# Improving the Preservation Quality of High Moisture By-Product Feedstuffs by Ensilage and Use of Additives

# IMPROVING THE PRESERVATION QUALITY OF HIGH MOISTURE BY-PRODUCT FEEDSTUFFS BY ENSILAGE AND USE OF ADDITIVES

by

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**A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Agricultural Sciences with Major Chair of Plant Production**

**Iwate University**

**March 2007**

# Declaration

I hereby declare that this dissertation, submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Agricultural Sciences with Major Chair of Plant Production and entitled **"Improving the preservation quality of high moisture by-product feedstuffs by ensilage and use of additives"**, represents my own work and has not been previously submitted to this or any other institution for any degree, diploma or other qualification.

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# List of Abbreviations

**A**

ADF, acid detergent fiber

**B**

B.O.D., biological oxygen demand

BC, buffering capacity

BW, body weight

**C**

CFU, colony forming unit

cm, centimeter

CP, crude protein

Cu, copper

**D**

DCP, digestible crude protein

DE, digestible energy

DM, dry matter

**E**

EE, ether extract

**F**

Fe, iron

FM, fresh matter

**G**

g, gram

GE, gross energy

**H**

h, hour

HCl, hydrochloric acid

$HClO_4$, perchloric acid

HMBF, high moisture by-product feedstuff

**K**

kg, kilogram

**L**

L, liter

LAB, lactic acid bacteria

LDH, lactate dehydrogenase

**M**

m, meter

meq, milliequivalent

Mg, magnesium

mg, milligram

MJ, mega joule

ml, milliliter

**N**

N, nitrogen

Na, sodium

NDF, neutral detergent fiber

**O**

$^oC$, degrees Celsius

OM, organic matter

**P**

PVC, poly vinyl chloride

PWRC, potential water retention capacity

**S**

SD, standard deviation

Se, selenium

**T**

TDN, total digestible nutrients

TN, total nitrogen

**V**

VBN, volatile basic nitrogen

VFA, volatile fatty acid

vs., versus

**W**

WRC, water retention capacity

WSC, water soluble carbohydrate

wt, weight

w:w, weight per weight

**Z**

Zn, zinc

μ m, micrometer

# Chapter 1

# 1. General Introduction

## 1.1. Importance of agricultural by-products

Agricultural by-product feedstuffs have gained global socio-economic importance in recent years due to their increasing relevance to livestock feeding systems in most developing countries. A by-product feedstuff by definition is 'a secondary product obtained during harvesting or processing of a principal commodity and has value as an animal feed' (Grasser et al., 1995) or 'a product that has value as animal feed and is obtained during the harvesting or processing of a commodity in which human feed or fiber is derived' (Fadel, 1999). These could be either from plant or animal origin. Crop residues derived from the production of maize, rice, wheat, barley, beet pulp, bagasse, etc.; cakes remaining after the extraction of oil from plant sources such as soybean, palm kernel, copra, corn gluten and groundnut; and agro-industrial sources such as potato pulp, brewer's grain, apple pomace, etc. constitute about 65% of the one billion metric tons of world dry matter tonnage of by-product feedstuffs of crop origin (Fadel, 1999). Future projections suggest that by-product feedstuff production will increase in developed countries and will remain the same or increase slightly in developing countries relative to population growth.

Although the use of cereal grains as supplements for livestock feed is a common practice in developed countries, this is hardly possible in developing countries, especially for the small holder producer, due to the use of such grains in human diets. Alternatively, there is growing emphasis on the recovery, recycling and upgrading of wastes in today's society driven by rising costs and often decreasing availability of raw materials together with the growing concern about environmental pollution. This is particularly true for the food and food processing industry in which wastes, residues and by-products can be recovered and often recycled to higher value and useful products. However, transport costs and sales problems due to the low quality of these residues in developed countries have led to alternative utilization concepts such as their use as building materials or conversion concepts like composting and biogas production (Laufenberg et al., 2003). Nevertheless, there is patronage of low moisture crop residues in most developing countries largely in their use as supplementary feed for ruminants mainly in pasture-based livestock feeding systems, and the most extensively used in this respect is straw, attracting a lot of reviews and evaluations. On the contrary, high moisture agricultural by-product feedstuff mostly from fruit and vegetable sources like citrus pulp, pineapple waste and reject bananas, most of which cannot be quantified, are often available but underutilized in ruminant nutrition or scarcely fed in dried form.

## 1.2. Definition of HMBF

The term high moisture by-product feedstuff (HMBF) is used in this dissertation to denote ‘any product or commodity that is obtained during the harvesting, production, or processing of feed or fiber containing a moisture content of more than 750 g/kg in fresh matter, and has value as an animal feed’. For the sake and scope of this investigation, this has been scaled down to include largely by-products from fruit and vegetable sources (either as by-product rejects or culls of no commercial value), canneries, or breweries.

## 1.3. Relevance of HMBF in current production systems

Although there is no data in the literature that quantify the amount of HMBF produced on a global level, reports (Grasser et al., 1995; van Horn and Hall, 1997; Fadel, 1999) have indicated that HMBF constitute a sizable portion of by-product feedstuffs produced worldwide. The obvious advantages of feeding livestock with by-products are the low cost, the reduction in reliance on grains that could be used as feed, the elimination of costly waste management programs (Grasser et al., 1995), and the high energy value of some by-products. The utilization of HMBF in livestock feeding systems in particular is however, constrained and limited in scope due to their bulky nature, short shelf life, and most often distance between production centers and livestock farms. Moreover, Laufenberg et al. (2003), in enumerating the current systems in place for the utilization of by-products from vegetable sources (those containing high moisture, emphasis mine) and the various concepts in use indicated that possibilities for the conversion and utilization or recycling of most of these wastes as animal feed are few. Since global production of by-products is, and would continue to preponderate in the near future (Fadel, 1999), the importance of animals as consumers of these by-products cannot be over-emphasized.

## 1.4. Preservation techniques for HMBF

Techniques for the conservation of feed are numerous and include, among others, drying, salting, sweetening, freezing, radiation, or changing of the pH (as in pickling or ensiling). However, preservation of fresh feed in particular involves the application of two basic measures: the prevention of enzyme activity in the product, and the protection of the product from external deterioration factors such as bacteria, molds, yeasts, insects, rodents, etc. (Ashbell, 2003).

Conservation by ensilage has been practiced for many centuries. Ensilage is defined as 'the material produced by the controlled fermentation of a crop of high moisture' or 'the preservation of a crop by natural fermentation under anaerobic conditions' (McDonald et al., 1991a). This definition, although restricting the material to 'crop', could be employed to encompass wet by-products such as HMBF. The advantages of preservation or conservation by ensilage are numerous and well established, and it is not the intention of this thesis to review them again. However, what is new and, in my opinion, an emerging necessity is the technology to preserve through ensiling, of agricultural by-products that are rich sources of nutrients and potential feed for livestock, yet contain very high moisture contents. Although developments in wet by-product preservation by ensilage have been reported (Ashbell and Donahaye, 1984; Ashbell et al., 1987; Weinberg et al., 1988; Bampidis and Robinson, 2006), literature on this aspect of study is thin.

## 1.5. Environmental concerns on disposal of HMBF

The disposal of by-products, especially those containing high moisture including HMBF, if not done properly, imposes a huge burden on the environment; if ensiling of HMBF is not conducted properly, this may add to the burden through effluent production. Effluent is the single most important factor affecting ensilage of wet crops, not only due to losses encountered in the silo (the fermentation vessel), but also due to the considerable loss of nutrients in effluent and drainage through silage, with the attendant environmental consequences. Silage effluent is considered one of the most common agricultural pollutants of water courses (Woolford, 1978). In a comparative study of various farm wastes with that of domestic sewage (Spillane and O'Shea, 1973), it was concluded that the biological oxygen demand (B.O.D.) or the water-polluting potential of organic matter of silage effluent far exceeded that of other pollutants as given in the table below.

**Table 1-1**. Biological oxygen demand (B.O.D.) of some agricultural wastes and domestic sewage (adapted from Spillane and O'Shea, 1973)

| | B.O.D. (mg $O_2$/L) |
|---|---|
| Silage effluent | 90,000 |
| Pig slurry | 35,000 |
| Cow urine | 19,000 |
| Cow slurry | 5,000 |
| Domestic sewage | 500 |

The obvious challenges militating against the interest in research into ensiling of particularly wet materials are:

i. the risk of high effluent production with attendant environmental consequences

ii. loss of nutrients through ensiling

iii. difficulties in producing good quality silages from the wet materials

iv. the aerobic instability or low shelf life of the silages

## 1.6. Objectives of the study

Methodologies involved in addressing problems related to preservation by ensilage of wet materials are varied but often subjective and product specific, or largely refer to grasses and crops, and thus highlight the need for strategies in addressing problems specific to HMBF.

The broad objectives of this study were therefore:

a. to investigate the ensiling potential of some selected HMBF
b. to improve preservation and extend shelf life of HMBF
c. to control effluent and nutrient losses during the course of ensiling HMBF
d. to evaluate the nutritive value of HMBF
e. to identify the main factors affecting the ensiling of HMBF, to determine their relationships, and to establish a method suitable for ensiling HMBF

To realize these objectives, four major experiments were conducted; the first involved a study of pre-ensiling characteristics of a selection of HMBF, followed by three others involving the ensiling of representatives of HMBF (potato pulp, daikon or oriental radish by-product, brewer's grain and apple pomace), determination of the aerobic stability, and nutritive value *in vivo* using sheep.

Important factors affecting the ensiling of HMBF and their relationships are discussed.

# Chapter 2

## Pre-ensiling characteristics of a representative of HMBF

### 2.1. Introduction

To fully comprehend the suitability for ensilage of any crop, and for that matter, HMBF, it is imperative that a study of the pre-ensiling characteristics of the material is conducted to provide an insight into the potential for ensilage. This would provide a basic and a preliminary assessment of its suitability for the ensiling process and an important point of reference. Of critical importance are the chemical and microbiological characteristics of the material, factors that intrinsically dictate the outcome of the ensiling process.

Considerable attempts have been made to score some vegetable wastes and fruits according to their nutritional potential (Teli et al., 1983; Gupta et al., 1993; Haque et al., 1997) and some by-products according to their ensiling characteristics (Ishigoro, 1994). However, no comprehensive approach has been made to study the specific ensiling characteristics of HMBF.

The objective of this study was to investigate the ensiling potential of a representative of HMBF from vegetable and fruit sources through chemical and microbiological characterization as a prelude to their use as silage materials.

## 2.2. Materials and methods

### *2.2.1. Pre-ensiling characteristics*

The HMBF investigated were those from vegetable sources (cabbage, cucumber, carrot pulp, and daikon [Oriental radish] *Raphanus sativus* L.), fruit sources (banana peel, apple pomace, orange peel, and pineapple skin) and one from an agro-industrial source (potato pulp).

A total of at least nine samples with three replications for each of the above-mentioned commodities except potato pulp were purchased from local supermarkets in Hokkaido, northern Japan, at different times during the harvesting season in 2004 and taken to the laboratory for the analyses of the ensiling characteristics. To ensure variability, each type of commodity was selected based on area of production in Japan. Vegetables were cut into pieces to about 2 cm long and 2 cm thick with a kitchen knife, while in the case of fruits, their juices were extracted to provide a semblance of their by-product similar to that in the cannery or extraction factories. Potato pulp, a by-product from the extraction of starch from raw potatoes, was obtained from three different factories in Hokkaido, northern Japan at different times during the production season in 2004. These were mixed, and representative samples were taken for dry matter determination. Part of the mixture was blended with an electric blender and sampled for the following determinations: 70 g was macerated with 140 ml of de-ionized water for measuring pH, 10 g for determination of the buffering capacity, and 10 g for the determination of microflora (lactic acid bacteria, LAB; yeast numbers). Water retention capacity (WRC) and water-soluble carbohydrates (WSC) were determined from dried samples.

### *2.2.2. Chemical analyses*

The dry matter content of the fresh materials was determined by freeze-drying for at least 24 h. The pH of aqueous extracts of the material was measured using a pH meter (model HM-30G, TOA Electronics Ltd, Tokyo, Japan). WSC were determined by the anthrone method as described by Morimoto (1971). WRC, employed as a novelty to determine the moisture absorptive capacity of silage / absorbent material, was determined according to the method of Robertson et al. (2000), with slight modifications. One gram of air-dry weight of material was put into a 50 ml centrifuge tube and hydrated with 30 ml distilled water containing 0.02 g sodium azide per 100 ml as a bacteriostat. Samples were equilibrated for 18 h at room temperature, transferred to a glass filter with a pore size of 100-160 μm, (1G P160, Sibata Company, Tokyo, Japan) and drained under a pressure of 2 g/cm$^2$ with a pressure pump (Compact air pump, NUP-1, AS-ONE Company, Tokyo, Japan). The glass filter was weighed, oven-dried at 135$^o$C for two hours, and weighed again. The WRC of the material was calculated as the amount of water retained by the pellet (g/g dry weight) after transfer to the glass filter. The potential WRC (PWRC), a novel terminology used in this study, is defined as 'the amount of moisture in grams that a HMBF can retain per 100 grams of its own fresh matter weight' and is calculated as WRC x DM of the material and given in g/100g FM. Buffering capacity (BC) was determined according to the method of Playne and McDonald (1966). This included the maceration of 10 g fresh weight of sample with 250 ml of distilled water, titration of the macerate with 0.1 N hydrochloric acid to pH 3, then titration to pH 6 with 0.1 N sodium hydroxide. The BC was expressed as milliequivalent of alkali required to change the pH from 4 to 6 per kg of dry matter, after correction for the titration value of 250 ml water blank. Microbial analyses (LAB and yeast counts) were carried out on representative samples of the materials as follows: 10 grams of sample were weighed into a sterile zippered bag, 90 ml of sterile water was added and homogenized by shaking for 3 min. The sample was serially diluted, and 1 ml and 0.2 ml of dilutions were taken for LAB and yeast determinations, respectively. Lactic acid bacteria were enumerated on pour plate Difco YMS agar (Difco Laboratories, Becton Dickson and Company, Sparks, Maryland, USA) supplemented with 1 mg/ml of both cycloheximide and sodium azide, respectively, to inhibit yeast growth. The plates were incubated at 32$^{o}$C for 72 h and LAB colonies counted directly on the plates. Yeasts were enumerated on spread-plate malt extract agar (Difco Laboratories, Becton, Dickson and Company, Sparks, USA) and incubated at 28$^{o}$C for 4 days, and colonies were counted directly on the agar plates.

### *2.2.3. Statistical analyses*

Means from the data of each material and the standard deviations were calculated using Excelstats for Windows 2004 (Microsoft Corporation, USA).

## 2.3. Results

The chemical and microbiological (LAB and yeast populations) compositions relevant to ensilage are given in Tables 2-1 to 2-8.

The selected products had high moisture contents, with those from vegetable sources a level over those from fruit sources (Table 2-1). Potato pulp was lowest in this respect with a high standard deviation, an indication of the variability of the sources from where the materials were procured.

**Table 2-1**. Moisture content of selected HMBF from vegetable, fruit and agro-industrial sources

| | Moisture (g/kg) | SD |
|---|---|---|
| Daikon | 951 | 10.7 |
| Cabbage | 933 | 7.5 |
| Cucumber | 967 | 2.0 |
| Carrot pulp | 912 | 3.6 |
| Banana peel | 908 | 9.1 |
| Apple pomace | 845 | 71.6 |
| Orange peel | 811 | 5.1 |
| Pineapple skin | 806 | 50.7 |
| Potato pulp | 789 | 57.2 |

g/kg, gram per kilogram; SD, standard deviation.

Values of pH of the selected products were variable, with those from vegetable sources higher in comparison with those from fruit sources (Table 2-2).

The BC, or the ability of a material to resist changes in pH, was higher in the vegetable sources (Table 2-3). Cabbage and daikon were particularly high, with the latter having a high standard deviation, indicative of the variation in the samples analyzed.

**Table 2-2**. pH values of selected HMBF from vegetable, fruit and agro-industrial sources

| | pH | SD |
|---|---|---|
| Daikon | 5.57 | 0.97 |
| Cabbage | 6.18 | 0.25 |
| Cucumber | 6.23 | 0.17 |
| Carrot pulp | 5.84 | 0.04 |
| Banana peel | 5.52 | 0.12 |
| Apple pomace | 4.26 | 0.25 |
| Orange peel | 4.41 | 0.03 |
| Pineapple skin | 3.75 | 0.09 |
| Potato pulp | 5.31 | 0.37 |

This result pre-supposed difficulty in the ensilage of daikon from a theoretical point of view. The opposite is true for potato pulp, which was conspicuously low in this characteristic.

**Table 2-3**. Buffering capacity (BC) of selected HMBF from vegetable, fruit and agro-industrial sources

| | BC ( meq/kg DM)[§] | SD |
|---|---|---|
| Daikon | 703.4 | 255.6 |
| Cabbage | 433.3 | 190.3 |
| Cucumber | 1276.3 | 135.0 |
| Carrot pulp | 562.3 | 94.7 |
| Banana peel | 618.2 | 73.9 |
| Apple pomace | 114.8 | 34.4 |
| Orange peel | 199.3 | 57.6 |
| Pineapple skin | 364.4 | 204.1 |
| Potato pulp | 21.5 | 9.1 |

[§] meq/kg DM, milliequivalent per kilogram dry matter.

LAB and yeast populations in the materials investigated were particularly low, with no clear consistency between vegetable and fruit materials. Potato pulp was highest and lowest in LAB and yeast, respectively (Tables 2, 4-5).

**Table 2-4**. Lactic acid bacteria (LAB) population of selected HMBF from vegetable, fruit and agro-industrial sources

| | LAB ($\log_{10}$ CFU/g FM) | SD |
|---|---|---|
| Daikon | 4.82 | 2.09 |
| Cabbage | 3.24 | 1.03 |
| Cucumber | 4.91 | 1.42 |
| Carrot pulp | 4.56 | 0.49 |
| Banana peel | 5.31 | 0.60 |
| Apple pomace | 3.40 | 0.37 |
| Orange peel | 2.59 | 0.16 |
| Pineapple skin | 5.32 | 0.08 |
| Potato pulp | 6.4[§] | 1.1 |

CFU/g FM, colony forming units per gram fresh matter.

[§] Based on Saito et al., 2006.

**Table 2-5**. Yeast population of selected HMBF from vegetable, fruit and agro-industrial sources

| | Yeast ($\log_{10}$ CFU/g FM) | SD |
|---|---|---|
| Daikon | 5.45 | 1.00 |
| Cabbage | 4.42 | 0.52 |
| Cucumber | 5.30 | 0.85 |
| Carrot pulp | 5.10 | 0.61 |
| Banana peel | 4.57 | 0.54 |
| Apple pomace | 3.21 | 0.71 |
| Orange peel | 2.58 | 0.70 |
| Pineapple skin | 4.57 | 0.31 |
| Potato pulp | 2.4[§] | 1.2 |

CFU/g FM, colony forming units per gram fresh matter.

[§] Based on Saito et al., 2006.

All materials except potato pulp were rich in WSC (Table 2-6). This indicated that sugar was the main available substrate and would be sufficient to maintain and direct the pattern of lactic acid fermentation during silage.

**Table 2-6**. Water soluble carbohydrates (WSC) of selected HMBF from vegetable, fruit and agro-industrial sources

| | WSC (g/kg DM) | SD |
|---|---|---|
| Daikon | 382 | 90.4 |
| Cabbage | 377 | 20.2 |
| Cucumber | 198 | 30.9 |
| Carrot pulp | 547 | 110.3 |
| Banana peel | 278 | 45.3 |
| Apple pomace | 445 | 71.0 |
| Orange peel | 417 | 57.1 |
| Pineapple skin | 322 | 36.6 |
| Potato pulp | 64 | 16.8 |

The PWRC of the materials was low in cucumber and daikon (the lowest in moisture contents) but high in orange peel and potato pulp (Table 2-7). There was no clear consistency among other materials in this respect.

**Table 2-7**. Potential water retention capacity (PWRC) of selected HMBF from vegetable, fruit and agro-industrial sources

| | PWRC (g/100g FM) | SD |
|---|---|---|
| Daikon | 16.9 | 1.24 |
| Cabbage | 44.2 | 0.47 |
| Cucumber | 16.0 | 0.80 |
| Carrot pulp | 55.8 | 0.94 |
| Banana peel | 39.9 | 0.43 |
| Apple pomace | 40.5 | 1.46 |
| Orange peel | 83.0 | 1.02 |
| Pineapple skin | 57.4 | 0.28 |
| Potato pulp | 72.8 | 0.75 |

## 2.4. Discussion

The chemical and microbiological characteristics relevant to ensilage of the materials studied showed some variations in terms of availability or not, versus the suitability to the ensiling process. The moisture content of the materials from vegetable sources were higher due to the mode of determination, ( i.e., without juice extraction) and this provided a realistic approach of their state prior to use as silage materials, in contrast with those from fruit and agro-industrial sources which principally undergo some sort of an extraction process. Potato pulp was highly variable in terms of its moisture content, and this was partly dependent on the mode of starch extraction and the product line of the extraction factory. On use as silage materials, highly absorbent materials need to be employed to soak or retain excess moisture to forestall high silage effluent production. The pH, BC, and, to a large extent, the WSC of materials from the vegetable sources were higher than those from fruit sources, providing a rather unpredictable and contrasting indication of their role in an ensiling process. In a typical silage fermentation process, a quick reduction of pH during the initial stages of the fermentation process is necessary to neutralize and prevent the proliferation of undesirable microorganisms with a gradual increase in lactic acid production from the available substrate (WSC) which is mainly sugar. Materials with high buffering capacities may delay the reduction in pH even in the presence of sufficient WSC. Addition of an inoculum of LAB, prior to ensiling of material from vegetable sources may be a viable proposition. On the contrary, fruit by-products and potato pulp, from a theoretical point of view, provided strong indications of being ideal silage materials judging from their chemical analysis, as evidenced by their low pH and buffering capacities and relatively high WSC contents (except potato pulp). Usually a material containing 150 g/kg in dry matter available WSC would be enough for a good fermentation process. Potato pulp would need an 'alternate' source of substrate to sustain effective silage fermentation. The LAB populations of the materials investigated (excluding potato pulp) were generally low and did not exceed the minimum threshold of $10^6$ organisms/g dry matter required to quick-start a highly effective lactic acid fermentation process. However, low initial population of LAB may not be a critical criterion for the success of the fermentation process. What is crucial is the efficiency of the indigenous LAB to initiate a rapid fermentation and sustain a rapid fall in pH (Woolford, 1984a). Yeast load was quite low in the investigated materials, especially in potato pulp. This augurs well for good silage fermentation. Again, what is critical is the yeast population at the end of the ensiling process (i.e., after opening of the silage). The rule is that if the number of yeasts exceeds the level of $10^5$ organisms/g dry matter, rapid fungal deterioration would occur after opening of the silage (Jonsson and Pahlow, 1984). From a microbiological point of view, potato pulp may be an ideal silage material.

The potential water retention capacity (PWRC), a terminology employed as a novelty in this study is, to a large extent, a function of moisture content (See Chapter 7). An important feature in the ensiling of particularly wet crops is the ability of the material to retain its moisture during the course of ensilage. Most wet crop silage materials are unable to retain moisture when ensiled, and consequently the system of drying prior to ensiling has been widespread. The PWRC describes the capacity of a material to retain or release its moisture under different silo conditions during the course of ensilage. This is an important factor *vis-à-vis* effluent production. The higher the PWRC, the stronger the ability of the ensiled material to retain effluent, and vice versa.

Judging by the PWRC of the materials investigated and barring any external factors, orange peel and potato pulp have the potential to produce less effluent than the other materials investigated during ensilage. The validity of this assertion is, however, subject to *ipso facto* ensiling of the two materials.

## 2.5. Summary

High moisture by-product feedstuffs (HMBF) contain ensilable characteristics such as water soluble carbohydrates sufficient for lactic acid fermentation. Vegetable by-products are high in buffering capacity and pH compared with those from fruit sources. Due to the high moisture contents, means of controlling effluent production during their use as silage materials are imperative.

# Chapter 3

## Potato pulp

### 3.1. Introduction

Potatoes are the most important agricultural product in Japan in terms of annual production, after rice and sugar beets. Each year, over two and a half million metric tons of potatoes are harvested (Statistical Handbook of Japan 2006), leading to a simultaneous production of potato pulp, a by-product of the potato starch industry. The pulp, a remnant of the cell wall of potato tuber and fruit liquid, contains other components such as starch, cellulose, hemicelluloses, pectin, proteins, and mineral salts (Mayer and Hillebrandt, 1997). Even after separation of the fruit liquid from the particulate fraction (pulp), the pulp contains up to 90% water. Thus it is a bulky by-product of considerable environmental concern. Several strategies for the reduction of the huge amount of this by-product are available including its use as fertilizer, substrate for cultivation of fungi, syrup for use in the cosmetic industry (Mayer and Hillebrandt, 1997), or as ruminant feed (Hanada et al., 2004; Pen et al., 2005; Okine et al., 2005). Still, several tons of potato pulps are returned to the soil with adverse effects not only to the topsoil but also to the environment, due to the high content of mineral salts. Ensiling potato pulp would be a more economical alternative to drying for preservation and consequent use as livestock feed especially for ruminants.

In the experiments to be described, a series of strategies involving the use of bacterial and fungal inoculants were employed to preserve fresh potato pulp by ensilage. Following that description is a discussion of the role of these inoculants in the enhancement of the preservation quality of potato pulp silage.

## 3.2. Ensiling potato pulp with or without microbial inoculants

The use of microbial inoculants to improve the fermentation quality of crops during ensilage has undergone a tremendous evolution in recent times. Several commercial products aimed at increasing lactic acid production in crop silages are on the market today, including *Lactobacillus rhamnosus*, which is widely used in Japan. The use of fungi, an aerobic inoculant in an anaerobic process such as ensiling was, until recently (Oda et al., 2002; Okine et al., 2005; Okine et al., 2007), a novelty.

Studies have been conducted on the use of the fungus *Rhizopus oryzae* in lactic acid production (Skory, 2000) and in the pharmaceutical industry for its therapeutic qualities (Peimin et al., 1997). Although the use of starch, which is a major chemical component in potato pulp, as a substrate for the production of lactic acid is considered to be rare (Woolford, 1984c), it has been shown that *Rhizopus oryzae* can produce lactic acid using cornstarch as a substrate (Peimin et al., 1997), and that production of lactic acid by *Rhizopus* cultures is often preferred to bacterial fermentations because of the ease and ability of the fungus to utilize both complex carbohydrates and sugars (Skory, 2000). This is due to the fact that it contains the enzymes pectinase and lactate dehydrogenase (LDH), which can hydrolyze carbohydrates into glucose resulting in the production of lactic acid (Skory, 2000; Erdogan and Mahinur, 2000). However, information on the use of *Rhizopus oryzae* as a fermentation stimulant in potato pulp silage is scanty. Oda et al. (2002) isolated the *Rhizopus oryzae* strain IFO 4707 as the best among thirty-eight others for its ability in rapid reduction in hardiness and pH of potato pulp, followed by gradual synthesis of lactic acid. There are no direct indications that *Rhizopus oryzae* organisms are hazardous (Coenen et al., 1997) and their use as inoculants could enhance the fermentation quality of potato pulp silage (PPS).

The objective of this study was to investigate the effect of the two inoculants, *Lactobacillus rhamnosus* and *Rhizopus oryzae*, separately and in combined application on changes in fermentation quality and nutrient composition with duration of storage of potato pulp silage.

## 3.2.1. Materials and methods

### *3.2.1.1. Silage preparation*

The study involved two experiments: In Experiment 1, fresh potato pulp was inoculated with *Lactobacillus rhamnosus* (L) and *Rhizopus oryzae* IFO 4707 (R). The two inoculants, both freeze-dried cultures, were added to potato pulp in the ratio of 0.5 and 1.0 g/kg in fresh matter, respectively, enough to produce at least $1x10^6$ colony forming units (CFU) $g^{-1}$ as per manufacturer's statement. A combined application of the two inoculants (R + L, included at 0.5 and 1.0 g/kg in fresh matter, respectively) was also prepared and set against potato pulp without additive (control). Four treatments viz; control (PP), L alone treated silage (PL), R alone treated silage (PR) and R+L treated silage (RL) were thus prepared. These were ensiled in 400 ml laboratory glass bottles with 12 bottles for each treatment. The average fresh weight of potato pulp in each bottle was 450 g, thus the average packing density obtained was 1,125 kg FM $m^{-3}$. These were pressed to ensure compaction, closed with cap and sealed with vinyl tape to avoid entry of air then kept in a room (ambient temperature 15 ± 5$^{o}$C). Potato pulp material (day 0) was sampled for subsequent analysis and three bottles per treatment were opened on the 2$^{nd}$ , 8$^{th}$ , 32$^{nd}$ and 50$^{th}$ day post-ensiling to investigate the changes in the fermentation characteristics and starch, sugar and pectin content of the resultant silages.

In Experiment 2, four treatments (PP, PL, PR and RL), containing the same inoculants and ratios of preparations as in Experiment 1, were ensiled in polyethylene bags. Four hundred kilograms fresh weight of potato pulp was prepared for each treatment. For the sake of packing convenience, each treatment was separated into four polyethylene bags, 100 kg apiece. Each polyethylene bag was inserted into a plastic container (120 L capacity) prior to filling. After each bag was filled, air was evacuated the container and carefully sealed. The packing density was about 30% less than that of Experiment 1. These were kept in a large open container with an ambient temperature of $12 \pm 5^{o}C$. The silos were opened 50 days after filling and sampled for chemical analyses.

### *3.2.1.2 Chemical analyses*

The dry matter (DM) content of the potato pulp materials and silages was determined as described in Chapter 1. Organic matter (OM) content was obtained by difference following ashing at $550^{o}C$ for 3 h. The crude protein (CP) and ether extract (EE) were determined using a Kjeldahl and a Soxhlet apparatus, respectively (AOAC, 1991). Neutral detergent fiber (NDF) assayed with a heat stable amylase and expressed inclusive of residual ash and acid detergent fiber (ADF) expressed inclusive of residual ash and without the use of sodium sulfite, were determined according to Van Soest et al. (1991). Sugar (net sugar, principally monosaccharides) was determined from dried samples by extraction with 800ml/L ethanol at a constant temperature of $80^{o}C$ and acid hydrolysis with anthrone solution (containing anthrone and thiourea in a solution of concentrated sulfuric acid). Hemicellulose was calculated as the difference between NDF and ADF. Starch was determined from the dried residue following sugar extraction with 600ml/L perchloric acid ($HClO_4$) and continuous boiling for 2 h. The starch concentration was then determined by its color reaction with glucose at room temperature using a test kit (Glucose B Test Wako, Wako Pure Chemical Industries Ltd, Tokyo, Japan). The sugar and starch concentrations were measured using a spectrophotometer with a standard solution from the test kit at peak transmissions of 650 mμ and 420 mμ, respectively. These methods are fully described by Abe (1988). The pH of aqueous extracts of pre-ensiling material and silages were measured using a pH meter (model HM-30G, TOA Electronics Ltd, Tokyo, Japan). Lactic acid was determined from aqueous extracts of materials and silages by the colorimetric method of Baker and Summerson (1961), in which lactic acid in the extracts was converted into acetaldehyde by treatment with concentrated sulfuric acid, and then determined by its color reaction with *p*-hydroxybiphenyl. The color was read in a spectrophotometer at a peak transmission of 560 mμ. Pectin was determined by the HCl extraction method of Phatak et al. (1988). The method included removal of protein from a defatted dried material using protease and dispersion in a solution of sodium phosphate buffer at pH 7.5 and incubation overnight. This was followed by filtration to isolate the residue and subsequent dispersion in water adjusted to a low pH (1.5) with HCl and continuous shaking at 80$^{o}$C for 4 h. This was followed by filtration and precipitation of the filtrate with 4 volumes of 950ml/L ethanol, centrifugation, and lyophilization to isolate pectin. The concentrations of acetic acid, a volatile fatty acid (VFA), were analyzed by gas-liquid chromatography (Shimadzu GC-14 A, Kyoto, Japan) equipped with a flame-ionization detector and a capillary column (ULBON HR-52, 0.53mm i.d. x 30m x

3.0 μm) by using 2-ethyl-*n*-butyric acid as the internal standard. The gross energy content of potato pulp, after drying at 60$^{o}$C in a forced-draught oven for 48 h, was determined by an adiabatic bomb calorimeter (CA-4P, Shimadzu, Tokyo, Japan).

### *3.2.1.3. Statistical analyses*

Silage fermentation data were analyzed using ANOVA in a randomized block design, using the General Linear Model Procedure of SAS (1996) computer package. Contrasts between the pre-ensiling quality and treated silages were performed, and means differences were compared for all variables and $P<0.05$ considered as statistically significant.

## 3.2.2. Results

### *3.2.2.1. Chemical characteristics of potato pulp*

The main chemical characteristics of potato pulp are presented in Table 3-1. A discussion on some of these characteristics has been made previously in Chapter 2. An attempt is however made here to highlight in much broader terms the composition of the by-product.

**Table 3-1**. Chemical composition and microbiological characteristics of potato pulp (Figures are means of at least three separate determinations and are given in g/kg dry matter, unless otherwise stated)

| | Range | Mean | SD |
|---|---|---|---|
| Moisture | 730-845 | 789 | 57.2 |
| Organic matter | 974-983 | 980 | 4.9 |
| pH | 4.89-5.59 | 5.31 | 0.4 |
| Buffering capacity (meq/kg DM) | 13.6-23.1 | 21.54 | 9.1 |
| Lactic acid (Log10 CFU g/FM)[§] | 4.6-8.4 | 6.4 | 1.1 |
| Yeast (Log10 CFU g/FM) [§] | 1.6-6.3 | 2.4 | 1.2 |
| Sugar | 5.0-23.2 | 11.4 | 10.2 |
| Crude protein | 47.0-49.2 | 48.4 | 1.2 |
| Neutral detergent fiber | 348-353 | 350 | 2.5 |
| Acid detergent fiber | 336-348 | 336 | 6.0 |
| Hemicellulose | 11.0-18.0 | 14.1 | 3.5 |
| Pectin | 207-181 | 177 | 5.5 |
| PWRC (g/100g) | 70.1-73.6 | 72.8 | 0.75 |

[§]Based on Saito et al, 2006.

### *3.2.2.2. Silage fermentation quality*

The fermentation qualities of potato pulp in both experiments after 50 days of ensilage are presented in Table 3-2. There were no significant treatment effects on the moisture content of the silages. In Experiment 1, the pH of PPS decreased sharply with or without the inoculants in the first few days of ensilage, but declined gradually after a week and continued to decrease until the 50th day of ensilage (Fig. 3-1a). Inoculation with R and L significantly ($P<0.05$) decreased the pH of PPS compared with the control. Lactic acid production increased in all treatments with days in ensilage (Fig. 3-1b), but was lower ($P<0.05$) with inoculation with R. Lactic acid production was higher ($P<0.05$) in PL than in PR after 50 days of ensilage, but not higher than the untreated silage. However, the combined application of the two inoculants did not have any significant effect on lactic acid production over the control. The acetic acid concentration, the only volatile fatty acid detected in PPS, increased with days in ensilage (Fig. 3-1c) and was higher ($P<0.05$) in PR and RL compared to the control. In Experiment 2, the pH decrease after 50 days of ensiling was similar to that of Experiment 1, although the values were slightly higher in Experiment 2. Lactic acid increased in all the silages to about six folds that of pre-ensiling content, but effect among treatments was not significant ($P>0.05$). The acetic acid concentration increased ($P<0.05$) in both PR and RL, but did not differ in PL, compared to the control, confirming the result in Experiment 1. The pH of pre-silage material in both experiments decreased ($P<0.001$) while lactic and acetic acid concentrations increased ($P<0.001$) in comparison with the silages.

**Table 3-2**. Pre-ensiling quality of potato pulp and fermentation quality in potato pulp silages without additive (PP), inoculated with *Lactobacillus rhamnosus* alone (PL), *Rhizopus oryzae* alone (PR) and a combination of the two inoculants (RL) after 50 days in two separate experiments (1 and 2). Values are given in g/kg dry matter, unless otherwise stated

| | | *Silages* | | | | | *Significance* | *Contrast*[§] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Fermentation quality* | *Potato pulp* | *PP* | *PL* | *PR* | *RL* | *SEM* | *level* | *1* | *2* | *3* | *4* |
| *Experiment 1* | | | | | | | | | | | |
| Moisture(g/kg FM[†]) | 830 | 839 | 835 | 835 | 831 | 3.84 | NS | *** | NS | NS | NS |
| pH | 5.51 | 3.36 a | 3.32 b | 3.21 c | 3.21 c | 0.004 | *** | *** | NS | * | * |
| Lactic acid | 3.0 | 63.2 ab | 67.3a | 55.31 c | 61.9 b | 1.03 | ** | *** | ** | *** | ** |
| Acetic acid | 0.0 | 6.5 b | 3.8 b | 11.9 a | 11.8 a | 1.42 | * | *** | NS | ** | * |
| Sugar | 4.9 | 14.5 | 10.1 | 13.2 | 28.3 | 0.03 | *** | *** | *** | *** | *** |
| Starch | 177 | 198 | 119 | 100 | 120 | 0.29 | *** | *** | *** | *** | *** |
| Pectin | 213 | 203 | 192 | 197 | 133 | 3.26 | *** | *** | ** | ** | NS |
| *Experiment 2* | | | | | | | | | | | |
| Moisture(g/kg FM[†]) | 830 | 842 | 839 | 840 | 840 | 1.75 | NS | *** | NS | NS | NS |
| pH | 5.51 | 3.57 a | 3.55 ab | 3.46 bc | 3.43 c | 0.034 | * | *** | NS | * | * |
| Lactic acid | 5.0 | 28.5 ab | 32.7 ab | 26.7 b | 35.8 a | 2.07 | NS | *** | NS | NS | NS |
| Acetic acid | 0.8 | 7.0 b | 8.1 bc | 10.7 ab | 12.9 a | 1.09 | * | *** | NS | ** | * |
| Sugar | 5.0 | 15.8 | 20.2 | 13.2 | 18.9 | 1.19 | *** | *** | NS | ** | NS |
| Starch | 206 | 195 | 196 | 208 | 214 | 6.45 | NS | NS | NS | NS | NS |
| Pectin | 213 | 213 | 215 | 207 | 199 | 8.26 | NS | NS | NS | NS | NS |

Except potato pulp, means with different letters in a row differ significantly (P <0.05); NS, not significant.

[†]Fresh matter;* P <0.05; ** P<0.01; *** P<0.001.

[§]1= Potato pulp vs. silages; 2 = PP vs. PL; 3 = PP vs. PR; 4 = PL vs. PR.

**Fig 3-1.**

(a)

(b)

(c)

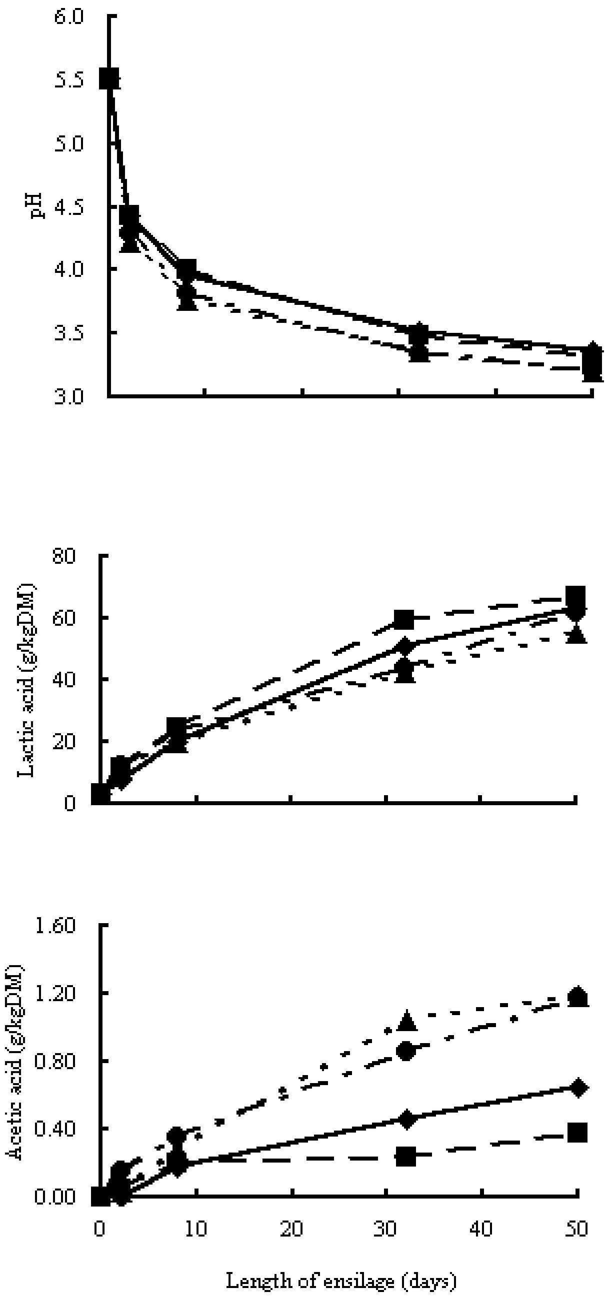


**Fig. 3-1.** a-c. Change in pH, lactic and acetic acid concentrations of potato pulp during 50 days of ensilage (Experiment 1). ( ◆) PP, potato pulp silage without additive; (■) PL, lactobacillus alone inoculated potato pulp silage; (▲) PR, rhizopus alone inoculated potato pulp silage; (●) RL, rhizopus + lactobacillus inoculated potato pulp silage.

**Fig. 3-2**.

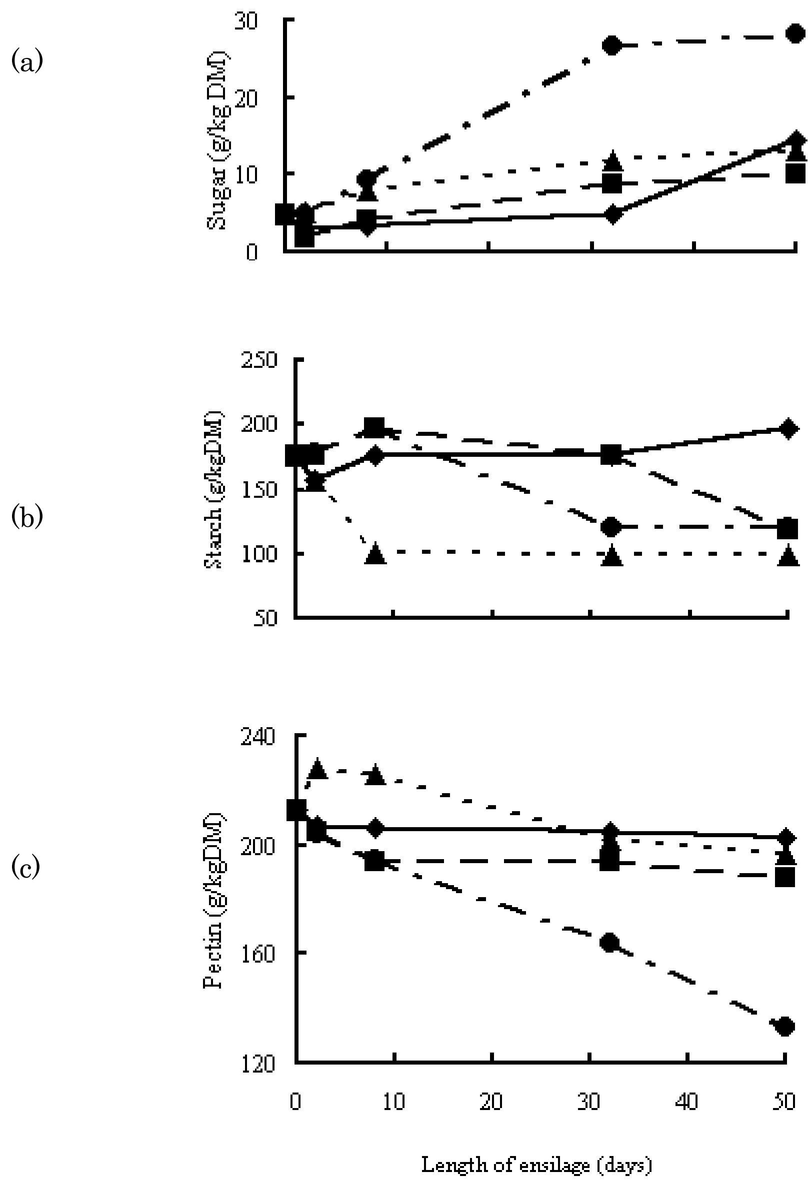


**Fig. 3-2** a-c. Change in sugar, starch and pectin during 50 days of (Experiment 1). ( ◆) PP = potato pulp silage without additive; (■) PL, lactobacillus alone inoculated potato pulp silage; (▲) PR, rhizopus alone inoculated potato pulp silage; (●) RL, rhizopus + lactobacillus inoculated potato pulp silage.

#### *3.2.2.3. Change in nutrient composition*

The change in the predominant carbohydrate fraction of potato pulp, starch, sugar and pectin in Experiment 1 is shown in Fig. 3-2 (a-c). Pectin, and to a large extent starch, decreased, while sugar increased, with length of ensilage. The initial sugar content in potato pulp (about 5 g/kgDM) increased to about four-fold in the control, PL and PR but five-fold in RL with significant difference (P<0.05) among the treatments after 50 days of ensilage (Table 3-2). Starch content showed a level of inconsistency with days in ensilage, and PP was the least degraded at the end of the ensiling period. Pectin content decreased consistently in RL (Fig. 3-2c) to about a half of its original content after 50 days of ensilage, with significant differences (P<0.05) among all treatments compared to the control (Table 3-2). In Experiment 2, however, there were no treatment effects on the predominant carbohydrate fraction of potato pulp silage (Table 3-2).

### 3.2.3. Discussion

The pH of all silages, irrespective of the type of inoculants added or silo used, was below 3.60, indicating a good fermentation process. However, R when used alone or in combination with L, significantly (P<0.05) decreased the pH of PPS in both investigations. The decrease with inoculation with R is in consent with the findings of Oda et al. (2002), who reported a rapid decrease in pH within one day of inoculation with *Rhizopus oryzae* in potato pulp. The defect in lactic acid production with R (in Experiment 1) may be based on two hypotheses: The first is probably due to the malfunction of LDH, the active enzyme for conversion of sugars to lactic acid in R in agreement with Skory (2000), who stated that the enzyme requires high concentrations of sugars for conversion into lactic acid. The low sugar content in potato pulp may have therefore hindered the activity of the enzyme. Secondly, since most fungi thrive well under aerobic conditions, the activity of R in the initial aerobic phase may have played a role in the early phase of ensiling, although Oda et al. (2002), observed lactic production with *Rhizopus oryzae* under airtight conditions in potato pulp. Their supposition that the narrow spaces present in potato pulp could permit aerobic fungal growth and consequently boost the activity of the fungus, however, runs contrary to the principles of ensilage that require anaerobic conditions in the silo.

Further, the ambient temperature during the ensiling period could have contributed in part. Hachmeister and Fung (1993), in their review of the *Rhizopus* strain (among them *Rhizopus oryzae*) used for the fermentation of tempeh (a traditional food in Indonesia), stated that temperature, relative humidity and length of fermentation are three crucial factors that dictate the outcome of tempeh fermentation and that optimum temperature requirements are in the range of 25 to 37$^{o}$C. Since there is no available literature on the use of R fermentation in PPS, it is our subjective conclusion that the low temperature range in the current experiments may have restricted effective fermentation and for that matter, lactic acid production in PPS. The higher fermentation quality in Experiment 1 compared to Experiment 2, as evidenced by lower pH values and higher lactic acid concentrations does reflect the ideal conditions in the former than the latter and could be attributed to the differences in the densities of the silages, the type of silos used, and differences in storage temperature, all of which may have affected the rate of fermentation. On visual appraisal, the quality of silages appeared good, and no differences in smell were observed.

The significant increase in lactic acid production in PL over PR in Experiment 1 (though not in Experiment 2 due to differences in ensiling conditions, as earlier suggested) does seem to confirm the efficiency of L, a lactobacillus strain over R, in agreement with Hang (1989), who, in a study with corn, reported that the production yield of *Rhizopus oryzae* in lactic acid (based on the total carbohydrate consumed) is relatively low in comparison with that produced by lactic acid bacteria. The effect of the combined application of the two inoculants on lactic acid production over the control silage was not observed, probably due to the counter effect of *Rhizopus oryzae*, which, as observed earlier, had shown defective lactic acid production. Future investigations should therefore focus on the optimum conditions necessary for increased lactic fermentation with *Rhizopus oryzae* alone in PPS.

The acetic acid contents in the silages were generally low compared to the lactic acid contents, indicative of the good preservation quality of the silages. Other volatile fatty acids that are usually produced during fermentation in silages were not detected in PPS. Oda et al. (2002) made similar observations. The increases in acetic acid concentration in PR and RL over the control and PL after 50 days of ensilage, though indicative of the good quality of the former over the latter, is of little significance since other VFA especially butyric acid, which are usually associated with badly preserved silages, were not present. The changes in the carbohydrate fraction of potato pulp seemed to assume a consistent pattern with advance in the period of ensilage. The most obvious of this pattern was observed in RL on the degradation of pectin, which led to a concomitant increase in sugar concentrations after 50 days (in Experiment 1) and could be due to the combined effect of the two inoculants. The increases in the concentrations of sugar with days in ensilage were probably due to slight increases in concentrations of unspecified monosaccharides during fermentation, due to the attack by enzymes on starch and pectin and possibly due to chemical interactions between other sugars. Hence, sugars, or specifically monosaccharides, which are the end products of enzymatic degradation, increase with fermentation, whereas starch concentrations are reduced by fermentation (Van de Riet et al., 1987). Others had supported this assertion earlier, including De Man (1957), who indicated that lactic acid fermentation took place in potato pulp when stored in pits using carbohydrate substrates, mainly galactan and also from pectin. Increases in sugar contents in Experiment 2 without a corresponding change in starch and pectin contents could, therefore, have originated from other sources such as cellulose or hemicellulose, which are present in potato pulp (Mayer and Hillebrandt, 1997). The degradation of starch in the current study showed some level of inconsistency among treatments, and this could be due to the slow degradability of potato starch in general (Monteils et al., 2002). It could be noted that the decrease in pectin and the resultant increase in sugar with RL after 50 days of ensiling in Experiment 1 (Fig 3-1 a, c) did not have any significant effect on lactic acid concentration (Table 3-2). This could be explained by the counter effect of R, which had shown defective lactic acid production in the current study as observed earlier. The breakdown of starch and pectin with the use of inoculants as observed in the current experiment could, and in agreement with Dongowski and Stoof (1993), lead to reduction of the water holding capacity of PPS and consequently effluent production; therefore, ensiling of potato pulp on a large scale would have to take this into account.

### 3.2.4. Conclusion

Ensiling potato pulp with *Lactobacillus rhamnosus* and *Rhizopus oryzae* and their combined application reduced the pH and improved the fermentation quality of the silage. However, potato pulp can ensile well with or without the bacterial inoculants. Lactic acid production was low with inoculation with *Rhizopus oryzae* alone, and further studies are required to ascertain the optimum conditions for the lactic acid production potential in potato pulp silage.

## 3.3. Effect of temperature and storage duration on potato pulp ensiled with fungal inoculants

Ensiling of potato pulp is considered a viable method for its preservation and subsequent use as feed for ruminants due to its high nutritional value (Okine et al., 2005; Aibibula et al., 2004). Although the use of bacterial inoculants in the enhancement of fermentation quality of silages is well established (Guan et al., 2002; Meeske et al., 1999; Gordon, 1989a), few studies (Okine et al., 2006a; Oda et al., 2002) have, however, investigated the use of fungal or aerobic inoculants in an anaerobic process such as ensiling.

Although the use of the fungus *Rhizopus oryzae* in aerobic fermentations is well documented (Skory et al., 1998; Skory, 2000; Erdogan and Mahinur, 2001), our (Okine et al., 2005) previous investigation on the use of *R. oryzae* in PPS fermentation did not confer any advantages over the silage without the inoculant in lactic acid production. Of the reasons considered for the lack of effect was the ambient temperature during the ensiling period. The success of microbial inoculants used to enhance ensiling fermentation is partly dependent on the temperature, since different inoculants have various temperature optima (McDonald et al., 1991b). Hachmeister and Fung (1993) reported that temperature (between 27 and 35$^{o}$C) and length of fermentation were crucial factors in *Rhizopus* fermentation of tempeh, a mold-modified indigenous fermented food made from soybeans or cereal grains in southeast Asian countries. In temperate regions like Hokkaido, in northern Japan, where potato pulp is produced abundantly annually and where preservation by ensiling is an option, temperature may be important in determining the efficacy of the fungus in PPS fermentation. The fungus *Amylomyces rouxii* is taxonomically identical to *R. oryzae* in characteristics and ability to produce lactic acid (Abe et al., 2004). Both were used in this study because of their ability to hydrolyze starch into glucose and their use as starters for potato pulp fermentation (Abe et al., 2003; Abe et al., 2004).

This study investigated the effect of various temperature regimes and duration of storage on the fermentation quality of potato pulp silage inoculated with the two fungi.

## 3.3.1. Materials and methods

Two fungi inoculants, *Rhizopus oryzae* IFO 4707 (IR) and *Amylomyces rouxii* CBS 438-76 (IA), originally from freeze-dried cultures, were each grown on media containing malt extract agar for fungi and incubated at 25$^{o}$C in Petri dishes 72 h prior to use. The fungi, including the media, were added separately to fresh potato pulp at the rate of 1.0 % (w/w) in fresh matter, enough to produce at least $1x10^{6}$ colony forming units (CFU) $g^{-1}$ on use as per manufacturer's statement. Three treatments made up of potato pulp without additive (control, C), IR treated silage (IPR) and IA treated silage (IPA) were prepared, and ensiling was done in polyethylene silos (300 mm x 200 mm x 0.15 mm). Eighty-one silos were made, 27 for each of the three treatments. Each silo was filled with 500 grams of potato pulp, and 5 grams fresh weight of the medium containing either IR or IA were added to 54 silos and mixed thoroughly before filling. To ensure compaction, the silos were pressed gently and a vacuum air deflator was used to remove any air from the silos followed immediately by heat-sealing and further strengthening with adhesive tape. Twenty-seven silos from each of the three treatments were kept under constant temperature regimes of 4, 12 and 25 ± 1°C, respectively. Pre-silage material was sampled on day 0 for immediate determination of pH and dry matter (DM) content. Three bags per treatment from every temperature regime were randomly selected and opened on days 7, 24 and 40 post-ensiling and sampled to investigate treatment effects on the fermentation quality through measurement of DM, pH, lactic acid, volatile fatty acids (VFA), sugar and starch contents of the resultant silages.

#### *3.3.1.1. Chemical analyses*

Potato pulp material and silages were dried for at least 24 h using a freeze-dryer, and sub-samples were ground to pass through a 1mm screen for subsequent analyses. Dry matter was determined from sub-samples by drying in a forced-draught oven at a constant temperature of 135°C for 2 h. The pH, lactic acid, sugar and starch contents of pre-silage material and silages were determined as described earlier. The concentrations of acetic acid were analyzed by gas-liquid chromatography (Shimadzu GC-14 A, Kyoto, Japan) equipped with a flame-ionization detector and a capillary column (ULBON HR-52, 0.53mm i.d. x 30m x 3.0 μm) and using 2-ethyl-*n*-butyric acid as the internal standard.

### *3.3.1.2. Statistical analyses*

Silage fermentation data were analyzed in a 3 x 3 factorial design using ANOVA in the general linear model procedure of SAS (1996). Main effects and interaction between treatments and contrasts were performed for responses of inoculation to temperatures and durations of storage. Significant mean differences in effects were separated using the least significant difference test and P-values less than 0.05 were considered statistically significant. In the regression equations, the measured parameters (pH and lactic acid) served as the dependent variables (Y), and storage temperature ($^{o}$C) and duration of storage (days) were the independent variables.

## 3.3.2. Results

### *3.3.2.1. Fermentation quality*

The effects of inoculation, storage temperatures, and duration of storage and their interactions are given in Table 3-3. The pre-silage material contained 227.0 g/kg DM, 1.90, 1.31, 322.0 and 6.0 g/kg DM as lactic acid, acetic acid (the only VFA detected in PPS), starch and sugar, respectively, while values for the final (day 40) control silage at 25$^{o}$C were 229.0 g/kg DM and, respectively, 73.1, 10.7, 308.0 and 3.1 g/kg DM for the same previous parameters. The pH was 5.62 and 3.32 for pre-silage material and silage, respectively, indicating a good fermentation process. The changes in pH, lactic acid, and acetic acid contents of the silages, as affected by the fungal inoculants under different storage temperatures and durations of storage, are given in Table 3-3.

### *3.3.2.2. Effect of inoculants*

There were no treatment effects on the DM of silages which were between 210.0 and 227.0 g/kg. The inoculants had no significant effect on the fermentation quality of PPS relative to the control silage, although numerically they reduced the pH and increased the lactic acid content. Acetic acid production was also not affected by inoculation.

### *3.3.2.3. Effect of storage temperature*

The effect of storage temperature on the fermentation quality of PPS was obvious. Generally, the pH decreased ($P<0.01$) while lactic and acetic acid contents increased

(P<0.01) with increase in storage temperatures. There were no significant effects on lactic and acetic acid contents of the silages at 4 and 12°C. However, at 25°C both lactic and acetic acid contents increased (P<0.01).

### *3.3.2.4. Effect of duration of storage*

The effect of duration of storage on the fermentation in PPS was similar to that of the storage temperature, decreasing (P<0.01) the pH and increasing (P<0.01) lactic acid production with progressive duration of storage. Acetic acid content was low at day 7, but increased and then decreased at 24 and 40 days of ensilage, respectively.

### *3.3.2.5. Starch and sugar contents in silages*

The changes in starch and sugar contents are given in Table 3-3. Starch levels fluctuated with no clear tendency in the effect of inoculation, storage temperature level, and duration of storage. The only obvious effect, though not statistically significant, was that of numerical decreases for IPR and IPA relative to the control, an indication that the inoculants somewhat enhanced starch degradation in PPS. Effects of temperature and duration of storage were significant (P<0.05) for sugar, but the inoculant effect was not. At 25°C the sugar level was low in comparison with that at lower temperatures of 4 and 12°C, while it increased significantly (P<0.05) at days 24 and 40, in comparison with day 7.

**Table 3-3**. Chemical characteristics of potato pulp material, main effects and interaction between inoculants, storage temperatures and duration of storage in fermentation characteristics of potato pulp silage after 40 days of ensilage (Values are least square means of three determinations given in g/kg dry matter, unless otherwise stated)

| | RM | Inoculants[1] | | | Temperature[2] | | | Duration of storage[3] | | | | Significance[4] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | IPR | IPA | T4 | T12 | T25 | D7 | D24 | D40 | SEM | I | T | D | I vs. T | I vs. D | T vs. D |
| DM (g/kg) | 227.0 | 214.0 | 211.1 | 213.3 | 212.0 | 212.1 | 213.2 | 214.0 | 215.1 | 210.3 | 3.60 | NS | NS | NS | NS | NS | NS |
| pH | 5.62 | 3.97 | 3.94 | 3.95 | 4.41a | 3.96b | 3.49c | 4.30a | 3.86b | 3.69c | 0.05 | NS | *** | *** | NS | NS | *** |
| Lactic acid | 1.90 | 24.6 | 26.8 | 24.8 | 11.1b | 18.9b | 46.2a | 13.4b | 21.6b | 41.3a | 3.63 | NS | *** | *** | NS | NS | *** |
| Acetic cid | 1.31 | 10.6 | 12.6 | 10.0 | 08.3b | 09.3b | 15.5a | 08.0b | 19.3a | 05.8b | 1.51 | NS | ** | *** | * | ** | NS |
| Starch | 322.0 | 283.0 | 270.3 | 264.1 | 244.2 | 288.2 | 285.1 | 261.2 | 277.0 | 279.1 | 13.60 | NS | NS | NS | NS | NS | NS |
| Sugar | 6.0 | 8.0 | 9.4 | 8.2 | 8.8a | 11.6a | 5.3b | 5.7b | 10.1a | 9.7a | 1.11 | NS | *** | * | NS | NS | ** |

RM: pre-silage material; DM: dry matter; SEM: standard error of the means.

[1] C: silage without additive (control); IPR: Rhizopus oryzae inoculated potato pulp silage; IPA: Amylomyces rouxii inoculated potato pulp silage.

[2] T4, T12, T25, storage temperatures of 4, 12 and 25°C, respectively.

[3] D7, D24, D40, duration of storage of 7, 24 and 40 days, respectively.

[4] I: inoculation; T: storage temperature; L: duration of storage; I vs. T: I×T interaction; I vs. D: I×D interaction; T vs. D: T×D interaction.

Except for RM, figures followed by different letters in a row for inoculants, temperature and duration of storage differ significantly ($P<0.05$).

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS: not significant.

### *3.3.2.6. Interaction between inoculants, storage temperature and duration of storage*

Interactions between inoculation and both temperature and duration of storage were absent for all measured parameters except for acetic acid (Table 3-3). The pH, lactic acid content and sugar concentration showed significant ($P<0.01$) interactions for both temperature and duration of storage.

### *3.3.2.7. Contrasts between effects of inoculation, storage temperatures and duration of storage*

Contrasts between control and inoculated silages showed no statistical differences between IR and IA or the average response of inoculation relative to uninoculated silages (Table 3-4). There were, however, clear significant ($P<0.01$) effects between pre-silage material and silages with respect to temperature and duration of storage in all the measured parameters except for acetic acid and sugar concentrations. Of particular significance was the linear relationship ($P<0.001$, Table 3-4) between storage temperature and duration of storage for pH and lactic acid concentration. These relationships could be summarized in the following multi-linear equations:

$$pH = -0.046\,T - 0.021\,D + 5.10 \qquad R^2 = 0.81 \quad (P<0.01)$$

$$LA = 0.16\,T + 0.08\,D - 1.40 \qquad R^2 = 0.66 \quad (P<0.01)$$

where T, ensiling temperature ( $^{o}C$ ); D, duration of storage (days); LA, lactic acid content (% DM) and R, correlation co-efficient.

Starch content in pre-silage material decreased ($P<0.05$) with storage temperature and duration of storage (Table 3-4). However, significant ($P<0.05$) but inconsistent relationships were observed for acetic acid and sugar concentrations.

### 3.3.3. Discussion

This study was conducted to investigate the possible conditions necessary for improving fermentation in PPS inoculated with fungi as a follow-up to our previous study (Okine et al., 2005). The silo chosen for the study has been shown to be acceptable for ensiling without affecting the preservation quality (Ashbell et al., 2001). The chemical analysis of the pre-silage material and the final control silage showed that the changes in DM, pH and lactic and acetic acids were comparable to our previous study.

In the present investigation, the inoculant effect on PPS fermentation, especially lactic acid production, was minor, in confirmation of our previous results. We hypothesized that application of the fungi to potato pulp in conditions sufficient to maintain their growth would yield better inoculant responses in fermentation end products as compared to the freeze-dried form employed in the previous study. This was built on the premise that the initial aerobic phase of the fungi was likely to influence their efficacy, since oxygen is indispensable for fungal growth. Evacuation of the bags after filling, though

**Table 3-4**. Contrasts between control and inoculated silages and effects between pre-silage material and silages with respect to storage temperatures and duration of storage in potato pulp fermentation characteristics

| | Contrast[1] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Inoculants | | | Temperature | | | Relationship | | Duration of storage | | | Relationship | |
| | C/IPR | C/IPA | C/IRA | RM/T4 | RM/T12 | RM/T25 | Lin. | Quad. | RM/D7 | RM/D24 | RM/D40 | Lin. | Quad. |
| Dry matter | NS | NS | NS | *** | *** | *** | NS | NS | *** | *** | *** | NS | NS |
| pH | NS | NS | NS | *** | *** | *** | *** | ** | *** | *** | *** | *** | ** |
| Lactic acid | NS | NS | NS | *** | *** | *** | *** | ** | *** | *** | *** | *** | ** |
| Acetic acid | NS | NS | NS | * | ** | NS | NS | ** | *** | NS | NS | ** | *** |
| Starch | NS | NS | NS | * | * | *** | * | NS | ** | ** | ** | NS | NS |
| Sugar | NS | NS | NS | *** | NS | * | * | *** | *** | *** | NS | *** | * |

[1] C/IPR: control silage vs. *Rhizopus oryzae* silage; C/IPA: control silage vs. *Amylomyces rouxii* silage; C/IRA: control silage vs. *R. oryzae* and *A. rouxii* silage.

RM/T4: RM/T12, RM/T25: pre-silage material vs. storage temperatures of 4, 12 and 25°C, respectively.

RM/D7: RM/D24, RM/D40: pre-silage material vs. storage duration of 7, 24 and 40 days, respectively.

Lin.: linear relationship; Quad.: quadratic relationship.

* P<0.05; ** P<0.01; *** P<0.001; NS: not significant.

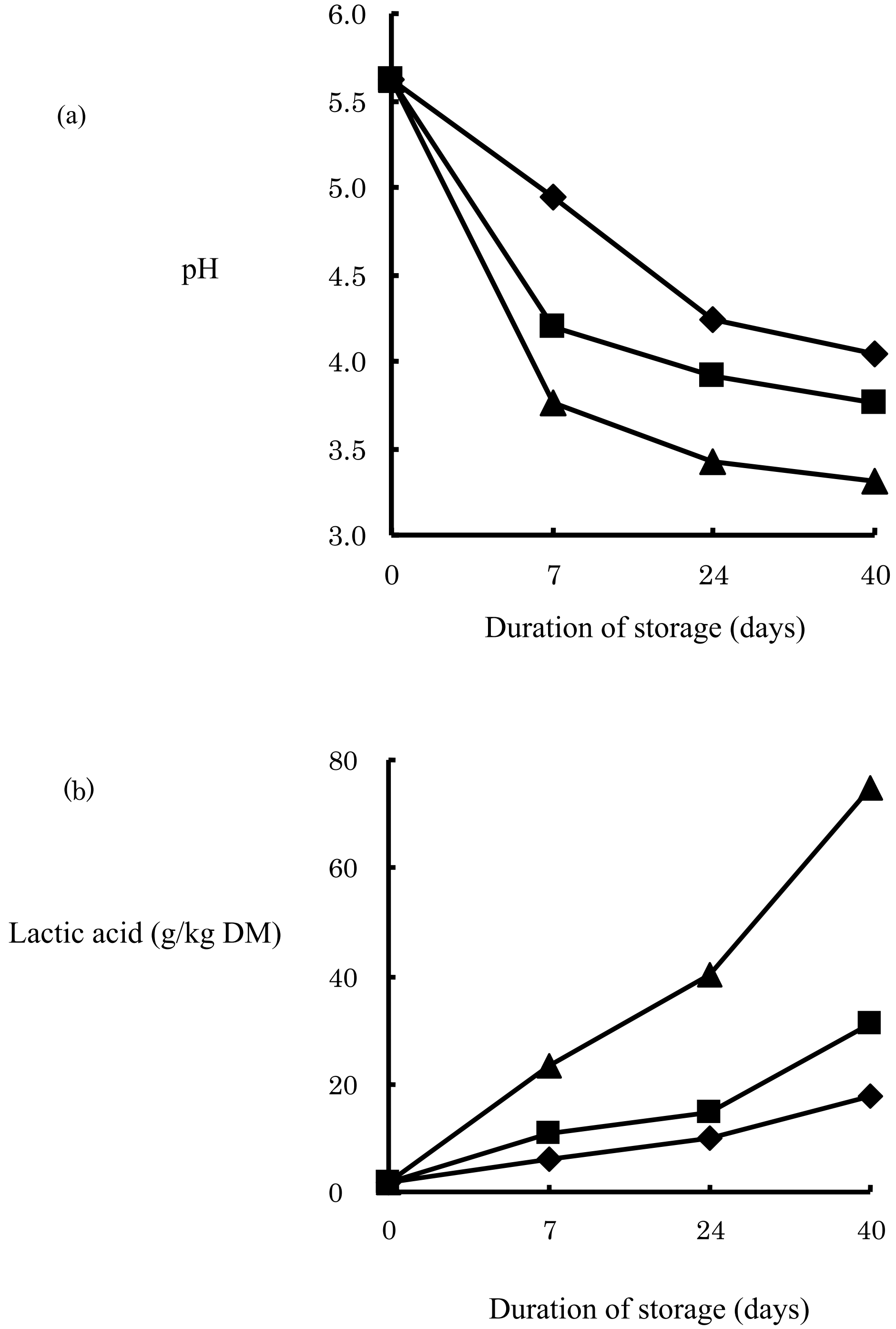


**Figure 3-3.** Change of pH (a) and lactic acid concentration (b) during storage under different temperatures in potato pulp silage. ( ◆) 4°C; (■) 12°C; (▲) 25°C. [Values represent least square means (n=81)].

necessary for anaerobiosis, may have inhibited slow diffusion of oxygen into the surface and consequently fungal activity, thereby impairing the inoculants' potential for lactic acid production. However, this assumption does not explain the high final concentrations of lactic acid at the end of the period of investigation (Fig. 3-3). Mayer and Hillebrandt (1997) and later Saito et al. (2006), in investigations of microbial characteristics of raw potato pulp sampled from different varieties over a period of time, observed that the dominant microbe present was lactic acid bacteria (up to $10^8$ CFU/g FM). This large load of lactic acid bacteria under favorable silo conditions, such as sustained anaerobiosis and sufficient substrate availability, would be enough to maintain fermentation even in the absence of a lactic acid fermentation stimulant. This report seems to support our previous conclusion (Okine et al., 2005) that potato pulp does ensile well without bacterial inoculants.

Ensiling is based on fermentation, which involves biological and enzymatic activity and as such is strongly influenced by temperature. There was a common trend in the fermentation pattern of PPS irrespective of the inoculants used. The pH decreased, while the lactic acid content increased, with rise in storage temperatures and progressive duration of storage. The multi-linear equation elucidates this relationship. On the basis of the equation, to attain a pH of 3.50, for example, it takes only 21.4 days at a temperature of 25$^o$C, while it requires 49.9 and 67.0 days at 12 and 4$^o$C, respectively. Similarly, to attain a lactic acid content of 35.0 g/kg DM it takes 11.3, 37.3 and 53.3 days at 25, 12 and 4$^o$C, respectively. The pH was lower at elevated storage temperatures and extended periods of storage, while lactic acid production accelerated at higher storage temperatures and extended periods of storage (Fig. 3-3). There was a higher correlation for pH decrease than for the rise in lactic acid production, evident from the correlation co-efficients ($R^2$ = 0.81 vs. 0.66). The insignificant differences in lactic acid content between 4 and 12$^{o}$C and also at days 7 and 24 (Table 3-3) elicited the relevance of a combined effect of both storage temperature and duration of storage on PPS fermentation.

Although the acetic acid content fluctuated inconsistently, the increase ($P<0.01$) at 25$^{o}$C followed an increase in lactic acid content (Table 3-3), reflecting the conversion of the latter to the former and/or the permeability of some oxygen into the bags, in agreement with Ashbell et al. (2001). These effects, however, did not adversely affect the quality of the silages and may have protected them from development of butyric acid and molds, which were not detected in any of the silages. The starch content was not affected by the inoculation, temperature, or duration of storage. The hypothesis that *Rhizopus* cultures enhance potato pulp starch degradation or conversion to sugar for use as a substrate for lactic acid fermentation could not be validated by the present results. Although inoculated silages had numerically (Table 3-3) higher values relative to the control, starch in potato pulp was degraded with/without the fungal inoculants. The slow degradation of potato starch (Monteils et al., 2002) may be responsible for this, or during fermentation enough sugar may have been produced from hydrolysis of other carbohydrate sources such as pectin (De Man, 1957; Okine et al., 2005) through the enzyme pectinase (produced by the fungi) in amounts sufficient to maintain fermentation and this obviated the need to hydrolyze starch. The significant decrease in sugar levels (from 12 to 25$^{o}$C) indicated that high ensiling temperatures increased the rate of sugar consumption as a substrate for lactic acid production.

### 3.3.4. Conclusion

These data suggest that the fermentation quality of potato pulp silage is influenced most by ensiling temperatures and the duration of storage and least by inoculation with fungal inoculants. The use of the *Rhizopus oryzae* and *Amylomyces rouxii* in potato pulp silage fermentation should be weighed against their cost and other nutritional benefits.

## 3.4. Effluent production and losses in potato pulp silage

In the previous experiments, the role of microbial inoculants in enhancing fermentation in potato pulp has been acknowledged. However, the use of inoculants, especially fungi, is related to the breakdown of pectin and starch in potato pulp silage, which may decrease the water holding capacity of PPS with undesirable consequences like increased effluent production and nutrient losses.

The objective of the following experiment (Experiment 3) was to quantify effluent losses in potato pulp silage inoculated with or without fungi.

### 3.4.1. Materials and methods

Fresh potato pulp with estimated moisture content (based on visual appraisal) of 900 g/kg was dehydrated by bagging and suspension in semi-porous polyethylene bags overnight. Three microbial inoculants; *Lactobacillus rhamnosus* (L), *Rhizopus oryzae* IFO 4707 (R) and *Amylomyces rouxii* CBS 438-76 (A), whose characteristics have been previously (Experiments 1 and 2) mentioned, were used. The inoculants were added at the rate of 0.5, 1.0 and 10 g/kg in fresh weight of potato pulp, respectively, and enough to produce at least $1x10^6$ CFU $g^{-1}$ on application and according to manufacturer's instructions. These were set against potato pulp without inoculant as control. The two previous inoculants were added to fresh potato pulp in their freeze-dried forms. The later, originally in freeze dried form was incubated in a medium containing agar at 25°C for 48 hours prior to addition to potato pulp. Treatments were thus made up of the control without inoculant (EC), *Lactobacillus rhamnosus* added to potato pulp (EL), *Rhizopus oryzae* added to potato pulp (ER) and *Amylomyces rouxii* added to potato pulp (EA). All the three inoculants were mixed with wheat bran (1.0 g/kg in fresh matter weight of potato pulp material) prior to addition to potato pulp for the sake of application and mixture convenience. About 10 kilograms fresh weight (with three replications from each treatment) were ensiled in silos (1 m in height, 0.10 m in diameter and 0.05 m thick) made of cylindrical polyvinyl chloride (PVC) plastic pipe with two ends. The end was open, and the base was closed. The base of each pipe comprised a sealed conical narrow end with a drain opening fitted with rubber tube and a cork which when relaxed, enabled the flow of effluent into a calibrated glass flask fitted with a rubber stopper. This prevented infusion of ambient air into the silo via the tube during effluent measurement. A perforated stainless steel plate, further covered with cheesecloth, was inserted into the base of each pipe, creating a spacer between silage material and effluent. Each silo was filled and pressed with weights to compaction and the upper part was capped with a polyethylene bag and enforced with vinyl tape to prevent the infiltration of air. The silos were placed horizontally on a stand to facilitate collection of effluent, then stored at room temperature and effluent was collected each day for the first 3 days of ensilage. Then, at 3-day intervals, pH was measured immediately upon opening, and samples were taken and stored at -20$^{o}$C until analyzed. The silos were opened on the 30$^{th}$ day of ensilage, weighed and sampled for subsequent analyses.

### *3.4.1.1. Chemical analyses*

The fermentation characteristics (moisture, pH, lactic acid and VFA), WRC, organic matter, sugar, and gross energy were determined using procedures previously described.

### *3.4.2.1. Statistical analyses*

Same as in the previous experiment.

## 3.4.2. Results

### *3.4.2.1. Fermentation quality*

The fermentation quality of potato pulp material and silages are presented in Table 3-5. Even after dehydration for 24 hours, the moisture content was still high (840.1 g/kg). The moisture content of the silages, though not statistically significant, numerically decreased in ER and EA. The pH of silages was low in the inoculated treatments relative to the uninoculated control silages and was particularly lowest in ER and EA. However, values were generally below 3.60 and consistent with the two previous experiments. Lactic acid concentration increased ($P<0.05$) in the inoculated silages except with ER. Sugar concentration increased significantly ($P<0.05$) in ER and EA, respectively. Water retention capacity decreased after ensiling and was more pronounced with inoculation, especially in EA.

### *3.4.2.2. Effluent production and nutrient losses*

Effluent volume increased significantly ($P<0.05$) in ER and EA, ranging from 619 to 1,816 grams at the end of 30 days of ensilage (data not shown), corresponding to 66.1-174.8 g/kg fresh weight of ensiled material, and was highest in EA. This had consequent adverse effects on the silages in losses of DM, OM, and gross energy.

**Table 3-5**. Chemical and silage characteristics of potato pulp after 30 days of ensiling. [(Experiment 3). Values are means of three determinations and given in g/kg DM, unless otherwise stated] [1]

| | | Silages | | | |
|---|---|---|---|---|---|
| | PP | EC | EL | ER | EA |
| Moisture (g/kg) | 840.1 | 830.2 | 831.1 | 823.3 | 811.0 |
| pH | 5.53 | 3.35b | 3.39a | 3.28c | 3.25d |
| Lactic acid | - | 60.7c | 77.7b | 62.3c | 114.3a |
| Acetic acid | - | 4.91c | 6.12b | 4.63c | 9.71a |
| Sugar | 23.1 | 19.2c | 20.3c | 23.0b | 25.5a |
| Water retention capacity (g/g) | 2.41 | 1.94a | 1.84b | 1.81b | 1.73c |
| Effluent (g/kg of material) | - | 66.1bc | 62.9c | 83.2b | 174.8a |
| *Total losses during ensilage* | | | | | |
| DM | - | 119.1bc | 138.0b | 114.2c | 204.3a |
| OM | - | 119.3bc | 137.2b | 113.1c | 201.1a |
| Energy | - | 116.0b | 120.2b | 93.2b | 184.3a |

[1] PP, potato pulp pre-silage material; EC, potato pulp silage without additive; EL, ER, EA, potato pulp inoculated with *Lactobacillus rhamnosus*, *Rhizopus oryzae* and *Amylomyces rouxii*, respectively.
Except PP, means within a row with uncommon letters differ significantly ($P<0.05$).

### 3.4.3. Discussion

Although the moisture content decreased in the silages compared to the original material, it was more evident in ER and EA. This was due to the effect of R and A which may have broken the cohesion of the cell wall structure of potato pulp (Dongowski and Stoof, 1993), enabling the release of water and effluent and consequently reducing the moisture content of the resultant silages. Reduction in pH of potato pulp in the silage fermentation without or with bacterial inoculants has already been acknowledged and discussed in detail in the previous experiments. The results of the present study (Experiment 3) lend further support to that view. Although increase in lactic acid with inoculation with L was expected and in tandem with Experiment 1, the high increase with inoculation with A, relative to the control uninoculated silage was a novelty in this study. Several reasons could be attributed to this, among them, the rate, state and mode of application (incubation with a medium prior to inoculation as against direct application in freeze-dried form) and pre-mixture with wheat bran. Addition of wheat bran has been reported to increase lactic acid content of raw comminuted potatoes (Weise, 1964). Conspicuously low in lactic acid production was ER. This result validated earlier conclusions (Okine et al, 2005; Okine et al, 2007) on the defect of *Rhizopus oryzae* in lactic acid production in potato pulp silage. Increases in sugar with inoculation with ER and EA reflected the rate of the fermentation process and to a large extent, end products of hydrolysis of carbohydrates in potato pulp such as starch and pectin (Okine et al., 2005).

The significant increase in effluent production with inoculation with A could be attributed to the breakdown of starch and pectin as theorized in previous studies (Experiments 1 and 2) and a direct consequence of reduction of the WRC of the silages. The increase in effluent production in PPS with A was so consistent that had the period of investigation been extended, more effluent would have been produced. ER also produced a substantial amount of effluent compared with EC, a result that could be explained by the action of the enzymes pectinase and LDH on the residual starch in potato pulp, which, according to Dongowski and Stoof (1993), decreases the water binding capacity of potato pulp after the action of cell wall degrading enzymes. The results of this study suggested that A was more effective in breaking the water holding capacity of PPS, compared with the other inoculants. As a consequence, losses were higher in EA, a result that could adversely affect the nutritive value of the silages. The combination of high sugar concentration and effluent production with inoculation with R and A would not only have negative impacts on the environment but also on the aerobic stability of the silages, since the residual sugars would serve as substrate for the proliferation of spoilage organisms such as molds, further impairing the nutritive value of potato pulp silage.

### 3.4.4. Conclusion

Ensiling potato pulp with *Rhizopus oryzae* and *Amylomyces rouxii* increased effluent volume and silage losses in potato pulp silage. Until evidence to the contrary arises, inoculation with fungal additives would seem to yield no benefits in potato pulp silage, and where their application is necessary, the use of in-silo absorbents prior to ensiling, is imperative.

## 3.5. Summary

Ensiling potato pulp with *Lactobacillus rhamnosus* and *Rhizopus oryzae* and their combined application reduced the pH and improved the fermentation quality of the silage. However, potato pulp can ensile well with or without the bacterial inoculants. Lactic acid production was low with inoculation with *Rhizopus oryzae* alone. A subsequent study suggested that the fermentation quality of potato pulp silage was influenced most by ensiling temperatures and the duration of storage and least by inoculation with fungal inoculants such as *Rhizopus oryzae* and *Amylomyces rouxii* in potato pulp silage. Moreover, ensiling potato pulp with *Rhizopus oryzae* and *Amylomyces rouxii* increased effluent volume and silage losses in potato pulp silage. Therefore, inoculation with fungal additives would yield little benefits in potato pulp silage and where their application is necessary, the use of in-silo absorbents prior to ensiling, is warranted.

# Chapter 4

## Daikon (Oriental radish)

### *4.1. Introduction*

Daikon, or Oriental radish (*Raphanus sativus* L.), is a popular root vegetable in Japan and East Asia. It goes by different names depending on the country of origin; *lor bark* (Cantonese Chinese), *Mu* (Korean), *labanos* (Filipino) or *cu-cai trang* (Vietnamese), and is grown all year round in areas having an oriental population. Daikon is consumed in different forms be it fresh, soaked, brined or dried. In Japan, more daikon is produced than any other vegetable after potatoes and yearly production runs into nearly two million tons with an increasing tendency during the past five years (Statistical Handbook of Japan, 2005). Out of this about 20 g/kg representing the crowns, leaves, damaged or infested root parts and culls that are of no commercial value are disposed off during processing as waste. A similar situation may exist in other regions where daikon is produced. These unwholesome parts are returned to the soil with detrimental consequences to the topsoil due to their high content of mineral salts. Daikon has high crude protein, digestible energy and low ether extract contents (154, 15 and 15 g/kg in dry matter, respectively) and contains easily digestible nutrients (National Agricultural Research Organization, 2001) thus, a potential animal feed resource. An intrinsic characteristic of daikon is its very high moisture content (about 940 g/kg in fresh matter), which is a major limitation to its use as livestock feed. Drying of daikon by-product for use in ruminant diets is an option but the high cost involved makes this uneconomical. Ensiling with drier materials for subsequent use as ruminant feed is a viable alternative from nutritional, preservation and environmental perspectives. However, the taste of, the taste of fresh daikon involves some irritation and a burning sensation on the tongue (Lindsay, 1985) and has a characteristically pungent smell and aroma (Friis and Kjaer, 1966) that may limit its use as livestock feed. To date and to the best of our knowledge, the only quantitative data available on the ensiling of daikon or its by-product, its fermentation pattern or its use as feed are our reports (Okine et al., 2006a, Okine et al., 2006b).

The objective of this investigation was to explore the possibility of using a material very low in dry matter content (such as daikon by-products) as animal feed with emphasis on ensiling as a means of preservation and, to assess the role of absorbents on effluent retention in the silages.

The study involved a series of experiments conducted separately over two years. First an investigation of the chemical composition and pre-ensiling characteristics of daikon, followed by ensiling of daikon by-products initially with wheat straw as a sole in-silo absorbent, and then with other absorbents: dried beet pulp, dried bean stalks and husks and wheat bran. A study of the aerobic stability or shelf life of daikon silages was investigated in both experiments.

## 4.2. Ensiling of daikon by-product with wheat straw (Experiment 4-1)

### 4.2.1. Materials and methods

#### *4.2.1.1. Chemical characteristics of daikon*

A total of twelve samples of fresh daikon (roots) were purchased from local supermarkets at different times during the harvesting season between July and October 2004 for the analyses of the chemical composition and ensiling characteristics.

Procedures used in the determination of the ensiling characteristics have already been described in Chapter 2. The chemical composition (organic matter, OM; crude protein, CP; neutral detergent fiber, NDF; ether extract, EE; gross energy, GE; water-soluble carbohydrates, WSC) was determined from dried samples.

### *4.2.1.2. Ensiling of daikon by-product*

Fresh daikon by-product (roots and leaves) were chopped to fit into two types of silos used for this purpose namely pipe and bucket type silos.

#### *4.2.1.2a. Pipe silos*

Twelve silos, (1 m in height, 0.10 m in diameter and 0.05 m thick) made up of cylindrical polyvinyl chloride (PVC) previously described in Chapter 3, were used. Daikon was chopped to about 2 cm length and 2 cm thickness (together with the leaves using a forage cutter and mixed). This size was determined through a short experiment as the most appropriate size for filling in terms of convenience, type of the silos employed, and effect on minimizing effluent output. The moisture content of daikon was adjusted to 850 g/kg in fresh matter by addition with wheat straw which had been chopped to about 2 cm prior to mixture with the daikon. The mixing ratio of daikon to wheat straw was 4:1 (w:w). A commercial lactic acid bacteria inoculant, *Lactobacillus plantarum* (LP, Ecosyl Products Ltd, Tokyo, Japan) was added at the rate of 10 g/kg, calculated to contain at least $1x10^6$ CFU per gram in fresh matter as per manufacturer's instruction. The contents were thoroughly mixed prior to filling. Four treatments were prepared with three replications for each treatment consisting of control (DN-N), with LP inoculation (DN-L), adjusted moisture level without LP (DA-N), and with LP

(DA-L). Each silo was filled and pressed with weights to compaction, with an average 8 or 6 kg in fresh matter of pre-silage material for non-moisture and adjusted moisture levels, respectively. After filling, the upper part of each pipe was capped with a polyethylene bag and enforced with vinyl tape to prevent the infiltration of air. The silos were rested horizontally on a stand to facilitate collection of effluent, stored at room temperature and effluent volume was measured after 1, 2, 3 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 28 and 30 days. On each effluent measurement, the cork fitted on the drainage tube was relaxed, enabling effluent flow, then tightened, flask removed and replaced immediately with a fresh one. The silos were opened and weighed on the 30$^{th}$ day of ensilage, and representative samples were taken for immediate determination of pH and juice extraction for subsequent chemical analyses. Composite samples of the silages (a total of 5 kg fresh matter) of each of the four treatments were transferred into separate open containers, kept at room temperature and representative samples taken from each container daily for 6 days for the determination of the aerobic stability using changes in pH, lactic acid and VFA as indices.

### *4.2.1.2. Bucket silos*

Thirty-six 20-liter polyvinyl chloride (PVC) buckets, each equipped with a septum-enforced lid and nozzle enabling gas escape only, were used as silos and prepared simultaneously with the pipe silos. Treatments, including rate of addition of LP and moisture adjustment level, were the same as that of the pipe silos. Filling was done by uniform mixture of straw and daikon and compacted by pressing with weights. The average weight was 20 and 17 kg in fresh matter for non-moisture and adjusted moisture levels, respectively. The silos were stored at room temperature and three buckets from each treatment were opened after 7, 14 and 30 days post-ensiling and sampled to investigate proceeding changes in fermentation quality, development of LAB and yeast flora.

### *4.2. 1.3. Chemical analyses*

The DM, pH, lactic acid and VFA concentrations, WSC, ether extract, NDF, ADF, and gross energy contents of material and silages were determined by methods previously described (Chapters 2 and 3). Volatile basic nitrogen (VBN) was determined by the complete distillation method as described by Morimoto (1971). Organic matter content was obtained by difference following ashing at 600$^{o}$C for 3 h. Total nitrogen (TN) was measured through Kjeldahl method and crude protein (CP) was calculated as TN x 6.25, all according to AOAC (1990).

### *4.2.1.4. Statistical analyses*

Silage fermentation data were analyzed using ANOVA in a randomized block design using General Linear Model Procedure of SAS (1996). Contrasts between fermentation quality of silages (inoculated with/without LP, and non/adjusted moisture, and the average response of the two factors to ensilage) were performed and means differences compared for all variables and $P<0.05$ considered as statistically significant.

### 4.2.2. Results

#### *4.2.2.1. Chemical characteristics of daikon*

The chemical composition and characteristics relevant to ensilage are given in Table 4-1. Daikon was characteristically low in DM, had high OM, CP and gross energy contents and low NDF, ADF and ether extract thus, a potential animal feed resource. The buffering capacity ranged 467-1001 meq/kg DM with a high standard deviation, which made it theoretically difficult to ensile in comparison with grass materials in general.

**Table 4-1**. Chemical and microbiological characteristics of daikon (n = 12). (Figures are given in g/kg DM, unless otherwise stated)[§]

| | Range | Mean | SD |
|---|---|---|---|
| Dry matter (g/kg ) | 42.7- 63.5 | 49.0 | 10.7 |
| Organic matter | 836 - 884 | 863.0 | 20.3 |
| pH | 5.85 - 6.20 | 6.05 | 0.18 |
| Water soluble carbohydrates | 262 - 480 | 382 | 90.4 |
| Buffering capacity (meq/kg DM) | 467-1001 | 703 | 255 |
| Crude protein | 131-210 | 180 | 35.0 |
| Neutral detergent fiber | 151-194 | 184 | 21.5 |
| Acid detergent fiber | 144 - 180 | 169 | 16.8 |
| Hemicellulose | 7.5 - 21.0 | 14.9 | 5.6 |
| Ether extract | 7.7 - 16.4 | 11.9 | 3.5 |
| Gross energy (MJ/kg DM) | 17.0 - 17.7 | 17.4 | 0.28 |
| Lactic acid bacteria ($Log_{10}$ CFU/g FM) | 3.3 - 7.8 | 4.8 | 2.09 |
| Yeast ($Log_{10}$ CFU/g FM) | 4.7 - 6.9 | 5.5 | 1.00 |

[§]DM, dry matter; meq/kg DM, milliequivalent per kilogram DM; SD, standard deviation; MJ, mega joule; CFU, colony forming units; FM, fresh matter.

#### *4.2.2.2 Ensiling of daikon by-products*

Pre-ensiling characteristics indicated that daikon by-product had high pH, contained varying levels of LAB and yeast numbers per gram of fresh matter and contained enough WSC to maintain lactic acid fermentation.

The chemical compositions of the pre-silage materials and silages in Experiment 4-1 are presented in Table 4-2. The DM of daikon by-product was elevated from 64.0 to 175.1 g/kg, and this had subsequent effects on the chemical composition of both pre-silage material and silages. Dry matter-adjusted material was low in sugar, buffering capacity and total nitrogen contents but had higher pH, WRC and gross energy values. The fermentation characteristics of the silages were good as indicated by low pH values and high lactic acid contents relative to the pre-silage materials. The silages contained some amounts of ethanol, fair amounts of acetic and propionic acids and traces of valeric acid but no butyric acid. Volatile basic nitrogen was low in all treatments. Within adjusted and non-adjusted moisture, inoculation with LP had no effect on all measured silage parameters except pH and lactic acid contents (main fermentation end products). Contrarily, contrasts between inoculation with/without LP, and non/adjusted moisture, and the average response of inoculation with LP and moisture adjustment on the silages were highly significant ($P<0.01$) except for pH.

**Table 4-2**. Chemical composition of daikon by-product material and silages after 30 days of storage in pipe silos (Experiment 4-1) [1]

| | Pre-silage material | | Silages | | | | Pooled | | Contrasts[#] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | DN | DA | DN-N | DN-L | DA-N | DA-L | SEM | P value | 1 | 2 | 3 |
| Dry matter (g/kg) | 64.0 | 175.1 | 48.1b | 47.3b | 132.0a | 131.3a | 2.33 | 0.0001 | ** | ** | ** |
| pH | 4.14 | 4.17 | 3.58a | 3.50b | 3.65a | 3.51b | 0.02 | 0.0020 | NS | NS | NS |
| Lactic acid (g/kg DM) | 21.4 | 9.2 | 132.3b | 179.8a | 63.5c | 68.7c | 3.06 | 0.0001 | ** | ** | ** |
| *Volatile fatty acids (g/kg DM)* | | | | | | | | | | | |
| Acetic | - | - | 12.7a | 10.2b | 5.4c | 4.1c | 0.62 | 0.0001 | ** | ** | ** |
| Propionic | - | - | 9.7a | 7.8a | 1.9b | 1.4b | 1.10 | 0.0014 | ** | ** | ** |
| Butyric | - | - | NF | NF | NF | NF | - | - | - | - | - |
| Valeric | - | - | 2.5a | 2.4a | 1.0b | 1.1b | 0.28 | 0.0070 | ** | ** | ** |
| Ethanol (g/kg DM) | - | - | 3.3a | 2.9a | 0.90b | 0.85b | 0.16 | 0.0001 | ** | ** | ** |
| Sugar (g/kg DM) | 274.0 | 53.0 | 11.8a | 11.5a | 2.6b | 1.9b | 0.47 | 0.0001 | ** | ** | ** |
| Water retention capacity (g/g) | 2.59 | 3.20 | 2.38ab | 2.02b | 2.72a | 2.54a | 0.13 | 0.0299 | ** | ** | ** |
| Buffering capacity (meq/kg DM) | 830.7 | 341.2 | - | - | - | - | - | - | - | - | - |
| Total nitrogen (g/kg DM) | 31.6 | 13.4 | 31.9a | 32.6a | 12.7b | 11.3b | 0.16 | 0.0001 | ** | ** | ** |
| Volatile basic nitrogen (g/kg TN) | - | - | 34.8 | 27.7 | 34.7 | 33.0 | 2.55 | 0.2394 | NS | NS | NS |
| Density (kg/$m^3$ fresh matter) | 942 | 683 | - | - | - | - | - | - | - | - | - |
| Gross energy (MJ/kg DM) | 17.5 | 18.3 | 17.3b | 16.9b | 18.2a | 18.0a | 0.22 | 0.0112 | ** | ** | ** |

[1] DN, daikon without moisture adjustment; DA, daikon adjusted moisture content with wheat straw; DN-N, DN-L, daikon silage with and without *Lactobacillus plantarum* (LP), respectively; DA-N, DA-L, daikon silage adjusted moisture content with wheat straw with and without LP, respectively. Except DN and DA, figures are means of three values. Means followed by different letters in a row differ significantly ($P<0.05$).

DM, dry matter; meq/kg DM, milliequivalent per kilogram DM; SEM, standard error of the means; MJ, mega joule; TN, total nitrogen.

[#] 1, DN-N vs. DA-N; 2, DN-L vs. DA-L; 3, average response of inoculation and moisture adjustment to ensilage; NS, not significant; *, $P<0.05$; **, $P<0.01$.

*4.2.2.3. Change in fermentation characteristics, LAB and yeast population with days in ensilage*

Changes in pH, lactic acid concentrations and development of LAB and yeast numbers during the course of ensilage in the bucket silos are shown in Fig. 4-1 and 4-2. The pH decreased sharply, followed by increases in lactic acid for all treatments during the first 7 days of ensilage, but continued steadily in the decrease and increase, respectively, till the end of the 30 th day. Moisture adjusted silages had lower lactic acid contents while inoculation with LP increased concentrations of lactic acid relative to the uninoculated control silages.

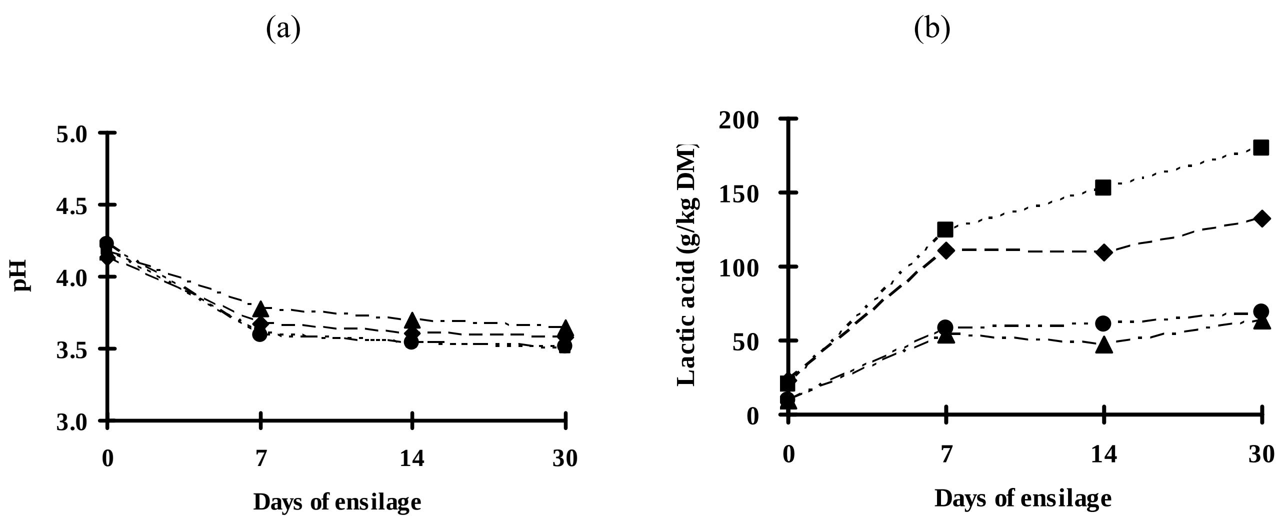


**Fig. 4-1.** Changes in pH (a) and lactic acid concentration (b) in daikon by-product silages days of ensilage in Experiment 4-1. (◆) daikon control; (■) daikon inoculated with *Lactobacillus plantarum*, (LP); (▲), daikon adjusted moisture with wheat straw only; (●) daikon adjusted moisture with wheat straw and inoculated with LP.

Lactic acid bacteria population increased in non-adjusted moisture in contrast with adjusted moisture during the first 7 days of storage, but both treatments decreased gradually till the end of the storage period. Yeast population followed a similar pattern and at the end of the duration of storage, had reduced considerably to about 30%. Inoculation with LP had lower LAB and yeast populations relative to the uninoculated silages.

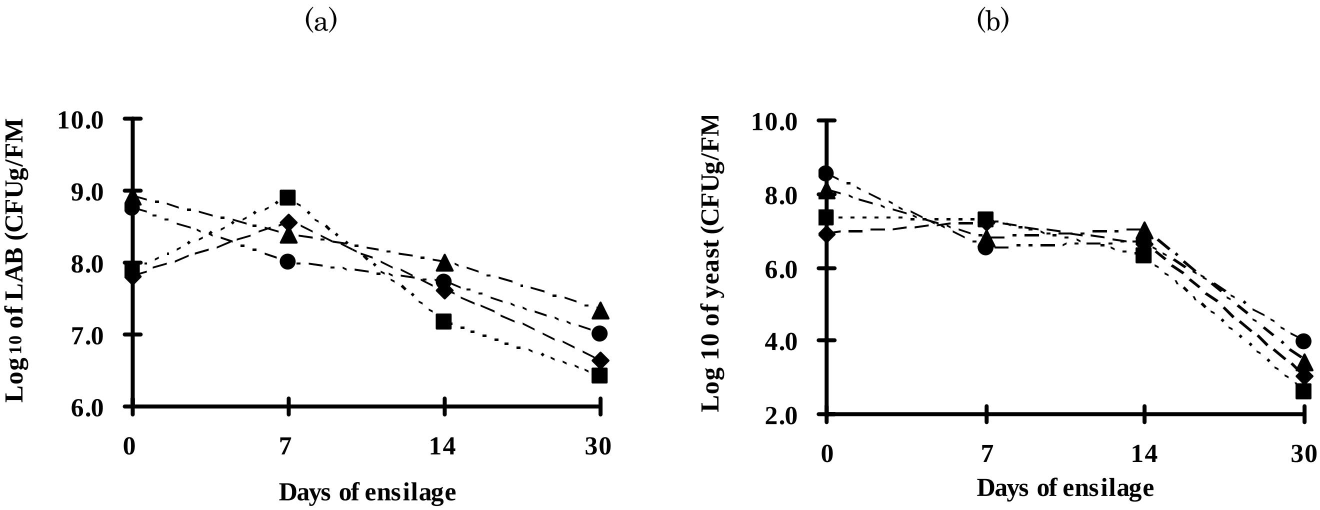


**Fig. 4-2**. Development of lactic acid bacteria (LAB) (a) and yeast (b) populations in daikon by-product silages days of ensilage in Experiment 4-1.
(◆) daikon silage control; (■) daikon inoculated with *Lactobacillus plantarum*, (LP); (▲), daikon adjusted moisture with wheat straw only; (●) daikon adjusted moisture with wheat straw and inoculated with LP.

### *4.2.2.4. Effluent production and nutrient losses during ensilage*

There was high effluent production from the non moisture-adjusted silages (with or without LP inoculation) with progression of days of ensilage especially during the first 6 days (Fig. 4-3), and at the end of 30 days had reached a peak of 378.4 and 507.7 g/kg fresh weight of ensiled material, respectively (Table 4-3). High dry matter, energy and nitrogen losses followed as a consequence. Adjustment with wheat straw minimized (P<0.01) these losses considerably. Total weight losses as effluent (calculated as the difference between initial and final total weight of silage minus effluent) were significantly (P<0.01) higher for non-adjusted moisture silages.

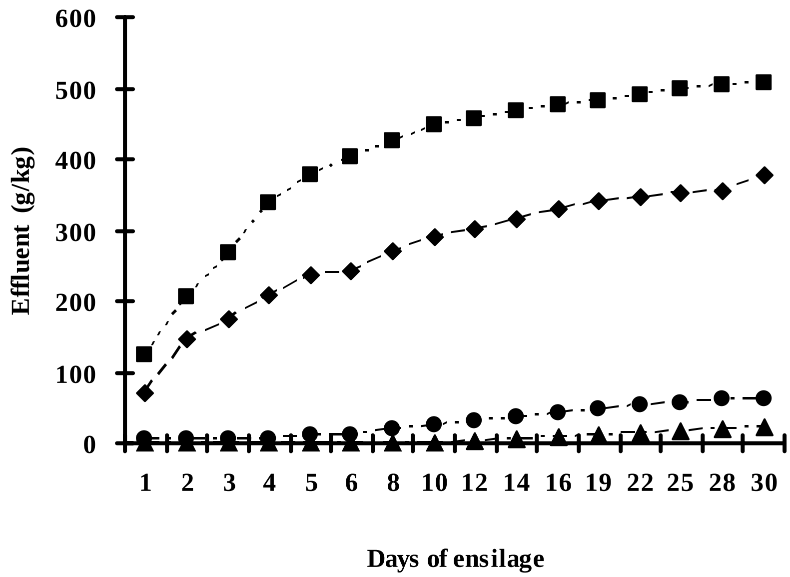


**Fig. 4-3**. Effluent volume in daikon by-product silages during 30 days of ensilage. (◆), daikon silage control; (■), daikon silage inoculated with *Lactobacillus plantarum*, (LP); (▲), daikon adjusted moisture with wheat straw only; (●), daikon adjusted moisture with wheat straw and inoculated with LP.

The opposite was true for gas losses. Significant increases (P<0.01) in effluent production were observed only for LP inoculated relative to the uninoculated silage; otherwise, inoculation had no effect on losses within moisture adjusted and non-adjusted silages.

**Table 4-3**. Total losses of dry matter, gross energy and nitrogen, effluent volume, weight losses as effluent and gas (g/kg of total weight losses) in daikon by-product after 30 days of ensilage (Experiment 4-1)

| | Silages[§] | | | | |
|---|---|---|---|---|---|
| | DN-N | DN-L | DA-N | DA-L | P value |
| Dry matter (g/kg) | 554.4a | 532.4a | 58.9b | 120.7b | 0.0001 |
| Gross energy (g/kg) | 560.5a | 535.8a | 61.5b | 122.3b | 0.0002 |
| Nitrogen (g/kg) | 500.1a | 518.4a | 141.1b | 248.5b | 0.0026 |
| Effluent (g/kg ensiled material) | 378.4b | 507.7a | 21.3b | 62.9b | 0.0001 |
| *Weight losses* | | | | | |
| Effluent (g/kg total weight loss) | 519.0a | 530.7a | 25.0b | 43.0b | 0.0001 |
| Gas (g/kg total weight loss ) | 481.0b | 469.3b | 975.0a | 957.0a | 0.0001 |

[§] Same as in Table 4-2.
Figures are means of three values. Means followed by different letters in a row differ significantly (P<0.05).

### *4.2.2.5. Aerobic stability of silages*

Aerobic stability of the silages in Experiment 4-1 was determined over 6 days with changes in fermentation end products as indices (Ashbell et al., 1991) and is presented in Figures 4-4 and 4-5. The pH of non-adjusted moisture silages was somewhat stable till the 5$^{th}$ day of aerobic exposure in contrast with that of the moisture adjusted silages which increased rapidly. There was a similar rate of decrease in lactic acid content in both moisture adjusted and non-adjusted silages. However, a common pattern was observed for LP inoculated silages (adjusted or non-adjusted moisture) in sharp increases and decreases in pH and lactic acid contents, respectively, after the 5$^{th}$ day of aerobic exposure. There were gradual decreases in residual VFA (acetic acid) and

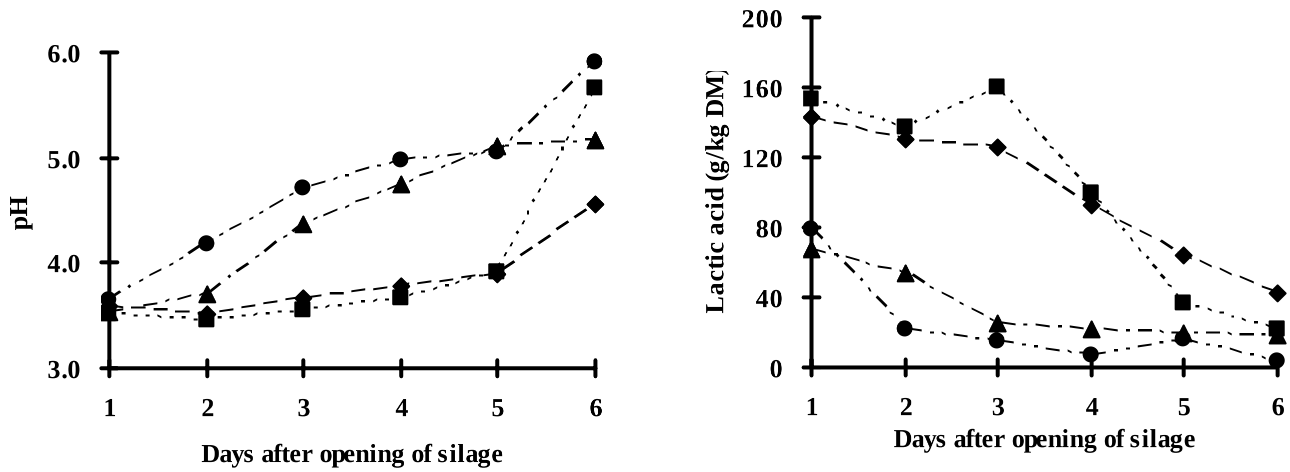


**Fig. 4-4.** Changes in pH (a) and lactic acid concentration (b) in daikon by-product silages during 6 days of aerobic exposure in Experiment 4-1. (◆), daikon silage control; (■), daikon inoculated with *Lactobacillus plantarum*, (LP); (▲), daikon adjusted moisture with wheat straw only; (●), daikon adjusted moisture with wheat straw and inoculated with LP.

increases (propionic acid) in all silages with progression of days of aerobic exposure of silages. Butyric acid, however, increased sharply after the 5 th day, indicative of the increase in putrefactory microorganisms in the silages.

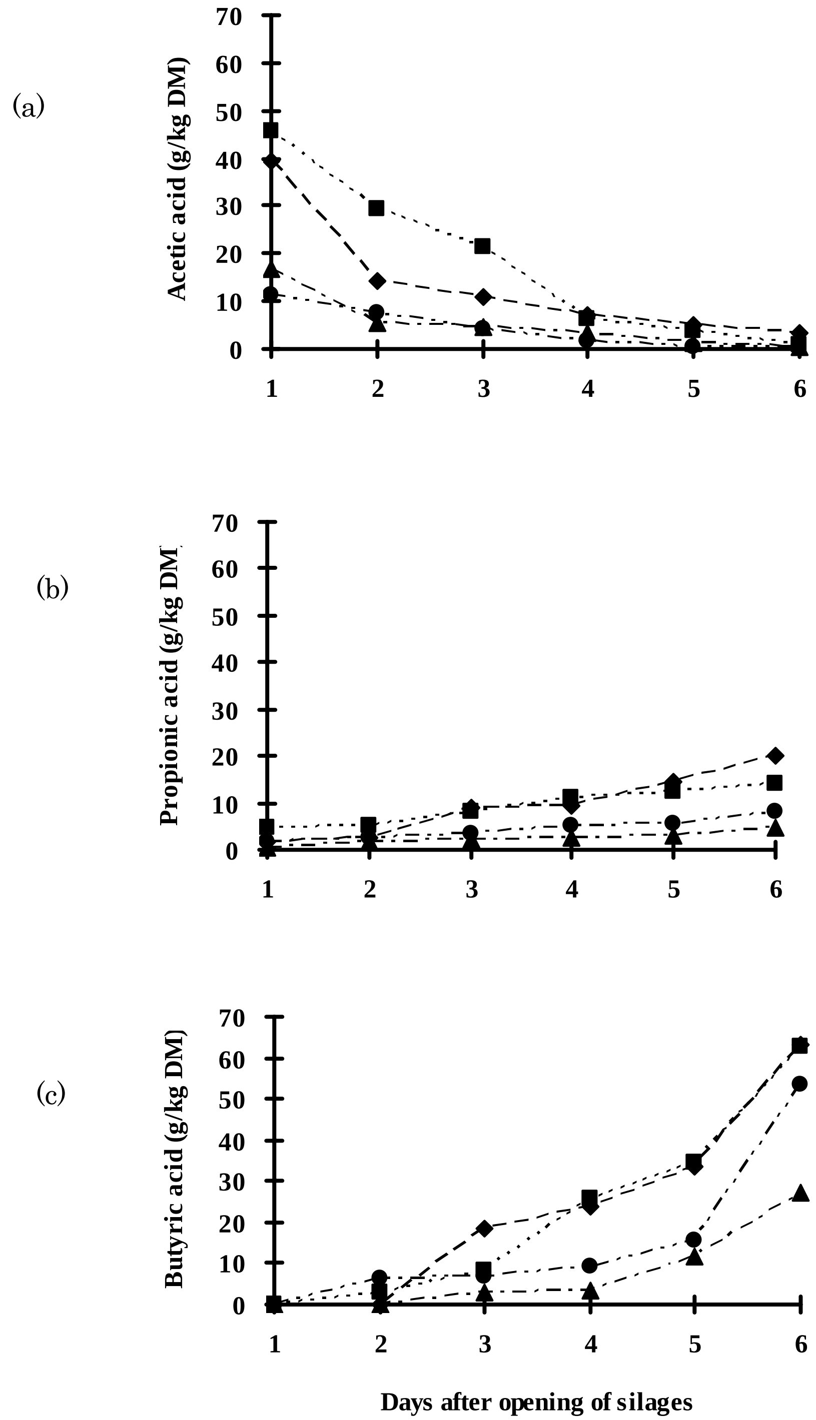


**Fig. 4-5** (a-c). Changes in volatile fatty acid concentrations in daikon by-product silages during 6 days of aerobic exposure in Experiment 4-1. (◆), daikon silage control; (■), daikon inoculated with *Lactobacillus plantarum*, (LP); (▲), daikon adjusted moisture with wheat straw only; (●), daikon adjusted moisture with wheat straw and inoculated with LP.

## 4.3. Ensiling daikon with other absorbents (Experiment 4-2).

The objective was to investigate the effect of other in-silo absorbents on the fermentation quality, effluent retention and aerobic stability of daikon by-product silage.

### 4.3.1. Materials and methods

Daikon by-product was adjusted to lower moisture (four treatments with three replications each) with dried beet pulp (DBP), dried bean stalks and husks (DBH), wheat straw (DWS) and wheat bran (DWB) as in-silo absorbents(Table 4-4). The daikon: absorbent mixing ratio was 4:1 (w:w) for DBP, DBH and DWS. The ratio for DWB was 7:3 (w:w) due to its perceived low water retention capacity. Ensiling was done in 18-liter capacity PVC cylindrical bucket silos (Takagi Company, Tokyo, Japan). Each bucket is equipped with a perforated stand at the base, which served as a separator between silage material and effluent, and a drain tap which enabled the flow and measurement of effluent. Each treated material was put into a polythene bag, pressed and inserted upside- down into the bucket silo and a weight (calculated to exert a pressure of 40 g/cm$^3$ per fresh weight of material) was placed on the silo. The purpose was to simulate conditions in a commercial silo and to investigate effluent retention of the treatments under pressure. The packing weights and densities are presented in Table 4-4. The silos were kept at room temperature (18-24$^o$C) and opened after 30 days of storage. Representative samples were taken from each treatment for the determination of the aerobic stability 3, 5 and 7 days after opening the silages using procedures similar to those in the previous experiment.

#### *4.3. 1.1. Chemical analyses*

Analyses of chemical components in pre-silage materials and silages were done as previous described in this chapter.

#### *4.3.1.2. Statistical analyses*

Silage fermentation data were analyzed using General Linear Model Procedure of SAS (1996). Statistical differences, where applicable, were declared at $P<0.05$.

### 4.3.2. Results

#### *4.3.2.1. Fermentation quality, effluent and losses during ensilage*

In Experiment 4-2, treatments were adjusted to lower dry matter contents based on observations of effluent production in silages from Experiment 4-1. The adjustments augmented the WRC of all the pre-silage materials except DWB (Table 4-4). Silage fermentation quality was relatively depressed compared with that of Experiment 4-1. There were significant ($P<0.05$) treatment effects on pH and lactic acid but none on VFA concentrations. Silage losses were mostly as effluent, and were significant ($P<0.05$) among treatments, with DWS producing zero effluent. Other treatments had fair amounts of effluent, with DWB producing the most.

**Table 4-4**. Pre-silage and silage chemical characteristics of daikon by-product ensiled with different absorbents (Experiment 4-2) §

| | DBP | DWS | DBH | DWB | SEM | P value |
|---|---|---|---|---|---|---|
| *Pre-silage characteristics* | | | | | | |
| Dry matter (g/kg) | 18.4 | 18.9 | 17.6 | 28.7 | - | - |
| Water retention capacity (g/g ) | 4.25 | 4.08 | 3.95 | 2.49 | - | - |
| Packing weight (kg/silo) | 12.1a | 5.1c | 6.4b | 12.0a | 0.06 | 0.0001 |
| Packing density ($kg/m^3$) | 671.1a | 282.3c | 356.1b | 664.4a | 3.36 | 0.0001 |
| *Silage characteristics* | | | | | | |
| Dry matter (g/kg) | 196.2c | 229.1b | 206.3c | 253.1a | 4.06 | 0.0010 |
| pH | 3.80b | 3.97a | 4.00a | 3.78b | 0.03 | 0.0020 |
| Lactic acid (g/kg DM) | 44.5a | 17.4c | 34.6b | 36.4b | 2.29 | 0.0020 |
| *Volatile fatty acids* (*g/kg DM*) | | | | | | |
| Ethanol | 0.01 | 0.20 | 0.04 | 0.07 | 0.06 | 0.2135 |
| Acetic | 0.2 | 0.2 | 0.4 | 0.2 | 0.45 | 0.5473 |
| Propionic | 0.0 | 0.1 | 0.1 | 0.1 | 0.10 | 0.4851 |
| Valeric | 0.0 | 0.1 | 0.1 | 0.1 | 0.64 | 0.2543 |
| Effluent (g/kg ) | 152.0b | 0.0d | 103.1c | 201.1a | 12.55 | 0.0002 |
| *Losses* | | | | | | |
| Effluent (g/kg total losses) | 935.9a | 0.0c | 889.6a | 714.4b | 28.03 | 0.0001 |
| Gas (g/kg total losses) | 64.1c | 1000.0a | 110.4c | 285.6b | 28.03 | 0.0001 |

§DBP, daikon + dried beet pulp; DWS, daikon + wheat straw; DBH, daikon + dried bean stalks and husks; DWB, daikon + wheat bran.
SEM, standard error of the mean. Except pre-silage dry matter and water retention capacity, values are means of three determinations; means followed by different letters in a row differ significantly (P<0.05).

*4.3.2.2. Aerobic stability of the silages*

The changes in pH and lactic acid contents of the silages after exposure for 7 days are given in Fig. 4-6. The pH rose sharply from 3.80 to about 5.00 after 3 days of opening of the silages, and continued to rise steadily thereafter. In 7 days, the silages were all above 6.0 with DBH recording the lowest. The opposite pattern was observed for lactic contents in quick declinations in all silages after 3 days. At the end of the period of investigation, values were below 10.0 g/kg DM, irrespective of the type of silage. Of the different treatments, DWS and DBH were the most unstable during the 7 day period.

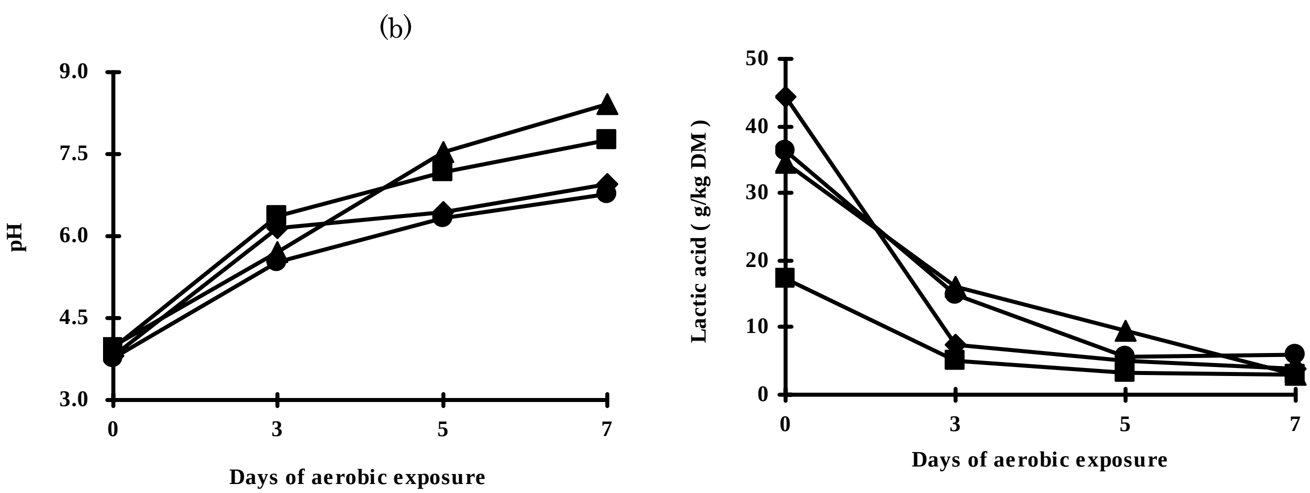


**Fig. 4-6.** Changes in pH (a) and lactic acid concentrations (b) in daikon by-product silages during 6 days of aerobic exposure in Experiment 4-2. (◆), daikon + dried beet pulp silage (DBP); (■), daikon + wheat straw silage (DWS); (▲), daikon + dried bean stalks and husks silage (DBH); (●), daikon + wheat bran silage (DWB).

### 4.3.3. Discussion

Despite the high contents of OM, CP, WSC, GE and the low content of EE that underline its prospect as animal feed resource, pre-ensiling characteristics, especially the high moisture content, pH, buffering capacity and yeast population of daikon pre-supposed difficulties in its preservation by ensilage. Adjustment of the moisture contents and inoculation with LP were measures to address these presumed deficiencies and to improve the suitability for ensiling. Dry matter adjustment of pre-silage material in Experiment 4-1 led to reduced concentrations of sugar, buffering capacity and total nitrogen due to low content of the absorbent (wheat straw) in these chemical components (not shown in data). Adjustment of DM content increased the WRC of daikon pre-silage material. The term WRC, defined as the amount of water retained by a known weight of fiber under the condition used (Robertson et al., 2000) or in other words, g moisture/g dry matter is employed as a novelty in this study. A major feature in the ensiling of particularly high moisture materials is the DM/WRC relationship *vis-à-vis* effluent production. From studies in our laboratory using low dry matter agriculture by-products, the WRC of silage materials is highly influenced by, and positively correlated to, the dry matter content (unpublished observation),. In the present study, an upward adjustment of the DM of pre-silage material in Experiment 4-1 from 64.0 to 175.1 g/kg (370 g/kg increase) led to about 810 g/kg increase in the WRC and an obvious effect on effluent retention in the silages (Fig. 4-3). Inoculation with LP reduced ($P<0.01$) the pH and final concentrations of lactic and acetic acids of daikon non-adjusted silages, obviously due to the initially high sugar concentration in them as typical of a lactic acid culture. This led to concomitantly, high effluent outputs with days in ensilage (Fig. 4-3) and consequently high nutrient losses (Table 4-3) relative to the moisture-adjusted silages. The amount of effluent produced from non-adjusted moisture silages with/without LP showed the non-feasibility of ensiling daikon by-product alone and underscored the utmost necessity of incorporation with absorbents prior to ensiling. The concentrations of VFA, apart from acetic acid, did not reveal differences between LP inoculations or otherwise, and were indicative of the relatively good preservation of the silages. This was particularly true with the absence of butyric acid, which is usually associated with badly preserved silages. Contents of silage VBN were low in all treatments and below the 80 g/kg of total N threshold suggested by Henderson (1993) of well preserved silage, a further indication of effective fermentation in the silages. The transitional behavior of the fermentation acids in the silages (bucket silos in Experiment 4-1) caused sharp decreases in pH and corresponding increases in lactic acid concentrations after 7 days of ensilage were typical of a good fermentation process (Woolford, 1984b). Between 14 and 30 days, a steady reduction in pH indicated stability, while gradual progression of lactic acid concentrations followed, reflective of substrate depletion with duration of storage. Growth of LAB peaked in the first 7 days in corroboration with the rise in lactic acid concentration in all silages (except moisture-adjusted silages), followed by decreases in LAB numbers. This phenomenon has been reported by several workers (Ashbell et al., 1987; Jonsson and Pahlow, 1984; Woolford, 1984b). The qualitative changes in lactic acid bacteria are generally attributed to their acid tolerance and acidifying potential (Woolford, 1984b). The behavior of lactic acid bacteria during the later stages of ensilage revealed little information on the state of preservation and, as concurred by (Woolford, 1984b), pH is a more reliable indicator of whether or not further microbial development will occur. Moreover, lactic acid bacteria can produce acid after cessation of growth or when the population is decreasing (Woolford, 1984b). The attainment of a stable pH of below 3.80 after the first 7 days to a large extent suppressed LAB growth. Yeast population decreased with time due to sustained anaerobic conditions in the silage, and with inoculation, due to the elevated concentrations of lactic acid, in consent with Jonsson and Pahlow (1984), who reported that anaerobic ensiling results in continuous reduction in the number of yeast, especially lactate-utilizing yeasts. In the present study, high lactic acid concentrations especially during the first 7 days of ensilage may have inhibited certain metabolic yeast groups resulting in the decline in yeast numbers and not simply low pH. The low yeast numbers, however, augurs well for daikon silage fermentation, since their presence would otherwise in high numbers indicate the infiltration of air during the course of ensilage. The behavior of LAB and yeast in moisture-adjusted silages during the first week of storage cannot not be explained.

In the first five days of aerobic exposure, the moisture adjusted silages in Experiment 4-1 deteriorated faster due to higher dry matter content and to a lesser degree, to lower packing density of the pre-silage material which enabled easy infiltration of air causing rapid spoilage. After the fifth day, LP inoculated silages had faster rates of spoilage as indicated by high pH and butyric acid concentrations, probably due to increased activity of lactate assimilating yeasts (Filya et al., 2000; Jonsson and Pahlow, 1984). Care is therefore needed after unloading of daikon silage to forestall such losses.

In Experiment 4-2, moisture levels were adjusted based on observations from the previous experiment. The hypothesis was that increasing the DM content to a level above that in Experiment 4-1 would lead to an increase in the WRC of the mixtures and consequently, to effluent retention in the silos. Although DM and WRC values were elevated, effluent outputs in DBP and DWB were more than the projected, suggesting that packing weight, density, and pressure exerted on the silages, or other factors such as fiber content (Offer and Al-Rwidah, 1989; O'Keily, 1991) and/or physical characteristics of ensiled material (Jones and Jones 1996) may have played a role in their ability to retain effluent. Daikon/wheat straw stemmed effluent production completely, while DWB, in spite of the high pre-silage DM, produced the most effluent. Straws are effective absorbents, but wheat bran and cereals in general have poor water absorptive capacities (Jones and Jones, 1996). It was obvious from the two experiments, however, that silage materials (singularly ensiled or with an absorbent material) with high water retention capacities tend to control effluent more effectively than those with lower water retention capacities. Although DBP had significantly lower pH and higher lactic acid contents compared with other treatments, silage fermentation in Experiment 4-2 was generally restricted due to effect of the absorbents.

Aerobic stability of daikon silages with different absorbent in Experiment 4-2 was similar in character to the daikon wheat straw adjusted moisture of Experiment 4-1 as evidenced by sharp increases and decreases in pH and lactic acid contents, respectively, after 3 days of aerobic exposure of the silages. The rate of rise in pH and decline in lactic acid contents indicated a phenomenon that is common to HMBF; a possible proliferation of yeast numbers. Yeast, especially lactate assimilating types are mostly responsible for rapid decline in silage quality at feed out stage (Filya et al., 2000; Jonsson and Pahlow, 1984). DBW and DBH deteriorated faster, compared to DWB and DBP on aerobic exposure due to the state of the added absorbents (wheat straw and bean stalks) prior to addition to daikon by-product. The two absorbents are usually gathered from the field after harvesting of wheat and beans and thus normally come with some soil which may serve as a contaminant, in contrast with wheat bran and dried beet pulp, which are 'cleaner' products from factories. Another factor responsible for the quick deterioration of the two silages was their low packing densities which create air pockets that, with the least available substrate, provide fertile grounds for yeast growth and metabolism and consequently, enhance spoilage.

## 4.4. Summary

These data have indicated that ensiling of daikon by-product is a viable venture and that the high moisture and buffering capacity not withstanding, daikon can be ensiled successfully using appropriate technology. Due to the high moisture content of daikon by-product, the use of bacteria or microbial inoculants is strongly discouraged since they increase effluent production. The use of in-silo absorbents, especially those capable of stemming effluent flow prior to ensiling of daikon by-product is *sine qua non* to the ensilage process. The use of appropriate in-silo dry materials with the dual responsibility of stemming effluent flow and minimizing the effect of silage spoilage during feed out stage would be a great contribution to extending the shelf life of daikon by-product silage.

A study of the nutritive value of daikon by-product silage *in vitro* and *in vivo* is highly warranted.

# Chapter 5

## Ensiling of brewer’s grain and apple pomace

### 5.1. Introduction

In the previous chapters, the ensilability of two representatives of HMBF from agro-industrial root and vegetable sources has been discussed. One of the fallouts of ensiling HMBF is effluent production. Effluent is produced from HMBF, whether ensiled alone (as in the potato pulp experiments) or with absorbents (as in daikon by-product), and the rate at which they are produced largely depends on the physical and chemical characteristics of the material used, the dry matter, and degree of consolidation of the silage material.

The previous experiments investigated the effect of chemical factors such as microbial inoculation and the use of absorbents in the control of effluent in HMBF. In the following experiments, the role of two other factors, pressure and packing density and their role on effluent production and retention using apple pomace and brewer’s grain as representatives of HMBF is discussed.

## 5.2. Brewer's grain

The use of brewer's grain as animal feed is not new. It has been recognized as animal feed and a lot of reports on its nutritive value have been reported (Alawa et al., 1988; McCarthy et al., 1990; Chiou et al., 1998).

The purpose of this investigation, though, was to investigate the role of physical factors such as pressure and packing density on effluent flow and retention in brewer's grain as a representative of HMBF, ensiled without or with dried beet pulp and wheat bran as in-silo effluent absorbents.

### 5.2.1. Materials and methods

Fresh brewer's grain procured from a local beer distillery was used in this study. The grain used in the distillery factory was wheat, and as such, the spent grains bore a typical brown color. Three treatments were prepared with brewer's grain without an additive (Control, C), with dried beet pulp (BP) and with wheat bran (WB). The mixing ratios of brewer's grain to dried beet pulp and wheat bran were 4: 1 for BP and WB (w: w basis), respectively. A freeze-dried commercial lactic acid bacteria inoculum, *Lactobacillus plantarum* (Ecosyl Products Ltd, Tokyo, Japan) was added to all the treatments including C at the rate of 0.75 g/100kg in fresh matter (as per manufacturer's instruction) to hasten lactic acid fermentation. These treatments were separately and uniformly mixed and ensiled in bucket silos with drain taps for effluent measurement as previously described in Experiment 2 of Chapter 4. Three pressure regimes (P0, P1 and P2) were allotted to each treatment by placing weights (0, 15 and 30 kg) on top of each silo of the three treatments (C, BP and WB). A total of 27 silos were thus prepared consisting of three treatments under three pressure regimes with three replications. Effluent was measured during 30 days of ensiling period. Samples from all treatments were taken after opening of the silages for subsequent chemical analyses.

#### *5.2.1.1. Chemical analyses*

General fermentation characteristics were analyzed with procedures described previously in earlier chapters.

#### *5.2.1.2. Calculations*

Packing density was calculated as mass of fresh pre-silage material (kg)/volume of the silo ($m^3$). Pressure at the bottom of the silages was calculated as force (weight of material)/area ($cm^2$) of silo.

#### *5.2.1.3. Statistical analysis*

Silage fermentation data were analyzed using ANOVA in a randomized block design using the General Linear Model Procedure of SAS (1996) computer package. Contrasts between treatment and pressure levels were performed and means differences were compared for all variables. Significant differences among treatments were accepted at $P<0.05$.

### 5.2.2. Results

The chemical characteristics of pre-silage material and silages are presented in Table 5-1. The material used in the current experiment was quite low in moisture (760.1 g/kg). Adjustments with dried beet pulp and wheat bran further reduced ($P<0.05$) the moisture content to about 700 g/kg. This adjustment in turn reduced ($P<0.05$) the packing density of each silo from 957 kg/m$^3$ to an average of 850 kg/m$^3$ and was significant ($P<0.05$) among adjusted and non-adjusted moisture treatments. PWRC was higher in WB but highest in BP, relative to C. The pH of all silages were generally low (below 3.80), indicating an acceptable fermentation process. Lactic acid concentrations were, however, low in all treatments despite addition of an inoculum of *Lactobacillus plantarum* as a fermentation booster. The effect of pressure exerted at the bottom of the silos was obvious as the weights on the silos were increased. This had remarkable increases ($P<0.05$) in effluent outputs in the silages especially in the control silages. Addition of dried beet pulp to the brewer's grain stemmed effluent production completely, regardless of the pressure level.

### *5.2.2.1. Effect of absorbents*

Addition of the absorbent (treatments) materials led to clear decreases ($P<0.05$) in pH, lactic acid contents and effluent production in the silages. Effects on moisture, pressure and packing density were inconsistent, with significant differences ($P<0.05$) only between C and the absorbent added treatments (BP and WB).

### *5.2.2.2. Effect of pressure*

Effect of pressure levels on fermentation characteristics was inconsistent. The only obvious ($P<0.05$) effect was on lactic acid and effluent production at P2; otherwise moisture, pH, or packing density did not change significantly.

**Table 5-1**. Effect of absorbents and pressure levels on chemical characteristics of brewer's grain after 30 days of ensilage.

| | Treatments | | | Pressure levels | | | | Contrasts[#] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *C* | *BP* | *WB* | *P0* | *P1* | *P2* | SEM | *1* | *2* | *3* | *4* | *5* | *6* |
| *Pre-silage characteristics* | | | | | | | | | | | | | |
| Moisture (g/kg) | 760.1 | 698.3 | 701.2 | - | - | - | - | - | - | - | - | - | - |
| pH | 5.03 | 5.17 | 6.00 | | | | | | | | | | |
| PWRC (g/100g) [§] | 57.6 | 89.4 | 71.9 | - | - | - | - | - | - | - | - | - | - |
| *Silage characteristics* | | | | | | | | | | | | | |
| Moisture (g/kg) | 736.5a | 679.8b | 683.5b | 707.5a | 702.1a | 690.4b | 2.40 | ** | ** | NS | NS | ** | ** |
| pH | 3.41c | 3.75a | 3.65b | 3.59a | 3.60a | 3.61a | 0.14 | ** | ** | ** | NS | NS | NS |
| Lactic acid (g/kg DM) | 12.6c | 13.4b | 15.8a | 14.4a | 13.9b | 13.5b | 0.14 | ** | ** | ** | ** | ** | NS |
| Effluent (g/kg ensiled material) | 20.6a | 0.0c | 5.6b | 7.7b | 8.9ab | 9.5a | 0.49 | ** | ** | ** | NS | * | NS |
| Pressure ($g/cm^2$)[§] | 45.7a | 42.9b | 42.9b | 22.5c | 43.9b | 65.1a | 0.11 | * | * | NS | ** | ** | ** |
| Packing density ($kg/m^3$) [§] | 957.0a | 850.2b | 849.6b | 881.9a | 889.5a | 886.3a | 4.32 | ** | ** | NS | NS | NS | NS |

C, brewer's grain without additive; BP, WB, brewer's grain added with dried beet pulp and wheat bran, respectively; P0, P1,P2, pressure levels exerted at the bottom of the silages; PWRC, potential water retention capacity.

Values apart from pre-silage characteristics, are least square means (n=27). Different letters following figures within a row are statistically significant (P<0.05); NS, not significant P>0.5; *, P<0.05; P<0.01.

[#] 1, C vs. BP; 2, C vs. WB; 3, BP vs. WB; 4, P0 vs. P1; 5, P0 vs. P2; 6, P1 vs. P2.

[§] At day 0 of ensiling period.

### 5.2.3. Discussion

The low moisture content of the brewer's grain used in the current experiment would not have been the most ideal representative material of HMBF for this study given the objective of the experiment, but appropriate in the context of variety, since different materials produce different results which enhance the validity of the results.

Although pH values were generally low, the fermentation quality of the silages was restricted as indicated in the general low lactic acid concentrations, a result that obliterated the rather significant differences among the treatments. The low lactic acid content in the current experiment is in consent with Nishino et al., (2001), on brewer's grain sampled from different factories in Japan. Several factors could be attributed to this, among them the chemical characteristics of the material. Wheat grain, the source material from which the by-product was derived, generally contains mainly starch as the principal carbohydrate source and has very little sugar (data not shown). After extraction of starch during the distillery process, the main nutrients remaining are mostly proteins and fiber (about 270 and 630 g/kg DM, respectively (NARO, 2001; Nishino et al., 2001). These, especially the former, are antagonistic to lactic acid fermentation due to the high buffering capacities of proteins in general (McDonald and Henderson, 1962). Brewer's grains undergo heating processes which generally neutralize or nullify the effect of enzymes that may aid the ensiling process. Additionally, yeast is added during the brewery process which may have been present in large numbers in the by-product thereby impeding lactic acid production during ensilage. The fact that even addition of an inoculum of *Lactobacillus plantarum* did not enhance lactic production is an indication of this fact.

On visual appraisal, however, the quality of the silages were not drastically different from the pre-silage material, a fact that strengthens a personal assumption that brewer's grain is better utilized fresh. Packing density and pressure have positive relationships with effluent production in silages, especially those ensiled from low dry matter materials (McDonald et al., 1960; Zimmer, 1974; Peters and Weissbach, 1977), and the results of this study amply demonstrated that. However, a combination of the two factors and PWRC appears to have had the most potent effects. The relationship between these factors and effluent production is fully discussed in Chapter 7.

## 5.3. Apple pomace

Apple pomace is a by-product resulting from the extraction of juice from fresh apples. It comprises the seeds, peel, core and stem along with insoluble material. The pomace is characteristically high in moisture, above 700 g/kg (Kennedy et al, 1999), but a rich source of sugar and pectin and it has been acknowledged as ruminant feed (Smock, 1950; Fontenot et al., 1977; Toyokawa et al., 1977; Alibes et al., 1984).

The purpose of this study was to investigate the effectiveness of in-silo absorbents at ensiling, on the fermentation, effluent control and aerobic stability of apple pomace silage.

### 5.3.1. Material and methods

Fresh apple pomace was procured from an apple juice factory using local apple varieties and located in the Tohoku (northern) region of Japan. Dried beet pulp, rice bran and wheat bran were used as in-silo absorbents to adjust the pomace to lower moisture levels at ratios of 87.5:12.5, 80:20 and 76: 24 (pomace: absorbent on w:w basis) for the three materials, respectively. Four treatments consisted of apple pomace without additive (AP), with dried beet pulp (ABP), with rice bran (ARB), and with wheat bran (AWB) with three replications for each treatment. These were filled into bucket silos with a drain tap and ensiled following procedures similar to those described in the previous experiment. A weight of 15 kg was placed on each silo to provide an average pressure of 43 g/cm$^2$ at the bottom on all silos. The silos were kept at room temperature for a 30-day ensiling period with regular measurement of effluent. After opening, silages were sampled for chemical analyses of the fermentation quality. Additionally, composite samples were taken from each treatment and exposed but protected from contamination of flies and rodents, at room temperature and sampled on the 2 nd, 4 th, 6 th and the 7 th day of exposure for the determination of the aerobic stability of the silages. With the exception of pre-silage moisture and pH, which were determined from bulk samples, all treatments were sampled from each silo for chemical analysis.

#### *5.3.1.2. Chemical analyses*

Pre-silage and silage chemical analyses were determined using procedures described in the previous experiment.

#### *5.3.1.3. Statistical analyses*

Silage fermentation data were analyzed using Excelstats for Windows 2004 (Microsoft Corp, USA). Significant differences among means were accepted at $P<0.05$ level.

### 5.3.2. Results

The chemical characteristics of apple pomace and silages are given in Table 5-2. It is evident that given the high moisture of the pomace material (918.7 g/kg), adjustments to lower levels were inevitable. ABP, ARB and AWB were adjusted to 821.2, 708.2 and

733.7 g/kg, respectively. These adjustments, in turn, reduced the PWRC values as shown in Table 5-2. The original low pH of the pre-silage material (3.75) facilitated the ensiling process with even lower values (below 3.60) in all the silages. Lactic acid content in the silages indicated a satisfactory fermentation process and was highest in AP, followed by ARB, AWB and ABP in that order. VFA in the silages were generally low, with no differences ($P>0.05$) among treatments. This result, especially for acetic and butyric acids, was indicative of the low incidence of secondary fermentation in the silages. Bulk density at ensiling was lower in ABP and AWB, respectively, relative to AP and ARB.

Effluent output was highest in AP (391.1 g/kg FM of ensiled material), followed by AWB and ARB, respectively. ABP silage had the lowest effluent production.

Aerobic stability of the silages was determined with changes in pH and lactic acid contents during the 7-day exposure period (Fig. 5-1) as indices. There was a steady rise in pH of all silages until the $6^{th}$ day of aerobic exposure. On the $7^{th}$ day, the rise was sharp except for AP. Conversely, lactic acid decreased in a similar pattern. At the end of the period of investigation, AWB and ABP were the most unstable, judging from the pH and lactic acid contents.

**Table 5-2**. Chemical characteristics of apple pomace before and after ensiling for 30 days. (Values are given in g/kg DM, unless otherwise stated)

| | Treatments[§] | | | |
|---|---|---|---|---|
| Characteristics | AP | ABP | ARB | AWB |
| *Pre-silage* | | | | |
| Moisture | 918.7 | 821.2 | 708.2 | 733.7 |
| pH | 3.75 | 3.99 | 4.74 | 4.20 |
| PWRC (g/100g ) | 50.9c | 90.7a | 76.0ab | 88.2b |
| Packing density (kg/m$^3$) | 886.9a | 818.8b | 914.1a | 776.3b |
| *Silage* | | | | |
| Moisture | 892.0a | 834.4b | 697.9d | 744.8c |
| pH | 3.43b | 3.53a | 3.51a | 3.48ab |
| Lactic acid | 116.6a | 62.6d | 70.9b | 68.9bc |
| *Volatile fatty acids* | | | | |
| Acetic | 16.8ab | 19.8a | 14.4ab | 11.3b |
| Propionic | 3.40 | 2.41 | 2.05 | 1.75 |
| Valeric | 3.01 | 4.49 | 3.71 | 5.59 |
| Butyric | 2.37 | 1.32 | 0.34 | 0.22 |
| Effluent (g/kg FM) | 391.1a | 37.2d | 73.2bc | 93.0b |

[§]AP, apple pomace without additive; ABP, ARB, AWB, apple pomace added with dried beet pulp, rice bran and wheat bran, respectively; PWRC, potential water retention capacity.

Except pre-silage moisture and pH, values are means of three silos; letters following figures within a row indicate a statistical difference ($P<0.05$).

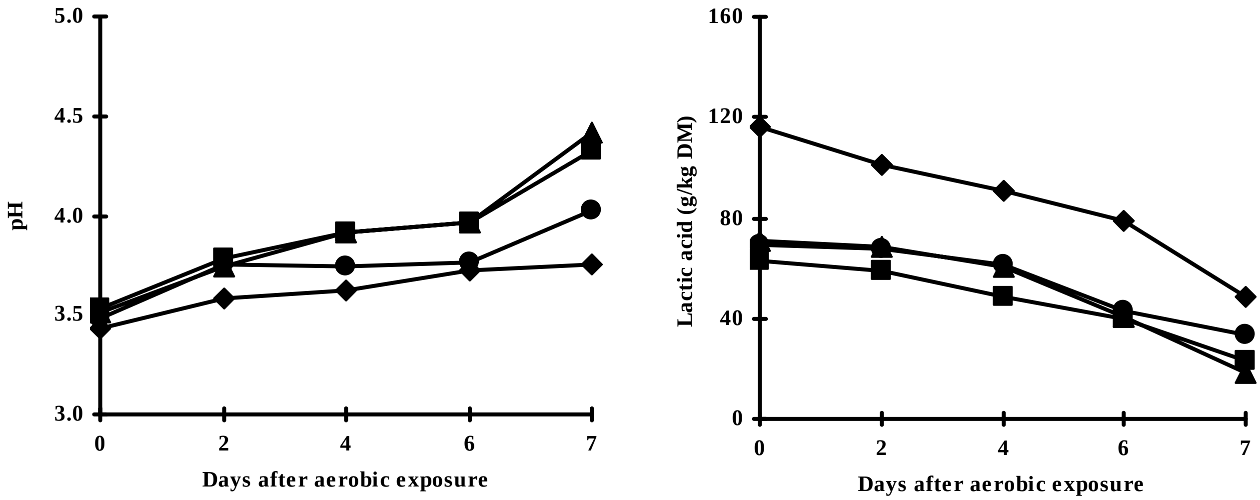


**Fig 5-1**. Changes in pH and lactic acid concentrations in apple pomace silages during 7 days of aerobic exposure. (◆), apple pomace without additive, (AP); (■), apple pomace + dried beet pulp, ABP; (▲), apple pomace + rice bran, ARB; (●), apple pomace + wheat bran, AWB.

## 5.3.3. Discussion

In the current study, adjustments of the moisture content were made based on results from the previous one. Dried beet pulp had proven its capacity in completely stemming effluent flow in the brewer's grain experiment, and the assumption was that applying similar mixing ratios between fresh material and absorbents would yield similar results. Results from effluent outputs in the current experiment were, however, contrary to the projected, indicating that calculations based solely on dry matter/moisture assumptions are insufficient. The PWRC results, in confirmation of previous results, gave a strong indication that barring other major determining factors such as the moisture content, effluent production from any given silage material is largely a function of its PWRC (See Chapter 7).

The good fermentation quality of the AP silage (especially the low pH content) is in consent with earlier reports (Kennedy et al., 1999; Rumsey and Lindahl, 1982; Osaka, 2001; Pirmohammadi et al, 2006) on apple pomace silage. This, in addition to its low buffering capacity (Chapter 2) and high lactic acid contents, in agreement with Anrique and Viveros (2002), Alibes et al., (1984) and Wyss (2003) indicated that apple pomace is an easy-to-ensile material. The high lactic acid content in AP silage was primarily due to the high WSC (Chapter 2). Other treatments had fairly low pH and high lactic acid contents due to effect of the high percentage of apple pomace material of the mixtures. The generally low contents of acetic and butyric acids typified the good fermentation quality of the silages.

Packing density was positively related to effluent production in the silages, as previously discussed in the study of brewer's grain silage. Despite the high pre-ensiling packing density, AP silage produced the most effluent. The spongy-like nature of apple pomace fiber, with its 'easy-to-absorb' and 'easy-to-release' moisture capacity, may be largely responsible for this. Moreover, fibers derived from fruits and vegetables have a high proportion of soluble dietary fibers (Herbafood, 2002) and possess functional properties such as swelling and binding capacities. These properties are related to the porous matrix structure formed by polysaccharide chains which can hold large amounts of water through hydrogen bonds (Dawkins et al., 2001; Kethireddipalli et al., 2002). From this study, however, the high effluent from AP silage may be related to the pressure exerted on the silo through the weight, rendering the binding capacity of apple pomace vulnerable. In contrast, AWB, with significantly lower density, produced more effluent than ABP. Cereals like wheat bran are known to have poor absorptive capacities (Jones and Jones, 1996). However, given the low moisture and packing density, the relatively high effluent produced from AWB provides a rather contrasting relationship which warrants further investigation.

The silages were relatively stable until the $6^{th}$ day of aerobic exposure, probably due to the low pH and high lactic acid contents which may have protected them from spoilage microorganisms and consequently enhanced the shelf life. On the $7^{th}$ day, ABP and AWB were the most unstable, probably due to their lower packing densities which may have made them susceptible to more air penetration, infiltration and proliferation of aerobic bacteria causing deterioration of the quality (Chapter 4). The aerobic stability of the silages, if anything, has proven that apple pomace silage could have a longer shelf life compared with other HMBF studied so far.

## 5.4. Summary

High packing densities and pressures exerted on fresh brewer's grain and fresh apple pomace, ensiled alone or in combination with absorbents prior to ensiling, generally increased effluent production from the silages. This was, however, influenced by the moisture content, the potential water holding capacity and type of material and absorbent used.

More experiments using different absorbents and pressure levels are needed to clarify this hypothesis.

# Chapter 6

## Nutritive value of HMBF

### 6.1. Introduction

In the previous chapters, the possibility of extending the shelf life by ensilage of various HMBF has been discussed. The nutritive value of silage depends on three major factors: voluntary intake, its nutrient digestibility and feed efficiency (Woolford, 1984d). The ambit of the silage-animal interface is, however, intrinsically the nutritive value or the acceptability by livestock who are the final determinants of the quality of the silages. Animal responses are therefore vital in the final equation of the fermentation quality of the silages. Not all well fermented silages have increased intakes of silages; in fact, several other factors such as type of silage, length of storage, physiological state of the animal etc. all play a role. However, there is ample evidence that well preserved silages (grass or whole crop), including those treated with or without an inoculum of lactic acid bacteria, have higher digestibility (McDonald et al., 1991c) and voluntary dry matter intakes (Gordon, 1989a) and would be more palatable and preferred by animals as opposed to 'badly' preserved ones (Michalet-Doreau, 1975). Agricultural by-product silages, in contrast to grass or whole crop silages, are most likely to differ widely in terms of voluntary intake and palatability due to their 'reduced' nutrient state prior to ensilage. This may have an effect on the nutritive value and, consequently, on dry matter intake.

In the following experiments, the nutritive value of two representatives of HMBF silages were evaluated *in vivo* using sheep.

## 6.2. Nutritive value of potato pulp silage

The objective of this study was to evaluate the intake and nutrient digestibility of potato pulp ensiled without/with microbial inoculants.

### 6.2.1. Materials and methods

#### *6.2.1.1. Silage preparation*

Silage preparation including inoculants, type of silo, and duration of storage has been described in Experiment 2 of Chapter 3. The four silage treatments, potato pulp silages without additive control (PP), inoculated with *Lactobacillus rhamnosus* (L) alone (PL), inoculated with *Rhizopus oryzae* (R) alone (PR) and inoculated with a combination of R and L (RL), were placed into small polyethylene bags as 4 kg each, kept at -10$^{o}$C and thawed on use in a digestion trial.

#### *6.2.1.2. Digestibility trial: animals, feed and experimental design*

Four mature wether sheep (Suffolk breed) with initial live weights of 46 ± 4 kg were used in a 4 x 4 Latin square design digestion trial using the silages, Italian ryegrass (*Lolium multiflorum*) hay and soybean meal. After being de-wormed of endoparasites and treated against ectoparasites, the animals were kept in metabolic cages facilitating the separate collection of feces and urine. The procedures used in this trial were approved by Obihiro University of Agriculture and Veterinary Medicine Committee for Animal Use and Care, where the trial was conducted. A standardization period of 10 days was allotted to the animals during which all animals were fed control silage *ad* *libitum* to allow for 100 g/kg refusal with regular hay and soybean meal supplements. This was followed by adaptation and collection periods of 7 and 5 days, respectively, during which time experimental diets comprising the control and treated silages, hay and soybean meal, representing 600, 250 and 150 g/kg of daily ration, respectively, were offered at 20 g/kg BW twice daily at 08.00 and 17.00 h. The hay was chopped to about 2 cm lengths with a mechanical forage cutter and mixed with the silages and soybean meal prior to offer to the sheep. The hay and soybean meal supplementation rates (250 and 150 g/kg DM on as fed basis of daily intake, respectively) contained an average of 130 g/kg crude protein and 18 MJ/kg DM of gross energy respectively, enough to satisfy the DCP and energy requirements for sheep according to NRC feeding standard (NRC, 1985). Mineral block (Nihonzenyaku Ltd., Tokyo, Japan) and drinking water were provided *ad libitum.* The mineral block contained: Fe 1232 mg/kg, Cu 150 mg/kg, Zn 500 mg/kg, Mn 500 mg/kg, Se 15 mg/kg and Na 382 mg/kg. During each of the 5-day collection periods, feed offered from each animal were sub sampled, bulked and stored at -10$^{o}$C for subsequent chemical analyses. Total feces from each animal were weighed, and kept at -10$^{o}$C and bulked at the end of each collection period. Portions for DM determinations were taken from composite samples and the rest frozen at -10$^{o}$C until analyzed. At the end of a collection period, the animals were released into an enclosed pen for exercise for 24 hrs and fed a mixture of the four treatment diets. The following collection period was preceded by 7 days of adaptation period where the experimental diets were fed. This was to rid the rumen of traces of the previous diets and to allow the rumen bacterial population to respond to the change in diet. The process was repeated until the end of the fourth period.

### *6.2.1.3. Chemical analyses*

Analyses of chemical components in feed has been previously described (Chapter 3). Analyses of chemical components in feces were done from dried samples following the same procedures as in the feed analyses.

### *6.2.1.4. Calculations*

Digestible energy (DE) was calculated as the difference between gross energy in feed offered and in feces voided. Total digestible nutrients (TDN) in feed were calculated from the DE using the regression equation of Harris et al. (1972):

$$\text{TDN (for sheep)} = \frac{DE}{0.04409}$$

where TDN is the total digestible nutrients of feed in g/kg DM; DE is the digestible energy in MJ/kg DM.

The TDN of each treatment diet was calculated as the sum of TDN of the treatment feed, hay and SBM. The TDN of silages (corrected TDN of silages) was calculated as the difference between the TDN of diets and the sum of TDN of Italian rye grass hay and SBM (values based on Standard Tables of Feed Composition in Japan, 2001) of the treatment diets. The DE of silages was calculated in a similar manner.

The nutritive value (TDN and DE) of potato pulp silages were estimated by extrapolation from the TDN and DE values of the diets minus those of Italian rye grass hay and soybean meal in the diets based on NARO (2001) and dividing by the rate of inclusion of the individual silage.

### *6.2.1.5. Statistical analyses*

Silage fermentation data were analyzed using ANOVA in a randomized block design, while data from the digestibility trial were analyzed as a 4 x 4 Latin square design using the General Linear Model Procedure of SAS (1996) computer package. Means differences were compared for all variables and $P<0.05$ was considered as statistically significant.

## 6.2.2. Results

The chemical composition of potato pulp, silages and hay and soybean meal are shown in (Table 6-1). Potato pulp had low DM, CP, fiber and fat contents but was fairly high in organic matter, starch, pectin and gross energy. The silages were not markedly different from the material with the exception of sugar that increased about 4-fold that of the pre-ensiling content. The CP and NDF contents of the silages were generally low (average of 50 and 350 g/kg DM, respectively). Supplementation with soybean meal and Italian rye grass hay increased the CP and NDF considerably to about 130 g/kg DM and 380 g/kg, respectively (Table 6-2). Dry matter intake was higher ($P<0.05$) in PL compared with the control (Table 6-3). There were no statistical differences among treatments in the digestibilities of DM, OM, NDF and energy in the diets which were on average 0.70, 0.78, 0.64 and 0.74, respectively. Crude protein digestibility was higher ($P<0.05$) in sheep fed L inoculated compared to the control silage, but there were no significant effects among the inoculant added silages.

The DE and TDN in the diets averaged 13 MJ/kg DM and 709 g/kg DM, respectively, while those of PPS were on average 13.3 MJ/kg DM and 724 g/kg DM, respectively. There was no effect of inoculation on the nutritive value of the diets and PPS. No orts were recorded during the entire experiment.

**Table 6-1**. Chemical compositions of potato pulp, hay, soybean meal (SBM) and potato pulp silages without additive (PP), inoculated with *Lactobacillus rhamnosus* alone (PL), *Rhizopus oryzae* alone (PR) and a combination of the two inoculants (RL) in g/kg DM[§], except where indicated.

| | *Potato pulp* | *Hay* | *SBM* | Silages[1] | | | |
|---|---|---|---|---|---|---|---|
| | | | | *PP* | *PL* | *PR* | *RL* |
| Dry matter (g/kg) | 170 | 842 | 864 | 158 | 161 | 159 | 160 |
| Organic matter | 974 | 943 | 934 | 974 | 969 | 974 | 969 |
| Crude protein | 49.0 | 91.0 | 502 | 49.0 | 49.6 | 50.3 | 49.9 |
| Neutral detergent fiber | 353 | 617 | 119 | 353 | 350 | 365 | 340 |
| Acid detergent fiber | 342 | 334 | 78 | 337 | 334 | 334 | 327 |
| Ether extract | 6.2 | 13.0 | 17.0 | 6.1 | 6.5 | 6.1 | 6.3 |
| Starch | 206 | - | - | 195 | 196 | 208 | 214 |
| Pectin | 213 | - | - | 213 | 215 | 208 | 199 |
| Sugar | 5.0 | - | - | 16.0 | 20.0 | 13.0 | 19.0 |
| Gross energy (MJ/kg DM) | 17.1 | 19.0 | 20.0 | 17.7 | 17.3 | 17.4 | 17.4 |

Except with potato pulp, hay (Italian rye grass hay) and (SBM) soybean meal, values means are of four silos.

[§]DM = dry matter; MJ, mega joule.

[1] PP, potato pulp silage without additive; PL, PR, *Lactobacillus rhamnosus* (L) and *Rhizopus oryzae* (R) inoculated potato pulp silage, respectively; RL, R and L inoculated potato pulp silage.

**Table 6-2**. Chemical composition of diets used in the digestibility trial. Values are given in g/kg dry matter unless otherwise stated.

| | Diets[§] | | | |
|---|---|---|---|---|
| | *PP* | *PL* | *PR* | *RL* |
| Dry matter (g/kg) | 43.5 | 43.7 | 43.6 | 43.6 |
| Organic matter | 960 | 958 | 960 | 957 |
| Crude protein | 127 | 128 | 128 | 128 |
| Neutral detergent fiber | 384 | 382 | 391 | 376 |
| Acid detergent fiber | 297 | 296 | 295 | 291 |
| Gross energy (MJ/kg DM) | 18.3 | 18.1 | 18.2 | 18.2 |

[§]Diets comprise 600 g/kg DM of potato pulp silages, 250 g/kg DM of hay and 150 g/kg DM of soybean meal; figures represent the sum of values of single feeds; PP, potato pulp silage without additive; PL, PR, *Lactobacillus rhamnosus* (L) and *Rhizopus oryzae* (R) inoculated potato pulp silage, respectively; RL, R and L inoculated potato pulp silage.

**Table 6-3**. Dry matter intake, apparent nutrient digestibility and nutritive value of diets and potato pulp silages (PPS)

| | Diets[1] | | | | | |
|---|---|---|---|---|---|---|
| | *PP* | *PL* | *PR* | *RL* | *S.E.M* | *Significance* |
| Dry matter intake (kg /d) | 1.07a | 1.16b | 1.10ab | 1.13ab | 0.02 | * |
| *Apparent nutrient digestibility* | | | | | | |
| Dry matter | 0.758 | 0.783 | 0.778 | 0.767 | 0.09 | NS |
| Organic matter | 0.768 | 0.790 | 0.787 | 0.776 | 0.08 | NS |
| Crude protein | 0.616a | 0.678b | 0.652ab | 0.645ab | 0.16 | § |
| Neutral detergent fiber | 0.617 | 0.651 | 0.650 | 0.645 | 0.17 | NS |
| Energy | 0.724 | 0.748 | 0.747 | 0.732 | 0.11 | NS |
| *Nutritive value of diets* | | | | | | |
| Digestible crude protein (g/kg) | 80.0 | 94.0 | 85.0 | 84 | 4.99 | NS |
| Digestible energy (MJ/kg DM) | 12.9 | 13.2 | 13.2 | 12.9 | 0.24 | NS |
| TDN (g/kg DM) | 701 | 716 | 717 | 701 | 12.66 | NS |
| *Nutritive value of silages* | | | | | | |
| TDN of silages (g/kg DM)[#] | 711 | 736 | 737 | 711 | 12.66 | NS |
| Digestible energy of silages (MJ/kg DM)[#] | 13.1 | 13.6 | 13.6 | 13.1 | 0.24 | NS |

Means in a row with different letters differ significantly (P<0.05); NS, not significant; *, P<0.05.
[1]Same as in Table 6-2.
[#] Calculation based on Standard Tables of Feed Composition in Japan (2001).
[§] P = 0.1866, although means were separated using least square means with a level of 0.05 and P value was 0.1866, but difference between treatments were significantly different.

### 6.2.3. Discussion

The fermentation qualities of the silages, whether treated with the microbial inocula or not, were satisfactory and has been discussed previously (Chapter 3) in detail. Addition of hay and soybean meal to the silages prior to feeding increased not only the DM of the diets but the texture, which was highly appreciated by the sheep. The low CP and NDF contents in the silages were due to their low content in the potato pulp pre-silage material. In order to make up for the low N and fiber contents, soybean meal and Italian rye grass hay were introduced into the diets. These augmented the diet CP and NDF contents to levels in tandem with NRC feeding standard (NRC, 1985). The pulp material used in the present trial contained fair amounts of easily digestible carbohydrates such as starch and pectin, though the content of the former was low compared to the conventional potato pulp of about 370 g/kg DM (Mayer and Hillebrandt, 1997), and could be due to the mode and rate of starch extraction, which differs among factories. The diets contained an average gross energy of 18 MJ/kg DM, fairly sufficient for the energy requirements and ages of the sheep used in the trial. With the exception of PL which was higher ($P<0.05$) compared with PP, DM intake among silages was not affected by addition of the inoculants. Similar improvements in DM intakes of silages treated with an inoculum of lactic acid bacteria, especially with *Lactobacillus plantarum*, have been reported in grass silages in particular (Gordon, 1989a; Gordon, 1989b), especially where there were improvements in the quality of the silages, although others (Hooper et al., 1984; Steen et al., 1989) have reported increased DM intakes with commercial inoculants with no obvious improvement in fermentation, as was the case in this study. It is noteworthy that the quality of the silages used in the digestibility trial was good and was readily eaten by the animals.

Crude protein digestibility was higher ($P<0.05$) in sheep fed silage inoculated with L compared to those fed control, a result that could not be explained. Moreover, other treatments, though not statistically significant, had numerically higher CP digestibilities compared with the control. Evidence is scanty on the efficiency of nitrogen utilization between treated and untreated silages. However, the phenomenon observed in the current trial could be related to the general improvement in nitrogen retention with feeding of PPS that may have improved microbial nitrogen synthesis within the rumen and/or reduced silage nitrogen degradability within the rumen. In a later study, and in agreement with these results, Aibibula et al. (2004) found no differences in nitrogen retention in sheep fed *Lactobacillus rhamnosus* and *Rhizopus oryzae* inoculated PPS based diets. However, it was observed that the concentration of rumen ammonium nitrogen and serum urea nitrogen decreased in PPS based diets compared with alfalfa hay, which led to consequent decreases in nitrogen excretion into the urine of sheep. Rooke et al. (1989), however, observed a marked difference in both nitrogen digestibility and nitrogen retention in sheep between treated and untreated grass silages. Studies are therefore warranted to investigate the influence of bacterial inoculants, especially *Lactobacillus rhamnosus*, in N digestibility with PPS.

Woolford (1984d) observed that starch-based materials when available in large proportions and used as material for ensilage would influence the nutritive value if they survive the ensiling process, since they would be available to amylotic organisms in the rumen. In the present study, though, the high DM contents of NDF and pectin, in addition to the starch contents of potato pulp, generally contributed to the high TDN values of the diets. Dry matter digestibility and nutritive value were not affected by the inoculants (Table 6-3), a result that was confirmed in another study with sheep

(Aibibula et al., 2004). The DM digestibility in the present study is in consent with the findings of Aibibula et al. (2004), who reported a value of 0.75 in sheep fed potato pulp silage based diets. The DE in this study is comparable to the values of the National Agricultural Research Organization (NARO, 2001) of 12.7 MJ/kg DM, but slightly higher than those reported by Aibibula et al. (2004) and Hanada et al. (2004), which were 12.1 and 10.3 MJ/kg DM, respectively. The TDN values of PPS in the present trial are in good agreement with those of NARO (2001). The digestibility and nutritive value of PPS is comparable to other by products like beet pulp and citrus pulp (NARO, 2001; O'Mara et al., 1999; Deaville et al., 1994).

### 6.2.4. Conclusion

The nutritive value of potato pulp silage was not influenced by inoculation with *Lactobacillus rhamnosus* and *Rhizopus oryzae* or their combined application. The high nutritive value of potato pulp silage compares to other agricultural by-products such as citrus pulp and beet pulp and as such could be a useful feed ingredient in ruminant diets.

## 6.3. Intake and preference of daikon by-product silage

In earlier chapters (2 and 4), the feasibility of ensiling daikon (Oriental radish), *Raphanus sativus* L. has been discussed in detail. Its nutritive value until now (Okine et al, 2006b), was unknown. Daikon, apart from its high moisture content, which may be a limitation on its use as a feed for ruminants, contains a characteristically pungent smell and aroma (Friis and Kjaer, 1966) and a burning sensation on the tongue when eaten fresh (Lindsay, 1985). These characteristics may have an effect on voluntary intake of daikon by-product silage.

The objective of the current study was to explore the possibility of using daikon by-product silage as ruminant feed through intake and preference test using sheep.

### 6.3.1. Materials and methods

#### *6.3.1.1. Ensiling of daikon by product*

Daikon was adjusted to lower moisture into with three replications each with dried beet pulp (DBP), dried bean stalks and husks (DBH) and wheat straw (DWS) as in-silo effluent absorbents (see Table 4-4, Chapter 4). A fourth treatment, daikon/wheat bran silage (DWB), was omitted from the group meant for the animal preference test. The daikon: absorbent mixing ratio was 4:1 (w:w) for all treatments and the same protocol for mixture and the packing density. The only differences were the type of silo used and packing density. In this case, 70 kilograms of the three treatments, DBP, DBH and DWS were filled into separate polythene bags and inserted into large plastic container silos and pressed with weights similar to that of the bucket silos in Experiment 2 of Chapter 4. The silages were kept at room temperature and opened on the 30$^{th}$ day of ensilage.

### 6.3.1.2. Silage intake and preference test

Three mature wether sheep weighing 55±5 kg were used in a 3 x 3 Latin square design (3 sheep x 3 feeds x 3 feeding positions) to study feed intake and preference of the silages. The sheep were kept in metabolic cages for 19 days with an adjustment period of 10 days during which animals were fed alfalfa hay (in pellet form) as a basal diet (24.0 g/kg body weight (BW) in dry matter per day) with gradual introduction of a mixture of the experimental silages to allow for a 100 g/kg feed refusal. This was followed by three experimental periods of 3 days each as follows: each day 1.20 kg of each of the three types of silages (this amount was determined based on intake of silages during the adjustment period) was separated into three trays and offered simultaneously to a sheep at 08.00 h. A basal diet of alfalfa hay as in the adjustment period was offered at 16.00 h every day. Water and mineral block were provided *ad libitum*. The mineral block contained: Fe 1232 mg/kg, Cu 150 mg/kg, Zn 500 mg/kg, Mn 500 mg/kg, Se 15 mg/kg and Na 382 mg/kg. Voluntary intake of silages was recorded after every 1, 2, 8 and 24 h post feeding on all the test days. Silage preference was determined as the highest feed intake time after silage allocation.

### 6.3.1.3. Chemical analysis

Analyses of chemical components in feed have been previously described (Chapter 4).

### 6.3.1.4. Statistical analyses

Silage fermentation data were analyzed using ANOVA in a randomized block design using General Linear Model Procedure of SAS (1996) as previously described in

Chapter 4. Data from the intake and preference test were analyzed as a 3 x 3 Latin square design with type of silage, period and feed position as factors. Means differences were compared for all variables and $P<0.05$ was considered statistically significant.

## 6.3.2. Results

### *6.3.2.1. Silage intake and preference test*

The chemical compositions of feed used in the preference test are presented in Table 6-4. Alfalfa hay was offered at 24.0 g/kg BW in DM/day/sheep, while the silages were offered at 1.20 kg in fresh matter per animal. Thus, the diets contained on average 877 g/kg in dry matter, 887, 202, 464, 329 and 29 g/kg DM in OM, CP, NDF, ADF and EE, respectively, and 19.3 MJ/kg in gross energy (calculated from the chemical components of alfalfa hay and those of the single silages). These figures meet or exceed the daily maintenance requirements for sheep (NRC, 1985). Daikon/wheat straw and DBH silages had higher NDF and ADF but lower CP and EE contents compared with DBP silage.

Silage preference was, to a large extent, influenced by its chemical composition (Table 6-5). Silage intake was significantly higher ($P<0.01$) in DBP, followed by DBH and DWS in that order for the first 2 hours after feed allocation; however, at 8 and 24 h, there were no statistical differences in intakes of DBP and DBH silages, respectively. Daikon/wheat straw silage was least in terms of intake and preference. Intake of silages between periods (except after 1 h of feed allocation) and effect of feed positions were not significant ($P>0.05$). Alfalfa hay was readily consumed immediately when offered to the sheep and as such did not feature in the preference test.

**Table 6-4**. Chemical composition of alfalfa hay and daikon by-product silages used for feed palatability/preference test

| | | Silages[§] | | |
|---|---|---|---|---|
| | Alfalfa hay | DBP | DWS | DBH |
| Dry matter (g/kg) | 881 | 179 | 140 | 145 |
| Organic matter (g/kg DM) | 890 | 903 | 905 | 904 |
| Crude protein (g/kg DM) | 211 | 143 | 69 | 92 |
| Neutral detergent fiber (g/kg DM) | 428 | 414 | 725 | 595 |
| Acid detergent fiber (g/kg DM) | 301 | 265 | 492 | 528 |
| Ether extract (g/kg DM) | 27.0 | 16.5 | 52.5 | 39.3 |
| Gross energy (MJ/kg DM) | 18.6 | 19.4 | 18.7 | 18.6 |

[§] DBP, daikon + dried beet pulp; DWS, daikon + wheat straw; DBH, daikon + dried bean stalks and husks.

**Table 6-5**. Feed intake and preference by sheep of daikon by-product silages alone, as indicated by intakes[§] (kg FM/hour) over three test periods and feeding positions. Values represent least square means of three sheep

| | Silage [1] | | | Period[2] | | | Position[3] | | | | P value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time after feed offer (h) | DBP | DWS | DBH | 1 | 2 | 3 | A | B | C | SEM | Silages | Period | Position |
| 1 | 1.08a | 0.51c | 0.80b | 0.69b | 0.83ab | 0.89a | 0.79 | 0.81 | 0.80 | 0.462 | 0.0001 | 0.032 | 0.973 |
| 2 | 1.13a | 0.63c | 0.94b | 0.83 | 0.95 | 0.95 | 0.89 | 0.92 | 0.91 | 0.481 | 0.0001 | 0.699 | 0.899 |
| 8 | 1.19a | 0.85b | 1.13a | 1.02 | 1.08 | 1.07 | 1.05 | 1.06 | 1.05 | 0.329 | 0.0001 | 0.328 | 0.928 |
| 24 | 1.20a | 1.00b | 1.16a | 1.09 | 1.12 | 1.15 | 1.11 | 1.12 | 1.13 | 0.20 | 0.0001 | 0.656 | 0.856 |

[§]Alfalfa hay was offered at 24.0 g/kg BW in DM/day/sheep as basal diet and was readily consumed by sheep. All silages were offered simultaneously at 1.20 kg FM /silage/sheep/day.
[1] Same as in Table 6-4.
[2] Periods of three experimental days.
[3] A, arrangement of silages according to the following order DBP, DWS , DBH; B, arrangement order of DWS, DBH, DBP; C, arrangement order of DBH, DBP, DWS.
SEM, standard error of the means.
Means followed by different letters in a row differ significantly ($P<0.05$).

### 6.3.3. Discussion

Even after addition of the absorbents prior to ensiling, daikon by-product silages were still low in DM. Some amounts of effluent had settled at the base of the silos indicating that the absorbents had not effectively soaked up the moisture as expected. Higher daikon by-product:absorbent mixture ratios need to be considered in future experiments. Addition of alfalfa hay to the diet augmented the DM, nitrogen and energy contents of the diets and generally improved the texture of the diets in a way preferred by the animals. Daikon/dried beet pulp silage had the best feed intake and preference in comparison with the other treatments probably due to its fermentation quality, low fiber and physical characteristics (dry matter content, particle size and resistance to fracture), factors that have profound effects on feed intake (Inoue et al., 1994; Baumont et al., 2000). Feeds that can be ingested fast and are rapidly digested are very palatable, provided they do not contain toxic compounds. Moreover, intake of feed, when supplied indoors, depends mainly on its nutritive value, fill effect and its sensory properties, provided it does not contain toxic compounds (Baumont et al., 2000), characteristics that were obviously higher in DBP than the other treatments. Intake is a key factor in assessing feed preference (Moseley and Antuna Manendez, 1989); thus, the high rate of intake in DBP silage at the first two hours was a key factor in assessing its preference over the other treatments. At 8 and 24 h, intake did not differ between DBP and DBH silages, times when intake of the silages decreased continuously until satiety and preference became less obvious.

This study indicated that dried bean stalks and hulls (a by-product produced abundantly but rarely utilized as a feed in Japan) can double as an absorbent and a worthy feed mixture in daikon silage due to the high intake of DBH by the sheep. Daikon/wheat straw silage had the lowest intake due to its high fiber and low CP contents, factors that affect nutritive value and intake. It is noteworthy that daikon silages were readily eaten by sheep and orts recorded were only after 24 h and were the hard, stemmy and undevourable portions of DWS and DBH. Further, this study has somewhat proven that the pungent smell in fresh daikon could not be a limiting factor when offered as silage to sheep. The significant increments in intake between periods 1 and 3 during the first hour, and insignificant but numeric increases with hours that followed, indicated that sheep ate more of the silages after becoming used to them.

### 6.3.4. Conclusion

Daikon by-product silages adjusted with dried beet pulp, bean stalks and husks and wheat straw were readily eaten when offered to sheep. More feeding trials with different absorbents are required to ascertain the full potential of daikon by-product silages.

## 6.4. Summary

Ensiling of potato pulp without or with microbial inoculants does not affect its feeding value using sheep. Since potato pulp silage is deficient in crude protein, supplementation with feed sources rich in protein is necessary. The high nutritive value compares to other by agricultural by-products such as citrus pulp and beet pulp and as such could be a useful feed ingredient in ruminant diets.

Daikon by-product silage has the potential to be ruminant feed ingredient. It is imperative, however, that the by-product is adjusted with absorbents prior to ensiling and supplemented with hay or dry feed due to the high moisture content of the by-product. Since the silage was readily eaten when offered to sheep, more feeding trials with different absorbents and bigger ruminants are required to ascertain its full potential.

# Chapter 7

## Factors affecting the ensiling of HMBF and their relationships

### 7.1. Introduction

In the previous chapters, pre-ensiling characteristics, followed by the ensiling of representatives of HMBF and the role of absorbents in the control of effluent have been discussed. It is evident from the results that ensiling HMBF is a viable venture from preservation and animal feed resource perspectives. However, this process also entailed peculiar challenges not only in its feasibility as animal feed resource, but also in its relationship with the environment. In an era where the latter is of considerable concern, it is prudent that any endeavor towards enhancement of science be 'environmentally friendly' as possible. A major factor in the ensiling of HMBF, as previously discussed, is effluent. However, several other factors directly or indirectly affect this process. In this chapter, the role of some important factors affecting ensiling of HMBF is discussed with particular emphasis on its relationship to effluent production.

## 7.2. Material and methods

Data were pooled from a series of experiments conducted separately over a period of five years. They involved the ensiling of some HMBF (n=27) ensiled without or with absorbents. These have been divided into 5 groups (Table 7-1), each group consisting of the pre-silage material and moisture adjusted treatments using various absorbents as follows:

Group 1 (Potato pulp A): This included four treatments consisting of silage material without moisture adjustment (control, PP1) and material adjusted with wheat bran (absorbent) to three different moisture levels (PP2, PP3 and PP4).

Group 2 (Potato pulp B): This included 7 treatments consisting of potato pulp adjusted without or with three different absorbents as follows:

Control, without moisture adjustment (PPC); adjusted with wheat bran to two moisture levels (PPWB 1 and PPWB 2); adjusted with dried beet pulp to two moisture levels (PPBP 1 and PPBP 2); and adjusted with wheat straw to two moisture levels (PPWS 1 and PPWS 2).

Group 3: Brewer's grain consisting of three treatments adjusted without absorbent (C), with dried beet pulp (BP), or wheat bran (BW) (Chapter 5).

Group 4: Apple pomace consisting of four treatments adjusted without an absorbent (AP), with dried beet pulp (ABP), rice bran (ARB), or wheat bran (AWB (Chapter 5).

Group 5: Daikon by-product without absorbents consisting of three treatments; DKS, DKM, DKL and DN; with wheat straw absorbents (DA and DWS); with dried beet pulp (DBP), with bean stalks and hulls (DBH) and with wheat bran (DWB). All treatments in this group have been described previously in Chapter 4.

All groups were ensiled at ambient temperatures of between 18-25$^o$C. Data of chemical components of pre-silage materials including moisture, WRC, PWRC, NDF, and silage characteristics (pH, lactic acid content and effluent output of the silages) were the parameters considered. These parameters were chosen for the sake of consistency and availability of data. Factors such as those that have a direct bearing on the fermentation process (sugar, buffering capacity, microbial numbers and physical characteristics such as pressure or density) were excluded because they have either been previously discussed in detail, or are limited by number of available data.

### *7.2.1. Chemical analyses*

Parameters were analyzed with procedures similar to those described previously in earlier chapters. Water retention capacity (WRC) was adapted from the method of Robertson et al., (2000) and modified for the sake of convenience, and is described (see Chapter 2) in more detail here. One gram of air-dry weight of each HMBF material (dried at 60$^o$C for 48 h and milled to < 1 mm) was measured into a 50 ml centrifuge tube and hydrated with 30 ml distilled water containing 0.02 g sodium azide per 100 ml as a bacteriostat. The tube containing the sample was then equilibrated for 18 h at room temperature and its contents were transferred to a glass filter with a pore size of 100-160 μm, (1G P160, Sibata Company, Tokyo, Japan) and drained under a pressure of 2 g/cm$^2$ with a pressure pump (Compact air pump, NUP-1, AS-ONE Company, Tokyo, Japan) for 2 minutes (The vacuum time of 2 minutes was chosen as the most convenient following a short experiment using three different materials and three vacuum duration times). The glass filter containing the sample was weighed, oven-dried at 135$^o$C for two hours and weighed again. The WRC of the material was calculated as the amount of water retained by the pellet (g moisture /g dry weight) after transfer to the glass filter. Potential WRC (PWRC), defined as 'the amount of moisture in grams that a HMBF can retain per 100 grams of its own fresh matter weight', is calculated as WRC x DM of the material and is given in g/100g fresh matter.

To enable comparison between WRC and PWRC and in essence validate the PWRC concept, the moisture, WRC and PWRC of some HMBF are provided (Table 7-3).

### *7.2.2. Statistical analysis*

Data were analyzed using the statistical analyses software Excelstats for Windows 2004 (Microsoft Corporation, USA). Means from the effluent data were calculated from each group and analyzed separately. Regressions were performed to study the relationships between the studied parameters. A multi-linear equation describing the relationship between effluent (dependent variable) and moisture, PWRC and NDF (independent variables) was established.

## 7.3. Results

### *7.3.1. Moisture content of pre-silage material*

The chemical parameters of the HMBF materials and silage effluent outputs are presented in Table 7-1. Each group consisted of the control followed by adjustments into different moisture levels. In Group 1, the moisture content of potato pulp was adjusted from 88.1 to 64.3 % in fresh matter with wheat bran as a sole in-silo absorbent, while in Group 2 it was adjusted from 78.1 to 64.8 % in fresh matter with wheat bran, dried beet pulp, or wheat straw at two levels per absorbent. In the brewer's grain and apple pomace experiments (Groups 3 and 4), adjustments were made with dried beet pulp, wheat bran and rice bran, which reduced the moisture contents from 81.5 to 64.0 and 91.9 to 73.4 % in fresh matter for brewer's grain and apple pomace, respectively.

In the daikon by-product study (Group 5), data were pooled from three separate experiments involving the use of the by-product without/with adjustment with various absorbents. These reduced the high moisture of the material from 96.9 to 73.6 % in fresh matter. The pooled mean for moisture content in all the experiments was 78.3%.

### *7.3.2. Water retention capacity (WRC) and potential WRC*

The WRC varied with type of HMBF, absorbent used and moisture content of the material alone or the mixture, with a pooled mean of 3.43 g/g. However, a pattern was observed in reductions of WRC with increment in moisture contents of Groups 1, 2, 3 and 4. In Group 5, the opposite was true. The WRC values of the adjusted moistures (DA, DBP, DWS, DBH and DWB) were higher than that of DN, the source material. There

**Table 7-1**. Chemical parameters of some HMBF materials and effluent output from their silages§

| Grouping | HMBF | Moisture (% FM) | WRC (g/g DM) | PWRC (g/100gFM) | NDF (% DM) | Effluent (g/100gFM) |
|---|---|---|---|---|---|---|
| Group 1 | *Potato pulp (A)*[1] | | | | | |
| | PP1 | 88.1 | 2.98 | 35.5 | 32.8 | 11.3a |
| | PP2 | 79.4 | 2.44 | 50.2 | 35.3 | 8.9ab |
| | PP3 | 74.3 | 2.22 | 57.1 | 36.6 | 6.8b |
| | PP4 | 64.3 | 2.00 | 71.4 | 40.8 | 0.2c |
| Group 2 | *Potato pulp (B)*[2] | | | | | |
| | PPC | 78.1 | 2.43 | 53.1 | 32.6 | 0.7a |
| | PPWB 1 | 74.1 | 2.37 | 61.3 | 33.5 | 0.4a |
| | PPWB 2 | 68.4 | 2.25 | 71.0 | 39.3 | 0.0b |
| | PPBP 1 | 73.1 | 2.44 | 65.7 | 35.1 | 0.0b |
| | PPBP 2 | 68.0 | 2.45 | 78.3 | 37.5 | 0.0b |
| | PPWS 1 | 70.0 | 2.16 | 64.7 | 54.0 | 0.0b |
| | PPWS 2 | 64.9 | 2.17 | 76.3 | 58.0 | 0.0b |
| Group 3 | *Brewers grain (BG)*[3] | | | | | |
| | C | 81.5 | 3.11 | 57.6 | 38.3 | 18.4a |
| | BP | 66.4 | 2.66 | 89.4 | 40.5 | 0.0c |
| | WB | 64.0 | 2.00 | 71.9 | 42.0 | 4.9b |
| Group 4 | *Apple pomace (P)*[4] | | | | | |
| | AP | 91.9 | 6.27 | 50.9 | 46.6 | 39.1a |
| | ABP | 82.1 | 5.08 | 90.7 | 50.8 | 3.7c |
| | ARB | 70.8 | 2.60 | 76.0 | 35.6 | 7.3b |
| | AWB | 73.4 | 3.31 | 88.2 | 49.0 | 9.3b |
| Group 5 | *Daikon (D)*[5] | | | | | |
| | DKM | 96.9 | 7.02 | 21.5 | 21.0 | 53.9a |
| | DKS | 96.8 | 7.02 | 22.5 | 21.0 | 54.5a |
| | DKL | 96.6 | 7.03 | 23.6 | 21.0 | 49.6a |
| | D N | 93.4 | 2.40 | 15.8 | 21.1 | 37.8b |
| | DA | 83.7 | 3.52 | 57.4 | 69.0 | 3.2e |
| | DWS | 81.6 | 4.08 | 75.1 | 72.5 | 0.0e |
| | DBH | 79.5 | 3.95 | 80.9 | 59.5 | 10.3c |
| | DBP | 79.3 | 4.25 | 87.9 | 41.4 | 15.2c |
| | DWB | 73.6 | 2.49 | 65.6 | 50.7 | 20.1d |
| | *Statistical parameter* | | | | | |
| | Mean | 78.3 | 3.43 | 61.5 | 41.3 | - |
| | Standard deviation | 10.3 | 1.6 | 21.8 | 13.6 | - |

§ Effluent means (of three samples) within a column for each group with uncommon letters indicate a significantly effect at $P<0.05$; WRC, water retention capacity; PWRC, potential WRC; NDF, neutral detergent fiber; FM, fresh matter; DM, dry matter; g, gram.

[1] PP1, potato pulp control (A); PP2, PP3, PP4, (A) adjusted to various moisture levels with wheat bran.

[2] PPC, potato pulp control (B); PPWB1, PPWB2, (A) adjusted with wheat bran; PPBP1, PPBP, (B) adjusted with dried beet pulp; PPWS1, PPWS2, adjusted with wheat straw to different moistures, respectively.

[3] C, brewers grain control (BG); BP, WB, (BG) adjusted with dried beet pulp and wheat bran, respectively.

[4] AP, apple pomace control (P); AP, ABP, ARB, AWB, (P) adjusted with dried beet pulp, rice bran and wheat bran, respectively.

[5] DKM, DKS, DKL, DN, daikon by-product control (D); DA and DWS, DBH, DBP, DWB, (D) adjusted to moisture levels with wheat straw, bean stalks, dried beet pulp and wheat bran, respectively.

was however, a clear consistency in the pattern of PWRC, increasing with decrease in moisture content (Table 7-1).

### *7.3.3. Fiber content*

The fiber contents (NDF) were low in the materials but increased according to the fiber content of the absorbent introduced. As a result, absorbents high in fiber, such as wheat straw and bean stalks, tended to increase the NDF of daikon by-product (DA, DWS and DBH).

### *7.3.4. Effluent output and relationship with pre-silage material*

The silages underwent acceptable fermentation processes, as indicated by the average pH and lactic contents in Table 7-2. Effluent outputs varied with type, moisture and PWRC of the material. Differences in effluent outputs were obvious (P<0.01) with an increase in moisture or addition of absorbent within each group. Daikon by-product silages produced the most effluent relative to the other HMBF silages studied.

**Table 7-2**. Mean fermentation characteristics of the silages used in the study

| | Moisture range (% DM) | pH | Lactic acid (% DM) |
|---|---|---|---|
| Potato pulp A | 89-67 | 3.75 ± 0.14 | 5.59 ± 1.48 |
| Potato pulp B | 80-67 | 3.39 ± 0.15 | 2.17 ± 0.62 |
| Brewer’s grain | 84-67 | 3.60 ± 0.12 | 1.44 ± 0.16 |
| Apple pomace | 89-70 | 3.49 ± 0.05 | 7.97 ± 2.48 |
| Daikon by-product | 94-75 | 3.80 ± 0.18 | 4.73 ± 3.10 |

Values are means ± standard deviation.

Obvious relationships were observed between the pre-silage characteristics of the HMBF studied and effluent production in the resultant silages and are presented in Fig 7-1. There was a positive relationship between effluent and moisture but inverse relationships with PWRC and NDF of the HMBF materials studied, as given in the following multi-regression equation:

$$Y = 1.569M - 0.1059PWRC - 0.2159NDF - 62.246$$

$$(R^2 = 0.7185, n=81, (P<0.01)$$

where Y, effluent (g/100g fresh matter of material); M, moisture content of material (%); PWRC, (potential water retention capacity of material, in g/100g FM); NDF, neutral detergent fiber of material (% DM).

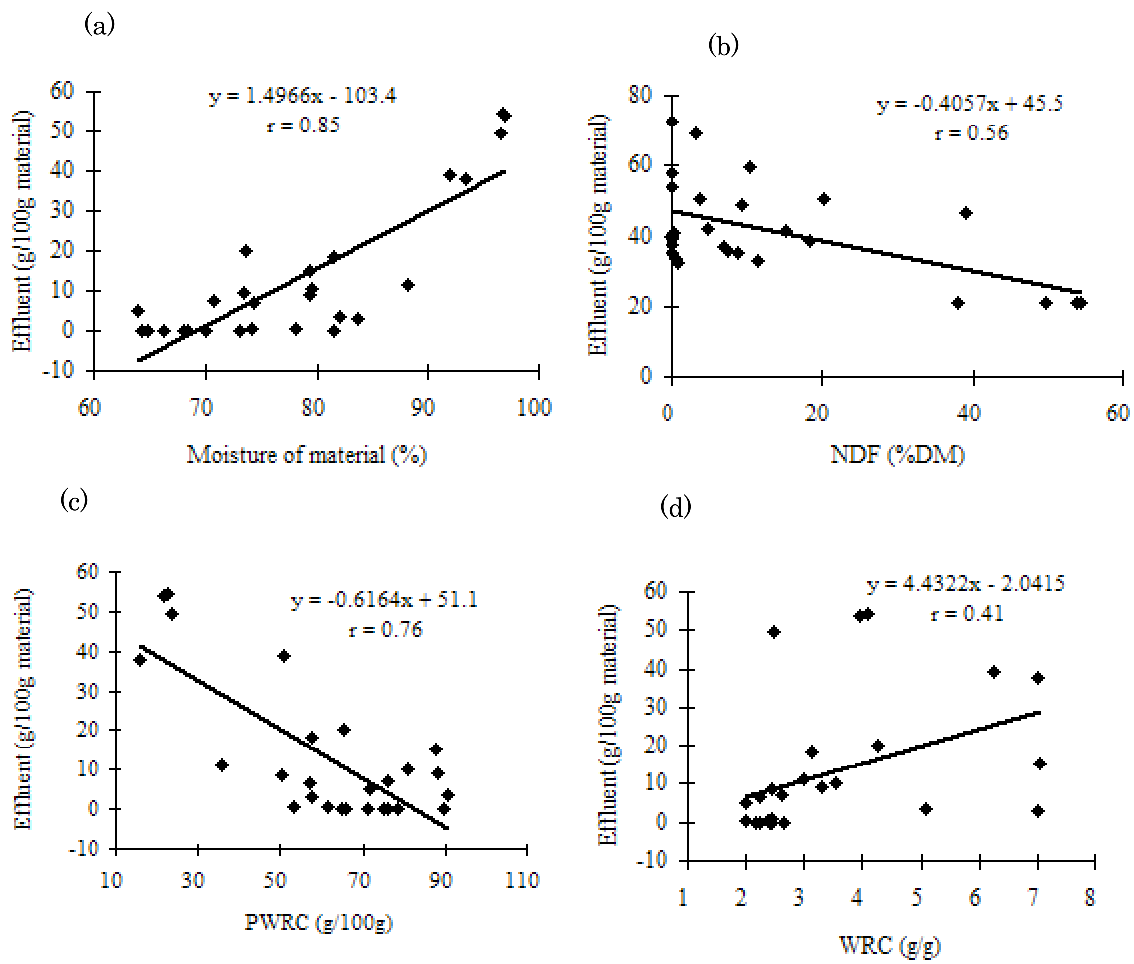


**Fig. 7-1**. Relationships between moisture (a), NDF (b), PWRC (c), and WRC (d) of some HMBF (n=27) and effluent output from their silages (n=81).

## 7.4. Discussion

Ensiling of wet materials is fraught with difficulties, not only in losses encountered in the silo, but also in the considerable loss of nutrients in effluent and seepage through silage, with the attendant environmental consequences. Most silage materials, especially grasses and whole crop materials, are unable to retain their moisture when ensiled, and consequently the system of drying prior to ensiling has been widespread. However, in cases where drying is unreliable or difficult, incorporation with drier materials is inevitable. In the ensiling of HMBF, one statement is valid: high moisture material = more silage effluent, and their relationship is conspicuously clear, as validated by the present results (Table 7-1). From an environmental perspective, the process by which the excess moisture in HMBF is contained during ensilage is of critical importance. Several methods have been suggested to address and control effluent production during ensilage from particularly low dry matter materials without compromising the quality of the resultant silage. Prominent among them is the inclusion of dry materials as in-silo effluent absorbents (Reaves and Brubaker, 1956; Done and Appleton, 1989; O'Keily, 1991; Ferris and Mayne, 1994, Okine et al., 2006b), which invariably control effluent flow and relationships between the dry matter of silage material and effluent have been established (Jones and Murdoch, 1954; Sutter, 1957; Castle and Watson, 1973). In the current study, a relationship between the moisture content of HMBF and effluent was established:

$$Y = 1.4966x - 103.4 \qquad (P<0.01),$$

where Y, effluent (g/100g of ensiled material) and x, the moisture content of pre-silage material (%), with a regression co-efficient (r) of 0.85 (Fig. 7-1a). In the given equation, when Y = 0, x = 69.1, i.e., the moisture content of an ensiled HMBF at which it is predicted that no effluent will be produced is 69.1 % (or 31.9 % DM). This is in good agreement with Sutter (1957), Zimmer (1967) and Bastiman (1976) for ensiled grasses and crops, who proposed dry matter content at zero effluent production to be between 29.9 and 30.7 %. From the pooled mean values of the moisture contents of materials ensiled in this study, the results are particularly significant. It is therefore prudent that the moisture content during ensiling of HMBF does not go beyond 70 %.

Water retention capacity (WRC), or the amount of water held by a known weight of fiber (Robertson et al., 2000), is one of the methods used to determine the hydration properties of dietary fiber in relation to nutrient absorption from the intestine and stool properties. In silage terminology (used interchangeably with 'water holding capacity'), it represents the amount of moisture in grams that a dry absorbent can hold or retain per its dry matter weight and is often used to describe the absorptive capacity of dry materials in silage effluent control (reviewed by Jones and Jones, 1996). This measurement, however, has some shortcomings. First, it accounts only for the amount of moisture that the absorbent material can 'hold' or 'absorb' without considering how much moisture the silage material can 'release' or 'retain' or is available for absorption. Fibrous materials are known to hold water differently depending on the chemical activity of the water, pore size distribution of the fiber matrix and trapped water within the cell wall lumen (Robertson and Eastwood, 1981a). Secondly, the ability to retain effluent under the influence of some chemical or physical conditions within the silo is more relevant than the ability of the absorbent material *per se*. The use of microbial additives prior to ensiling and/or the application of external forces, such as surface pressure on the silo as pertains during ensilage, affect effluent outputs from HMBF.

To elucidate this point, it is important to consider the measurement of WRC as proposed by Robertson et al. (2000) and shown in the scheme below (Fig. 7-2):

Hydrate dry sample in a centrifuge tube

↓

Centrifuge (3000 x g; 20min) [§]

↓

Remove excess supernatant/soluble material

↓

Record residue hydrated wt

↓

Record residue dry weight

↓

WRC (g/g) = (residue fresh weight – residue dry weight) / residue dry weight

**Fig. 7-2**. Scheme of water retention capacity (WRC) according to Robertson et al., 2000.

[§]Centrifugation was substituted with filtration for the sake of convenience as described in 'Materials and methods'.

The WRC values of HMBF in this study (Table 7-3) were quite low compared with other reports for vegetable and fruit by-products in general (Cho et al., 1997a, b; Thibault et al., 1992) but comparable to those of Auffret et al., (1994) for wheat bran and pea hulls, and (Dexter, 1961; Hillman and Thomas, 1974) for dried beet pulp, and they only reflect the source of fiber and the method used. Water retention in fiber has been determined using different methods such as centrifugation, filtration, or suction pressure (McConnell et al., 1974; Robertson and Eastwood, 1981b, c; Chen et al., 1984), with each method measuring the amount of water associated with a sample under the defined conditions. The calculation of WRC, although valid for the measurement of the absorptive element of an in-silo effluent absorbent, discounts the characteristics and fate of the moisture of silage material.

**Table 7-3**. Water retention capacity (WRC) of some HMBF and dry absorbents§

| | WRC (g/g DM) |
|---|---|
| *HMBF* | |
| Cucumber | 4.86 ± 0.80 |
| Water melon residue | 4.54 ± 1.26 |
| Daikon (root) | 3.58 ± 1.31 |
| Cabbage | 6.55 ± 0.47 |
| Carrot pulp | 6.33 ± 0.94 |
| Banana peel | 4.50 ± 0.43 |
| Apple pomace | 3.12 ± 0.54 |
| Peach pomace | 2.17 ± 0.15 |
| Pineapple (skin & pomace) | 2.96 ± 0.28 |
| Potato pulp | 3.55 ± 0.75 |
| *Absorbents* | |
| Dried beet pulp | 2.96 ± 0.58 |
| Wheat straw | 3.08 ± 0.40 |
| Bean stalks and husks | 2.58 ± 0.52 |
| Wheat bran | 1.32 ± 0.62 |
| Rice bran | 0.92 ± 0.23 |
| Wood shavings | 2.44 ± 0.46 |

§ Values are means of three determinations ± standard deviation.

To address this shortcoming, the terminology potential water retention capacity (PWRC) is proposed:

PWRC of silage material = WRC x specific DM of the material

If WRC = $\frac{W1+W2}{DM}$, and specific DM of material (in fresh matter) = $\frac{DM}{FM}$,

where W1, original moisture content of material and W2, absorbed moisture,

then, PWRC = $\frac{W1+W2}{FM}$

This equation takes into account the 'moisture' element of the material and the absorptive capacity of the dry in-silo absorbent and it provides a more realistic approach in determining the retentive capacity of the mixture *vis-a-vis* effluent output. This is particularly valid during the addition of absorbents prior to ensilage and gives a reliable prediction of effluent outputs from materials particularly high in moisture such as HMBF, since different materials have various dry matter contents. This is true, since during ensilage of HMBF, effluent output is intrinsically a function of its PWRC, as indicated by the following figures using different HMBF (Fig. 7-3 a, b).

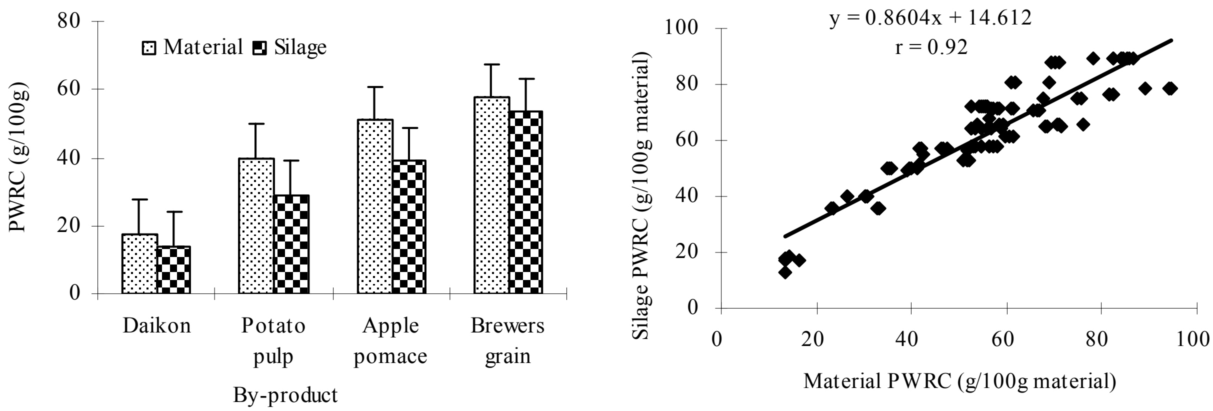


**Fig. 7-3.** PWRC of some HMBF materials and silages with error bars (a) and regression equation showing the relationship between HMBF and their silages (b) (n=81).

It is evident from the figures that after ensiling, the PWRC of HMBF reduces as effluent is released. The strong relationship between material and silage PWRC ($r = 0.92$, $P<0.01$) validates this theory. The data in Fig. 7-1c, d of the HMBF silage experiments showed a stronger effluent relationship with PWRC than with WRC ($r = 0.76$ vs. 0.41), a further indication that the former may be a more relevant index for the measurement of effluent retention in HMBF silages. (See Chapters 4 and 5 on PWRC effect on effluent outputs in some HMBF silages.) The regression equation between the PWRC of the HMBF and effluent outputs from the silages (see Fig. 7-1c) is given below:

$$Y = - 0.6164x - 51.1 \qquad (P<0.01)$$

where Y, effluent (g/100g FM of ensiled material) and x, PWRC (g/100g FM of pre-silage material).

From the given equation, the theoretical PWRC of a silage material at which effluent production would be zero is 82.9. To achieve this, a combination of factors such as the moisture content and the WRC of both silage material and the absorbent need to be considered. The use of absorbents high in WRC and low moisture materials favor an elevated PWRC of the mixture.

The fiber content of both material and absorbent also play an important role in both WRC and PWRC of materials in relation to effluent retention in silages. A typical illustration of this is given in Table 7-1. The WRC of the adjusted moistures DA, DBP, DWS, DBH and DWB were higher than that of DN, the source material, while the opposite was true for the other groups (PP1, PPC, C and AP (Table 7-1). This could be attributed to the high NDF of the absorbents used which increased the low original NDF of DN (daikon by-product) by ≥ twofold. In the other groups, addition of absorbents, however, did not affect the NDF contents of the mixtures. The PWRC values, however, were consistent in increases with an increase in NDF contents, and both had an inverse relationship with effluent outputs, as shown in the multi-regression equation described earlier. Since fibrous materials retain effluent better than non-fibrous materials (Jones and Jones, 1996), the PWRC/NDF relationship with effluent further validates the PWRC theory and could be an important parameter in ensiling of wet materials.

## 7.5. Summary

A major factor affecting the ensiling of HMBF is effluent production, which if not properly disposed, may have adverse consequences on the environment. Effluent is influenced mainly by such factors as the moisture and fiber (NDF) contents and the potential water retention capacity (PWRC) of pre-silage material. While the relationship with moisture is an inverse one, the effluent relationship with NDF and PWRC were positive.

The terminology PWRC is an important novel terminology in the ensiling of HMBF and a theory that warrants further validation.

# Chapter 8

## General Discussion

Feed conservation or preservation by ensilage is a viable method by which the shelf life of crops meant for feeding of ruminants can be extended. This practice has evolved tremendously in recent times due to, among others, the discovery of new preservation techniques, silage additives and advances in silo technology. Methodologies involved in addressing problems related to preservation by ensilage of wet materials are varied but mostly refer to grasses and crops. Preservation by ensilage of agricultural by-products, especially those that contain high moisture contents, is still in embryonic stage. The aims of the experiments were to investigate the ensiling potential of HMBF, improve the preservation by incorporation with additives, control effluent flow and minimize nutrient losses from their silages, extend their shelf lives, evaluate the nutritive value, and to identify the main factors that affect the ensiling of HMBF and their relationship and establish a suitable method for ensiling HMBF.

## 8.1. Pre-ensiling characteristics of HMBF

One of the aims of this research was to identify the suitability of type of HMBF for ensilage, through chemical and microbiological characterization as a prelude to the actual ensiling of the by-product. The chemical and microbiological characteristics relevant to ensilage of the materials studied here showed some variations in terms of availability or not, versus the suitability to the ensiling process. The moisture content of the materials was variable within and among the samples studied but generally higher in by-products from vegetable sources, compared with those from fruit and agro-industrial sources. Potato pulp and apple pomace were typical examples of this, partly due to the mode of extraction employed in the processing factory. The pH, buffering capacity, and, to a large extent the WSC of materials from vegetable sources, were higher than those from fruit sources, providing a rather unpredictable and contrasting indication of their role in an ensiling process. In a typical silage fermentation process, a quick reduction of pH during the initial stages of the fermentation process is necessary to neutralize and prevent the proliferation of undesirable microorganisms with a gradual increase in lactic acid production from the available substrate (WSC), which is mainly sugar. Materials with high buffering capacities may delay the reduction in pH even in the presence of sufficient WSC. Addition of an inoculum of LAB, prior to ensiling of material from vegetable sources may be a viable proposition. Conversely, fruit by-products and potato pulp, from a theoretical point of view, provided strong indications of being ideal silage materials judging from their chemical analysis, as evidenced by their low pH and buffering capacities and relatively high WSC contents (except potato pulp). Usually a material containing 150 g/kg in dry matter available WSC would be enough for a good fermentation process. The LAB populations of the materials investigated except potato pulp were generally low and did not exceed the $10^6$ minimum organisms/g dry matter threshold required to quick-start a highly effective lactic acid fermentation process. However, low initial population of LAB may not be a critical criterion for the success of the fermentation process. What is crucial is the efficiency of the indigenous LAB to initiate a rapid fermentation and sustain a rapid fall in pH (Woolford, 1984a). Yeast load was quite low in the investigated materials especially in potato pulp. This augurs well for good silage fermentation. Again, what is critical is the yeast population at the end of the ensiling process, i.e., after opening of the silage. The rule is that if the number of yeasts exceeds the level of $10^5$ organisms/g dry matter, rapid fungal deterioration would occur after opening of the silage (Jonsson and Pahlow, 1984). From a microbiological point of view, potato pulp may be an ideal silage material.

The potential water retention capacity (PWRC), a terminology employed as a novelty in this study is, to a large extent, a function of its moisture content (See Chapter 7). An important feature in the ensiling of particularly wet crops is the ability of the material to retain its moisture during the course of ensilage. The PWRC describes this capacity of materials ensiled either alone or with an absorbent, under different silo conditions during the course of ensilage, and it is an important index in ensiling of HMBF (Chapter 7). The higher the PWRC, the stronger the ability of the ensiled material to retain effluent, and vice versa.

## 8.2. Ensiling of HMBF

### *8.2.1. Potato pulp*

In all the three investigations on potato pulp silages, the pH was below 3.60, irrespective of the type of inoculants added or silo used, indicating a good fermentation process. There were, however, variations in character and efficiency of pH reduction of the inoculants used. Particularly effective was the fungus *Rhizopus oryzae* which, when used alone or in combination with *Lactobacillus rhamnosus*, decreased ($P<0.05$) the pH in two separate experiments. A third experiment confirmed this when *Amylomyces rouxii*, a fungus taxonomically identical to *Rhizopus oryzae* was used. This decrease after inoculation with the fungal inoculants is in good agreement with other reports (Oda et al., 2002; Okine et al., 2005), but it was rather overshadowed by a defect in lactic acid production. Hypotheses built on the lack of effect included the probable malfunction of LDH (the active enzyme responsible for conversion of sugars to lactic acid in *Rhizopus oryzae*), the lack of aerobic conditions, the ambient temperature during ensiling and the duration of storage. Subsequent experiments, however, proved that ambient ensiling temperatures and lengths of ensilage were more important in determining the rate of fermentation than inoculation with the fungal inoculants (Okine et al., 2007). Pre-ensiling characteristics of potato pulp material, such as the low buffering capacity (Chapter 2) and bacteria load (Saito et al., 2006) in the pulp, facilitated and enhanced its ensiling capacity.

In all three investigations, the low VFA in potato pulp silage, especially the low acetic acid content and non-appearance of butyric acid, the latter which is usually associated with badly preserved silages, further boosted the ensiling potential of potato pulp, in consent with Oda et al., (2002). The fate of nutrient composition during the ensiling process was to a large extent, affected by inoculation with the microbial inoculants. The carbohydrate fraction of potato pulp, starch, sugar and pectin seem to assume a consistent pattern with ensilage time. The most obvious of these patterns was observed in the effect of *Rhizopus oryzae* on the degradation of pectin, which led to a concomitant increase in sugar concentrations. The increases in the concentrations of sugar over time were probably due to slight increases in concentrations of unspecified monosaccharides during fermentation (Van de Riet et al., 1987) or galactan or pectin (De Man, 1957), or from other sources such as cellulose or hemicellulose, which are present in potato pulp (Mayer and Hillebrandt, 1997). The degradation of starch, however, was not affected by inoculation or duration of storage and could be due to the slow degradability of potato starch in general (Monteils et al., 2002).

Although ensiling of potato pulp was effective from a preservation standpoint, the process involved some difficulties with effluent and losses in nutrients as observed in the third experiment. This phenomenon was more pronounced with the use of the fungal inoculants. The increase in effluent production upon inoculation with *Amylomyces rouxii* could be attributed to the breakdown of starch and pectin as described in previous studies (Experiments 1 and 2 of Chapter 3) with consequent reductions of silage WRC. *Rhizopus oryzae* equally produced a substantial amount of effluent compared with the uinoculated control silage, a result that could be explained by the action of the enzymes pectinase and LDH on the residual starch in potato pulp, which, as supported by Dongowski and Stoof (1993), decreases the water binding capacity of potato pulp. The results of this study suggested that *Amylomyces rouxii* was more effective in breaking the water holding capacity of PPS, compared to the other inoculants, with consequent higher losses compared to other treatments, a result that could adversely affect the nutritive value of the silages.

### *8.2.2. Daikon*

The two separate experiments conducted on the ensiling potential of daikon by-product provided information on an otherwise novel by-product and was a test case for the validity of the hypotheses on ensiling particularly high moisture by-products. For various reasons, this was fraught with many challenges. Despite the high contents of OM, CP, WSC, GE and low EE that underline its prospect as animal feed resource, pre-ensiling characteristics, especially the high moisture content, pH, buffering capacity and yeast population of daikon pre-supposed difficulties in its preservation by ensilage. Adjustment of the moisture contents and addition of an inoculum of *Lactobacillus* *plantarum* prior to ensiling were measures to address these presumed deficiencies and to improve the suitability for ensiling. Dry matter adjustment in Experiment 4-1 increased the water retention capacity of daikon pre-silage material. A major feature in the ensiling of particularly high moisture materials is the DM/WRC relationship *vis-à-vis* effluent production. This study indicated that the WRC of silage materials is highly influenced by, and positively correlated to, the dry matter content. Upward adjustments of the DM of pre-silage material led to significant increases in the WRC and an obvious effect on effluent retention in the resultant silages. Inoculation with *Lactobacillus plantarum* though improved silage fermentation, led to high effluent outputs with consequent high nutrient losses and as such would not be recommended. Changes in the pH, organic acid content and microbial numbers (LAB and yeast populations) were all important indices in assessing the fermentation quality of daikon by-product silages and this is amply supported by other works (Jonsson and Pahlow, 1984; Woolford, 1984b; Ashbell et al., 1987).

Moisture adjusted daikon by-product silage deteriorated faster on aerobic exposure due to higher dry matter content and, to a lesser degree, lower packing density of the pre-silage material, and was further accelerated by inoculation with *Lactobacillus plantarum*. Application of the inoculum is therefore not recommended.

In Experiment 4-2, moisture levels were adjusted based on observations from the previous experiment. The hypothesis was that increasing the DM content a level above that in Experiment 4-1 would lead to an increase in the WRC of the mixtures and consequently, to effluent retention in the silos as well. Although DM and WRC values were elevated, effluent outputs in DBP and DWB were more than the projected, suggesting that packing weight, density, and pressure exerted on the silages, or other factors such as fiber content (Offer and Al-Rwidah, 1989; O'Keily, 1991) and/or physical characteristics of ensiled material (Jones and Jones 1996), may have played a role in their ability to retain effluent. Daikon/wheat straw stemmed effluent completely, while DWB, in spite of the high pre-silage DM, produced the most effluent. Straws are effective absorbents, but wheat bran and cereals in general have poor water absorptive capacities (Jones and Jones 1996). It is obvious from the two experiments, however, that silage materials (singularly ensiled or with an absorbent material) with high water retention capacities tend to control effluent more effectively than those with lower water retention capacities. Although DBP had significantly lower pH and higher lactic acid contents compared with other treatments, silage fermentation was generally restricted in Experiment 4-2 due to effect of the absorbents.

Aerobic stability of daikon silages with different absorbents in Experiment 4-2 was similar in character to the daikon wheat straw adjusted moisture of Experiment 4-1 in sharp increases and decreases in pH and lactic acid contents, respectively, after 3 days of aerobic exposure of the silages. The rate of rise in pH and decline in lactic acid contents indicated a phenomenon that is common to HMBF: a possible proliferation of yeast numbers. Yeast, especially lactate assimilating types, are mostly responsible for a rapid decline in silage quality at feed out stage (Filya et al., 2000; Jonsson and Pahlow, 1984). DBW and DBH deteriorated faster, compared to DWB and DBP on aerobic exposure due to the state of the added absorbents (wheat straw and bean stalks) prior to addition to daikon by-product. The two absorbents are usually gathered from the field after harvesting of wheat and beans and they normally come with some soil which may serve as a contaminant, in contrast with wheat bran and dried beet pulp, which are 'cleaner' products from factories. Another factor responsible for the quick deterioration of the two silages was their low packing densities, which create air pockets that, with the least available substrate, provide fertile grounds for yeast growth and metabolism and consequently enhancement of spoilage.

### *8.2.3. Brewer's grain and apple pomace*

The low lactic acid contents of the silages confirmed a phenomenon typical to the fermentation quality of brewer's grain silages in general and in consent with Nishino et al., (2001). Several factors could be attributed to this, including the characteristic of the material, the low buffering capacity and the yeast load in the material. However, the main aim of the experiment was to investigate the effects of packing density and pressure and their relationship with effluent production. The results in this study confirmed other reports on silages, especially those ensiled from low dry matter materials (McDonald et al., 1960; Zimmer, 1974; Peters and Weissbach, 1977). Rather significant, however, was the effect of the two factors in conjunction with the PWRC, which appeared to have had the most potent effects. This result was confirmed in the apple pomace silage experiment. Apart from pressure that had a significant influence on effluent outputs, PWRC gave a strong indication that, barring other major determining factors such as the moisture content, effluent production from any given silage material is largely a function of its PWRC (See Chapter 7).

Aerobic stability in the apple pomace silages confirmed earlier results (Chapter 4) that pH and lactic acid (fermentation end products) are important barometers of aerobic stability in HMBF silages, and that lower pre-silage packing density makes silages more prone to quick deteriorations in quality during the feed out stage.

## 8.3. Nutritive value of HMBF

The high moisture content of HMBF, as a matter of necessity, dictates that feeding with them be in conjunction with other dry feeds. In the digestion trial with potato pulp silages, addition of hay and soybean meal to the silages prior to feeding increased not only the DM of the diets but the texture, which was highly appreciated by the sheep. This also augmented the diet CP and NDF contents to levels in tandem with NRC feeding standards (NRC, 1985).The pulp material used in the present trial contained fair amounts of easily digestible carbohydrates such as starch and pectin, though the content of the former was low compared to the conventional potato pulp of about 370 g/kg DM (Mayer and Hillebrandt, 1997), and could be due to the mode and rate of starch extraction as described in (Chapter 6). The diets contained an average digestible energy of 13 MJ/kg DM, fairly sufficient for the energy requirements and ages of the sheep used in the trial. With the exception of *Lactobacillus plantarum* inoculated silage, DM intake among silages was not affected (P>0.05) by addition of the inoculants. Similar improvements in DM intakes of silages treated with an inoculum of lactic acid bacteria, especially with *Lactobacillus plantarum*, have been reported in grass silages in particular (Gordon, 1989a; Gordon, 1989b), especially where there were improvements in the quality of the silages, although others (Hooper et al., 1984; Steen et al., 1989) have reported increased DM intakes with commercial inoculants with no obvious improvement in fermentation, as was the case in this study. The silages were acceptable to the sheep, as they were readily eaten by the animals.

Crude protein digestibility was higher (P<0.05) in sheep fed silage inoculated with *Lactobacillus plantarum*, compared to those fed control, a result that could not be explained. This could be related to the general improvement in nitrogen retention with feeding of PPS that may have improved microbial nitrogen synthesis within the rumen and/or reduced silage nitrogen degradability within the rumen as implied by Aibibula et al., (2004), who found no differences in nitrogen retention in sheep fed *Lactobacillus rhamnosus* and *Rhizopus oryzae* inoculated PPS based diets.

The DM, NDF and CP contents of the diets, in addition to the starch and pectin contents of potato pulp generally contributed to the high TDN values of the diets. Dry matter digestibility and nutritive value were not affected by the inoculants in consent with other reports (Aibibula et al., 2004; Hanada et al., 2004). The digestibility and nutritive value of potato pulp silage is comparable to other by-products like beet pulp and citrus pulp (NARO, 2001; O'Mara et al., 1999; Deaville et al., 1994).

In the experiment with daikon by-product silages, addition of alfalfa hay to the diet augmented the DM, nitrogen and energy contents of the diets and generally improved the texture of the diets in a way preferred by the animals. No N supplements were necessary since the silages and hay contained CP levels that met or exceeded their requirement (NRC, 1985). Daikon/dried beet pulp silage had the best feed intake and preference in comparison with the other treatments, probably due to its fermentation quality, low fiber and physical characteristics (dry matter content, particle size and resistance to fracture), factors that have profound effects on feed intake (Baumont et al., 2000; Inoue et al., 1994). Intake was a key factor in assessing feed preference (Moseley and Antuna Manendez, 1989); thus, the high rate of intake in DBP silage in the first two hours was a key factor in assessing its preference over the other treatments. At 8 and 24 h, intake did not differ between DBP and DBH silages, times when intake of the silages decreased continuously until satiety and preference became less obvious. The use of cheap and available dry by-products as absorbents in the ensiling of daikon is noteworthy from an economic perspective.

## 8.4. Factors affecting the ensiling of HMBF

Several factors may directly or indirectly affect the flow or release of effluent from HMBF. However, two revelations are worth acknowledging in this study: the theoretical optimum moisture needed for zero effluent in HMBF silages, and, more significantly, the theory of PWRC. The moisture level for zero effluent production in HMBF (69.1%) is close to those of Sutter, (1957); Zimmer, (1967) and Bastiman (1976), which predicted between 29.9 and 30.7 % for ensiled grasses and crops on the optimum moisture content necessary to stem effluent in silages in general. As such the value in this study could therefore be an important index for ensiling HMBF.

The terminology PWRC, attempts to address a rather ignored and important aspect of effluent retention in silages. Although the absorptive capacities of several dry materials have been reported (Reaves and Brubaker, 1956; Done and Appleton, 1989; O'Keily, 1991; Ferris and Mayne, 1994), the relationship between the 'absorbent' and 'absorbed' has not been considered, until now. The contention here is that if dry absorbents are capable of holding water several times their weight in dry matter, how much moisture is released or 'allowed to be held' by the absorbed material? The PWRC theory provides an answer to this question. High moisture materials have varied capacities to release their moisture and therefore a provision must be made for this capacity when they are used as silage materials. The strength of this hypothesis, however, rests on further experimentation using different materials and a broad range of experimental data. The relationship between effluent and PWRC, both in the single and multi-regression equations (Chapter 7), validated the assumption that PWRC of the material is the most important determinant of effluent after moisture.

## 8.5. General Conclusions

To sum up, high moisture by-product feedstuffs could be preserved by ensiling based on the intrinsic characteristic of the material. The more the material is endowed with characteristics favorable to the ensilage process, the better the chances of success of preservation, and vice versa. Effluent production is an important factor affecting the ensiling of high moisture by-products feedstuff and, as such, the use of appropriate absorbents prior to ensiling is highly warranted in controlling effluent outputs from HMBF silages.

The aerobic stability of HMBF silages is highly variable, but mostly influenced by the pre-silage bulk density, pH and organic acid content of the silage. The lower the bulk density, the more susceptible the silage is to fast deterioration in quality. Rapid use of HMBF silages during the feed out stage is highly recommended.

The potential for use as ruminant feed is high and very promising due to the high nutritive value of some HMBF.

The potential water retention capacity, or the ability of the silage material to retain effluent, is a novel terminology and could be an important index in the ensiling of HMBF. The theoretical moisture content and PWRC at which there would be no effluent in HMBF silages were 69.1 % in fresh matter and 82.9 g/100g, respectively.

## 8.6. Further outlook

The theory of potential water retention capacity (PWRC) requires further substantiation with a wide range of absorbent materials and HMBF. Future experiments should also consider the possibility of extending the 'shelf' or 'bunker' life of HMBF. Solutions and strategies should include experimentation with materials with proven ability to control secondary fermentation and aerobic stability with a focus on biological rather than chemical means.

Studies on rates of inclusion in ruminant diets and animal responses are equally vital in ascertaining the nutritive value of HMBF.

# Chapter 9

## General Summary

Agricultural by-products, principally residues from farm harvests or processing factories, are an integral part of livestock feeding in most developing countries. The term high moisture by-product feedstuff (HMBF) is used in this thesis to describe 'any product or commodity that is obtained during the harvesting, production, or processing of feed or fiber and contains moisture content of more than 750 g/kg in fresh matter, and has value as an animal feed. There are several means of preservation or extension of the shelf life of HMBF including drying and freezing. However, preservation of HMBF by ensilage is, comparatively, a more viable and cost effective method. However, this, involves challenges both in terms of quality of the resultant silage and effect of the process on the environment, since effluent production is a major characteristic of HMBF silages. Methodologies involved in addressing problems related to preservation by ensilage of wet materials are varied but often subjective, product-specific, or largely refer to grasses and crops and thus highlight the need for strategies in addressing problems specific to HMBF.

The objectives of this study were therefore to investigate the ensiling potential of HMBF, improve the preservation by incorporation with additives, control effluent flows and minimize nutrient losses from their silages, extend their shelf life, evaluate the nutritive value, and to identify the main factors that affect the ensiling of HMBF and their relationship and establish a suitable method for ensiling HMBF.

To realize these objectives the following experiments were conducted:

## 1. Study of the pre-ensiling characteristics of a selection of HMBF

The objective of this study was to investigate the ensiling potential of representatives of HMBF from vegetable and fruit sources through chemical and microbiological characterization as a prelude to their use as silage materials. Materials investigated were from vegetable sources (cabbage, cucumber, carrot pulp and daikon or Oriental radish (*Raphanus sativus* L.), fruit sources (banana peel, apple pomace, orange peel, and pineapple skin) and one agro-industrial source (potato pulp). Pre-ensiling parameters investigated indicated that they were high in moisture and variable in pH, buffering capacity and microbial populations (lactic acid bacteria and yeast), but had water soluble carbohydrates sufficient for lactic acid fermentation. Vegetable by-products were generally high in buffering capacity and pH compared with those from fruit sources.

Due to the high moisture contents, means of controlling effluent production during their use as silage materials are imperative.

## 2. Ensiling of representatives of HMBF

*Potato pulp*: A series of strategies were used to preserve fresh potato pulp by ensilage. These involved the use of microbial inoculants in three separate experiments with a lactic acid bacteria inoculum (*Lactobacillus rhamnosus*) and two fungal inocula (*Rhizopus oryzae* and *Amylomyces rouxii)*, and investigations on their role in enhancing the fermentation quality in potato pulp silage.

Results indicated that although these inoculants improved ($P<0.05$) the fermentation quality, potato pulp can ensile well with or without microbial inoculants. A subsequent study suggested that the fermentation quality of potato pulp silage was influenced most by ensiling temperatures and the duration of storage ($P<0.01$) and least ($P>0.05$) by inoculation with fungal inoculants. Moreover, ensiling potato pulp with *Rhizopus oryzae* and *Amylomyces rouxii*, in particular, increased ($P<0.05$) effluent volume and silage losses in potato pulp silage. It was concluded that inoculation with fungal additives would yield little benefits in potato pulp silage and where their application is necessary, the use of in-silo absorbents prior to ensiling was required.

*Daikon* (*Oriental radish*)*:* The study involved two experiments. First, an investigation of the chemical composition and pre-ensiling characteristics of daikon, followed by ensiling of daikon by-product with wheat straw as a sole in-silo absorbent and an inoculum of lactic acid bacteria (*Lactobacillus plantarum*), while the second involved ensiling with other absorbents (dried beet pulp, dried bean stalks and husks and wheat bran). A study of the aerobic stability or shelf life of daikon silages was investigated in both experiments.

Results indicated that ensiling of daikon by-product was a viable venture and that the high moisture content (951 g/kg) and buffering capacity (703.4 meq/kg DM) not withstanding, the by-product could be ensiled successfully using appropriate technology. Due to the moisture content of the by-product, the use of microbial inoculants is strongly discouraged, since they increase ($P<0.01$) effluent production and nutrient losses. There were variations in the efficiency of the absorbents used; wheat straw was the most effective ($P<0.05$) but least in effect on aerobic stability of the silages. The use of appropriate in-silo absorbents, with the dual responsibility of stemming effluent flow and minimizing effect of silage spoilage during feed out stage (aerobic stability), is needed to extend the shelf life of daikon by-product silage.

*Brewer's grain and apple pomace*: The aim was to investigate the role of two physical factors (surface pressure and packing density) on the capacity of the two by-products ensiled without absorbents (as in brewer's grain) or with absorbents (as in apple pomace) on effluent production and retention using the two by-products as representatives of HMBF.

High packing density and pressures exerted on fresh brewer's grain and apple pomace ensiled alone or in combination with absorbents prior to ensiling, generally increased ($P<0.05$) effluent production from the silages. However, this was influenced by the moisture content, type of material, absorbent used and potential water holding capacity of the ensiled material.

## 3. Nutritive value of HMBF

The nutritive value of two representatives of HMBF silages, potato pulp and daikon by-product silages were evaluated *in vivo* using sheep. First, potato pulp, ensiled with or without microbial inoculants was used in a 4 x 4 Latin square design experiment to investigate the nutritive value of potato pulp silage. The silages were supplemented with Italian ryegrass hay (*Lolium multiflorum*) and soybean meal. Addition of the microbial inoculants did not affect ($P>0.05$) its feeding value for sheep. The high nutritive value (digestible energy, (DE) 13 MJ/kg DM; total digestible nutrients (TDN), 709 g/kg DM) of potato pulp silage compares to that of agricultural by-products such as citrus pulp and beet pulp and as such could be a useful feed ingredient in ruminant diets.

In the second study, a 3 x 3 Latin square design experiment was undertaken to investigate intake and preference of daikon by-product ensiled with dried beet pulp (DBP), wheat straw (DWS) and bean stalk/husks (DBH) as absorbents. Alfalfa hay was offered as a basal diet at 24.0 g/kg BW/sheep/day with the silages. Intake and preference was best in DBP, followed by DBH and DWS, in that order. Since the silages were readily eaten when offered to sheep, more feeding trials with different absorbents and bigger ruminants are required to ascertain the full potential of the by-product.

## 4. Factors affecting the ensiling of HMBF

A study of the relationship between effluent and its major determinants during the ensiling of HMBF was investigated. Data were pooled from a series of experiments involving the pre-silage materials of potato pulp, brewer's grain, apple pomace and daikon silages either alone or with various absorbents materials (n=27) and their silages (n=81).

Results indicated a high correlation ($P<0.01$) between silage effluent and moisture/potential water retention capacity (PWRC) of the pre-silage material. The theoretical moisture content and PWRC at which there would be no effluent in HMBF were 69.1% in fresh matter and 82.9 g/100 g, respectively.

# 要約

農作物の収穫および加工過程で生じる農産副産物は、多くの発展途上国において重要な飼料資源である。この論文では、高水分農産副産物(HMBF)という語句を、食料や繊維製品の生産過程で得られる植物由来の副産物で、水分含量が750g/kg 以上と高く、家畜の飼料として価値を有するものとして用いる。高水分農産副産物の保存や貯蔵期間の延長には、乾燥や凍結などさまざまな方法があるが、高水分農産副産物のサイレージ化は他の方法に比べ現実的であり、費用のかからない方法である。しかし、高水分農産副産物をサイレージ化する過程では排汁が生成しやすく、サイレージ品質の低下や環境への悪影響が懸念される。水分含量の高い原料を用いたサイレージ調製に伴う問題点の解決方法は様々あるが、適切な対処方法はそれぞれの原料で異なるため、個々の高水分農産副産物に対応できるような問題解決の戦略が必要となる。

本研究の目的は、高水分農産副産物のサイレージ化の可能性の検討、添加剤利用による保存性の向上、排汁生成ならびに栄養損失の抑制、貯蔵期間の延長ならびに高水分農産副産物サイレージの栄養価を評価することである。さらに高水分農産副産物のサイレージ化に影響を及ぼす主要因を明らかにするとともに、高水分農産副産物の適切なサイレージ化の方法を確立することである。

この目的を達成するために、下記に示した一連の実験を実施した。

## １．高水分農産副産物のサイレージ化適性に関する研究

この研究では、高水分副産物のサイレージ化適性を検討するため、野菜や果

物由来の副産物の化学的・微生物学的特性を調査した。研究で供した材料は、キャベツ、キュウリ、ニンジン粕、ダイコンの野菜由来の副産物とバナナの皮、リンゴ粕、オレンジ皮、ナイナップル粕の果物由来の副産物、さらにデンプン加工工程排出される残渣物であるジャガイモデンプン粕(ポテトパルプ)であった。これら材料の特徴を調べた結果、いずれの材料とも水分含量は高く、pH、pH 緩衝能および乳酸菌や酵母数などは材料によって大きく異なっていたが、いずれの副産物も乳酸発酵に必要な水溶性炭水化物は十分に含まれていた。果物由来の副産物に比べ野菜由来の副産物は、pH 緩衝能が大きく pH が高かった。これらの農産副産物は水分含量が高いため、排汁生成の抑制は重要な課題である。

## ２．高水分農産副産物のサイレージ化

**ポテトパルプ**：ポテトパルプを貯蔵するため、いくつかの方法を用いてサイレージ調製した。乳酸菌(*Lactobacillus rhamnosus*)と２種類の乳酸生成糸状菌(*Rhizopus oryzae*, *Amylomyces rouxii*)を添加剤として用いサイレージ調製し、これらの微生物添加剤がポテトパルプサイレージの発酵品質に及ぼす影響について調査した。

ポテトパルプをサイレージ調製する際に、乳酸発酵を促進させる微生物添加剤を使用することによりサイレージの発酵品質は向上したが、添加剤を使用しなくても良好な発酵品質のサイレージが得られることが示された。ポテトパルプサイレージの発酵品質は、微生物添加剤の使用の有無よりも貯蔵期間の温度や長さの違いによる影響のほうが大きいことが示された。さらに *Rhizopus oryzae* や *Amylomyces rouxii* といった乳酸生成糸状菌の添加は排汁の生成量を

増加させ、サイレージ損失につながることが示された。これらの結果からポテトパルプサイレージ調製における乳酸生成糸状菌の添加効果は小さく、これらの微生物添加剤を使用する際には、水分吸収剤を同時に添加して排汁生成を抑制する必要があると判断された。

**ダイコン**：野菜の選果場から排出されるダイコン(ダイコン選別残渣)のサイレージ調製に関して 2 つの実験を実施した。最初の実験ではダイコン選別残渣のサイレージ化適性を調べると共に、水分吸収剤および乳酸菌(*Lactobacillus plantarum*)添加がダイコン選別残渣サイレージの発酵品質に及ぼす影響について検討した。2 番目の実験では、水分吸収剤として乾燥ビートパルプ、マメの殻と茎およびフスマを用い水分吸収剤の違いがダイコン選別残渣サイレージの発酵品質および排汁生成量に及ぼす影響について検討した。また、いずれの試験においても好気的条件下におけるダイコン選別残渣サイレージの安定性について検討した。

これらの試験の結果、ダイコン選別残渣は水分含量や pH 緩衝能が高いが、適切な手法によりサイレージ化できる材料であることが示された。ダイコンは水分含量の高い副産物であるため、ダイコン選別残渣のサイレージ調製時における微生物添加剤の利用により排汁生成量や栄養損失の増加が認められた($P<0.01$)。水分吸収剤の添加効果は吸収剤によって異なり、小麦ワラは最も水分吸収能に優れていたが、小麦ワラを添加したダイコン選別残渣サイレージの好気的条件下における安定性は、他の水分吸収剤を添加したサイレージよりも劣っていた。これらのことからダイコン選別残渣サイレージの保存性を高める

ためには、排汁生成量の抑制とともにサイロ開封後における好気的発酵の抑制といった２つの目的を達成できるような添加剤の利用が必要であることが示された。

**ビール粕とリンゴ粕**：高水分農産副産物を用いたサイレージ調製時における物理的条件(サイレージ表面にかかる圧力と詰め込み密度)が排汁生成に及ぼす影響について、水分吸収剤を添加したとき(リンゴ粕を用いてサイレージ調製)としないとき(ビール粕を用いてサイレージ調製)で検討した。

その結果、ビール粕、リンゴ粕のいずれにおいても詰め込み密度やサイレージ表面の圧力が高いと、水分吸収剤の添加の有無にかかわらずサイレージからの排汁生成量が増加した($P<0.05$)。しかし、排汁生成量は材料の水分含量や種類、潜在水保持能力(potential water holding capacity, PWRC)によって左右されることが示された。

## ３．高水分農産副産物の栄養価

デンプン粕とダイコン選別残渣を用いて調製したサイレージをめん羊に給与し、栄養価を査定するため２つの実験を実施した。最初の実験では添加剤を用いずに調製したポテトパルプサイレージと微生物添加剤を用いて調製したサイレージの栄養価を調べるため、４×４のラテン方格法に基づいて消化試験を実施した。飼料はポテトパルプサイレージの他にイタリアンライグラス乾草(*Lolium multiflorum*)と大豆粕を給与した。その結果、サイレージ調製時における微生物添加剤の利用はポテトパルプサイレージの栄養価に影響を及ぼさな

かった(P>0.05)。ポテトパルプサイレージの可消化エネルギー含量は 13MJ/kgDM、可消化養分総量は 709g/kgDM であり、オレンジ粕やビートパルプのような副産物と同等のエネルギー価であった。これらのことからポテトパルプサイレージは反芻家畜のエネルギー飼料として利用価値があると判断された。

もう一方の試験では、水分吸収剤として乾燥ビートパルプ(DBP)、小麦ワラ(DWS)および豆の殻と茎(DBH)を用いてサイレージ調製したダイコン選別残渣サイレージの採食性を調べるため、めん羊を用いた３×３ラテン方格法による採食試験を実施した。めん羊にはサイレージとともに乾草を 24.0g/kgＢＷ/頭/日給与した。ダイコン選別残渣サイレージの摂取量は水分吸収剤として DBP を給与したサイレージで最も多く、次いで DWS、DBH を用いたサイレージであった。これらのサイレージはいずれも給与後、速やかに採食された。このようにダイコン選別残渣サイレージはめん羊による嗜好性が高いため、ダイコン選別残渣のもつ潜在的な飼料価値をさらに引き出すためには、さまざま水分吸収剤との組み合わせによるサイレージ調製試験や大型の反芻家畜による給与試験の実施が必要であろう。

## ４．高水分農産副産物のサイレージ化に影響を及ぼす要因

高水分農産副産物のサイレージ調製過程における排汁生成に影響を及ぼす要因明らかにするため、これまでに実施した試験のデータを用いて解析を行った。解析に用いたデータには、デンプン粕、ビール粕、リンゴ粕、ダイコンなどの高水分農産副産物とそれらに水分吸収剤を添加したサイレージ原料(n=27)とそれらを用いて調製したサイレージ(n=81)であった。

その結果、排汁量とサイレージ原料の水分およびPWRCとの間には高い正の相関が認められ、排汁抑制の理論値は原料の水分含量が 69.1%以下、PWRC は 82.9g/100g以下となった。

# Acknowledgements

I would like to acknowledge the support of the Ministry of Education, Science and Culture of Japan for the scholarship program without which this work would not have been possible.

With a deep sense of gratitude, I would like to express my sincere thanks to both of my supervisors, Professor Meiji Okamoto and Associate Professor Masaaki Hanada for their immense support and guidance during the course of my studies. The fatherly guidance of Professor Okamoto has been very inspirational and his constant supports in my studies a great source of motivation. I am deeply grateful. On the other hand, the profound knowledge, valuable suggestions, and insightful directions from Professor Hanada on my entire research work are greatly acknowledged. What I know today about research, I learned from him. I am sincerely thankful.

I am grateful to Professor Toshiyoshi Takahashi of Yamagata University for his valuable suggestions and useful advice on my graduate program and the final manuscript.

My sincere thanks to Professor Akio Hongo of the Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Science for the use of sheep during the animal trials, and for his counsel and useful remarks on the manuscript.

I am also indebted to Dr. Aibibula Yimamu, Mie University, Japan, for his immense help and selfless contributions towards my research work. The tremendous assistance offered during his presence in our laboratory is greatly appreciated.

I would also like to acknowledge the assistance of all my past and current Japanese colleagues and staff from the Laboratory of Grassland Utilization, including Ikehata

Keita, Murata Satoshi, Izumita Mai, Hamabe Kazutaka and others for their untiring assistance during the experiments. This also includes my foreigner colleagues, Huiquan Li, Maimaijiang Zunong (China) and Shin In Sun (Korea) for their company and contributions to my work.

I also want to thank my parents, Mohammed Okine and Aishetu Larley, and to share this moment with my brothers and sisters for their support during my studies.

Finally, I am grateful to my wife Hajara, for her untiring moral and loving support, encouragement and patience, and to my son Abdul Gafar who, even at his tender age, endured the loss of my company at times when I was needed most.

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