

EFFECTS OF ORGANIC ACID ON SILAGE FERMENTATION AND AEROBIC STABILITY OF WET BREWER'S GRAIN AT DIFFERENT TEMPERATURES

L. COSKUNTUNA¹, F. KOC¹, M. LEVENT OZDUVEN¹ and A. COSKUNTUNA²

¹*Namik Kemal University, Agricultural Faculty, Dept. of Animal Science, Tekirdag, Turkey*

²*Namik Kemal University, Agricultural Faculty Dept. of Plant Protection, Tekirdag Turkey*

Abstract

COSKUNTUNA, L., F. KOC, M. LEVENT OZDUVEN and A. COSKUNTUNA, 2010. Effects of organic acid on silage fermentation and aerobic stability of wet brewer's grain at different temperatures. *Bulg. J. Agric. Sci.*, 16: 651-658

The purpose of this research was to study the effect of organic acid on silage microbiology and aerobic stability of wet brewer's grain silages at different temperatures.

The silage samples ensiled in 1.0 l anaerobic jars, with and without an organic acid, at room (20°C) or elevated temperatures (30-37°C). After 45 days of ensiling, the silages were subjected to an aerobic stability test and room and elevated (30-37°C) temperatures.. Samples incubated at 20-30°C had the highest yeast and mould count, most profilic CO₂ production. The duration of exposure had a significant effect on aerobic stability, especially at 30°C.

As a result, organic acid showed a high antibacterial activity in wet brewer's grain silages. During the 45 days anaerobic period, organic acid decreased yeast and moulds populations, moreover improved aerobic stability at 37°C

Key words: Organic acid, wet brewer's grain silage, aerobic stability, temperature

Introduction

Brewers' grains are by-products of the brewing industry. These products are derived mainly from barley fermented to produce beer. They contain 230 to 290 g/kg CP of DM basis and are high in digestible fiber (Pereira et al., 1998 and Dhiman et al., 2003). Due to their fibrous nature and low energy content, brewers' grains are suitable for ruminants, particularly in dairy cows, to balance intake of large amounts of high

starch diets (Chiou et al., 1998; Dhiman et al., 2003; Xu et al., 2007).

Many by-products from the food and beverage industries have been used as animal feeds, and ensiling is sometimes used to preserve moist by-products for subsequent feeding. However, acceptable fermentation is often difficult to achieve when by-products are ensiled alone due to high-moisture contents and lack of sugar substrates. Moreover, acetic and butyric acid production may increase especially after prolonged

storage (Imai, 2001), and spoilage might occur when by-products are exposed to air after silo opening (Schneider et al., 1995; Niwa, 2001; Nishino et al., 2003).

Organic acids are one of the most efficient feed additives for mould prevention. For example, propionic acid, a biostatic agent used successfully for preservation of high moisture grain (Filya et al., 2004; Samli et al., 2008) has potential in suppressing microbial activity during this short storage interval. Formic acid, as silage additive, has anti-bacterial effect on many bacteria species, including lactic acid bacteria; thus, addition of formic acid into silage results in limited fermentation and reduction in organic acid content of silage (Rooke et al., 1988; Polan et al., 1988, Filya et al., 2004).

The purpose of this study was to investigate the effects addition of organic acid on silage fermentation and aerobic stability of wet brewer's grain at different temperatures.

Materials and Methods

Ensiling of wet brewer's grain

Wet brewer's grains were obtained from a private brewing factory. The composition of the utilized organic acid mixture (SÝLOFARM® LIQUID, Farmavet KOCAELI) was : formic acid 60%, sodium formate 20 % and water 20%. Silage materials were divided in two trial groups for the control and organic acid treatments. After organic acid was applied, silage materials ensiled 108 1.0 l glass jars (Weck, Wher-Oftringen, Germany) equipped with lid that enabled gas release only. Each jar was filled with about 550 g (wet weight).

The jars were stored under the following temperature regimes: (i) constant room temperature ($20\pm 1^\circ\text{C}$); (ii) constant moderate temperature ($30\pm 1^\circ\text{C}$) (iii) constant high temperature ($37\pm 1^\circ\text{C}$).

Three jars per treatment from every temperature were sampled on days 45 for pH, dry matter (DM), water soluble carbohydrate (WSC), lactic acid (LA) content measurement and lactic acid bacteria (LAB), mould, yeast and enterobacteria enumeration. At the

end of the experiment the silages were also subjected to an aerobic stability test, lasting 5 days, in a system developed by Ashbell et al. (1991). The system is constructed from two parts of recycled soft drink bottles (polyethylene terephthalate). The upper part (1 litre) is filled with about 250 g (wet weight) loosely-packed silage and the lower part, with 100 ml 20% KOH gas is exchanged through 1 cm holes in the upper part carbon dioxide produced during aerobic exposure is absorbed in the base and determined by titration with 1 N HCl. In addition, change in pH, yeast and mould counts, visual appraisal, also serve as indicators for aerobic spoilage. Visual appraisal of that samples exposed to air was performed by a panel of 3 according to the extent of mould cover, texture and their odour. The panel evaluation was converted into numeric scale from 1 to 5, with 1 being good quality silage with no apparent moulding and 5 being completely moulded samples (Filya et al., 2000).

Analytical procedure

Chemical analyses were performed on triplicate samples. Dry matter was determined by oven drying for 48 h at 60°C . pH in fresh material and silage samples was measured according to the British standard method (Anonymous, 1986). The ammonia nitrogen ($\text{NH}_3\text{-N}$) content of silages was determined, according to Anonymus (1986). The water soluble carbohydrate content of silages was determined by spectrophotometer (Shimadzu UV-1201, Kyoto, Japan) after reaction with an antron reagent (Anonymous, 1986). LA and acetic acid (AA) were determined by the spectro-photometric method (Koc & Coskuntuna, 2003).

Crude protein (CP), and crude fiber (CF) were determined following the procedure of Association of Official Analytical Chemists (AOAC, 1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the method of Goering and Van soest (1982).

Microbiological evaluation included enumeration of lactobacilli on pour-plate Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, UK), and yeast and moulds on spread-plate malt extract agar (Difco,

Detroit, MI, USA) adjusted to pH 3.5 by addition of 50 ml of percent lactic acid per litre incubated at 25°C for 72 h (Meeske et al., 1999). Colonies were counted directly on the agar plates.

Statistical Analysis

Statistical analysis of the silage chemical analysis results included a one-way analysis of variance and Duncan's multiple range test, performed with the Statistical Analysis System Software (SAS, Cary, NC).

Results

The WBG contained 218.6, 32.0 and 279.5 g kg⁻¹ DM, WSC and crude protein respectively, and the pH were 4.68. The log numbers of cfu g⁻¹ FM of LAB, yeasts, and enterobacteria in the fresh material were 4.72, 3.92 and 3.53 respectively.

The pH changes of the various treatments are given in Figure 1. The pH values of the organic acid silages were different to those of the control silages at the same temperature 45 days. Table 1 gives the chemical and microbiological analyses of the final wet brewer's grain silages. In the silages stored at the 30°C temperature, higher acetic acid was produced, which is consistent with their final higher pH values; in these silages, higher losses were observed (Figure 1). Residual WSC were found in all of the silages. Within a temperature regime there were significant differences between the control and organic acid silages on the pH, WSC, NH₃-N, NDF, ADF, ADL and LA contents (P<0.001). Statistical analysis revealed significant (P<0.001) organic acid effects on final pH, WSC, NH₃-N, CP, NDF, ADF, ADL and CF at 30°C - 37°C temperatures, lower pH, less AA contents and higher WSC were obtained. Significant temperature X organic acid interactions were obtained for pH, CP, NH₃-N, NDF, ADF, ADL and CF contents.

The numbers of microbiological composition of the wet brewer's grain silages are given in Table 1. By day 45, the number of LAB had decreased in control and organic acid silages, and more so at 30°C. Yeasts and enterobacteria were found in substantial numbers 20°C of the control and organic acid silages. Mould

were found only of the at 30°C silages. Significant temperature X organic acid interactions were obtained for all of the microbiological parameters (P<0.01).

Treatment Effects on Fungal Growth and Aerobic Stability

The results of the aerobic stability test are given in Table 2. Samples which were exposed to air at 20°C-30°C temperature tended to deteriorate more than those which were exposed to air at high temperature (37°C). This was indicated by the CO₂ production and large numbers of yeasts and moulds. In this experiment, aerobic deterioration was accompanied by change in pH, and the pH values after exposure to air effect the pH values of the silages before exposure to air. This was probably due to the high levels of residual WSC that the yeasts could use instead of LA, which did not result in a change in pH. The statistical analyses of the aerobic stability test revealed highly significant effects on CO₂ production of both the ensiling (P<0.01) and aerobic exposure (P<0.01) temperatures; organic acid did not have a statistical significant effect on CO₂ production, but more samples of the organic acid silages deteriorated upon exposure to air than of the control silages.

Table 3 shows the morphological characteristics of moulds isolates. Isolates A was suspected to be *Penicillium* spp. While, isolates B was *Aspergillus* spp.

Discussion

Ensiling is based on fermentation, which involves microbiological and enzymatic activity. Therefore, it is expected to be strongly influenced by temperature. In most of the literature describing ensiling experiments, there is a few reference to the temperature at which the ensiling was carried out. In farm-scale ensiling in a warm climate, however, the temperature within the silage often increases to around 30°C-40°C, due to plant respiration and microbiological activity, and it remains high for many months (Jiang et al., 1988; Ashbell and Weinberg, 1992; Weinberg and Ashbell, 1994; Filya et al., 2004; Kim and Adesogon, 2006). Air may penetrate the silage during storage and when

Table 1
Chemical and microbiological composition of the wet brewer's grain before exposure to air (chemical analysis is given as g kg⁻¹ DM, and microbiological data as log number of colony forming units g⁻¹ fresh matter)

Time days	Temperature	Organic acid	pH	WBG														
				DM	WSC	NH ₃ -N	CP	NDF	ADF	ADL	CF	LA	AA	LAB	Yeast	Mould	Enterobacteria	
0			4.68	21.86	32.00	-	27.95	75.84	26.13	11079	18.15	-	-	4.72	3.92	NF	3.53	
	20°C	-	4.29f	23.96a	16.97b	0.25c	27.98b	78.29	27.29	14366	15.18	0.59a	0.32	4.13c	4.14a	0.00c	3.30b	
		+	3.92d	22.38e	17.43a	0.35b	29.27a	70.04	31.66	25324	16.66	0.58a	0.34	4.97a	3.77c	0.00c	4.50a	
45	30°C	-	4.11b	22.86c	14.22d	0.27c	26.41b	73.61	37.40	43617	17.47	0.56b	0.56	1.99d	0.00d	1.70b	0.00c	
		+	3.78c	23.59b	14.67c	0.44a	28.58b	71.05	31.67	18415	16.69	0.49b	0.28	1.96d	0.00d	1.88a	0.00c	
	37°C	-	4.39a	22.64d	12.38f	0.24c	28.99b	69.47	28.41	26054	19.36	0.33c	0.39	0.00e	0.00d	0.00c	0.00c	
		+	3.73e	23.58b	13.16e	0.41a	27.62b	70.04	29.54	12540	40284	0.32c	0.46	4.74b	4.17a	0.00c	0.00c	
Standard error of mean				0.075	0.173	0.557	0.023	0.930	0.987	0.126	0.413	0.035	0.029	0.540	0.608	0.254	0.564	
Source of variation				<i>P level</i>														
Organic acid				<0.001	>0.05	<0.001	>0.05	<0.001	<0.026	0.142	<0.048	0.219	>0.05	<0.001	<0.001	<0.001	<0.001	<0.001
Temperature				<0.004	>0.05	<0.048	<0.001	>0.05	<0.001	<0.001	<0.016	<0.001	>0.05	<0.001	<0.001	<0.001	<0.001	<0.001
Organic acid X Temperature				<0.003	>0.05	0.140	<0.001	<0.001	<0.001	<0.004	0.0557	>0.05	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

^{a-d} Means in the same column with different superscripts differ significantly ($P < 0.001$); $P < 0.05$).

Table 2**Results of the aerobic stability test of wet brewer's grain silages after 5 days of exposure.**Yeasts and moulds are given as log cfu g⁻¹.

Days of ensiling	Organic acid	Temperature	pH	DM, %	CO ₂ g kg ⁻¹ DM	Mould	Yeasts ¹	Visual appraisal
45	-	20°C	6.31b	23.98a	30.57a	2.70c	NF	2
	+		9.21a	23.71c	29.74b	NF	NF	1
	-	30°C	5.25c	22.96e	22.62d	3.68a	NF	3
	+		3.52d	23.85b	23.20c	3.24b	NF	3
	-	37°C	4.06d	23.12d	15.26e	NF	NF	1
	+		3.51d	23.85b	15.24e	NF	NF	1
	<i>SEM</i>		0.612	0.118	0.330	0.490	-	
	Organic acid		0.423	<0.001	0.065	<0.001		
	Temperature		<0.001	<0.001	<0.001	<0.001		
	Organic acid x temperature		<0.001	<0.001	<0.001	<0.001		

¹ Not found² Visual appraisal is expressed using a scale of 1-5 where 1: good quality silage with no visible moulding,

2: a few small mould spots,

3: scattered mould spots, 4: silage with partially covered moulds, lumpy silages, 5: completely mould covered samples, unpleasant odour and silage particles sticking together.

Table 3**Morphological characteristics of moulds isolates**

Isolates	Colour of mycelium	Septation	Colour of spores	Types of spores	Spore head	Presence of special structure	Appearance of spore head	Suspected moulds
A	C	S	G	Con	CS	-	Smooth	<i>Penicillium</i> spp.
B	DiWh	S	Db	ConC	TS	FC	Rough	<i>Aspergillus</i> spp

Wh: white; DiWh: Dirty white; C: Colourless; S: Septate, Ns: Non septate; G: Green; Db: Deep brown, B: Black;

ConC: Conidia chains; Con: Conidia, Spo: Sporangia ; ConsC: Conidia chains; FC: Foot cell,

RS: Rhizoid and stolon

the silage is unloaded for feeding, it is fully exposed to air and can warm up again because of aerobic microbial activity. This could result in losses and affect the quality of the silage, and might have implications for its aerobic stability and the success of silage additive.

Aerobic deterioration of silage is a complex pro-

cess which depends on many factors. Usually it is initiated by aerobic yeasts which can use either residual WSC or lactic acid for their metabolism. Aerobic deterioration usually results in production of CO₂ and consequent DM losses. It is assumed that when high levels of residual WSC are available for the aerobic

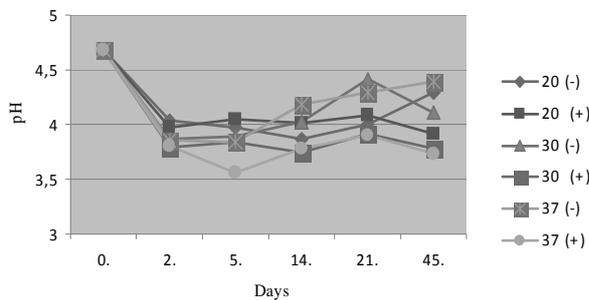


Fig. 1. pH change in WBG silages

yeasts, no change in pH will occur during aerobic deterioration; however, when lactic acid is their only energy source, the pH will increase. The wet brewer's grain silages had high contents of residual WSC and lactic acid and therefore, tended to spoil more upon aerobic exposure, as indicated by more intensive CO₂ production, moreover change in pH. test. The results reveal that aerobic deterioration of the wet brewer's grain silages were more intensive when the silage had a high temperature history and the test, too, was performed at high temperatures. Therefore, special care should be taken during silage making and unloading in a warm climate in order to minimize temperature increase and consequent losses.

Two moulds were isolated namely; *Penicillium* spp., *Aspergillus* spp. Many studies have shown that most silage samples have species from *Aspergillus* and *Penicillium* genera as predominant flora (Ono et al., 1999; Raid and Kucharek, 2005; Samapundo et al., 2005; Akintokin et al., 2007) whereas Sanli, 2001 found *Fusarium* and *Penicillium* species as prevalent in silage. *Aspergillus* and *Fusarium* toxins are the greatest concern for animal health. However, the high frequency of *Penicillium* spp. as well as the presence of *F. subglutinans*, must be considered a potential risk factor.

Previous studies have shown that an organic acid is one of the most effective silage additives for preventing mould growth (Filya et al., 2004; Samli et al., 2008; Koc et al., 2009). The present data are in agreement with this result, we also noticed that addition of an organic acid may result in a reduced mould growth, especially for low dry matter silage.

Conclusion

The result of the present study indicated that supplementation of wet brewer's grain silages with the investigated organic acid may prevent mould growth in particular silage samples. Warmer silages might also be more susceptible to aerobic deterioration, especially if the ambient temperature is high. Therefore, in warm climate, special care should be taken during all stages of silage making. The efficiency of organic acids for silage might also be affected by high ensiling temperatures, and this point should be considered during their formulation, silage dry matter content and doses.

Acknowledgements

The authors thank to Scientific Technical Research Council of Turkey (TUBITAK). Veterinary and Animal Husbandry Research Grant Committee for the financial support that made this study possible (Project no. TOVAG-107 O 641)

References

- Akintokun, A., A. O. Jolaosho and R. O. Afolabi, 2007. Effect of *L. plantarum* and Carbohydrase on Microbes and Composition of *T. procumbens* Silage. *Arch. Zootec.*, **56** (214): 145-156.
- Anonymous, 1986. Analysis of Agricultural Materials. Reference Book: 427. London. Pp. 248.
- AOAC, 1990. Official methods of analysis, 4th ed. Association Official Analytical Chemists, Inc. Arlington, Virginia USA
- Ashbell, G. and Z. G. Weinberg, 1992. Top silage losses in horizontal silos. *Canadian Agricultural Engineering*, **34**: 171-175.
- Ashbell, G., Z. G. Weinberg, Y. Hen and I. Filya, 2002. The effects of temperature on the aerobic stability of wheat and corn silages. *J. Ind. Microbiol. Biotechnol.*, **28**: 261-263.
- Ashbell, G., Z. G. Weinberg, A. Azrieli, Y. Hen and B. Horev, 1991 A simple systems to study the aerobic deterioration of silages. *Canadian Agricultural Engineering*, **33**: 391-393.
- Chiou, P. W. S., C. R. Chen, K. J. Chen and B.

- Yu, 1998. Wet brewers' grains or bean curd pomace as partial replacement of soybean meal for lactating cows. *Anim. Feed Sci. Technol.*, **74**: 123–134.
- Chuncheng, Xu, Yimin Cai, Naoko Moriya and Masuhiro Ogawa, 2007. Nutritive value for ruminants of green tea grounds as a replacement of brewers' grains in totally mixed ration silage. *Animal Feed Science and Technology*, **138**: 228–238.
- Dhiman, T. R., H. R. Bingham and H. D. Radloff, 2003. Production response of lactating cows fed dried versus wet brewers' grain in diets with similar dry matter content. *J. Dairy Sci.*, **86**: 2914–2921.
- Filya, I., G. Ashbell, Y. Hen and Z. G. Weinberg, 2000. The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage. *Animal Feed Science and Technology*, **88**: 39–46.
- Filya, I., E. Sucu and O. Canbolat, 2004. Researches on Using Organic Acids in the Silage Fermentation. *Uludag Uni. Agric. Fak.*, **18** (2): 35–45.
- Honig, H., 1991. Reducing losses during storage and unloading of silage. In Proceedings of the European Grassland Federation forge conservation towards 2000 Conference ed. Pahlow, G. and Honig, H. Pp. 11–128. Braunschweig, Germany.
- Imai, A., 2001. Silage making and utilization of high moisture by-products. *Grassl. Sci.*, **47**: 307–310.
- Jiang, S., J. C. Jofriet and J. Buchanan-Smith, 1988. Temperature observations in a bottom-unloading concrete silo. *Canadian Agricultural Engineering*, **30**: 249–255.
- Kim, S. C. and A. T. Adesogan, 2006. Influence of Ensiling Temperature, Simulated Rainfall, and Delayed Sealing on Fermentation Characteristics and aerobic Stability of Corn Silage. *J. Dairy Sci.*, **89**: 3122–3132.
- Koc, F. and L. Coskuntuna, 2003. The Comparison of the Two Different Methods on the Determination of Organic Acids in Silage Fodders. *Journal of Animal Production*, **44** (2): 37–47.
- Koc., F., B. Yildirim, M. L. Ozduven and L. Coskuntuna, 2009. The Effect of Organic Acid Usage of Fermentation and Aerobic Stability Wet Brewer's Grain Silages. 6. Zootekni Bilim Kongresi 24–26 Haziran 2009, Erzurum
- Mc Donald, P., A. R. Henderson and S. J. E. Heron, 1991. *The Biochemistry of silage*. 2nd Edn. Chalcombe Publications. Pp: 344.
- Meeske, R. and H. M. Basson, 1998. The effect of a lactic acid bacterial inoculant maize silage. *Anim. Feed Sci. Technol.*, **70**: 239–247.
- Meeske, R., H. M. Basson and C. W. Cruywagen, 1999. The effects of a lactic acid bacterial inoculant on the fermentation dynamics, of intake and digestibility of *Digitaria enriantha* silage. *Anim. Feed Sci. Technol.*, **81**: 237–248.
- Nishino, N., H. Harada and E. Sakaguchi, 2003. Evaluation of fermentation and aerobic stability of wet brewers grains ensiled alone or in combination with various feeds as a total mixed ration. *J. Sci. Food Agric.*, **83**: 557–563.
- Niwa, Y., 2001. Silage making and utilization of high-moisture by-products. 4. Making silage from tofu cake and utilization. *Grassl. Sci.*, **47**: 323–326.
- Ono, E. Y., Y. Sugiura, M. Homechin, M. Kamogae, E. Vizzoni, Y. Ueno and E. Y. Hirooka, 1999. Effect of climatic conditions on natural mycoflora and fumonisins in freshly harvested corn of the State of Parana, Brazil. *Mycopathologia*, **147**: 139–148.
- Pereira, J. C., M. D. Carro, J. Gonzalez, M. R. Alvir and C. A. Rodriguez, 1998. Rumen degradability and intestinal digestibility of brewers' grains as affected by origin and heat treatment and of barley rootlets. *Anim. Feed Sci. Technol.*, **74**: 107–121.
- Polan, C. E., D. E. Stieve and J. L. Garrett, 1998. Protein Preservation and Ruminal Degradation of Ensiled Forage Treated with Heat, Formic Acid, Ammonia, or Microbial Inoculant. *J. Dairy Sci.*, **81**: 765–776.
- Raid, R. and T. Kucharek, 2005. Florida plant disease management guide: Sweet corn. EDIS PDMG-V3-51, Florida, Cooperative Extension Service, IFAS, University of Florida, Gainesville.

- Rooke, J. A., F. M. Maya, J. A. Arnold and D. G. Armstrong**, 1988. The Chemical Composition and Nutritive Value of Grass Silages Prepared with no Additive or with the Application of Additives Containing either *Lactobacillus Plantarum* or Formic acid. *Grass Forage Sci.*, **43**: 87-95.
- Samapundo, S., F. Devlieghere, B. De Meulenaer and J. Debevere**, 2005. Effect of water activity and temperature on growth and the relationship between fumonisin production and the radial growth of *Fusarium verticillioides* and *Fusarium proliferatum* on corn. *J. Food Prot.*, **68**: 1054–1059.
- Samli, H. E., G. Sirci, J. L. Bermejo, F. Koc, A. A. Okur and N. Senkoylu**, 2008. Effect of addition of a commercial organic acid mixture on feed microbiology according to duration and conditions of storage. *Acta Agriculturae Scand Section A*; **58**: 186-190.
- Schneider, R. M., J. H. Harrison and K. A. Loney**, 1995. The effects of bacterial inoculants, beet pulp, and propionic acid on ensiled wet brewers grains. *J. Dairy Sci.*, **78**: 1096–1105.
- Statistical Analysis System**, 2005. SAS® User's Guide: Statistics. Version 6. SAS Institute. Cary. NC. USA.
- Van Soest, P. J.**, 1982 Analytical systems for evaluation of feeds. In *Nutritional Ecology of the Ruminant* ed. van Soest. P.J. pp. 75-94. Ithaca. NY: *Cornell University Press*.
- Weinberg, Z. G. and G. Ashbell**, 1994. Changes in gas composition in corn silages in bunker silos during storage and feed out. *Canadian Agricultural Engineering*, **36**: 155-158.
- Weinberg, Z. G., G. Szakacs, G. Ashbell and Y. Hen**, 2001. The effect of temperature on the ensiling process of corn and wheat. *J. Appld. Microbiol.*, **90**: 561-566.

Received December, 2, 2008; accepted for printing December, 12, 2009.