported in T1.

2.3 Chemical analyses
Pineapple waste samples were analyzed in duplicate. The reducing sugar content was determined on liquid aliquots of peel and core juice before and after T1 and T2 according to Lane and Eynon (1923) method with Crison compact titrator (Crison Instruments SA, Alella, Spain). Carrez I and Carrez II reactive for sugar analisys were provided by Carlo Erba reagents (Milan, Italy). The acidity and pH were measured using the Crison TitroMatic 1S (Crison Instruments SA, Alella, Spain).

2.4 Data analysis and statistics.
All determinations were done in duplicate and a factorial ANOVA with Tukey's test at $p \leq 0.05$ was used to measure the significance of the differences among the conditions tested within each treatment. The statistics package IBM SPSS Statistics 19 (IBM Corporation, New York, USA) was used.

3. Results and discussion

Acidity and pH of pineapple wastes before and after T1 are reported in Table 1. The results were in agreement with Abdullah and Hanafi's study (2008), and with Sasaki et al. (1991) who reported the pH of pineapple juice in the range value of 3.6-4.6.

Table 1: Chemical analysis of pineapple waste samples before (control) and after T1. Values are means ± SD (n=15). Within each row, different letters indicate statistically different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$

Analysis	Control peel	Peel	Control core	Core
Acidity	2.55±0.97 a	3.54±1.05 a	3.46±0.90 a	4.42±1.37 a
pH	4.16±0.45 a	3.89±0.10 a	3.93±0.10 a	3.85±0.08 a

The results of control samples indicated that peel and core from pineapple are a substrate suitable for cultivation of bacteria, i.e. potentially used as a carbon source for organic acid fermentation (Abdullah and Hanafi, 2008). Indeed, the saccharification process can be performed without changing the pH value of substrate, as pH of the wastes is ideal for the activity of cellulolytic and pectinolytic enzymes (Romsayud et al., 2009).

3.1 Hydrolysis of cellulose polymers and of sucrose in peel and core samples (T1)

Hydrolysis with cellulase, hemicellulase, and pectinase was tested at three temperatures (30-40-50 ºC), in a time range of 4-8-18-24 h, in order to gain the highest yield of reducing sugars (Table 2).

The enzymes tested were more effective in the peel samples, probably because of the higher cellulosic content compared with the core (Abdullah and Hanafi, 2008). Moreover, according with Tengbord et al. (2001) more than 8 h hydrolysis at 30 °C improved the final sugar yield. As a consequence, peel and core were submitted to 18 and 24 h hydrolysis at 40 and 50 °C (Figure 1). In agreement with De Prados et al. (2010), high temperature and long time were found to be favourable for maximum sugar yield.

Once determined the optimal time-temperature (24 h-50 °C) values for cellulolytic hydrolysis, the subsequent action of other enzymes was tested. From the data received from literature (Abdullah and Hanafi, 2008; Hemalatha and Anbuselvi, 2013) the amount of sucrose in the pineapple liquid waste is in the range of 16.75 to 40.10 g/L, in a significant percentage out of the total amount of reducing sugars (about 40 g/L) which primarily consists of glucose (>20 g/L) and fructose (<20 g/L). Thereby, invertase was tested to hydrolyze sucrose at the optimum enzyme conditions (Mohd Zaina et al., 2010), i.e. 3 h at 50 °C.

The results of the saccharification process with and without the addition of invertase are reported in Figure 2. As shown, the use of invertase enzyme allowed hydrolyzing the sucrose enabling to significantly increase the concentration of reducing sugars in core especially.

Table 2: Reducing sugars (g/kg_{fw}) of pineapple peel and core after 4-8-18-24 h of hydrolysis with cellulase, hemicellulase, and pectinase at 30 °C. Values are means ± SD (n=16). Within each row, different letters indicate statistically different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$

Sample	Initial	4 h	8 h	18 h	24 h
Peel	23.2±2.8 c	28.8±1.3 b	34.1±2.4 a	35.5±2.1 a	37.5±2.6 a
Core	20.2±1.7 b	21.6±0.8 b	27.9±1.1 a	28.0±0.9 a	29.3±1.3 a

These results were in agreement with Mohd Zaina et al. (2010) who used 3 h-50 °C invertase immobilized in PVA-alginate matrix and observed that sucrose was reduced from 58.7 g/L to 5.1 g/L in liquid pineapple waste. Nadzirah et al. (2013) reported that pineapple juice contains 12-15 % (w/V) sugars of which two-third are in the form of sucrose and the rest are glucose and fructose. They also found that sucrose in clear supernatant of pineapple peel and core is in the range of 2.58 to 3.87 % (w/V) and from 8.37 to 8.92 % respectively, highlighting sucrose as the major sugar in the pineapple waste extract.

3.2 Physical pre-treatments and use of amylolytic enzymes on peel and core samples (T2)

In order to enhance the yield of reducing sugars particularly from pineapple peels, physical pre-treatments to alter the structure of the fibrous matrix were arranged. As stated by Taherzadeh and Karimi (2007), the physical pre-treatment of pineapple fibre is crucial before enzymatic hydrolysis, especially regarding the peels.

The sample preparation described in *"raw material"* section implied the crushing of the pineapple wastes, as a reduction of particle size and cristallinity of lignocellulosic components in order to increase the specific surface and reduce the degree of polymerization (Sun and Cheng, 2002). This should to improve the degradation of the structure of the fibrous matrix, but it is often not sufficient alone to allow the complete hydrolysis of lignocellulosic components (Kuo and Lee, 2009).

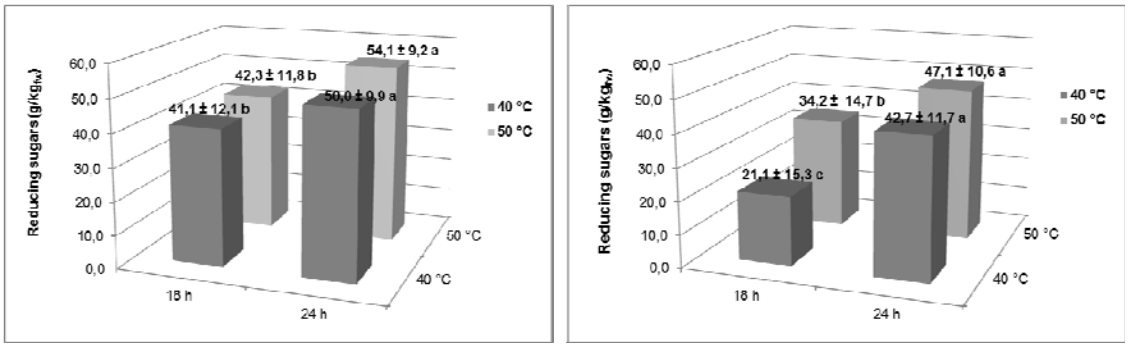

Figure 1: Reducing sugars (g/kg$_{fw}$) of pineapple peel (a) and core (b) after 18 and 24 h of hydrolysis at 40 and 50 °C. At each bar top, means ± SD (n=8) are reported with different letters indicating statistically different values according to post-hoc comparison (Tukey's test) at p ≤ 0.05

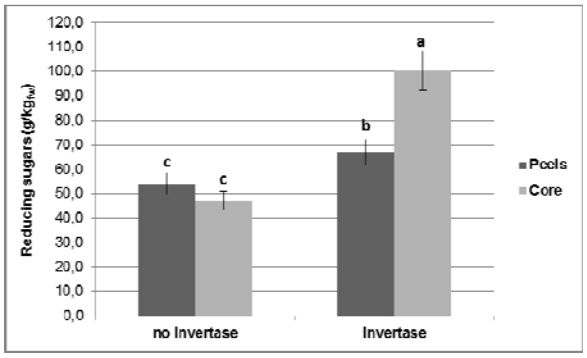

Figure 2: Reducing sugars (g/kg$_{fw}$) of pineapple peel and core at 50 °C for 24 h with and without invertase enzyme addition. Values are means ± SD (n=6). At each bar top, different letters indicate statistically different values according to post-hoc comparison (Tukey's test) at p ≤ 0.05

Since the lignin barrier, the complex structure of the cellulose and hemicellulose molecules, and the high crystallinity of lignocellulose restrict the enzyme action, inhibiting the direct hydrolysis with enzymes (Chandra et al., 2007), physical pretreatments coupled with enzymatic hydrolysis are required to aid the conversion of the hemicelluloses and cellulose to monosaccharides.

Based on these findings and on the results from Saha and Bothast (1999), pineapple wastes were treated for 10 and 30 min to 143.27 kPa and then they were incubated with cellulolytic enzymes and invertase under the optimal time-temperature values obtained from T1.

The results showed that the reducing sugars reached the value of 48.6±10.3 and 98.5±5.1 g/kg$_{fw}$ in peel and core respectively, when the enzymatic hydrolysis was preceded by the 10 min 143.27 kPa treatment. Not significantly different values were detected in peels (47.5±10.5 g/kg$_{fw}$) and a slightly, but significant greater sugar amounts were measured in core (105.1±4.7 g/kg$_{fw}$) when the treatment lasted in 30 min.

From these outcomes and in order to increase the accessibility to the enzymatic attack (Alvira et al., 2010), it was chosen to maintain the 10 min-143.27 kPa treatment before the addition of cellulolytic and invertase enzymes both to peel and core samples. Moreover, as the structural modifications of the fibrous matrix have a great effect on the subsequent steps, it was attempted to mix the amylolytic enzymes (thermostable α-amylase, glucoamylase, and pullulanase) with cellulase, hemicellulase, and pectinase and then to proceed with 3 h-50 °C incubation with invertase. This treatment used 0.025 % thermostable α-amylase (V_{enzyme}/kg$_{fw}$) and 0.05 % glucoamylase and pullulanase (V_{enzyme}/kg$_{fw}$). It was arranged by adding the thermostable α-amylase before and after the 10 min-143.27 kPa step, the subsequent glucoamylase

and pullulanase mixing with cellulase, hemicellulase, and pectinase enzymes for 50 °C-24 h hydrolysis followed by 3 h incubation with invertase at 50 °C.
As detailed in Figure 3, the use of amylolytic enzymes seems to be crucial only if the thermostable α-amylase was added before the 143.27 kPa step, allowing the yield of 96.6±7.6 g/kg$_{fw}$ and of 235.2±12.8 g/kg$_{fw}$ from the pineapple peels and core, respectively. This was unexpected since starch component is usually not detected in pineapple (Araya-Cloutier et al., 2012).

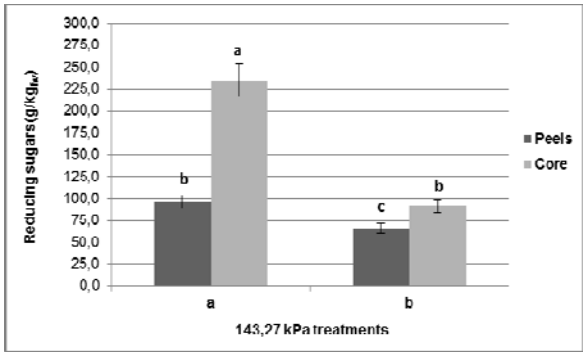

Figure 3: Reducing sugars (g/kg$_{fw}$) of pineapple peels and core after saccharification process. Values are means ± SD (n=10). At each bar top, different letters indicate statistically different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$

From these preliminary results, the action of thermostable α-amylase is effective only when this enzyme was added at the beginning of the process. Its contact with the fibrous matrix during the 143.27 kPa treatment seemed to be essential in order to achieve a significant improved sugar yield at the end of cellulolytic and sucrose hydrolyses. Further investigations are needed on this matter to explain the mechanisms that this enzyme seemed to have in promoting the sugar release.

4. Conclusion

The purpose of the present study was to deep saccharification process, the first step of vinegar production from pineapple wastes, looked into feasibility of producing the greatest yield of reducing sugars from peel and core of pineapples.
Pre-treatments and enzymatic saccharification procedures were evaluated for the conversion of pineapple waste fibres to monomeric sugars with different enzymes (cellulolytic, amylolytic and invertase). Firstly, cellulolytic enzymes followed by invertase were added achieving final reducing sugars of almost 67 and 100 g/kg of fresh weight of pineapple peel and core, respectively. Optimal time-temperature conditions of this enzymatic hydrolysis were proven to be 24 h-50 °C. Secondly, to open the bundles of lignocelluloses in order to access the polymer chains of cellulose and hemicelluloses to enzymatic action, a 10 min-143.27 kPa treatment was performed. Finally, it was turned out that addition of thermostable α-amylase during the 143.27 kPa pretreatment and the subsequent hydrolysis with a mix of cellulolytic and amylolytic enzymes allowed reaching about 100 and 330 g/kg$_{fw}$ of reducing sugars in pineapple peels and core, respectively.
These results indicated that enzymatic treatments of pineapple wastes had a significant effect on the saccharification process, but further investigations are needed especially regarding the action of thermostable α-amylase and the related operating parameters.

References

Abdullah A., Hanafi M., 2008, Characterisation of solid and liquid pineapple waste, Reaktor 12, 48-52.
Alvira P., Tomás-Pejó E., Ballesteros M., Negro M.J., 2010, Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review, Bioresour. Technol. 101, 4851-4861.
Araya-Cloutier C., Rojas-Garbanzo C., Velázquez-Carrillo C., 2012, Effect of initial sugar concentration on the production of L(+) Lactic acid by simultaneous enzymatic hydrolysis and fermentation of an agro-industrial waste product of pineapple (*Ananas comosus*) using *Lactobacillus casei* subspecies *rhamnosus*, Int. J. Biotech. Well. Indus. 1, 91-100.

Buckle K.A., 1989, Biotechnology opportunities in waste treatment and utilization for the food industry. In Biotechnology and The Food Industry. Eds. Rogers P.L., 261-277. Breach Science Publishers, New York, USA.

Chandra R., Bura R., Mabee W., Berlin A., Pan X., Saddler J., 2007, Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics?, Adv. Biochem. Eng. Biotechnol., 108, 67-93.

Dacera D.D.M., Babel S., Parkpian P., 2009, Potential for land application of contaminated sewage sludge treated with fermented liquid from pineapple wastes, J. Hazard. Mater. 167. 866-872.

De Freitas Borghi D., Guirardello R., Cardozo Filho L., 2009, Storage logistics of fruits and vegetables: effect of temperature, Chemical Engineering Transactions, 17, 951-956.

De Prados S.M., Fito P.L., 2010, Industrial pineapple waste as a feasible source to produce bioethanol. In International Conference on Food Innovation. Universidad Polinecnica de Valencia, Spain.

FAO, 2009, Food Outlook. Global Market Analysis. Global Information and Early Warning System. 2, 1-103.

Hemalatha R., Anbuselvi S., 2013, Physicohemical constituents of pineapple pulp and waste, J. Chem. Pharm. Res., 5, 240-242.

Huang Y.L., Chow C.J., Fang Y.J., 2011, Preparation and physicochemical properties of fiber-rich fraction from pineapple peels as a potential ingredient, J. Food Drug Anal., 19, 318-323.

Itelima J., Onwuliri F., Onwuliri E., Onyimba I., Oforji S., 2013, Bio-Ethanol production from banana, plantain and pineapple peels by simultaneous saccharification and fermentation process, International Journal of Environmental Science and Development, 4, 213-216.

Krueger D.A., Krueger G.R., and Maciel J., 1992, Composition of pineapple juice, J. AOAC Int., 75, 280-282.

Kumar P., Barrett D.M., Delwiche M.J., Stroeve P., 2009, Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production, Ind. Eng. Chem. Res., 48, 3713-3729.

Kuo C.H., Lee C.K., 2009, Enhancement of enzymatic saccharification of cellulose by cellulose dissolution pretreatments, Carbohydr. Polym., 77, 41–46.

Lane J.H., Eynon L., 1923, Determination of reducing sugars by means of Fehling's solution with methylene blue as internal indicator, J. Chem. Ind. Trans., 42, 32-36.

Larrauri J.A., Ruperez P., Calixto F.S., 1997, Pineapple shell as a source of dietary fiber with associated polyphenols, J. Agri. Food Chem., 45, 4028-4031.

Mohd Zaina N.A., Suhaimib M.S., Idris A., 2010, Hydrolysis of liquid pineapple waste by invertase immobilized in PVA–alginate matrix, Biochem. Eng. J., 50, 83–89.

Nadzirah K.Z., Zainal S., Noriham A., Normah I., Siti Roha A.M., Nadya H., 2013, Physico-chemical properties of pineapple variety N36 harvested and stored at different maturity stages, Int. Food Research J., 20, 225-231.

Nigam J.M., 1999, Continuous ethanol production from pineapple cannery waste, J. Biotechnol., 72, 197-202.

Romsayud A., Songkasiri W., Nopharatana A., Chaiprasert P., 2009, Combination effect of pH and acetate on enzymatic cellulose hydrolysis, J. Environ. Sci., 21, 965–970.

Saha B.C., Bothast R.J., 1999, Pretreatment and enzymatic saccharification of corn fiber, Appl Biochem. Biotechnol. 76, 65–77.

Sasaki K., Noparatnaraphorn N., Nagai S., 1991, Use of photosynthetic bacteria for the production of SCP and chemicals from agro industrial waste. In Bioconversion of Waste Material to Industrial Product, Ed. Martin. A.M. Elviser Applied Science, London, 225-233.

Schieber A., Stintzing F.C., Carle R., 2001, By-products of plant food processing as a source of functional compounds - recent developments, Trends Food Sci. Technol., 12, 401-413.

Sun Y., Cheng J., 2002, Hydrolysis of lignocellulosic materials for ethanol production: A Review, Bioresource. Technol. 83, 1-11.

Taherzadeh M.J., Karimi K., 2007, Enzyme-based hydrolysis processes for ethanol from lignocellulosic materials: a review, Bioresources, 2, 707-738.

Tengborg C., Galbe M., and Zacchi G., 2001, Resuce inhibition of enzimatic hydrolysis of steam-pretreated soflwood, Enzyme Microb. Technol., 28, 835-844.