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Lactic Acid Fermentation by Different *Lactobacillus* Species Using *Sorghum* Seed Extract as Carbon Source

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Abstract

Sorghum bicolor L., a plant of Gramineae family is a perenial grass which is grown in many parts of tropical and warm temperates of the world. Sorghum seeds are rich in carbohydrates and can be used as fermentation medium providing it is produced through proper extraction and sacharification processes. In this study, the fermentation capacity of an aqueous or ethanolic extract from sorghum seed was investigated in Lab-scale bioreactors by using three selected Lactobacillus species including L. delbrueckii, L. acidophilus and a new isolated strain identified as a Lactobacillus sp.

Among the selected bacterial species, Lactobacillus delbrueckii was found to have unique characteristics as it could tolerate different physico-chemical conditions. Compared to other species, L. delbrueckii could remain metabolically active in high concentration of NaCl and lactic acid, which is particular to the fermentation of sorghum seed extract neutrilized by NaOH. L.delbrueckii could also tolerate high temperature and, additionally, was able to produce more lactic acid in high concentration of carbohydrate during fermentation process (Yield 85-90%). In conclusion, the results of this study demonstrated that hydrolysed sorghum seed extract has enough nutrients to support the efficient growth of lactic acid bacteria and production of lactic acid under favorable conditions (37°C, 100 g/L initial carbohydrate concentration).

Keywords: *Sorghum Seed, Lactic Acid Fermentation, Lactobacillus Sp., Saccharification, Growth*

1. Introduction

Grain sorghum (*Sorghum biocolor L.*) is considered a rich source of carbohydrates which is used for various food and industrial applications (Correia et al., 2005). It is one of the most drought-tolerant cereal grain crops which requires little input during growth, but the yield may further imrove with good husbandry (Taylor et al., 2006).

Many reports contrast world-wide use of different parts of this plant in food and traditional medicine (Akah and Nwambie, 1994; Ciacci, 2007) Moreover, the emergence of *sorghum*-derived products in food and renewable energy make it a promising crop, able to diversify many

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aspects of industrial nutritional products and meet the challenges in feeding and green fueling (Dahlberg et al., 2011). *Sorghum grain* can be efficiently utilized in the production of bio-industrial products, including bioethanol (Rooney et al., 2000) and lactic acid (Zhan et al., 2003).

Lactic acid bacteria are used industrially for production of biologically based products such as dairy products, lactic acid, probiotics and so on (Guglielmotti et al., 2007). Lactic acid is the simplest hydroxy acid having an asymmetric carbon atom. It exists in two optically active isomeric forms with opposite rotations of polarized light, D-(+) lactic acid and L-(+) lactic acid (Correia et al., 2005).

Lactic acid can be produced either by chemical synthesis or by fermentative processes, however, the optically pure L-(+)- or D-(−)-lactic acid can only be prepared through fermentation processes, with a proper selection of microorganisms (Lin et al., 2007).

In recent years, the amount of lactic acid obtained by fermentation processes has increased. This organic acid has been used as a raw material for polylactic acid (PLA) and ethyl lactate production (R). PLA is a biodegradable thermoplastic resin that can be well substituted for petroleum-based thermal plastics, reducing environmental pollution and other problems associated with petroleum-based plastics (Kharas et al., 1994).

Correia et al. (2005) studied the effect of lactic fermentation on wet-cooked *sorghum.* When the wet-cooked *sorghum* seed extract was inoculated with lactic acid bacteria, only *L. fermentum* and the commercial yogurt inoculums were able to grow in *sorghum* media. In all fermentations, a decrease in pH was noticed and consequently an increase in titratable acidity was detected.

Zhan et al. (2003) studied the effect of *sorghum* genotypes and fermentation condition for ethanol and lactic acid production and found that for the *sorghum* varieties they studied, both genotypes and locations had significant effects on lactic acid yield. The unique health characteristics associated with *sorghum* seed made it a good candidate for the present study. Our attempt in this work was to study the fermentability of sorghum seed as a raw material and a carbon source for lactic acid production.

Sorghum seed extract was prepared with respect to physicochemical parameters related to the technical and operational downstream process. The present study was therefore undertaken to investigate the potential use of this extract as lactic acid fermentation substrate. To achieve this goal, three lactic acid bacterial species, *L. delbrueckii*, *L sp.* and *L. acidophilus* were tested on the extract in a controlled manner in order to develop and define an optimum condition for lactic fermentation.

2. Materials and methods

2-1. Saccharification of *sorghum* seeds

Sorghum seeds (*Sorghum biocolor L.*) cultivated in Isfahan university research farm were ground by hammer mill. The powdered seeds were treated with concentrated H_2SO_4 and heated at 95°C for 1 h. The reaction mixture was neutralized with lime: $Ca(OH)_2$ 35% and filtered. The filtrate was then evaporated under reduced pressure and the

residue was used in fermentation process. The overall process has been demonstarted in Fig. 1, where the selected operating conditions have been included. All steps were performed at bench scale using *sorghum* seeds.

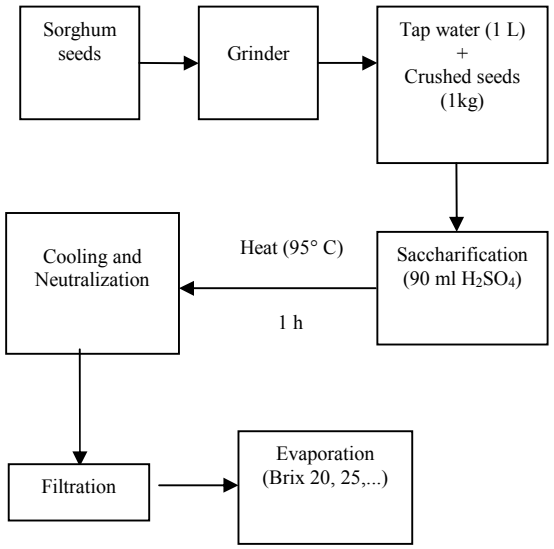

Figure 1. Flow chart for saccharification of *sorghum* seeds.

2-2. Bacterial strains

Two species of lactic acid bacteria (LAB) *Lactobacillus acidophilus PTCC 1643 and Lactobacillus delbrueckii* PTCC 1333 were purchased from Persian type culture collection (PTCC) in freeze-dried form. An isolated lactic acid bacterium which was identified in genius level (*Lactobacillus* sp.) obtained from Isfahan Biotechnology and Agriculture Research Center was also used in this study. *L. acidophilus* and *L. delbrueckii* were chosen according to their physiological characteristics such as their homofermentative features and the ability of maltose fermentation. Heterofermentation capacity was determined by using Durham tubes in MRS broth (see below). Growth was assigned positive if turbidity of the media progressed in the tubes during incubation period whereas gas accumulation in Durham tubes was considered as an indication for CO_2 production.The species which produced gas was taken as heterofermentative lactic acid.

2-3. Culture media and inocula preparation

The growth culture media used through this study were MRS agar (de Man, Rogosa and Sharpe agar) and MRS broth. These media were used for *Lactobacillus* sp. inoculum preparation. The inoculum was prepared by inoculating a full slant culture into 100 mL of sterile growth medium (MRS broth) in 500 mL flasks. The flasks were incubated at 37ºC with shaking at 160 rpm for 18 h.

Inoculum of each stratin was prepared in MRS broth. An 18 h culture of each isolate was used as the inoculum. Cells were centrifuged, resuspended in physiologic serum, and 100 µl of the suspension was inoculated into each test tube.

The main culture medium used in Lab-scale bioreactor was prepared from seed extract syrup. It consisted of: seed extract with different sugar concentrations (30-150 g L^{-1}), ammonium phosphate $(NH_4)_2 HPO_4$ (0.020 g) was added for each gram of glucose, with a pH of 5.7 ± 0.05 (Crueger, 1990). The effect of vitamins on lactic acid fermentation was determined by addition of multivitamins mixture containing: B_1 (0.5 mg mL^{-1}), B_2 (0.6

mg mL^{-1}), B_6 (0.4 mg mL^{-1}), B_{12} (1.5 mg mL^{-1}) and Panthotenic acid (1.0 mg mL^{-1}).

2-4. Assessment of some physiological features related to lactic acid fermentation

In order to determine the abilities of selected strains to survive and grow under the conditions they encounter during lactic acid fermentation, we tested their maltose utilization capacity, low pH tolerance as well as tolerance to NaCl.

2-4-1. Glucose and maltose utilization at different pH

A basal MRS medium (contained in g/L: Peptone proteose 10.0, yeast extract 8.0, sodium acetate 5.0, Triammonium citrate 2.0, Magnesium sulfate 0.25, Manganese sulfate 0.05, Dipotassium phosphate 2.0) was used in these series of studies. 20 g/L of glucose or 20 g/L of maltose were used as carbon sources. Universal test tube containing an inverted Durham tube was filled with 20 mL of the basal MRS medium and sterilized at 110°C for 10 min. To ensure the sterility of the culture media, the sterilized media were kept at 30 °C for 48h after sterilization. The study was performed with five different initial pH values, i.e., 4.0, 4.5, 5.0, 6.0 and 6.5. 1 M HCl and 1 M NaOH was used for pH setting.

2-4-2. Growth at different temperature and various concentration of sodium chloride

Basal MRS medium (see 2.4.1) containing 20 g/L glucose was used for evaluating the tolerance of selected lactic acid bacteria (LAB) to different temperature and concentration of lactic acid. The following temperatures were tested: 15, 20, 30, 37, 45 and 50°C. Shift of pH to acidic range would change the medium colour from purple to yellow which is assumed as an indicator for cellular carbohydrate metabolisms.

2-4-3. Growth at various concentration of acid lactic with different initial pH

The ability of glucose utilization in various lactic acid concentration with different initial pH was also studed by using basal MRS medium (see 2.4.1) containg 20 g/L of glucose. The concentrations of lactic acid tested were 0, 2.5, 5, 7.5, 10, 15% (w/v). The test tubes were inocubated at 37°C after inoculation.

After 48 h, the color change and turbidity of each test tube was noted as a simple indication of growth and finally the pH of each test tube was measured. Each treatment was performed in triplicate.

2-5. Shaking flask experiments

To maintain approximately the same conditions in the various experiments, pre-cultures were prepared in 500 mL shaking flasks, each containing 200 mL of growth media as described above. After incubation for 18 h at 37 °C with shaking at 160 rpm, the culture media were harvested and transferred to 500 mL shaking flask with fresh main culture medium in a volume ratio of 1:10. The fermentation temperature was 37 °C. The pH of the content of each flask was measured every 8 h. The samples were analyzed in duplicate.

2-6. Fermentation in a stirred tank reactor

In the present study fermentation was carried out simultaneously in two stirred tank bioreactors (AG CH-4301, INFORS Co. Switzerland). Each bioreactor was equipped with a culture vessel with a working volume of 2.7 L. The stirrer was a two stage-blade Rushton impeller. pH and temperature were

regulated automatically. Foam was detected by a probe capable of controlling antifoam addition. The exhaust gases pass through a condenser and then through a filter to avoid microbial contamination. During the course of experiments, the pH was adjusted automatically and fixed at 5.7 ± 0.05 by dosing NaOH. The fermentation temperature was 37 °C with an initial stirring of 160 rpm. The composition of the main culture medium was the same as the culture used in batch process. The main medium was pasteurized by Tyndallization method. The harvested cells from 200 mL of pre-culture (inoculums) were prepared as already described, and were then added to the previously sterilized vessels. A sample of about 20 mL was taken every two hours for further analyses. Each test was carried out in duplicate.The fermentation continued until no further decrease in pH was observed.

2-7. Determination of reducing sugars

Reducing sugars were determined by 3,5-Dinitrosalicylic acid (DNS) method (Bernfeld et al., 1995).

2-8. Determination of free glucose

Free glucose was measured in diluted extract by enzymatic method (Glucose oxidase kit, ChemEnzyme Co. Ltd).

2-9. Determination of Lactic acid concentration

The titratable acidity of fermented broth was determined according to titration method by NaOH (5N).

3. Results and discussion

The aim of this study was to investigate the possibility of using the syrup extract from *sorghum* seed as a carbon substarte for lactic acid production. For this purpose, various physicochemical parameters have been investigated and the results described hereunder.

3-1. Sorghum seed extraction

Extracts of sorghum seed were analyzed after acid-hydrolysis in order to determine the content of total carbohydrates. As shown in Table 1, the main available carbohydrate is glucose and the rest of carbohydrate was determined by 3,5- Dinitrosalicylic acid (DNS) reagent, maltose and other oligosaccharides. As shown in Table 1, by increase in the Brix of extract, the concentration of carbohydrate increases. This may reduce the risk of extract contamination due to osmotic pressure and lower water activity which inhibits microbial growth. As evidenced by the results in Table 1, no change was observed on leaving the concentrated extract at room temperature after 72 h, indicating that the growth of microorganisms had been inhibited by high concentration of carbohydrates. Therefore, the concentrated extract was selected for performing the experiments.

Table 1. Carbohydrate contents and pH of different *sorghum* seed extracts.

Brix	Total Carbohydrate (g/100 mL)	Glucose (g/100 mL)	pH[1]	pH[2]
Brix 15	9.54	4.12	6.65	4.51
Brix 20	13.45	6.37	5.53	4.31
Brix 25	23.0	15.30	5.4	3.4
Brix 35	34.52	28.73	5.38	3.21

Brix 43	40.83	34.5	5.25	5.25

pH [1] pH at starting fermentation reaction
pH [2] pH value of extract after 72 h. of fermentation reaction

3-2. Rapid screening for tolerance to low pH, high temperature, and high concentration of sodium chloride

Table 2 shows the tolerance of three selected LAB strains. One strain (*L. acidophilus*) grew at 15°C, while the other two strains were able to grow at higher temperatures ranging from 20 - 45°C. However, no growth was detected at 15°C. Wouters *et al*. (2000) noted that reduced glycolytic activity lead to reduced production of lactic acid in *L. lactis* at low temperatures. So it can be inferred that low temperature decreases the enzymatic activity which finally leads to slow metabolism of LAB.

The ability to grow at high temperature is a desirable trait in fermentation industry as it could result in higher rate of growth and hence increase lactic acid production. At the same time, a high fermentation temperature reduces contamination by other micro-organisms.

Table 2. Characteristics of the three homofermentative lactic acid bacteria.

Fermentation Conditions	Bacterial Species		
	L. acidophilus	***L. delburckii***	***L.* sp.**
Temperature (°C)			
15	+	-	-
20	+	+	+
30	+	+	+
37	+	+	+
45	-	+	+
50	-	-	-
Lactic Acid Concentration (%)			
2.5	+	+	+
5	+	+	+
7.5	+	+	-
10	+	+	-
15	-	-	-
Initial pH			
4	-	-	-
4.5	+	+	-
5	+	+	+
5.5	+	+	+
6	+	+	+
NaCl Concentration (%)			
1.5	+	+	+
2.5	+	+	+
5	+	+	+
7.5	-	+	-
10	-	-	-

+ indicates growth and production of acid by bacteria.
-indicates no growth and acid production by bacteria.

L. acidophilus and *L. delbrueckii* were the most tolerant strains in high concentrations of lactic acid as they were able to grow at 10% of initial lactic acid concentration, while (*L.*.) could sustain up to 5% lactic acid concentration. None of the species grew at 15% lactic acid concentrations. A higher tolerance to lactic acid is a desirable trait for an industrial strain of LAB as it could produce more lactic acid in the fermentation broth without prematurely affecting itself adversely (Adnan and Tan, 2007).

L. acidophilus and L. delbrueckii could grow at pH 4.5. The inability of *Lactobacillus* sp. to grow at low pH was consistent with its failure to grow at high lactic acid concentration and also its heterofermentative metabolic character.

3-3. Effect of pH on fermentation in batch culture

In order to investigate the effect of pH on fermentation, batch culture containing 50 g/L total carbohydrates was used. Table 3 shows the results of pH change in batch culture during fermentation. According to the results obtained, when pH isn't adjusted during fermentation process, there will be a rapid decline in pH during the first hours of fermentation. For all species no remarkable change in pH and carbohydrate consumption was observed within 24 h. It may be concluded that pH condition of about 4.0 is a limiting factor for all bacterial species.

On the other hand, it appears that *L. delbruckii and L. acidophilus* are more resistant to low pH. This characteristic may be considered as a confirmation for the results given in Table 2. As shown in Table 2 the latter can tolerate high concentration of lactic acid and low initial pH. Based on the results obtained, due to low buffering capacity of sorghum seed extract, fermentation process should be performed with some substances capable of keeping pH in a steady state.

3-4. Fermentation of *Sorghum* seed extract

3-4-1. Effect of carbohydrate concentrations on fermentation

Table 4 illustrates the effects of various carbohydrate concentrations on lactic fermentation in pH regulated conditions. As it can be seen, the initial concentration of total carbohydrate (TC) has a pronounced effect on initiation of lactic acid production for all the selected species. The phase lag of growth has been increased by increasing TC concentration, so that in concentrations above 100 g/L, the rate of consumption of carbohydrates declined significantly. This decrease in carbohydrates consumption may be due to the presence of high osmotic pressure in the medium. The optimum carbohydrate concentration for all species was determined to be 100 g/L. Since the yield of production with all bacterial species was maximum (about 85-90%) in this concentration, the total content of carbohydrates was found to be consumed after 72 h. *Lactobacillus* sp. was a heterofermentative strain, so its yield was less than 50%, while the other two species were homofermentative and therefore by the use of these two species, yields of higher than 80% were achieved for all

concentrations of carbohydrates below 100 g/L.

L. delbrueckii was found to be the most efficient species for production of lactic acid as indicated by the higher yield obtained by the use of this species after 72 h in 100 g/L of initial carbohydrate concentration.

Table 3. Rate of carbohydrates consumption by selected bacterial strains during fermentation.

Bacterial Species	Fermentation Time					
	24 h		48 h		72 h	
	pH [1]	TC [2] (gL^{-1})	pH [1]	TC [2] (gL^{-1})	pH[1]	TC [2] (gL^{-1})
L. sp.	4.3	47.1	4.12	46.54	4.09	46.4
L. acidophilus	4.12	48.9	4.05	48.3	4.0	48.0
L. delbrueckii	4.08	48.6	3.98	48.0	3.90	47.77

[1] Initial pH 5.7± 0.05

[2] Total unreacted carbohydrate concentration

Table 4. Effect of initial carbohydrate concentrations on lactic acid yield with the three selected bacterial strains.

Initial TC[1]	Bacterial Species	Total Carbohydrate (gL^{-1})				Final Lactic Acid (gL^{-1})
		12 h	24 h	48 h	72 h	
30 gL^{-1}	*L. sp.*	21.4	12.5	0	0	12.3
	L. acidophilus	19.8	8.3	0	0	21.6
	L.delbrueckii	22.3	10.6	0	0	21.3
50 gL^{-1}	*L. sp.*	42.3	22.5	0	0	21.4
	L. acidophylus	41.6	19.4	0	0	37.0
	L. delbrueckii	39.7	15.7	0	0	38.1
70 gL^{-1}	*L. sp.*	63.1	44.5	8.6	0	27.3
	L. acidophylus	62.8	38.9	7.6	0	55.3
	L. delbrueckii	60.2	37.7	3.2	0	5.65
100 gL^{-1}	*L. sp.*	95.2	87.6	40.9	0	42.2
	L. acidophylus	96.9	83.9	38.9	0	81.1
	L. delbrueckii	94.1	78.9	37.2	0	82.3
120 gL^{-1}	*L. sp.*	118.2	102.5	86.5	25.6	ND3
	L. acidophylus	113.2	105.7	84.3	23.4	ND
	L. delbrueckii	117.8	101.4	82.1	17.5	ND
150 gL^{-1}	*L*	149.2	139.0	121.6	104.1	ND
	L. acidophylus	148.6	134.2	111.2	100.5	ND
	L. delbrueckii	148.3	133.5	106.4	95.4	ND

[1] TC: Total Carbohydrate

[2] LA: Lactic acid
[3] ND, Not determined

3-4-2. Effect of multivitamins on fermentation at constant pH

Since the enrichment of fermentation culture media with nutrients such as vitamins is not cost-effective for production of low value products, the effect of multivitamin enrichment on fermentation process was investigated. Fig. 2 shows the effect of multivitamins on lactic fermentation by *L. delbrueckii.* There was no significant effect on the product yield obtained by using multivitamins. On the basis of the results obtained, it can be deduced that this species does not require incorporation of vitamins to the culture medium or the sorghum extract has enough nutrients to support the growth and production. It seems that the required growth factors are present in the seed extract medium, since most of the species of LAB are fastidious, and the selected species is also supposed to be a fastidious species.

4. Conclusions

Increasing demands for lactic acid as a food and pharmaceutical excipient and a raw material for biodegradable polylactate polymer production have made great effort in finding new and more efficient production processes.This study describes a part of upstream process for production of lactic acid by the use of sorghum seed extract as a unique medium or carbon source. An efficient method for the hydrolysis of sorghum seed extract was designed by using inorganic acid and heat; thus the concentrated extract is converted to a highly fermentable product and can be kept easily under room conditions without carbohydrate

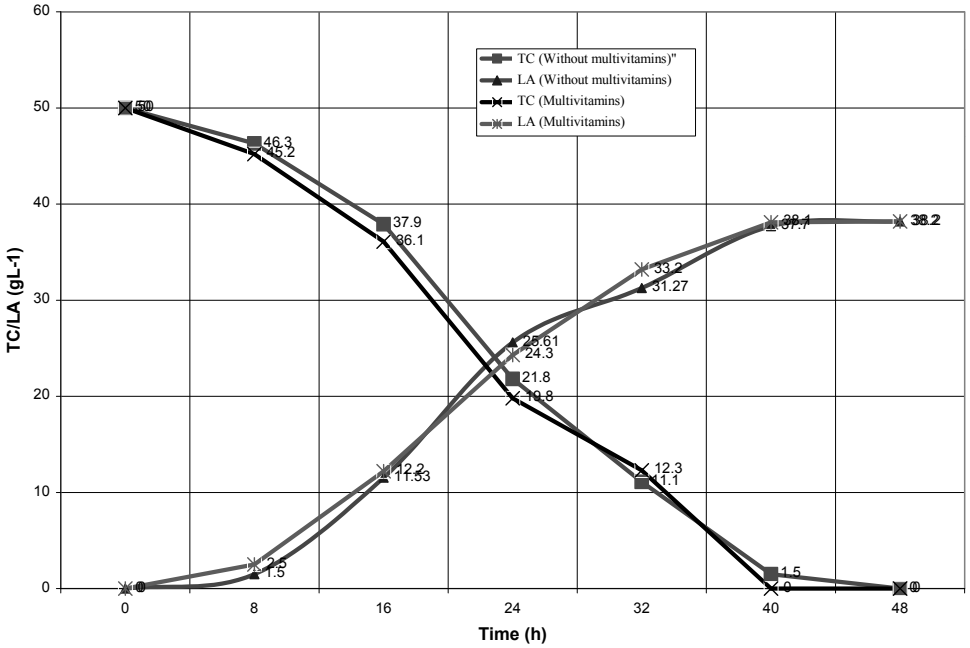

Figure 2. The influence of raw *sorghum* seed extract and *sorghum* seed extract supplemented with mutivitamins on lactic acid fermentation by *L. delbruekii.*

deterioration. The bacterial selection was based on a series of tests for industrially desirable traits, and finally the species were tested in Lab scale-bioreactor in order to evaluate them under certain productive and practical conditions. The results of this work suggest that sorghum seed extract can be efficiently used as a raw material for lactic fermntation. However, more experimental investigations are required to determine the contribution of various factors in fermentation conditions and their correlations in the overall yield of lactic acid. In addition, the downstream process for extraction of the produced lactic acid merits further investigation.

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References

[1] Adnan, A. F. M. and Tan, K. P., "Isolation of lactic acid bacteria from Malaysian foods and assessment of the isolates for industrial potential", Bioresource Technology, 98, 1380, (2007).

[2] Akah, P. A. and Nwambie, A. I., "Evaluation of Nigerian traditional medicinal plants used for rheumatic inflammatory disorders", J. Ethnopharmacol, 42, 179, (1994).

[3] Bernfeld, P., Colowick, S. P. and Kapan, N. O., Methods in enzymology, Academic Press, New York, p. 301, (1995).

[4] Ciacci, C., Maiuri, L., Caporaso, N., Bucci, C., Del Giudice, L., Massardo, D. R., Pontieri, P., Di Fonzo, N., Bean, S. R., Ioerger, B. and Londei, M., "Celiac disease: In vitro and in vivo safety and palatability of wheat free sorghum food products", Clinical Nutrition, 26 (6), 799, (2007).

[5] Correia, I., Nunes, A., Duarte, L. F., Barros, A. and Delgadillo, I., "Sorghum fermentation followed by spectroscopic techniques", Food Chemistry, 90, 853, (2005).

[6] Crueger, W. and Crueger, A., Biotechnology: A textbook of industrial microbiology, 2nd ed., T. D. Brock. Sunderland, Mass., Sinauer Associates, (1990).

[7] Dahlberg, J., Berenji, J., Sikora, V. and Latković, D., "Assessing sorghum [Sorghum bicolor (L) Moench] germplasm for new traits: Food, fuels & unique uses, Maydica, 85, (2011).

[8] Gonza´lez, M. I., lvarez, S. A., Riera, F. and Lvarez, R. A., "Economic evaluation of an integrated process for lactic acid production from ultra-filtered whey", Journal of Food Engineering, 80, 553, (2007).

[9] Guglielmotti, D. M., Marco´, M. B., Golowczyc, M., Reinheimer, J. A. and Quiberoni, A. L., "Probiotic potential of

Lactobacillus delbrueckii strains and their phage resistant mutants", International Dairy Journal, 17, 916, (2007).

[10] Kharas, G. B., Sanchez-Riera, F. and Severson, D. K., Polymers of lactic acid, In: Mobley, D. P. (Ed.), Plastics from microbes: Microbial synthesis of polymers and polymer precursors, Hanser Publishers, Munich, Germany, p. 93, (1994).

[11] Lin, J., Zhou, M., Zhao, X., Luo, Sh. and Lu, Y., "Extractive fermentation of l-lactic acid with immobilized Rhizopus oryzae in a three-phase fluidized bed", Chemical Engineering and Processing, 46, 369, (2007).

[12] Rooney, L. W. and Waniska, R. D., Sorghum food and industrial utilization, In: Smith, C. W., Frederiksen, R. A. (Eds.), Sorghum: Origin, history, technology, and production, Wiley, New York, 689, (2000).

[13] Taylor, J. R. N., Schober, T. J. and Bean, R. B., "Novel food and non-food uses for sorghum and millets", Journal of Cereal Science, 44, 252, (2006).

[14] Wouters, J. A., Kamphuis, H. H., Hugenholtz, J., Kuipers, P., De Vos, W. M. and Abee, T., "Changes in glycolytic activity of Lactococcus lactis induced by low temperature", Applied and Environmental Microbiology, 66, 3686, (2000).

[15] Zhan, X., Wang, D., Tuinstra, M. R., Bean, S., Seib, P. A. and Sun, X. S., "Ethanol and lactic acid production as affected by sorghum genotype and location", Industrial Crops and Products, 18, 245, (2003).