CHANGES AND LOSSES IN SILAGES DURING AEROBIC EXPOSURE

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Abstract

Silage making is based on anaerobic lactic acid fermentation of forage crops. Silages are exposed to air during preparation, storage, and feedout. Practically, it is almost impossible to achieve a complete avoidence of aerobic exposure in silage making process. During aerobic exposure of silages, aerobic microbial activity results in losses of nutritive value of silages. Along with abiotic factors, aerobic stability of silages depends upon crop composition and additives used. The objective of this study was to determine the changes and losses during aerobic exposure of silages of two wheat cultivars, Bet Hashita (BH) and Galil, harvested at flowering and milk stage of maturity, ensiled as direct-cut (DC) and wilted and inoculated with homofermentative lactic acid bacteria (LAB) (*Lactobacillus plantarum*). In addition, the relationship between wheat silage composition and aerobic stability was evaluated. Another objective of the study was to determine the changes and losses in sorghum and corn silages during aerobic exposure and to evaluate whether any of these silages contain factors contributing to aerobic stability by mutually spraying the aqueous extracts of these silages. We hypothesized that important intrinsic factors of wheat silage such as stage of maturity, type of silage (DC and wilted), and silage composition (DM, water-soluble carbohydrates [WSC], lactic acid, volatile fatty acids [VFA], etc.) act both separately and interactively to affect the aerobic stability of silages. In sorghum and corn silages, it was hypothesized that sorghum silages contain certain intrisic stabilizing agents responsible for its aerobic stability and that can be exploited and used in aerobically suceptible silages by spraying aqueous extract of sorghum silages.

Silages were prepared in 1.5 L anaerobic glass jars and after 4-6 months of storage at room temperature (26 ± 2^0C), silages were opened and subjected to aerobic stability test lasting 7 days. Chemical (pH, residual WSC, lactic acid, VFA, CO2 production, DM and NDF digestibility), temperature change, numbers of yeasts and molds, and sensory (visual and olfactory) parameters served as indicators to study the changes during aerobic exposure of silages.

In cultivar BH, flowering DC (287.0 g/ kg DM) and wilted silages (362.2 g/ kg DM) had ensiling DM losses of 11.5% and 8.0% respectively, whereas milk DC (398.4 g/ kg DM) and wilted silages (427.0 g/ kg DM) had only 3.7% and 4.7%, respectively, DM losses. Flowering DC and wilted silage were stable during aerobic exposure. Milk DC silages were stable but wilted silages slightly tended to spoil for 7 day of aerobic exposure (AE), having higher number of yeasts and molds and increased CO_2 production.

In cultivar Galil, flowering DC silages (199.2 g/ kg DM) had 11.8% ensiling DM losses but were stable during aerobic exposure. Inoculation resulted in aerobic deterioration, having 149.6 g/ kg DM CO_2 production, 12.7 % DM losses and heating to 32^{O}C during 4 day of AE. Flowering wilted silages were unstable during 7 day of AE, inoculation did not result further deterioration. Milk DC silages (299.2 g/ kg DM) had ensiling losses of 8.9% DM and were unstable after 7 day of AE, showing 67.2 g/ kg DM CO_2 level, 8.8% DM losses and 31^{O}C temperature. Inoculation further increased deterioration resulting in 82.3 g/kg DM CO_2 production. Milk wilted silages (437.3 g/ kg DM) had ensiling losses of 1.7% DM and showed higher level of spoilage both on day 4 (34.0 g/ kg DM CO_2 and 4.5% DM losses) and 7 (133.2 g/ kg DM CO_2, and 12.9% DM losses) of AE and inoculation further increased deterioration. It is concluded that in wheat silages, cultivar, stage of maturity at harvest, wilting, DM content affect the ensiling fermentation, silage composition, and aerobic stability. Stepwise regression analysis indicated that levels of acetic acid had a marked effect on wheat silage aerobic stability.

Sorghum silages (305.6 g/ kg DM) had higher ensiling losses of 11.0% DM whereas corn silages (319.4 g/ kg DM) had only 0.9% DM. Sorghum silages were more stable during aerobic exposure (7.4 g/kg DM CO_2 after 7 days), but corn silages were unstable after day 4 (38.3 g/ kg DM CO_2) and 7 (47.7 g/ kg DM CO_2) of AE. The aqueous extracts from corn and sorghum, silages contained 13.5 and 28.1 mg/ml polyphenols, respectively. As expected, spraying of corn silage extracts on sorghum silages further enhanced the aerobic deterioration of sorghum silages (15.6 g/ kg DM CO_2 and 3.9% DM losses on day 7). Unexpectantly, spraying of sorghum silages extract on corn silages did not improve the aerobic stability of corn silage, in stead, it further enchanced deterioration (101.0 g/ kg DM CO_2 on day 7). Although sorghum silages were more stable than corn silage and their extract contained higher

concentrations of polyphenols, spraying of sorghum silages extract did not improve the aerobic stability of corn silage. Further study is needed to explore the factors or compounds behind the aerobic stability of sorghum silages and to find a suitable way to exploit and utilize such compounds for the aerobic stability improvement of aerobically suceptible silages like corn.

Key words: Silage, wheat, corn, sorghum, DM losses, aerobic stability

Table of Contents

Topics **Pages**

Abbreviations:

AAB – Acetic Acid Bacteria

ADF – Acid Detergent Fiber

ADL – Acid Detergent Lignin

ADIN – Acid Insoluble Nitrogen

AFP – Antifungal Protein

BC – Buffering Capacity

BH – Bet Hashita

BOD – Biochemical Oxygen Demand

CFU – Colony Forming Units

DC – Direct-Cut

DM – Dry Matter

DMD – Dry Matter Digestibility

DMI – Dry Matter Intake

GAP – Glyceraldehydes Phosphate

LAB – Lactic Acid Bacteria

NDF – Neutral Detergent Fiber

NDFD – Neutral Detergent Fiber Digestibility

NPN – Non-Protein Nitrogen

NSC – Non-Structural Carbohydrates

NSP – Non-Starch Polysaccharides

OB – Oxygen Barrier

PPB – Propionic Acetic Bacteria

TMR – Total Mixed Ration

VFA – Volatile Fatty Acids

WSC – Water-Soluble Carbohydrates

List of Figures

List of Tables

I. INTRODUCTION

1. Silage making

Silage making is a method of moist forage preservation that is based on natural fermentation whereby lactic acid bacteria (LAB) convert water soluble carbohydrates (WSC) to organic acids, mainly lactic acid, under anaerobic conditions (Weinberg et al., 2002). The process of making silage is referred to as ensiling, which is achieved by compacting the chopped crops in a sealed structure, or a container called silo. The major aim of silage conservation is to maintain nutritional value, mainly fiber, non-structural carbohydrates, and protein as much as possible similar to the nutrients in the fresh plants before ensiling.

In modern agriculture, ensiling has become a popular technology for the preservation of different forage crops. This is because large amounts of forage and fodder crops are available only in particular seasons. Therefore, forages can be efficiently preserved as silage and can be provided to animals throughout the year to meet the nutritional needs of ruminants. Although forages can be preserved as hay, ensiling is less dependent on weather, and wider ranges of crop species, including those with thick stems may be ensiled. In addition, no feeding problems to animals, no need of feed processing, and harvesting of crops at less mature stage of growth are some of the advantages of well-preserved silage.

Silage making is a multi-step process, which is affected by many factors, such as stage of maturity of the forage at harvest, the type of fermentation that occurs in silo and type of storage structure and methods of harvesting and feeding. Furthermore, biological processes during ensiling such as plant respiration, plant enzyme activity, buffering capacity (BC) and aerobic microbial activity as mentioned by Muck (1988) are the important factors that may have negative effects on ensiling of a crop, which will be discussed later. Harvesting of crops, wilting, chopping, use of additives, transportation of crops to silo, compaction in silo, sealing and feeding are the important steps in silage making; a brief description on them is given below.

Silage making process combines both field operations and fermentation processes in silo. Harvesting of crops at right stage of maturity is the first step during ensiling. Stage of maturity affects chemical composition of crops such as moisture content, concentration of fermentable substrate in the form of WSC, fiber content and

buffering capacity. Sometimes the crop is picked up immediately after harvesting, transported to silo and ensiled; this is referred to as direct-cut (DC) silages. In other cases, harvested biomass is left in the field to wilt, prior to being picked up in order to increase the dry matter (DM) content of crops and the resulting silage is called pre-wilted silages. DM content is one of the important factors that affect the amount of effluent produced during storage (Weinberg and Ashbell, 2003). Therefore, wilting plays an important role for making high quality silage by obtaining DM content concentration to a desired level (usually 300 to 400 g kg^{-1}) that ultimately ensures an achievement of good fermentation and elimination or reduction of effluent losses.

Desired size of ensiling materials is obtained by chopping forage biomass before ensiling and it is the last field operation in the process of ensiling. Along with effective consolidation of ensiling materials, chopping increases the surface area of the plant particles for the fermentation and sap exudate supplies necessary nutrients for the fermenting bacteria. The fineness of chop of forage can also alter the effectiveness of forage fiber for maintaining and stimulating adequate chewing activity and ultimately proper rumen function. This is referred to as physically effective particle size (Mertens, 1997). After chopping, silage additives for example urea, ammonia, bacterial inoculants, enzymes, acids etc. can be used to increase the nutritional value of silage, to improve fermentation and to enhance aerobic stability of silages. There are several categories of silage additives: fermentation inhibitors, fermentation enhancers, inhibitors of aerobic spoilage, nutritional enhancers, and absorbents for low DM silages.

The next step in the ensiling process is to transport the already chopped crop from field to silo by a variety of vehicles. Although it is simple process, it has a significant effect on total cost of ensiling because of the high moisture content and the low density of the chopped crop (Weinberg and Ashbell, 2003). After transportation of chopped materials to the silo, filling should be done as soon as possible to expel air from crop particles and to minimize the losses resulting from plant respiration and activity of aerobic microorganisms. Compaction also affects DM losses of silage by changing the silage density. Higher DM losses of silage have been reported with lower silage density (Pitt, 1986). After transportation, ensiling materials are filled into the silo and compacted well to remove air and avoid further air penetration. When compaction is finished and optimum height of crop in the silo is achieved, the next

step in the ensiling process is covering and sealing with plastic sheeting of various thickness (0.1-0.2 mm) which protects penetration of air into the silage surface. Recently an oxygen barrier (OB) plastic has been developed as a sealing material, which minimizes top losses (Holmes and Bolsen, 2009). After the sufficient storage time, prepared silage is taken out from the silo and fed to animals. It should be done very carefully exposing required amount of silages for shorter time as possible to avoid yeasts and mold growth.

1.1. The biochemical processes during ensiling (fermentation pathways):

Quality of silage is largely affected by the type of fermentation that occurs during silage preservation. Therefore, a good knowledge about different fermentation processes is important for the manipulation, control, maintenance, and improvement of silage quality. The main fermentation process that occurs during ensiling is the production of lactic acid from WSC, although, volatile fatty acids (VFA) such as acetic, propionic and butyric acids are sometimes also produced. As a result, the pH of ensiled plant material decreases and then moist forage is preserved, given no air penetrates into the silage. This is because reduction in pH prevents the growth of acid intolerant spoilage microorganisms (Woolford, 1984). The ensiling process can be divided into four distinct phases: aerobic, fermentation, stable, and feedout.

1.1.1. Aerobic phase or plant respiration:

Just after ensiling, air is still trapped between the plant particles and the plant biomass continues to respire and produces CO_2, water and heat by using sugars and oxygen. Therefore, before the depletion of air from the silage, there is continuous metabolism (mainly respiration and proteolysis) of plant cells from the activity of plant enzymes (McDonald et al., 1991). During the aerobic phase, aerobic microorganisms (both obligate and facultative) such as yeasts, molds and some bacteria are still active until oxygen is depleted and the pH begins to decrease. Another important change that occurs during the early stage of ensiling is proteolysis *i.e.* breakdown of plant proteins by the hydrolysis of peptide bonds or de-amination. The acid environment developed in silage eventually reduces the activity of enzymes that break down proteins. In this phase, the prolonged aerobic respiration can induce substantical respiratory heating and DM loss, which may have negative impact on fermentation.

1.1.2. Fermentation Phase:

(a) Lactic acid fermentation:

Once oxygen is eliminated from the silage mass, an anaerobic fermentation starts where the growth of lactic acid producing bacteria occurs. In the beginning of this phase, different facultative and obligate anaerobic microorganisms such as enterobacteria, clostridia, certain bacilli, and yeasts can theoretically compete with the LAB flora for the nutrients (Pahlow et al., 2003). LAB ferment soluble carbohydrates and produce lactic acid as an end product, thus resulting a drop in pH. The disappearance of enterobacteria and the development of a dominant LAB population are the major microbial change during this step in properly ensiled and successfully fermented silages (Pahlow et al., 2003).

Lactic acid fermentation is the most efficient and desirable fermentation that results in fast decrease in the pH of the silage, thus minimizing fermentation losses. When sufficient lactic acid is produced, all microbial activity is suppressed, primarily through the effect of undissociated lactic acid, and the silage can be stored until required for feeding under anaerobic conditions (Rooke and Hatfield, 2003).

(b) Lactic acid bacteria (LAB):

LAB are gram positive, non-sporulating rods or cocci, catalase negative, usually non-motile, acid tolerant, fermentative (lactic acid as the major product) and prefer growing under anaerobic conditions but are aerotolerant. *Lactobacillus, Pediococcus, Leuconostoc, Enterococcus, Lactococcus* and *Streptococcus* are 6 important genera of LAB that are related with silage (Pahlow et al., 2003).

Depending on how LAB ferment hexose sugars they can broadly be divided into two major categories: homofermentative and heterofermentative LAB. Homofermenters produce from hexose sugars just lactic acid and include some species of *Lactobacillus* like *L. plantarum, Pediococcus species* and *Enterococccus species* (Contreras-Govea and Muck, 2006). Obligate homofermentative LAB ferment hexoses almost exclusively (>85%) to lactic acid but they are not able to ferment pentoses due to lack of phosphoketolase enzyme. Homofermenters contains enzyme aldolase with ability to ferment glucose more directly to lactic acid.

The other category, heterofermenters produce lactic acid, acetic acid or ethanol and carbon dioxide (Contreras-Govea and Muck, 2006). The heterofermentative LAB

includes the genera *Leuconostoc* and few species of *Lactobacillus* e.g. *L. buchneri.* Heterofermenters use an alternate pentose phosphate pathway to convert six carbon sugars (hexoses) to five carbon sugars (pentoses) by the enzyme phosphoketolase (Carr et al., 2002). The resulting pentose-5-phosphate is cleaved into one molecule of glyceraldehydes phosphate (GAP) and one molecule of acetyl phosphate. GAP is then further metabolized to lactate as in homofermentation and acetylphosphate is reduced to ethanol via acetyl CoA and acetaldehyde intermediates.

(c) Clostridial or butyric acid fermentation:

Clostridia are the major group of microorganisms causing silage deterioration. If the fermentable substrate is limited or inefficient fermentation, enterobacteria may not be suppressed and clostridia can proliferate in ensiled material (Rooke and Hatfield, 2003). They are active in moist silages (<30% DM) with high BC. This insufficient acidification may lead to a secondary fermentation during the ensiling process. Then, residual plant sugars as well as the initially formed lactic acid and acetic acids are fermented to butyric acid by saccharolytic clostridia; the common saccharolytic clostridia species include *C. tyrobutyricum* and *C. butyricum* (Pahlow et al., 2003). Then butyric acid produced by proliferation of clostridia leads to rising in pH because butyric acid is weaker than either lactic acid or acetic acid (the pK values of lactic, acetic and butyric acids are 3.8, 4.76, and 4.82, respectively). With increase in pH, this condition favors the growth of proteolytic clostridia which are less acid tolerant than saccharolytic clostridia (Woolford, 1984). These proteolytic clostridia ferment proteins and amino acids into amines, amides and ammonia, further increasing pH of the ensiled forage. The proteolytic clostridia species include *Clostridium bifermentans* and *Clostridium sporogenes,* whereas *Clostridium perfringens* (syn. *welchii*) shows both saccharolytic and protelolytic properties (Woolford, 1984). The clostridia are important for dairy industries as they pass from the ruminant's digestive tract to the milk and spoil semi-hard cheeses. For example the nature of *Clostridium tyrobutyricum* to convert lactic acid into butyric acid and ability of its spores to survive pasteurization enables it to grow in semi-hard cheeses resulting in the development of off-flavors and excessive gas formation (the late-blowing defect) (Vissers et al., 2006).

1.1.3. Storage:

During storage the silage is mainly stable and little changes occur given the silo is well sealed and no air penetrates into the silage. In this phase, highly acid tolerant yeast species can survive in an inactive stage, along with bacilli and clostridia.

1.1.4. Feedout:

During feedout, the silage is re-exposed to air enabling undersirable aerobic microorganisms, particulary yeasts, molds, and acetic acid bacteria (AAB) to become active and spoil the silage. The presence of air allows these organisms to multiply, resulting heating of silage and bringing chemical changes such as a rise in pH and loss of nutritive matters.

1.2. Plant fibers and digestibility:

Digestibility in forage is associated by cell wall composition and strucutre. During primary growth of cell wall formation, cell wall is a composite structure composed of polysaccharides, proteins and phenolic acids in which pectins, xylans (hemicellulose) and cellulose are all deposited but not lignin. Once cell enlargement is completed, secondary wall is formed which is composed of cellulose, hemicellulose and lignin. Lignin is a complex indigestible substance and it is considered as a major structural component of mature plants. Cell walls are partially digested by ruminal microorganisms and affect forage intake and digestibility. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) are the important measures to determine the quality of forage crops, which are analyzed according to the method proposed by Van Soest et al. (1991).

NDF gives a close estimate of fiber constituents of feedstuffs as it measures the total amount of cellulose, hemi-cellulose, lignin, silica, tannins, and cutins and some of the pectin. Enhanced NDF digestibility of forage has been shown to significantly increase the DMI and milk yield (Oba and Allen, 1999). The NDF has been shown to be negatively correlated with DMI, as the NDF in forages increases, animals are able to consume less forage (Hayirli et al., 2002). ADF is a fiber measurement extracted with acidic detergent and includes fibrous, least digestible portion of roughage. It consists of the highly indigestible parts of the forage, including lignin, cellulose, silica, and insoluble forms of nitrogen (ADIN) and acid-insoluble ash.

Chemically, lignin is a condensed phenylpropanoid polymer of high molecular weight derived primarily from three cinnamyl alcohols (monolignols): p-coumaryl, coniferyl,

and sinapyl alcohols. From nutritional point of view, lignin is considered as a negative index of forage quality in ruminants. Lignin itself is covalently linked to at least hemicellulose and, probably also to cellulose (Van Soest, 1982). The cross linking of lignin with other cell wall components minimizes the accessibility of cellulose and hemicellulose to microbial enzymes.

2. Silage aerobic stability:

The effective preservation of forage crops as silage depends upon the ability of LAB to produce sufficient acid that inhibits activity of undesirable microorganism under anaerobic conditions (Cai et al., 1999). The degree of anaerobiosis reached in the sealed silo is the most important single factor influencing the preservation efficiency of forage ensiling, whereas air is detrimental to silage because it enables spoiling microorganisms to become active (Woolford, 1990). Aerobic stability is a term that indicates the length of time that silage remains cool and does not spoil after it is exposed to air. At the start of fermentation, residual air, which is still trapped between the silage particles, allows the respiration process to continue using sugars essential for acid production and that process produces heat and increased temperature (Kunkle et al., 2006). If the temperature exceeds $50^{O}C$ due to aerobic activity, that could result the formation of Maillard products (Goering, 1973), which reduces protein digestibility (Muck et al., 2003). Such an effect is called 'browning' or Maillard reaction. Furthermore, exposure of forage mass to air prolongs the activity of undesirable aerobic microbes such as yeasts and molds, which thrive in air and delay the growth of most desirable lactic acid producing bacteria, resulting in deterioration of silage quality. During unloading (removal of silage for feeding) of commercial silages, air penetrates 1-2 m from the face (unloading side) into the silage (Weinberg and Ashbell, 1994). Therefore, silage particles are practically exposed to air 3-5 days before feeding (depending upon silage removal rate, 20-40 cm per day). In addition, rapid removal of air from the forage mass and prevention of air infiltration into the silage during storage and feedout are the important factors that determine the quality of the silage. Therefore, oxygen trapped during ensiling, penetration of oxygen into silo mainly due to improper sealing during storage and exposure of silage during unloading and feedout are the responsible factors that result in aerobic deterioration of silage. At feeding, air penetrates the silage and promotes the growth of aerobic, acid-

tolerant microorganisms, such as yeasts and molds, which oxidize the fermentation products present in the silages (Danner et al., 2003).

2.1. Microorganisms related with silage aerobic instability:

Yeasts and molds in silage are the major microorganisms, which are related with aerobic deterioration of silages (Woolford, 1990). During aerobic exposure, yeasts are the primary microbes that assimilate lactic acid in the presence of air, resulting in an increase in pH and spoilage of the silage. Yeasts are unicellular, eukaryotic, heterotrophic microorganisms classified in kingdom fungi. *Candida, Pichia (=Hansenula), Issatchenkia and Saccharomyces* are the major species of the yeasts that cuase such spoilage in silage (Woolford, 1990; Inglis et al., 1999). In whole-crop maize silage, just after 2 weeks of ensiling, many yeast species such as *Candida holmii, C. lambica, C. milleri, Hansenula anomala* and *Saccharomyces dairensis* have predominately been identified (Middelhoven et al., 1990). In addition, many new yeast species are being found in silages. For example, a new yeast species: *Kazachstania aerobica* sp. nov., with close phyologenetic relationships to *K. servazzii* and *K. unispora*, has been identified from aerobically deteriorating corn silage (Lu et al., 2004). Another yeast *Saccharomyces bulderi* sp. nov. also has been identified from maize silage as a new yeast species which is closely related with *S. barnettii* and *S. exiguus* (Middelhoven et al., 2000). Some yeasts species present in silage have capability of lactic acid and acetic acid assimilation. The degree or extent to which yeast can metabolize lactic acid is of great concern because high production of lactic acid is presumed to be the goal of silage fermentation.

Molds are similar to yeasts besides that they form multicellular filamentous colonies. Most yeasts and molds are aerobic and require oxygen for their growth but they prefer different ranges of water activity (a vapor pressure of water above a sample divided by that of pure water at the same temperature; dimensionless quantity) for their growth. Moisture requirement for mold is quite low (water activity of 0.85 or less) but yeasts require slightly higher water activity. Yeasts and molds are generally reproduce asexually by budding and occasionally some species reproduce by binary fission or producing spores. During fermentation, moderate growth of yeast occurs until oxygen is expired in silage and at feedout, yeasts are re-exposed to oxygen and then their growth becomes exponential. Various facultatively anaerobic and acid-tolerant yeasts are also involved in the process of silage fermentation. Yeasts ferment

sugars to ethanol and CO_2 that reduces the sugars availability for acid production and increases DM loss during ensilage (Lu et al., 2004). Although yeasts and molds are major microorganisms associated with aerobic instability of silages, some other microorganisms may also be involved in the silages during aerobic exposure. The degradation of lactic acid and resulting increase in pH also allow opportunistic bacteria (e.g. *Bacilli*) and molds (e.g. *Aspergillus*, *Fusarium*, and *Pencillium*) to grow and further deteriorate the quality of silage (McDonald et. al., 1991). In addition, Spoelstra et al. (1988) showed the role of AAB, genus *Acetobactor* for the onset of aerobic deterioration of maize silages. These microbes have the capacity to oxidize ethanol to acetic acid and further degrade lactic and acetic acids to carbon dioxide and water, causing spoilage in silage. Although clostridia are strictly anaerobic bacteria, studies have shown that their spore outgrowth can take place during aerobic deterioration of silages (Pahlow et al., 2003; Tabacco et al., 2009). The coexistence of aerobic and anaerobic niches in the silage where clostridia profit from the oxidation of the preserving acids by aerobic microorganisms could be a reason for clostridial growth during aerobic deterioration (Jonsson, 1989).

2.2. Consequences of silage aerobic instability:

Prolonged exposure or infiltration of air during storage or feedout into the silage can lead to aerobic spoilage. The carbohydrates and organic acids are respired due to aerobic microbial activity resulting in the loss of DM and energy along with the production of heat (Muck, 1988). Ranjit and Kung (2000) showed that DM losses from corn silage exposed to air for just 1 to 2 days was found to be as high as 6%. Ashbell and Kashanchi (1987) studied the in-silo losses from wheat ensiled in bunker silos in a subtropical climate. They reported that mean DM losses in the middle of the bunker were between 2.8 to 16%; near the walls between 10.1 and 22.7%; in the cover layer (middle) between 13.9 and 26.7% and in the shoulders (the upper corner), it was between 20.4 and 75.8%. These zones represent different suceptibility to air exposure. Ashbell and Weinberg (1992) also reported top DM losses from middle and shoulder part of horizontal bunker silos, ensiled with wheat and corn. Chen and Weinberg (2009) showed that DM and NDF digestibility of silage samples taken from the shoulders of bunker silos were substantially lower as compared with that of samples taken from the center or near the walls.

Apart from the economic loss of nutrients, a depression in nutrient intake and a decrease in production have been obtained while spoiled silage was fed to ruminants although exact causes of reduced intake and performance are not fully understood. The products produced by detrimental yeasts might alter rumen fermentation, the direct consumption of spoiled nutrients may reduce performance, and undesirable end products (e.g. mycotoxins) produced from further spoilage by molds and other organisms may also be problem (Kung, 2005).

2.3. Factors affecting aerobic stability:

In the fermentation processes, high concentrations of lactic acid are highly desirable because it quickly drops the pH of silage but it has poor antifungal properties. On the other hand, acetic and propionic acids have good antifungal attributes (Moon, 1983), whose concentrations can be increased by adding the acids or using specific microbial inoculants, such as *L. buchneri.* Although butyric acid, the end product of clostridial fermentation, is one of strongest antifungal acids, it is not desirable in silage fermentation because of the other detrimental effects (*i.e*. large DM loss and protein degradation) (Kung, 2005). In addition, length of fermentation may also affect on aerobic stability of silages during aerobic exposure. Gonzalez and Rodriguez (2003) showed a higher instability in round bale silages exposed to air after long period of fermentation (100 d as compared with 53 d). However, Rodriguez (1996) (cited by Gonzalez and Rodriguez; 2003) showed the less stability of sorghum silage exposed to air after 40 d of fermentation as compared to 100d.

Abiotic factors such as storage methods, environment temperature, rate of unloading and feedout, and quality of sealing materials affect on silage fermentation, aerobic stability and then silage quality. Gonzalez and Rodriguez (2003) reported that bales stored under shade are less aerobically deteriorated as compared with storage under direct sunlight, showing the greater effect of environmental temperature and direct solar radiation on the microorganisms associated with aerobic deterioration (yeasts and molds). Henderson et al. (1979) also showed the possibility of high temperature in promoting yeast and mold populations. Ashbell et al. (2002) reported the significant effect of storage temperature on the aerobic stability of silages, showing most intensive aerobic deterioration at 30^{O}C. Ashbell and Weinberg (1992) showed that the thicker the plastic sheeting through which air may penetrate into the silage, the smaller the losses in the top parts of the silage. Kim and Adesogan (2006)

showed the detrimental effects of high ensiling temperature and stimulated rainfall on the fermentation process and silage quality. Hence, warm weather is stimulatory to microbial growth so aerobic spoilage is a larger problem during the summer months.

2.4. Assessment of aerobic stability of silages:

An accurate assessment of silage aerboic deterioration is prerequisite before taking any decision on management of problems associated with silage aerobic instability. Some of indicators for aerobic instability of silage can be visualized during farm visits. For example, color of silage, odor (moldy smell or not), visual observation of molds (probably yeasts as well), higher temperature while feeling, moist silage as a result of aerobic activity are some of the parameters for aerobic instability of silage, one could easily observe in a commercial silage farm. Woolford et al. (1977) developed a laboratory scale system to measure the aerobic deterioration of silages, which establishes a relationship among DM losses, temperature rise, and CO_2 production. Henderson et al. (1979) suggested the use of change in pH and temperature as indicators for aerobic deterioration of silage sample. Pahlow (1981) introduced a new technique for the estimation of aerobic stability of silages by measuring their biochemical oxygen demand (BOD). He also measured yeasts and bacterial population, which could initiate aerobic deterioration in silage. Brookes (1990) described a method for the determination of aerobic stability of silage, using infrared gas analyses of metabolically produced CO_2 in a humidified air stream over 5-7 days and number of yeasts was also enumerated. Ashbell et al. (1991) used a simple system constructed from polyethylene terephthalate bottles (used soft drink bottles) to study the aerobic deterioration of silages. This systems measures CO_2 produced in aerobically exposed silage samples due to aerobic activity of yeasts and molds. The system is described in details in the materials and methods section.

From all of the above, it can be concluded that visual appraisal, CO_2 production, temperature, pH, and other chemical and microbiological parameters are the major indicators to study the aerobic stability of silages and extent of aerboic deterioration.

2.5. Management of silage aerobic instability:

Minimizing oxygen exposure to silage is a major essential factor for good quality silages. Therefore, any management practice that helps to exclude oxygen from silages is an important factor for the avoidance and inhibition of growth of yeasts and

molds. Certain yeast species secrete protein toxin that kills sensitive strains of the same and other species of yeasts, which could be important and useful approach to inhibit the growth of many lactate-utilizing yeasts and so prevent silage from aerobic deterioration. For example, the growth of *S. cerevisiae* IFO 0304 was repressed by a killer protein produced by *Kluyveromyces lactis* IFO 1267 (Kitamoto et al., 1993).

The factors such as harvesting of forages at optimum moisture levels, correct of particle size of forage mass, quick filling, consolidation (compaction) and sealing, adequate rate of removal of silage from the silo each day can have positive effects in minimizing silage's exposure to air that would eventually help to maintain the quality of silage (Kung, 2005). Quality of sealing material may also affect on silage aerobic stability. Borreani et al. (2007) showed that use of newly developed OB films reduced DM losses and improved aerobic stability of corn silage as compared with conventional polyethylene films. In addition, many silage additives have been used to improve aerobic stability of silages.

Effects of additives on aerobic stability:

Microbial inoculants:

Type and number of microorganisms that dominate the fermentation process is an important factor in silage making. Silage can be inoculated with specific microorganisms to enhance the speed and efficiency of fermentation that fasten the accumulation of acids and lowers pH at earlier stages of ensiling which ultimately improves forage conservation.

When heterofermentative bacteria are used in silage inoculants, they may improve aerobic stability of silages by producing acetic acid that inhibits yeasts and molds. Ranjit and Kung (2000) showed that corn silage inoculated with $1*10^6$ CFU (colony forming units)/g of *L. buchneri* resulted in a more heterolactic fermentation and dramatically improved its aerobic stability. Driehuis et al. (2001) reported that inoculation of *L. buchneri* with or without homofermentative, LAB enhances the aerobic stability of wilted grass silages. *L. buchneri* also produces 1,2-propandiol during anaerobic degradation of lactic to acetic acid (Oude Elferink et al., 2001), which inhibits fungi. The combination of *L. buchneri* and *L. plantarum* improved the aerobic stability of corn silages (Weinberg et al., 2002; Filya, 2003). Hu et al. (2009) showed that corn silages treated with *L. buchneri* 40788 had higher concentrations of

acetic acid, lower yeast population, and improved aerobic stability as compared with untreated silage regardless of silage DM contents.

On the other hand, in homofermentative fermentation, only lactic acid is produced, on which aerobic yeasts can thrive; they are followed by molds. For example, high level of WSC and lactic acid and low level of VFA in silages inoculated with homofermentative LAB at ensiling were found to be more susceptible to aerobic exposure (Weinberg et al., 1993). Filya (2003) also found that wheat, sorghum and corn silages were aerobically unstable when incoulated solely with *L. plantarum.* Hence, it can be concluded that although homofermentative inoculats improve fermentation characteristics of silage by rapidly decreasing pH, they can enhance the aerobic deterioration of whole crop cereal silages. In contrast, use of heterofermentative inoculants not only improves fermentation characteristics but also show favorable effects on aerobic stability of silages. Few studies have been done on effect of propionic acid bacteria (PAB) on the aerobic stability of silages. Weinberg et al. (1995) reported that PAB (*Propionibacterium shermanii*) can survive in and improve the aerobic stability of only slow-fermenting silages which are prone to aerobic deterioration. In rapid fermenting silage, a fast decrease in pH to below 4.5 may provide unfavorable enviorment to PAB to produce propionic acid (Pahlow and Honig, 1994). Filya et al. (2004) showed an effectiveness of *Propionibacterium acidipropionici* in protecting wheat, sorghum, and maize silages exposed to air under laboratory conditions.

In addition to microbial inoculants, experiments with non-protein nitrogen (NPN), molasses, inorganic and organic acids, and enzyme application have been conducted to study their effects on aerobic stability of silages. In NPN additives, anhydrous NH_3, urea, mixtures of water or molasses and NH_3, or urea and minerals, have been added to forages at the time of ensiling to increase the CP content (as urea or NH_3) and to improve aerobic stability of silages (Kung et al., 2003). Ammonia-N treatment (0.3% fresh wt. basis) improved the aerobic stability of whole-plant corn silage (82 hours) with lower number of yeast and molds as compared with untreated silage (35.3 hours) (Kung et al., 2000). Ashbell and Weinberg (1993) also reported an increment in aerobic stability of wheat and sorghum silage treated with ammonia, but in the same study ammonia treated silage showed enhanced aerobic deterioration of corn silage. A special attention should be paid while applying ammonia because it is a hazardous gas. Along with improvement in fermentation quality of foxtail millet

(*Seteria italica*), molasses treatment also enhanced aerobic stability of silage by inhibiting temperature increase of the silage (Arbabi and Ghoorchi, 2008). Inorganic acids such as hydrochloric acid, sulfuric acid, and phosphoric acid are generally applied to moist crops. Besides their role as acidifying agents, they do not show specific antimicrobial properties (Drysdale, 1987) and sometimes only reduction in pH may not be sufficient to inhibit growth and multiplication of all undesirable microorganisms. Chamberlain and Quig (1987) reported that when the sulfuric acid-treated silage dropped the pH to 3.5, the activities of coliform bacteria were not completely eliminated and yeasts were encouraged to grow.

Although, acidity of organic acids is weaker as compared with inorganic acids (they have higher pK values), organic acids act both as acidifying as well as antimicrobial agents hence provide a good platform in controlling fermentation during ensiling process. The aerobic stability of silages was improved with the application of formic acid (Crawshaw et al., 1981; Keady and Murphy, 1996). Nevertheless, Pitt et al. (1991) showed a shorter duration of aerobic stability (5.75 d) after exposure to air in silage treated with formic acid as compared to untreated silage (8 d). According to Crawshaw et al. (1981), formic acid was effective in reducing aerobic deterioration if bacteria (but not yeasts and molds) were the cause of aerobic deterioration. VFA (acetic, propionic and butyric acids) are antimycotic (Moon, 1983); propionic acid has been studied to inhibit the growth of yeasts and molds, acting as an enhancer of aerobic stability. Selwet (2008) showed that the growth of fungi was inhibited most strongly in corn silage by the mixture of formic acid, propionic acid and ammonium salts thus improved the aerobic stability. On the other hand, formaldehyde treated silage is found to be more sensitive to aerobic deterioration as compared to untreated silage because of its restriction on fermentation. For example, Barry et al. (1980) found that silages treated with formalin at various rates from 5.4 to 9 L/t had restricted fermentation and all of the treated silages show surface spoilage in silos. In addition to above mentioned additives, the effect different enzymes on aerobic stability of silages also have been done. Weinberg et al. (1995) also obtained a higher intensity of aerobic deterioration, when they applied high rates of cell-wall hydrolyzing enzymes on pea and wheat silages (>0.1% on wet basis) which increased the levels of WSC.

2.6. Research needs:

Different aspects of silage aerobic stability such as, factors affecting silage aerobic stability, consequences of aerobic instability, indicators to assess instability and effects of different additives on silage aerobic stability have been mentioned so far. However, there is not enough information on the effects of silage type, cultivar, stage of maturity, DM content and silage composition affect aerobic stability of silage. In this context, this study attempted to quantify losses during aerobic exposure of model silages. In addition, this research mainly foucuses on effect of harvesting wheat at different stages of maturity, type of silages *i. e.* ensiled as DC or wilted to different DM contents and their interactions on magnitude of aerobic deterioration. Hence, the research provided data, which can be useful in decision making for silage management in order to minimize aerobic losses and maintain a quality and nutritive value of silage.

II. RESEARCH OBJECTIVES

1. Goal:

The main objective of the current research was to determine changes and losses in wheat, corn and sorghum silages during aerobic exposure. Specifically, it was planned to determine the effects of different individual factors (cultivar, stage of maturity at harvet, type: DC and wilted, inoculated and control; and silage composition) and their interactive contribution on aerobic stability of wheat silages.

2. Specific objectives:

- To determine the effects of stage of maturity at harvest and wilting on the aerobic stability of wheat silages and on changes in composition, microbial populations and *in vitro* digestibility during aerobic exposure.
- To establish a relationship between silage composition and aerobic stability of wheat silages.
- To establish a relationship between an inoculation (homofermentative LAB) and stage maturity, type (wilted and direct-cut) and DM content of wheat silages.
- To establish a relationship between water activity and DM content of wheat silages.

- To determine changes and losses and the aerobic stability of corn and sorghum silages.
- To determine factors which enhance aerobic stability of corn and sorghum silages.

3. Hypotheses:

(a) The important intrinsic factors of silage such as type of silage (DC and wilted), stage of maturity, cultivar and silage composition (DM, WSC, lactic and VFA) act both separately and interactively to affect the aerobic stability of wheat silages.

(b) Polyphenol compounds in sorghum silages may be the factors responsible for aerobic stability and spraying of aqueous extract of sorghum silages on aerobically susceptible silages like corn could improve their aerobic stability.

III. MATERIALS AND METHODS

1. Experimental:

In June 2008, the following forage crops were used in the study: whole-crop wheat, cultivar Bet Hashita (BH) (early maturing cultivar) at flowering and milk stage, both DC and wilted, corn and sorghum. The crops were chopped to 3-5 cm pieces (using a Wintersteiger® chopper, Ried, Austria) and ensiled in 10 anaerobic glass jars (1.5 liter) which allow one way gas release (Weck®, Wehr-oftlingen, Germany). Wilting was achieved by spreading out a thin layer of freshly harvested crops on nets, which allowed to dry in the sun for 8 hours. Each jar was filled with about 550-700 g (on wet basis) chopped materials without headspace. The jars were stored for 4-6 months at room temperature (26^0C±2) until use.

In 2009 additional ensiling experiments were performed with wheat, cultivar 'Galil' (late maturing cultivar) at the flowering and milk stages of maturity, both DC and wilted, with and without LAB inoculant (*Lactobacillus plantrum* MTD1, Ecosyl, UK). The following treatments were used: 12 jars control (no additives) and 6 jars with the inoculant, in each experiment. The LAB inoculant was applied as follows: on the day of the experiment, 0.2 g of the inoculum powder was suspended in 20 ml distilled-water and sprayed over 5 kg of the chopped forage spread over a 1 × 4 m

area, followed by through mixing. The bacterial counts in the inoculant powder were 9×10^{11} CFU g^{-1} powder. Thus, 3.6×10^{7} CFU g^{-1} fresh crop were applied.

All jars of the same experimental batch were opened on the same day (0). The fresh silages from all jars were analyzed for DM, pH, water activity (a_w), ash, residual WSC, lactic acid and volatile fermentation products. Three jars were sampled also for microbial analysis (LAB, yeast and mold enumeration). The rest of the silages from the other jars were mixed thoroughly and the pooled sample was used for aerobic stability test in bottle systems and by temperature measurement. The pooled sample was distributed to 12 aerobic stability bottle systems according to Ashbell et al. (1991) (Fig. 1) which were incubated at 30^{O}C for 2 to 7 days. Three bottles were sampled on days 2, 3, 4 and 7 for chemical and microbiological analysis in each experiment, except for sprayed corn and sorghum samples, which were sampled on days 4 and 7 only. The analysis included determination of the pH value, CO_2 production and enumeration of yeasts and molds for all samples; determination of lactic acid, VFA, WSC was performed only on days 4 and 7 after aerobic exposure. Temperature measurement was performed twice a day (8 and 16 hours interval) in triplicate on pooled sample by taking about 150 gm of silage in holed plastic bag, which was kept in insulated boxes and connected with to thermocouple.

Aerobic stability test:

For aerobic stability test, the bottles are made of polyethylene terephthalate (Fig. 1). The pooled silage sample was loosely packed (250 – 275 gm on wet basis) in the upper part of the bottles and 100 ml of KOH (25%) solution was placed in lower part of the unit to absorb the CO_2 produced as a result of aerobic activity that may take place in a silage samples exposed to air. The gas CO_2 is 1.5 times denser as compared with air and therefore, sinks to the bottom of bottle from silage samples and is absorbed in the KOH.

To quantify CO_2 produced as a result of aerobic exposure, ten ml of KOH was taken from lower bottle of the aerobic stability testing unit, diluted with 90 ml distilled water, and titrated with 1N HCl solution. Then volume of 1N HCl required to drop pH from 8.1 to 3.6 was recorded and this value was used to calculate the amount of CO_2 produced (gram CO_2 per Kilogram DM).

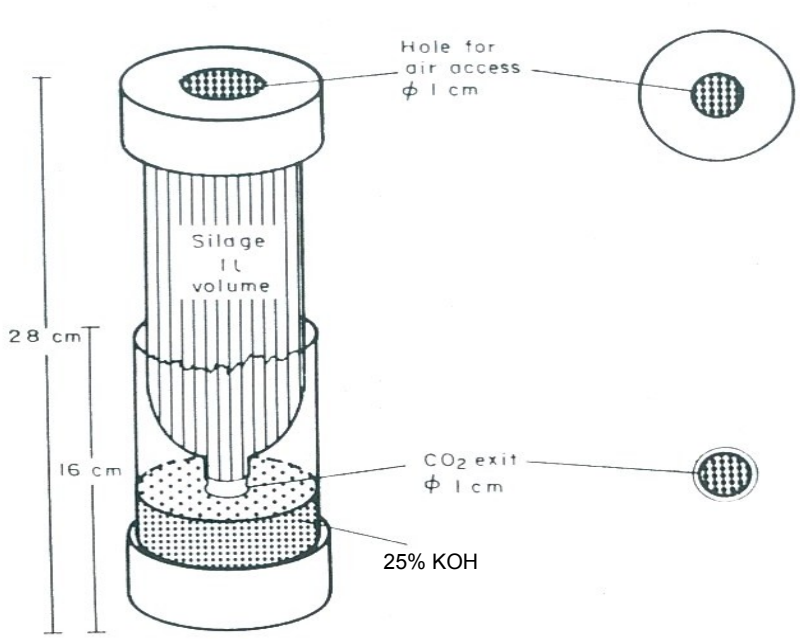

Fig. 1: System for aerobic stability determination (Ashbell et al., 1991).

The chemical reactions that occur during aerobic stability test and titration are given below:

$KOH + CO_2 \longrightarrow K_2CO_3 + H_2O$ (Reaction during aerobic exposure)

(In lower bottle of aerobic stability testing unit) (Produced as a result of aerobic exposure)

$K_2CO_3 + HCl \longrightarrow KHCO_3 + KCl$ (Until pH 8.1)

$KHCO_3 + HCl$ (1N) $\longrightarrow CO_2 + H_2O + KCl$ (Until pH 3.6)

Reactions during titration

The amount of HCl (1N) consumed during titration to expel CO_2 was used in the following formula determine the amount of CO_2 (g/kg DM):

$$CO_2 = 0.044\ T \bullet V / (A \bullet W \bullet DM)$$

Where,

0.044 = The weight of one equivalent of CO_2 (kg)

T = Volume HCl (1N) used in titration (ml),

V = Total volume 25% KOH (usually 100 ml),

A = Volume of KOH sampled for analysis (usually 10 ml),

W = Mass of fresh silage filled in bottle (Kg)

DM = Fraction of dry matter.

Mutual extract spraying experiment:

In order to find out whether aerobically stable silages contain intrinsic stabilizing agents, the following experiments were performed in which aqueous extracts were prepared from corn and sorghum silages; the extracts were used for cross spraying the silages just prior to the beginning of the aerobic stability test. For that end, additional 6 aerobic stability bottles were prepared for corn or sorghum silages. The extracts were obtained at a ratio of 1:4 (50 gm: 200 ml distilled water), which were extracted for 3 min. in a Stomacher blender (Stomacher, model 400, Seward, UAC house, London, UK). The extracts were cross-sprayed on the respective silages at the rate of 6.7% (133 ml extract solution in 2 kg silage) by weight and the sprayed silages were used for aerobic stability test in bottle systems. The extracts were also analyzed for polyphenols as representatives of antioxidants by the method of Kanner et al. (1994).

2. Analytical:

DM was determined by weight difference after oven-drying for 48 h at 60^{O}C. DM losses were calculated from weight and moisture in the original silages and silages that were subjected to aerobic exposure. Ash was obtained after burning for 3 h at 600^{O}C in a muffle oven. The pH value was measured on the filtrate of 40 g wet material blended for 3 min in a Stomacher blender with 360 ml distilled water. Water activity (a_w) was measured by using water activity meter (A_W chamber model enBSK, Novasina AG, Lachen, Switzerland) with 3 different randomly taken samples after opening of jars on day 0 or on the fresh crops in the experiments in 2009. WSC were determined by the phenol sulphuric acid method according to Dubois et al. (1956). Lactic acid was determined by a spectrophotometric method, according to Barker and Summerson (1941). The method was modified in the laboratory to fit lactic acid determination in silages.

Volatile fermentation end-products (ethanol and volatile fatty acids) were determined with a gas chromatograph equipped with a semicapillary nitroterephthalic acid-modified polyethylene glycol column (FFAP, Hewlett-Packard, Waldborn, Germany), over a temperature range of 40 to 230^{O}C according to Weinberg et al. (2009). Polyphenol contents in sorghum and corn extracts were determined according to the Folin-Ciocalteu procedure (Kanner et al., 1994) and calibrated against gallic acid. NDF was assayed with a heat-stable amylase and expressed inclusive of residual

ash (aNDF – NDF) according to Van Soest et al. (1991) with an Ankom fiber analyzer (Ankom Technology, Macedon, NY). *In vitro* DM digestibility (DMD) and NDF digestibility (NDFD) were determined according to the 2-stage fermentation technique of Tilley and Terry (1963), with all samples in triplicate. Rumen fluid was obtained from a ruminally fistulated dry Holstein cow fed 6 kg of DM of wheat hay and 4 kg of DM of TMR (total mixed rations) containing 30% concentrated grains, 35% wheat and corn silages, 15% soybean and sunflower meals, and 20% by-products (cottonseed, wheat bran, and gluten feed).

The microbiological examination included the enumeration of lactobacilli on pour plate Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, UK), and of yeast and molds on spread – plate malt extract agar (Difco) acidified with lactic acid to pH 4.0. Plates were incubated for 3 days at 30^{O}C before the colonies were counted. Total counts of bacteria in sprayed and non-sprayed corn silage during 7 day of aerobic exposure were performed on pour plate plate count agar (Scharlau Chemie, S.A. Barcelona, Spain).

3. Statistical analysis:

One-way analysis of variance was applied to the results of each silage type separately with day as main effect (treatment) in General Linear Model of SAS (SAS, 1982). Within each cultivar, two-way (factorial) analysis of variance was performed for the pooled results of fermentation profiles and aerobic stability parameters from day 4 and 7 with stage of maturity, wilting, inoculation (only in cultivar Galil), and their interactions as main effect. Significant differences between means were identified by using the Tukey's Studentized range test in which $P<0.05$ was decleared as significant. In addition, regression models were estabilished between overall CO_2 production (day 4 and 7 separately), and DM content, acetic and butyric acids, and residual WSC content of silages by using Proc Stepwise of SAS (SAS, 1982). The comparison between composition of sorghum and corn silages were performed by independent t-test where $P<0.05$ was considered as significant. The comparison on results of polyphenol content of sorghum and corn silages were performed by t-test at 5% level of significance level.

IV. RESULTS

1. Wheat silages (cultivar BH):

Tables 1 and 2 summarize the changes and losses in flowering DC and wilted wheat (cultivar BH), from the fresh crop through silage and untill 7-day of aerobic exposure. During ensiling, the pH decreased, WSC content decreased, while organic acid particularly lactic acid increased in both types. Both DC and wilted silages also had increased ash and NDF content. Both silages had contained considerable level of butyric and acetic acids. The DC silages reduced DMD during ensiling but wilted silages did not. In both silage types, they had significant level of DM losses.

During aerobic exposure, both DC and wilted silages were very stable, with no or slight change in silage composition. However, silages, which were aerobically exposed for 7 days, showed a higher CO_2 production without change in pH. Silages did not heat during aerobic exposure, as the silage temperature was not higher than ambient. Negative DM losses obtained during aerobic exposure, may be attributed to technical limitation of DM determination and the un-homogeneous nature of the dried forage samples. Therefore, negative losses are considered as zero. The results of the aerobic stability tests agree with the low numbers of yeast and mold throughtout the duration of the test.

Tables 3 and 4 summarize the changes and losses in the DC and wilted wheat (cultivar BH), from milk stage through silage and until 7-day of aerobic exposure. The major point is that both DC and wilted crops were very dry (>400 g/kg DM). In both DC and wilted, organic acids particularly lactic and acetic increased, but WSC content did not decrease during ensiling. Although pH decreased in both silage types; it was unusually high as 4.7 and 4.9 for DC and wilted, respectively. NDF content, DMD and NDFD did not change significanlty in both types of silages during ensiling whereas ash content increased only in the DC silages. Ensiling DM losses were negative in both types, and were considered as zero.

During aerobic exposure, both DC and wilted silages exposed to air were very stable until day 4 of aerobic exposure, with no or little change in silage composition. However, silages which were aerobically exposed for 7 days showed a higher CO_2 production in the DC silage and unsignificantly higher in the wilted silage as compared to their repective silages exposed for days 2, 3 and 4. During day 7, DC

Table 1. Changes in chemical and microbiological composition during ensiling and aerobic exposure of wheat silages from the flowering stage, direct-cut and wilted (cultivar BH).

Chemical parameters are in g/kg DM and microbiological data are in log CFU/g DM (means±s.d.)

Type	Day	pH	LAB	Yeasts	Molds	Ash	WSC	LA	Ethanol	Acetic acid	Propionic acid	Butyric acid
Direct-cut	Fresh crop	6.1±0.1^{a}	1.9^{a}	3.8^{a}	6.0^{a}	62.5^{b}	136.5^{a}	-	-	-	-	-
	Silage	4.3±0.1^{b}	3.2±0.9^{a}	0.73±1.3^{b}	NF	75.9±2.0^{a}	40.6±11.1^{b}	35.7±8.5^{a}	8.9±5.0	8.4±2.5	1.8±1.1	16.3±2.6
	AE2	4.2±0.1^{b}	-	NF	NF	73.9±0.8^{a}	-	-	-	-	-	-
	AE3	4.3±0.0^{b}	-	NF	1.5±1.3^{b}	74.0+2.6^{a}	-	-	-	-	-	-
	AE4	4.3±0.0^{b}	-	NF	NF	75.8±0.3^{a}	31.7±9.8^{b}	44.2±8.4^{a}	5.6±1.9	5.5±0.4	0.8±0.2	11.9±1.4
	AE7	4.3±0.0^{b}	-	NF	1.7±2.6^{b}	75.9±2.6^{a}	32.1±22.2^{b}	20.0±4.5^{b}	5.1±1.8	5.2±1.1	0.8±0.2	11.5±3.9
Wilted	Fresh crop	6.4±0.1^{a}	NF	4.2^{a}	5.6^{a}	62.7^{b}	73.40	-	-	-	-	-
	Silage	4.4±0.1^{c}	4.3±0.3^{a}	NF	NF	70.6±3.2^{a}	43.8±17.4	27.4±2.8	8.1±5.0	6.2±2.0	0.9±0.9	4.2±1.2
	AE2	4.6±0.0^{b}	-	NF	0.7±1.2^{b}	-	-	-	-	-	-	-
	AE3	4.4±0.0^{c}	-	NF	NF	-	-	-	-	-	-	-
	AE4	4.4±0.0^{c}	-	NF	NF	70.1±1.1^{ab}	42.2±1.4	33.6±1.3	7.4±3.8	5.1±0.2	1.5±1.1	5.5±0.1
	AE7	4.4±0.0^{c}	-	NF	NF	70.8±1.0^{a}	44.6±5.6	32.1±7.4	3.4±1.1	4.2±0.5	1.2±0.8	4.5±1.1

Within each column and silage type, means followed by different letters are significantly different (P<0.05).
WSC, water-soluble carbohydrates; LA; lactic acid; LAB, lactobacilli; NF, not found; AE, aerobic exposure.

Table 2. Losses during ensiling and aerobic exposure of wheat silages from flowering stage, direct cut and wilted (means±s.d.)

Type	Day	CO_2 (g/kg DM)	DM (g/kg)	% DM loss	NDF (g/kg DM)	DMD (g/kg DM)	NDFD (g/kg DM)
Direct-cut	Fresh crop	-	316.0^{a}	-	558.5±5.1^{b}	722.9±25.3^{a}	542.9±36.1
	Silage	-	287.0±3.9^{c}	11.5±1.2^{a}	581.9±9.4^{a}	676.8±13.1^{b}	549.9±12.3
	AE2	0^{b}	291.9±2.1^{bc}	0.0^{b}	-	-	-
	AE3	1.9±0.4^{ab}	291.9±2.1^{bc}	0.0^{b}	-	-	-
	AE4	0.5±0.4^{b}	299.3±5.2^{b}	0.0^{b}	582.5±5.1^{a}	693.6±6.5^{b}	571.3±14.5
	AE7	2.7±1.4^{a}	296.4±1.6^{bc}	0.0^{b}	585.3±6.8^{a}	687.4±10.0^{b}	561.9±5.4
Wilted	Fresh crop	-	385.0^{a}	-	573.9±1.7	663.6±0.9	521.6±0.8
	Silage	-	362.2±6.8^{b}	8.0±2.0^{a}	585.1±10.6	676.5±33.9	537.7±32.3
	AE2	0.4±0.3^{b}	371.8±2.3^{ab}	0.0^{b}	-	-	-
	AE3	0.3±0.3^{b}	367.7±0.8^{b}	0.0^{b}	-	-	-
	AE4	0.6±0.3^{b}	369.3±5.1^{ab}	0.0^{b}	587.8±2.0	687.8±10.2	566.8±12.4
	AE7	1.8±0.2^{a}	373.6±4.6^{ab}	0.0^{b}	590.1±4.0	674.0±21.2	554.3±23.6

Within each column and silage type, means followed by different letters are significantly different (P<0.05).
DMD, dry matter digestibility; NDFD, NDF digestibility; AE, aerobic exposure.

Within column % DM loss, negative values obtained for day AE2 to AE7 for both direct and wilted are considered as zero.

Table 3. Changes in chemical and microbiological composition during ensiling and aerobic exposure of wheat silages from the milk stage, direct-cut and wilted (cultivar BH)

Chemical parameters are in g/kg DM and microbiological data are in log CFU/g DM (means±s.d.)

Type	Day	pH	LAB	Yeast	Mold	Ash	WSC	Lactic acid	Ethanol	Acetic acid	Propionic acid	Butyric acid
Direct-cut	Fresh crop	6.32^{a}	NF	3.1^{abc}	5.2^{a}	54^{b}	87.8	-	-	-	-	-
	Silage	4.7±0.1^{b}	5.8±0.3^{a}	NF^{c}	0.7±1.2^{b}	63.7±2.5^{a}	92.2±8.8	16.4±2.3	11.2±2.4^{a}	6.0±1.9	0.61±0.6	4.0±1.0
	AE2	4.7±0.0^{b}	-	NF^{c}	0.7±1.2^{b}	-	-	-	-	-	-	-
	AE3	4.7±0.1^{b}	-	0.9±1.5^{bc}	0.7±1.2^{b}	-	-	-	-	-	-	-
	AE4	4.7±0.0^{b}	-	3.6±0.3^{a}	0.7±1.2^{b}	63±2.1^{a}	68.4±18.5	13.1±3.8	6.2±1.6^{b}	4.0±0.3	0.14±0.1	3.8±0.7
	AE7	4.7±0.0^{b}	-	2.1±1.9^{bc}	NF	62.3±1.3^{a}	77.8±12.3	14.8±2.5	5.1±1.9^{b}	4.5±1.5	0.37±0.1	3.7±0.8
Wilted	Fresh crop	6.17^{a}	NF	4.3^{d}	4.6^{b}	62.6	63.4^{b}	-	-	-	-	-
	Silage	4.9±0.1^{b}	6.5±0.4^{a}	NF^{e}	NF	66.9±2.1	101.9±9.6^{a}	14.9±2.8	8.8±2.6^{a}	5.7±1.0^{a}	0.39±0.6	0.66±0.6
	AE2	4.9±0.1^{b}	-	6.2±0.1^{c}	NF	-	-	-	-	-	-	-
	AE3	4.8±0.1^{b}	-	6.9±0.1^{b}	0.9±1.6^{c}	-	-	-	-	-	-	-
	AE4	4.9±0.0^{b}	-	7.4±0.2^{ab}	3.1±0.5^{b}	65.8±1.5	91.6±9.8^{a}	13.2±1.6	5.7±0.2^{a}	4.9±0.5^{ab}	0.0	0.0
	AE7	5.0±0.0^{b}	-	7.6±0.4^{a}	7.1±0.3^{a}	67.1±1.1	78.0±7.9^{ab}	11.9±3.1	0^{b}	3.6±0.8^{b}	0.0	0.0

Within each column and silage type, means followed by different letters are significantly different (P<0.05).
WSC, water-soluble carbohydrates; LAB, lactobacilli; NF, not found; AE, aerobic exposure.

Table 4. Losses during ensiling and aerobic exposure of wheat silages from milk stage, direct cut and wilted (means±s.d.).

Type	Day	CO_2 (g/kg DM)	DM (g/kg)	% DM loss	NDF (g/kg DM)	DMD (g/kg DM)	NDFD (g/kg DM)
Direct-cut	Fresh crop	-	406±5.3	-	576.2±3.4	663.9±9.0	491.5±11.8
	Silage	-	398.4±6.4	$3.7±1.6^{a}$	570.7±15.0	651.0±19.7	473.5±25.5
	AE2	$0.5±0.5^{b}$	413.6±1.5	0.0^{b}	-	-	-
	AE3	$0.2±0.3^{b}$	416.1±2.6	0.0^{b}	-	-	-
	AE4	$0.6±0.1^{b}$	420.0±3.7	0.0^{b}	565.1±6.4	647.0±10.0	454.2±6.2
	AE7	$3.3±1.4^{a}$	420.9±3.2	0.0^{b}	566.6±2.1	654.2±3.8	473.2±6.9
Wilted	Fresh crop	-	$442±3.6^{ab}$	-	$551.0±6.7^{b}$	$679.3±4.1^{a}$	497.3±5.6
	Silage	-	$427.0±8.3^{b}$	$4.7±1.9^{a}$	$549.1±5.8^{b}$	$656.2±9.6^{ab}$	472.8±36.4
	AE2	1.0±0.1	$446.2±0.9^{a}$	0.0^{b}	-	-	-
	AE3	0.2±0.3	$446.3±2.5^{a}$	0.0^{b}	-	-	-
	AE4	1.3±0.3	$452.2±4.3^{a}$	0.0^{b}	$549.1±6.2^{b}$	$650.1±17.2^{b}$	449.0±16.4
	AE7	40.1±41.1	$439.8±3.2^{ab}$	0.0^{b}	$567.0±8.6^{a}$	$645.6±19.2^{b}$	460.2±12.3

Within each column means followed by different letters are significantly different ($P<0.05$).
DMD, dry matter digestibility; NDFD, NDF digestibility; AE, aerobic exposure.
Within % DM loss, negative values obtained for day AE2 to AE7 for both the direct-cut and wilted are considered as zero.

silages did not change in pH and did not heat as the silage temperature was similar or not higher than the ambient. Wilted silages had increased NDF content and had higher temperature than ambient along with higher numbers of yeasts and molds. Number of yeasts and molds agree with the CO_2 production in the wilted silages.

Table 5 summarizes the effect of stage of maturity and wilting on the composition of freshly harvested wheat crop and silage throughout aerobic exposure (cultivar BH). For the aerobic exposure parameters, pooled results from days 4 and 7 were used. Crops harvested at milk stage had higher DM content than at flowering. Wilting increased DM content regardless of stage of maturity. DC crop at flowering had higher WSC content and DMD. Flowering wilted and milk DC had higher NDF content. LAB numbers were below detectable levels, whereas mold numbers were substantial, especially in the DC fresh wheat of the flowering stage. During ensiling, silages from flowering stage had lower pH and residual WSC, increased ash content, higher lactic acid prodution and NDFD as compared to milk stage. Within each maturity stage, wilted silage had lower ash content and less butyric acid production. The highest numbers of LAB were found in wilted silage from milk stages whereas yeast and mold numbers were below detectable levels in all types.

During aerobic exposure, silages from flowering stage were more stable as indicated by lower pH values and higher lactic acid as compared to the silages from milk stage. The silages from flowering stage also showed higher ash and NDF content, DMD and NDFD. Within each maturity stages, DC silages had lower pH, higher ash content and a higher concentration of butyric acid as compared to wilted silages. In overall, flowering silages relatively tended to be stable during aerobic exposure; milk wilted silages had higher numbers of yeasts and molds and higher but unsignificant level of CO_2 production than other types. During aerobic exposure, most of the chemical parameters such as DM content, pH, ash content, WSC and lactic acid concentration, propionic and butyric acid, NDF content, DMD and NDFD and DM losses were significantly affected ($P<0.01$) by stage of maturity. Wilting had significant effect ($P<0.01$) for DM content, pH and butyric acid concentration. The interaction of maturity stage by wilting had significant effect for DM, pH, and ash content at $P<0.01$ and for propionic, butyric acids, and NDF content at $P<0.05$.

Table 5. The effect of stage of maturity and wilting on the composition of the fresh wheat crop, silage, and aerobic exposure parameters, (day 4 and 7) (cultivar BH).

Chemical parameters are in g/kg DM. Microbiological data are in CFU/ g DM. Yeast and mold numbers are from day 7 of aerobic exposure from each experiment and they are given as log CFU/g DM.

Forage	Type	DM	pH	Ash	WSC	LA	Ethanol	HAc	HPr	HBu	NDF	DMD	NDFD	LAB	Yeast	Mold	% DM loss	CO_2 g/kg DM
Fresh crop	F DC	316.3^{d}	6.1	62.6	136.5	-	-	-	-	-	558.5^{b}	722.9^{a}	542.9	1.9	3.8	6.0	-	-
	F wilt	385.0^{c}	6.4	62.7	73.4	-	-	-	-	-	573.9^{a}	663.7^{b}	521.7	NF	4.2	5.6	-	-
	M DC	406.0^{b}	6.3	54.0	87.8	-	-	-	-	-	576.2^{a}	663.9^{b}	491.5	NF	3.1	5.2	-	-
	M wilt	442.0^{a}	6.2	62.6	63.4	-	-	-	-	-	551.0^{b}	679.3^{b}	497.3	NF	4.3	4.6	-	-
Silage	F DC	286.9^{d}	4.3^{c}	75.9^{a}	40.6^{b}	35.7^{a}	8.9	8.4	1.8^{a}	16.3^{a}	581.9^{a}	676.8	549.9^{a}	3.2±0.9	0.73±1.3	NF	11.5^{a}	-
	F wilt	362.2^{c}	4.4^{c}	70.6^{b}	40.8^{b}	27.4^{a}	8.1	6.2	0.9^{ab}	4.2^{b}	585.1^{a}	676.5	537.7^{a}	4.3±0.3	NF	NF	8.0^{b}	-
	M DC	398.4^{b}	4.7^{b}	66.9^{c}	92.2^{a}	16.4^{b}	11.2	6.0	0.6^{ab}	4.0^{b}	570.7^{a}	651.0	473.5^{b}	5.8±0.3	NF	0.7±1.2	3.7^{c}	-
	M wilt	427.0^{a}	4.9^{a}	63.7^{d}	101.9^{a}	14.8^{b}	8.7	5.7	0.4^{b}	0.7^{c}	549.1^{b}	656.2	472.8^{b}	6.5±0.4	NF	NF	4.7^{c}	-
AE	F DC	297.8^{d}	4.3^{d}	75.9^{a}	38.5^{b}	32.1^{a}	5.3	5.3	0.8^{ab}	11.7^{a}	583.9^{a}	690.5^{a}	566.6^{a}	-	NF	1.7±1.5	0.0	1.6
	F wilt	371.5^{c}	4.4^{c}	70.5^{b}	43.4^{b}	32.9^{a}	5.4	4.7	1.4^{a}	5.0^{b}	589.0^{a}	680.9^{a}	560.5^{a}	-	NF	NF	0.0	1.2
	M DC	420.5^{b}	4.7^{b}	66.5^{c}	74.2^{a}	14.0^{b}	5.6	4.2	0.3^{b}	3.7^{b}	565.9^{b}	650.6^{b}	462.2^{b}	-	2.1±1.9	NF	0.0	2.0
	M wilt	446.0^{a}	5.0^{a}	63.4^{d}	84.8^{a}	12.6^{b}	2.9	4.2	0.0^{b}	0.0^{c}	558.1^{b}	647.8^{b}	454.6^{b}	-	7.6±0.4	7.1±0.3	0.0	20.7
	Stage[1]	***	***	***	***	***	NS	**	***	***	***	***	***	-	-	-	***	NS
	Type[2]	***	***	*	NS	NS	NS	NS	NS	***	NS	NS	NS	-	-	-	*	NS
	Stage*Type	***	***	***	NS	NS	NS	NS	**	**	**	NS	NS	-	-	-	NS	NS

For the fresh crop, silage and AE separately, means within each column followed by different letters are significantly different ($P<0.05$). [1]flowering, mik; [2]direct-cut, wilted.

NS, non-significant; *, <0.1; **, <0.05; ***, <0.01.

AE, aerobic exposure; F, Flowering; M, Milk; DM, dry matter; WSC, water-soluble carbohydrates; LA, lactic acid; HAc, acetic acid; HPr, propionic acid; HBu, butyric acid; NDF, neutral detergent fiber; DMD, DM digestibility; NDFD; NDF digestibility; LAB, lactobacilli.

Within the column % DM loss, negative values obtained for all silages exposed aerobically are considered as zero.

[1] Flowering DC, Flowering Wilt, Milk DC, Milk Wilt;

[2] Day 4, Day 7

2. Wheat silages (cultivar Galil):

Table 6 and 7 summarize the changes and losses in DC and wilted wheat crop (cultivar Galil) from the flowering stage from the fresh crop through silage and until 7 day of aerobic exposure. During ensiling, in the DC control silage, the pH decreased, WSC content decreased, organic acids particularly lactic and butyric acids increased. In the DC inoculated silage, the pH decreased and lactic acid production increased to a higher extent as compared with the control silage. In addition, DC inoculated silage had lower butyric acid concentration, showing efficient homolactic fermentation as compared with the control silage. The control flowering DC silages had higher ensiling DM losses as compared to inoculated silage.

In case of wilted silages, both control and inoculated silages decreased pH, inceased ash content and lactic acid prodution. Inoculated silages had lower ethanol concentration, whereas other parameters were similar in control and inoculated silages.

During aerobic exposure, the DC control silages were very stable with almost no change in silage compostion and silage temperature was similar or below to ambient except for 48 hours of aerobic exposure where silage temperature was slightly higher than ambient (Fig. 2). In case of wilted silages, control silages, although, showed some CO_2 production and rise in temperature (Fig. 3) from day 2, did not change their composition untill day 4 of aerobic exposure. However, on day 7 of aerobic exposure, wilted control silages were already spoiled as reflected by much increased pH, higher numbers of yeasts and molds, and intensive CO_2 production along with a rise in temperature (Fig. 3). These silages also increased ash content and decreased the concentrations of WSC and lactic acid.

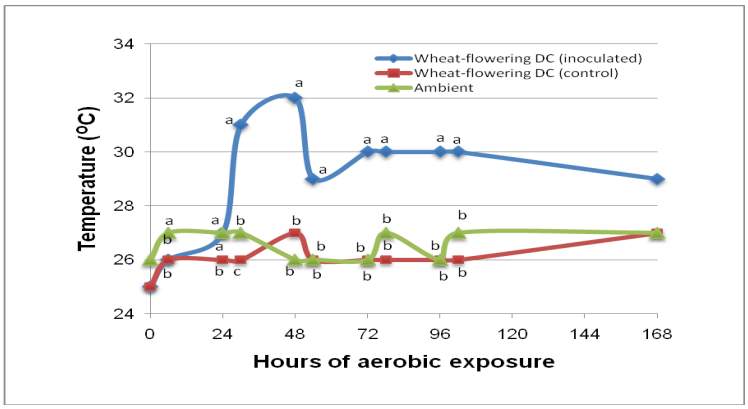

Fig. 2. Changes in temperature during aerobic exposure of DC control and inoculated wheat silages from flowering stage (culivar Galil). Within sampling hours, means followed by different letters are significantly different (P<0.05).

Table 6. Changes in chemical and microbiological composition during ensiling and aerobic exposure of wheat silages from the flowering stage, direct-cut and wilted (cultivar Galil).

Chemical parameters are in g/kg DM and microbiological data are in log CFU/g DM (means±s.d.)

Type	Day	pH	LAB	Yeasts	Molds	Ash	WSC	LA	Ethanol	Acetic acid	Butyric acid
Direct-cut	Fresh crop	6.2±0.0^{b}	3.0^{b}	3.6±0.1^{d}	3.7±0.0^{b}	83.9±0.0^{c}	46.4±3.1^{a}	-	-	-	-
	C silage	4.4±0.1^{c}	7.5^{a}	3.6±0.6^{d}	NF	96.4±2.1^{ab}	0.0±0.0^{b}	54.6±10.4^{b}	4.4±0.7^{a}	3.2±2.8^{ab}	3.5±0.5^{a}
	I silage	3.7±0.0^{d}	5.9^{a}	7.1±0.6^{b}	1.2±1.7^{c}	90.1±3.1^{bc}	9.9±12.3^{b}	115.0±10.3 a	3.2±0.6^{a}	0.2±0.4^{b}	0.0±0.0^{b}
	C AE2	4.3±0.3^{c}	-	4.0±0.9^{d}	NF	-	-	-	-	-	
	C AE3	4.3±0.0^{c}	-	5.1±0.2^{cd}	NF	-	-	-	-	-	-
	C AE4	4.4±0.1^{c}	-	5.2±0.3^{bcd}	NF	95.3±2.0^{ab}	0.0±0.0^{b}	57.8±1.2^{b}	3.7±2.0^{a}	2.3±2.2^{ab}	3.1±1.5^{a}
	C AE7	4.4±0.1^{c}	-	6.2±1.3^{bc}	0.9±1.6^{c}	95.8±1.2^{ab}	0.0±0.0^{b}	56.0±4.3^{b}	2.1±1.8^{ab}	10.8±9.7^{a}	4.0±0.0^{a}
	I AE4	7.2±1.4^{a}	-	10.2±0.2^{a}	6.6±0.3^{a}	95.5±1.7^{ab}	12.2±5.6^{b}	16.5±7.8^{c}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{b}
	I AE7	7.5±0.2^{a}	-	9.8±0.1^{a}	7.6±0.4^{a}	99.7±2.1^{a}	36.1±9.0^{a}	0.0±0.0^{c}	0.0±0.0^{b}	0.7±1.2^{b}	0.0±0.0^{b}
Wilted	Fresh crop	6.1±0.1^{b}	3.2±0.0^{c}	4.3±0.2^{c}	3.2±0.2^{bc}	74.4^{c}	77.6±18.3^{a}	-	-	-	
	C silage	4.2±0.1^{c}	6.5±0.3^{a}	5.9±0.7^{b}	NF	85.3±1.6^{b}	64.0±12.2^{a}	53.1±14.8^{a}	9.6±2.3^{a}	1.0±0.4^{ab}	
	I silage	3.8±0.1^{c}	4.4±0.5^{b}	5.3±0.4^{bc}	NF	82.3±2.5^{b}	56.4±13.4^{a}	55.0±3.8^{a}	3.4±0.8^{b}	3.1±1.6^{a}	
	C AE2	4.1±0.0^{c}	-	8.3±0.4^{a}	NF	-	-	-	-	-	
	C AE3	4.2±0.0^{c}	-	8.4±0.0^{a}	NF	-	-	-	-	-	
	C AE4	4.3±0.1^{c}	-	8.7±0.2^{a}	0.7±1.3^{c}	82.9±0.8^{b}	63.9±3.1^{a}	54.4±4.4^{a}	0.9±1.5^{bc}	0.6±0.5^{b}	
	C AE7	8.6±0.1^{a}	-	8.6±0.3^{a}	6.0±3.3^{ab}	90.8±0.9^{a}	0.0±0.0^{b}	0.0±0.0^{b}	0.0±0.0^{c}	0.0±0.0^{b}	
	I AE4	4.0±0.1^{c}	-	8.6±0.2^{a}	NF	82.3±1.3^{b}	60.9±11.3^{a}	68.7±5.4^{a}	10.7±0.5^{a}	0.5±0.5^{b}	
	I AE7	6.3±0.6^{b}	-	8.6±0.7^{a}	7.8±0.7^{a}	86.2±0.9^{ab}	16.0±16.3^{b}	11.5±3.0^{b}	0.0±0.0^{c}	0.1±0.1^{b}	

Within each column and silage type, means followed by different letters are significantly different (P<0.05).

LAB, lactobacilli; WSC, water-soluble carbohydrates; NF, not found; C, control; I, Inoculated; AE, aerobic exposure.

No propionic acid was detected in all silages.

No butyric acid was detected in silages and during aerobic exposure for wilted.

Table 7. Losses during ensiling and aerobic exposure of wheat silages from flowering stage, direct cut and wilted (means±s.d.)

Type	Day	CO_2 (g/kg DM)	DM (g/kg)	% DM loss	NDF (g/kg DM)	DMD (g/kg DM)	NDFD (g/kg DM)
Direct-cut	Fresh crop	-	221.9±2.2^{a}	-	641.4±0.9^{d}	676.5±9.8^{a}	625.2±10.7^{a}
	C silage	-	199.2±3.4^{c}	11.8±1.5^{a}	666.4±9.2^{bc}	621.1±15.6^{b}	584.3±21.6^{bc}
	I silage	-	213.1±2.9^{b}	4.6±1.3^{b}	645.8±11.4^{cd}	660.3±12.1^{a}	604.9±13.3^{ab}
	C AE2	1.4±0.5^{b}	204.2±1.7^{c}	0.0^{c}	-	-	-
	C AE3	1.5±0.8^{b}	205.5±3.5^{c}	0.0^{c}	-	-	-
	C AE4	4.4±2.5^{b}	204.6±1.4^{c}	0.0^{c}	669.9±2.8^{b}	612.5±14.2^{b}	554.1±20.0^{c}
	C AE7	6.8±1.2^{b}	206.0±1.5^{bc}	0.0^{c}	677.1±9.7^{b}	601.0±9.6^{b}	548.8±14.1^{c}
	I AE4	72.4±4.1^{ab}	200.7±2.0^{c}	7.1±0.9^{b}	718.7±5.9^{a}	618.9±5.5^{b}	614.9±3.3^{ab}
	I AE7	149.6±75.4^{a}	190.4±3.8^{d}	12.7±1.9^{a}	730.9±8.8^{a}	560.6±9.3^{c}	549.1±8.6^{c}
Wilted	Fresh crop	-	379.3±3.3^{a}	-	654.2±3.7^{c}	677.6±13.5^{a}	621.5±15.2
	C Silage	-	378.8±4.9^{a}	1.4±2.7^{b}	660.9±12.2^{c}	665.0±14.9^{ab}	615.4±12.8
	I Silage	-	381.0±10.9^{a}	0.1±2.9^{b}	649.0±7.5^{c}	672.5±11.2^{a}	619.7±19.5
	C AE2	3.4±1.2^{c}	378.0±3.4^{a}	0.5±0.9^{b}	-	-	-
	C AE3	4.6±0.2^{c}	381.2±10.0^{a}	0.0±2.6^{b}	-	-	-
	C AE4	15.5±3.9^{c}	378.2±1.9^{a}	1.0±0.5^{b}	669.5±7.3^{c}	641.2±8.8^{bc}	585.1±8.4
	C AE7	76.6±5.5^{a}	352.3±11.2^{b}	9.6±3.1^{a}	756.4±8.3^{a}	589.0±14.9^{d}	586.1±14.2
	I AE4	9.2±0.7^{c}	374.6±0.4^{ab}	2.7±0.2^{b}	656.4±8.3^{c}	666.8±19.7^{ab}	606.3±19.8
	I AE7	53.0±9.4^{b}	374.0±12.6^{ab}	4.4±3.2^{ab}	718.7±10.2^{b}	621.3±12.2^{c}	591.6±13.6

Within each column and silage type, means followed by different letters are significantly different (P<0.05).

DMD, dry matter digestibility; NDFD, NDF digestibility; C, control; I, Inoculated; AE, aerobic exposure.

Within column % DM loss, negatives values obtained for C AE2 to C AE7 in direct-cut silages are considered as zero.

These silages had higher DM losses, increased NDF content, and decreased DMD. On the other hand, DC inoculated silages exposed for both days 4 and 7 were very unstable during aerobic exposure indicated by increased pH, higher CO_2 production, and increased temperature (Fig. 2) along with higher numbers of yeasts and molds. They also increased NDF content, decreased DMD, decreased lactic and volatile fatty acids, decreased ethanol, and had higher DM losses. However, wilted inoculated silages exposed for 4 days, although showed a rise in temprature (Fig. 3) and decreased acetic acid concentration, did not change in composition, indicating aerobic stability. However, wilted inoculated silages on the 7 day of aerobic exposure showed increased pH, decreased WSC, and decreased lactic acid, ethanol and aectic acid along with higher temperature than ambient (Fig. 3). These silages had higher CO_2 production, increased NDF content and decreased DMD. Most of the aerobic stability parameters of the control and inoculated silages prepared from the wilted flowering stage were comparable on day 7 of the aerobic exposure.

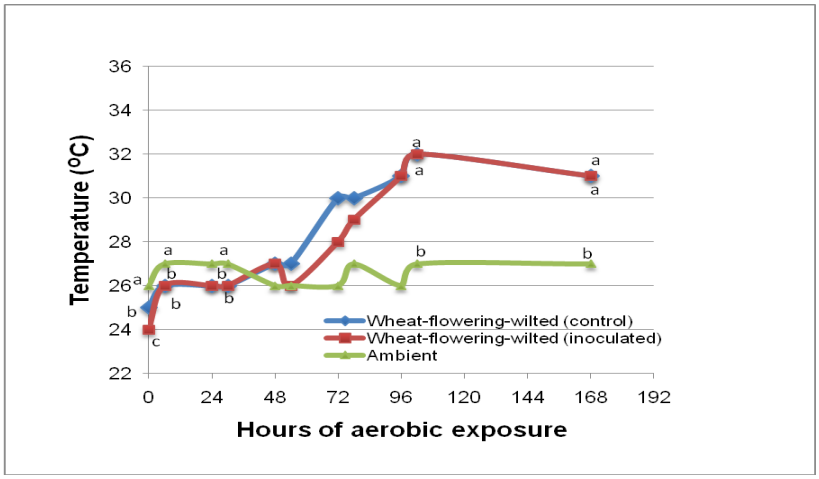

Fig. 3. Changes in temperature during aerobic exposure of wilted control and inoculated wheat silages from flowering stage (cultivar Galil). Within sampling hours, means followed by different letters are significantly different (P<0.05).

Tables 8 and 9 summarize the changes and losses in DC and wilted wheat silages (cultivar Galil) from the milk stage from the fresh crop through silage and until 7 day of aerobic exposure. During ensiling, for both DC and wilted, control and inoculated silages decreased pH and increased lactic acid concentration. Both DC control and inoculated silages decreased WSC but the level of WSC was higher in control silages. The DC inoculated silages had more ethanol and much less acetic acid as compared

Table 8. Changes in chemical and microbiological composition during ensiling and aerobic exposure of wheat silages from the milk, direct-cut and wilted (cultivar Galil).

Chemical parameters are in g/kg DM and microbiological data are in log CFU/g DM (means±s.d.)

Type	Day	pH	LAB	Yeasts	Molds	Ash	WSC	LA	Ethanol	Acetic acid
Direct-cut	Fresh crop	6.0±0.1^{ab}	3.1±0.8^{c}	5±0.1^{ef}	5.5±0.0^{ab}	69.2^{b}	92.9±20.7^{a}	-	-	-
	C silage	3.9±0.1^{c}	8.8±0.3^{a}	3.8±1.0^{f}	NF	78.9±2.8^{ab}	59.3±14.7^{b}	72.4±8.2^{ab}	15.2±2.6^{bc}	10.3±2.6^{ab}
	I silage	3.7±0.0^{c}	6.0±0.1^{b}	6.5±0.1^{de}	1.1±1.6^{bc}	80.3±2.7^{ab}	10.9±10.0^{c}	88.7±13.2^{a}	55.5±10.9^{a}	0.3±0.3^{c}
	C AE2	3.8±0.1^{c}	-	7.3±0.2^{cd}	NF	-	-	-	-	-
	C AE3	3.7±0.0^{c}	-	8.2±0.1^{abc}	NF	-	-	-	-	-
	C AE4	3.8±0.1^{bc}	-	7.5±0.7^{bcd}	0.7±1.3^{c}	77.4±1.7^{ab}	49.0±26.3^{b}	58.1±6.7^{bc}	9.5±10.2^{bc}	15.3±10.7^{a}
	C AE7	5.0±0.9^{bc}	-	8.8±0.4^{ab}	4.2±3.6^{abc}	82.7±5.3^{a}	16.1±5.0^{c}	26.9±10.2^{c} d	0.7±1.2^{c}	4.0±5.0^{bc}
	I AE4	4.2±0.2^{bc}	-	9.3±0.1^{a}	NF	81.2±2.3^{a}	4.9±4.3^{c}	32.3±2.5^{c}	22.2±8.6^{b}	0.6±1.0^{bc}
	I AE7	7.3±0.3^{a}	-	9.6±0.2^{a}	7.9±0.1^{a}	85.7±3.8^{a}	7.8±7.9^{c}	0.0±0.0^{d}	2.9±5.0^{c}	0.0±0.0^{c}
Wilted	Fresh crop	6.0±0.1^{a}	4.3±1.0^{a}	5.2±0.1^{d}	5.3±0.0^{c}	60.8^{d}	162.0±45.8^{a}	-	-	-
	C Silage	3.9±0.0^{c}	5.2±0.9^{a}	4.4±0.2^{d}	NF	68.5±1.9^{c}	180.7±20.1^{a}	43.6±7.9^{a}	6.0±1.5	1.0±0.9
	I Silage	3.8±0.1^{c}	4.8±0.6^{a}	5.0±0.4^{d}	2.1±0.1^{d}	65.6±1.6^{cd}	166.5±30.7^{a}	47.3±4.1^{a}	4.8±4.1	0.7±1.1
	C AE2	3.9±0.1^{c}	-	8.1±0.4^{c}	NF	-	-	-	-	-
	C AE3	3.9±0.0^{c}	-	8.6±0.5^{bc}	NF	-	-	-	-	-
	C AE4	4.0±0.1^{c}	-	9.0±0.1^{ab}	NF	70.3±1.2^{bc}	127.5±11.4^{a}	36.2±3.3^{a}	4.2±0.2	0.1±0.1
	C AE7	5.4±1.0^{ab}	-	8.7±0.2^{abc}	8.9±0.1^{a}	78.1±3.7^{a}	40.1±14.5^{b}	9.7±8.4^{b}	0.8±0.8	0.0±0.0
	I AE4	4.6±0.8^{bc}	-	9.2±0.1^{ab}	7.9^{b}	70.5±1.7^{bc}	56.3±37.4^{b}	17.1±15.1^{b}	5.2±4.9	0.3±0.6
	I AE7	5.9±0.9^{a}	-	9.5±0.1^{a}	8.9±0.1^{a}	74.9±2.1^{ab}	24.3±6.6^{b}	3.6±6.2^{b}	0.0±0.0	0.0±0.0

Within each column means followed by different letters are significantly different (P<0.05).

WSC, water-soluble carbohydrates; LAB, lactobacilli; C, control; I, inoculated; NF, not found; AE, aerobic exposure.

No propionic or butyric acids were detected during ensiling and throughtout the aerobic stability test.

Table 9. Losses during ensiling and aerobic exposure of wheat silages from milk stage, direct cut and wilted (means±s.d.)

Type	Day	CO_2 (g/kg DM)	DM (g/kg)	% DM loss	NDF (g/kg DM)	DMD (g/kg DM)	NDFD (g/kg DM)
Direct-cut	Fresh crop	-	322.6±1.6^{a}	-	541.6±10.7^{d}	697.1±19.3^{a}	579.3±24.1^{a}
	C silage	-	299.2±4.1^{bc}	8.9±1.7^{a}	579.8±7.5^{c}	654.1±15.5^{ab}	544.9±17.3^{ab}
	I silage	-	288.0±2.3^{cd}	13.2±0.7^{a}	586.5±5.5^{c}	626.5±16.0^{bc}	512.2±23.1^{b}
	C AE2	1.6±0.2^{b}	318.8±2.3^{a}	0.0^{c}	-	-	-
	C AE3	10.0±2.1^{b}	310.7±2.2^{ab}	0.0^{c}	-	-	-
	C AE4	18.5±2.3^{ab}	308.4±1.8^{ab}	0.0^{c}	585.3±9.9^{c}	628.5±14.4^{bc}	514.3±16.6^{b}
	C AE7	67.2±27.6^{a}	281.2±17.5^{de}	8.8±6.4^{a}	639.5±25.7^{b}	600.5±37.1^{cd}	519.3±31.4^{b}
	I AE4	38.8±3.5^{ab}	285.8±5.3^{de}	1.9±1.8^{b}	641.2±7.9^{b}	598.3±10.0^{cd}	525.1±14.4^{b}
	I AE7	82.3±12.0^{a}	272.4±4.4^{e}	8.0±2.0^{a}	695.8±14.3^{a}	577.6±18.9^{d}	541.9±12.2^{ab}
Wilted	Fresh crop	-	440.6±2.6^{a}	-	512.9±10.0^{c}	677.7±7.2^{a}	522.1±10.0^{a}
	C Silage	-	437.3±4.9^{a}	1.7±1.1^{bc}	523.3±32.4^{c}	659.5±15.1^{ab}	508.8±30.1^{a}
	I Silage	-	435.5±4.9^{ab}	1.9±1.4^{bc}	534.3±12.6^{c}	650.8±15.0^{ab}	498.9±12.7^{a}
	C AE2	3.2±2.1^{c}	445.5±4.1^{a}	0.0^{c}	-	-	-
	C AE3	11.0±4.1^{c}	437.1±6.7^{a}	1.1±1.8^{c}	-	-	-
	C AE4	34.0±14.9^{bc}	424.0±4.6^{ab}	4.5±1.2^{b}	562.5±10.5^{bc}	633.0±16.5^{b}	492.6±19.4^{a}
	C AE7	133.2±47.4^{a}	399.0±2.0^{c}	12.9±1.1^{a}	650.5±33.6^{a}	572.2±23.8^{c}	497.4±13.1^{a}
	I AE4	45.1±2.3^{bc}	397.6±5.4^{c}	11.9±2.0^{a}	607.3±16.4^{ab}	591.9±8.1^{c}	480.9±14.6^{a}
	I AE7	94.6±1.2^{ab}	390.8±6.2^{c}	14.8±1.4^{a}	652.0±9.1^{a}	580.7±17.7^{c}	494.7±20.8^{a}

Within each column, means followed by different letters are significantly different (P<0.05).

DMD, dry matter digestibility; NDFD, NDF digestibility; C, control; I, inoculated; AE, aerobic exposure.

Within column DM loss, negative values obtained for C AE2 to C AE4 for direct-cut and for C AE2 for wilted are considered as zero.

with the control silages. Both DC silages had increased NDF content and similar ensiling DM losses but DMD and NDFD were decreased only in inoculated silages.

During aerobic exposure, until day 4 of aerobic exposure, for both DC and wilted, control silages did not change their composition significantly. However, for both DC and wilted, control silages were already spoiled on day 7 of aerobic exposure indicated by high numbers of yeasts and molds, intensive CO_2 production, increased pH with reduction in lactic acid and WSC concentration, and rise in temperature (Fig. 4 and 5). These silages also had higher DM losses as compared to respective silages exposed for days 2, 3 and 4. During 7 day of aerobic exposure, wilted control silages

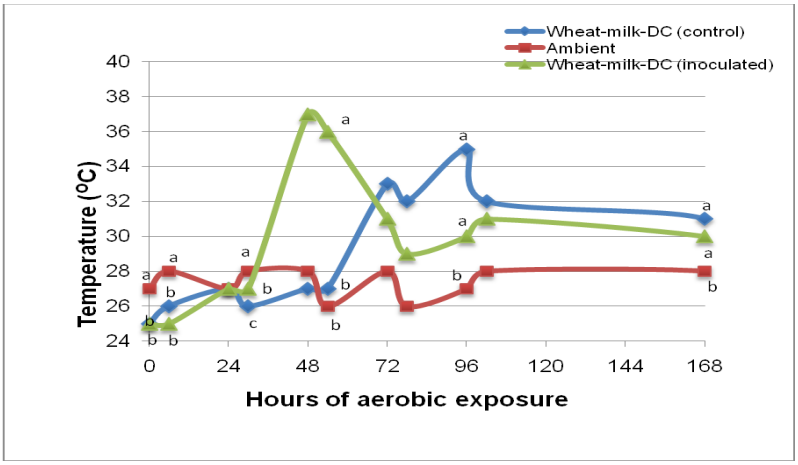

Fig. 4. Changes in temperature during aerobic exposure of DC control and inoculated wheat silages from milk stage (cultivar Galil). Within sampling hours, means followed by different letters are significantly different (P<0.05).

had higher ash content and lower DMD as compared to fresh and other aerobically exposed silages (control) for days 2, 3 and 4. In case of DC inoculated silages, on the day 4 of aerobic exposure, silages had higher numbers of yeasts, higher DM losses, and higher temperature than ambient (Fig. 4). The temperature increased at earlier time and to higher degrees than the control silages. These silages had increased NDF content. The wilted inoculated silage, on the 4th day of aerobic exposure were unstable as indicated by decreased WSC and lactic acid concentration, and higher DM losses in addition to higher numbers of yeasts and higher temperature as in DC silages.

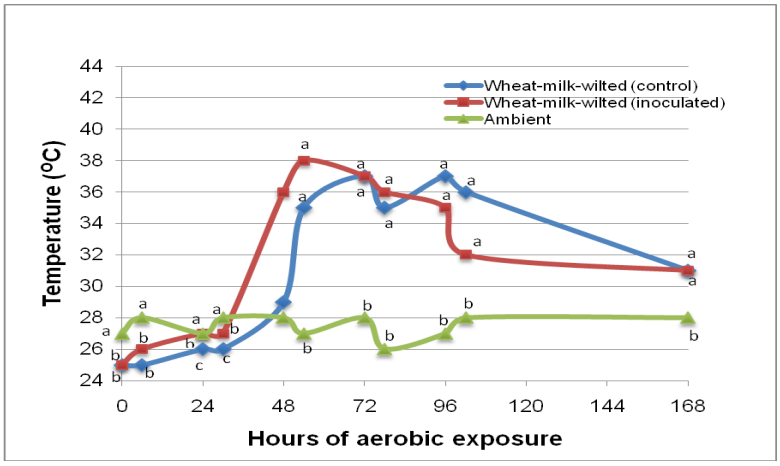

Fig. 5. Changes in temperature during aerobic exposure of wilted control and inoculated wheat silages from milk stage (Galil). Within sampling hours, means followed by different letters are significantly different (P<0.05).

During 7th day of aerobic exposure, DC inoculated silages had higher pH, higher numbers of yeasts and molds with a higher temperature than that of ambient (Fig. 4). In case of wilted inoculated silages, inoculation did not have major effects on aerobic deterioration although temperature had increased (Fig. 5).

Table 10 summarizes the effect of stage of maturity and wilting on the composition of freshly harvested wheat crop (cultivar Galil), and both control and inoculated silages (cultivar Galil) and on aerobic exposure parameters of both control and inoculated silages during 4 and 7 days of aerobic exposure. Crops at flowering stage had higher NDF content and NDFD as compared to milk stage. Wilting increased DM content and WSC regardless of stage of maturity.

During ensiling, silages from flowering stage had higher pH, increased ash content, and increased NDF content and NDFD as compared with the silages from milk stage. Regardless of maturity stage, wilting increased DM content, lowered ash content, increased WSC content and wilted silages had lower ensiling losses. Within the flowering stage, wilting lowered silage pH, increased ethanol concentration, decreased butyric acid concentration, and increased DMD. In case of milk stage, wilting decreased lactic acid, acetic acid and ethanol concentration, decreased NDF content and NDFD.

In case of inoculated silages, silages from flowering stage had higher NDF content, DMD and NDFD than silages at milk stage. Regardless of maturity stage, wilting increased DM content, WSC level and lowered ensiling losses. Within the flowering stage, wilting increased silage pH and acetic acid concentration. In case of

Table 10. The effect of stage of maturity and wilting on the composition of the fresh wheat crop, silage (control and inoculated) and during aerobic exposure (day 4 and 7) (cultivar Galil).

Chemical parameters are in g/kg DM. Microbiological data are in CFU/ g DM and from day 7 for aerobically exposed silages.

Forage	Type	DM	pH	Ash	WSC	LA	Etoh	HAc	NDF	DMD	NDFD	LAB	Yeasts	Molds	% DM loss	CO_2
Fresh	F DC	221.8^{d}	6.2	83.9	46.4^{b}	-	-	-	641.4^{a}	676.5	625.2^{a}	3.0±0.4	3.6±0.1	3.7±0.0	-	-
	F wilt	379.3^{b}	6.1	74.7	77.6^{ab}	-	-	-	654.2^{a}	677.6	621.5^{a}	3.2±0.0	4.3±0.2	3.2±0.2	-	-
	M DC	322.6^{c}	6.0	69.2	92.9^{ab}	-	-	-	531.6^{b}	697.1	579.3^{b}	3.1±0.8	5±0.1	5.5±0.0	-	-
	M wilt	440.6^{d}	6.0	60.8	162^{a}	-	-	-	512.9^{b}	677.7	522.1^{c}	4.3±1.0	5.2±0.1	5.3±0.0	-	-
C silage	F DC	199.2^{d}	4.4^{a}	96.4^{a}	0.0^{c}	54.6^{b}	4.4^{c}	3.2^{b}	666.4^{a}	621.1^{b}	584.3^{a}	7.5±0.7	3.6±0.6	NF	11.8^{a}	-
	F wilt	378.8^{b}	4.2^{b}	85.3^{b}	64.0^{b}	53.1^{b}	9.6^{b}	1.0^{b}	660.9^{a}	665.0^{a}	615.4^{a}	6.5±0.3	5.9±0.7	NF	1.4^{c}	-
	M DC	299.2^{c}	3.9^{c}	78.9^{c}	59.3^{b}	72.4^{a}	15.2^{a}	10.4^{a}	579.8^{b}	654.1^{a}	544.9^{b}	8.8±0.3	3.8±1.0	NF	8.9^{b}	-
	M wilt	437.2^{a}	3.9^{c}	68.5^{d}	180.8^{a}	43.6^{b}	6.0^{c}	1.0^{b}	523.3^{c}	659.5^{a}	508.8^{c}	5.2±0.9	4.4±0.2	NF	1.7^{c}	-
I silage	F DC	213.1^{d}	3.7^{b}	90.2^{a}	9.9^{c}	115.0^{a}	3.2^{b}	0.2^{b}	645.8^{a}	660.3^{ab}	604.9^{a}	5.9±0.4	7.1±0.6	1.2±1.7	4.6^{b}	-
	F wilt	381.0^{b}	3.8^{a}	82.3^{a}	56.4^{b}	55.0^{c}	3.4^{b}	3.1^{a}	649.0^{a}	672.5^{a}	619.7^{a}	4.4±0.5	5.3±0.4	0±0	0.1^{c}	-
	M DC	288.0^{c}	3.7^{b}	80.3^{a}	11.0^{c}	88.7^{b}	55.5^{a}	0.3^{b}	586.5^{b}	626.5^{c}	512.2^{b}	6.0±0.1	6.5±0.1	1.1±1.6	12.2^{a}	-
	M wilt	435.6^{a}	3.8^{ab}	65.6^{c}	166.5^{a}	47.4^{c}	4.8^{b}	0.7^{b}	534.3^{c}	650.8^{b}	498.9^{b}	4.8±0.6	5.0±0.4	2.1±0.1	1.9^{c}	-
AE C silage	F DC	205.3^{d}	4.4^{b}	95.5^{a}	0.0^{c}	56.9^{a}	2.3	6.5^{ab}	713.0^{a}	606.8	551.5^{b}	-	5.7^{b}	0.5^{b}	0.0^{c}	5.6^{b}
	F wilt	365.3^{b}	6.5^{a}	86.8^{ab}	32.0^{b}	27.2^{b}	0.4	0.3^{b}	673.5^{b}	615.1	585.6^{a}	-	8.7^{a}	3.0^{ab}	5.3^{ab}	46.1^{a}
	M DC	294.8^{c}	5.2^{ab}	67.1^{b}	38.4^{b}	36.7^{b}	5.5	9.6^{a}	623.5^{c}	595.1	515.0^{c}	-	8.2^{a}	2.5^{ab}	3.2^{b}	49.0^{a}
	M wilt	411.5^{a}	4.7^{b}	74.2^{ab}	83.8^{a}	22.9^{b}	2.5	0.03^{b}	606.5^{c}	602.6	495.0^{c}	-	9.1^{a}	4.0^{a}	8.7^{a}	83.6^{a}
AE I silage	F DC	195.6^{d}	7.4^{a}	97.6^{a}	24.2^{ab}	8.3^{b}	0.0^{b}	0.4^{a}	724.8^{a}	589.7^{b}	582.0^{a}	-	9.8^{a}	7.6	9.9^{b}	111.0^{a}
	F wilt	374.3^{b}	5.2^{b}	84.3^{a}	38.5^{a}	40.1^{a}	5.3^{b}	0.3^{a}	687.6^{b}	644.1^{a}	599.0^{a}	-	8.6^{c}	7.8	3.5^{c}	35.5^{b}
	M DC	279.1^{c}	5.8^{b}	83.5^{b}	6.4^{b}	16.2^{b}	12.6^{a}	0.3^{a}	668.5^{c}	587.9^{b}	533.5^{b}	-	9.6^{b}	7.9	5.0^{c}	60.5^{ab}
	M wilt	394.2^{a}	5.3^{b}	72.7^{c}	40.3^{a}	10.3^{b}	2.6^{b}	0.2^{a}	629.7^{d}	586.3^{b}	487.8^{c}	-	9.5^{b}	8.9	13.4^{a}	74.8^{ab}

For the fresh crop, silages and aerobic exposed silages, separately, means within each column and crop/ silage followed by different letters are significantly different ($P<0.05$). F, Flowering; M, Milk; C, control; I, inoculated; DC, direct-cut; DM, dry matter; WSC, water-soluble carbohydrates; LA, lactic acid; HAc, acetic acid; NDF, neutral detergent fiber; DMD, DM digestibility; NDFD; NDF digestibility; LAB, lactobacilli. Butyric acid was found in the control silages of flowering stage dircut cut at 3.5 g/kg DM whereas, no butyric acid was found in all inoculated silags. Propionic acid were not detected in both control and inoculated silages. During aerobic exposure, butyric acid was found in the control silages of flowering stage dircut cut at 3.5 g/kg DM whereas, no butyric acid was detected inoculated silages. No propionic acid was detected in both control and inocualted silages of flowering stage direct-cut during aerobic exposure. With column % DM loss, a negative obtained for control F DC during aerobic exposure is considered as zero.

milk stage, wilting increased ethanol concentration, decreased ash and NDF content and increased DMD.

During aerobic exposure, silages from flowering stage had lower WSC and higher ash content, NDF and NDFD as compared to silages from milk stage. In both maturity stages, wilting decreased acetic acid concentration and increased DM, ash content, WSC and DM losses. Within flowering stage, wilting increased pH, decreased lactic and butyric acid concentrations, and increased CO_2 and DM losses. These silages also had lower NDF content with increment in NDFD. Silages from the inoculated flowering stage had increased ash, NDF, and NDFD as compared to milk stage. In both maturity stages, wilting increased DM, decreased NDF content and increased WSC concentration. Within flowering stage inoculated silages, wilting decreased pH, increased lactic acid concentration, increased DMD and DM losses, and decreased CO_2 production. In milk stage, wilting decreased ethanol concentration, ash content, and NDFD, and increased DM losses. The results indicate that inoculation with homofermentative LAB enhanced the aerobic deterioration of the silages of the DC flowering stage. However, inoculation did not have a major effect on the other types of silages (flowering wilted, and DC and wilted silages from the milk stage) the control silages of which were already spoiled on days 4 and 7 of the aerobic exposure.

Table 11 summarizes the statistical analysis on effect of stgae of maturity, wilting, inoculation, and their interactions on aerobic exposure parameters in silages (cultivar Galil) exposed for days 4 and 7. DM content, DMD and NDFD were highly affected ($P<0.01$) by stage of maturity. Ash content and lactic acid concentration were affected by stage of maturity at 0.05 level significance. Wilting had significant effect ($P<0.01$) on DM and ash content, and WSC level. Lactic acid concentration was highly affected ($P<0.01$) by inoculation. Inoculation had significant effect ($P<0.05$) for acetic and propionic acid, NDF and NDFD content, and CO_2 production ($P<0.05$). The interaction of maturity stage by wilitng was significant ($P<0.01$) for DM content and NDFD. The interaction of maturity stage by inoculation was significant ($P<0.05$) for DM content and WSC concentration. The interaction of wilting by inoculation was significant ($P<0.01$) for pH, lactic acid level, and CO_2 production. The cumulative effect of maturity stage, wilting and inoculation were found to be significant for DM, pH and lactic acid concentration ($P<0.05$). The meaning of significant interactions

Table 11. Statistical analysis on effect of maturity, wilting, and inoculation on aerobic exposure parameters (Day 4 and 7) (cultivar Galil).

Factors	DM g/kg	pH	Ash g/kg DM	WSC g/kg DM	LA g/kg DM	Ethanol g/kg DM	Hac g/kg DM	Hpr g/kg DM	Hbu g/kg DM	NDF g/kg DM	DMD g/kg DM	NDFD g/kg DM	% DM loss	CO_2 g/kg DM
Stage[1]	***	*	**	*	**	*	NS	NS	*	***	*	***	NS	NS
Type[2]	***	NS	***	***	NS	NS	NS	**	NS	*	NS	NS	NS	NS
Inoc[3]	NS	*	NS	NS	***	NS	**	**	*	**	NS	**	NS	**
Stage*Type	***	NS	NS	NS	NS	**	NS	NS	NS	NS	NS	***	NS	*
Stage*Inoc	**	NS	NS	**	NS	NS	NS	NS	NS	NS	*	NS	NS	*
Type*Inoc	NS	***	NS	NS	***	NS	NS	**	NS	NS	*	NS	NS	***
Stage*Type*Inoc	**	**	NS	NS	**	*	NS	NS	NS	NS	NS	NS	NS	*

NS, non-significant; *, <0.1; **<0.05; ***<0.01. [1]flowering, milk; [2] Direct-cut, wilted; [3] Inoculated, Control. DM, dry matter; WSC, water-soluble carbohydrates; LA, lactic acid; HAc, acetic acid; HPr, propionic acid; HBu, butyric acid; NDF, neutral detergent fiber; DMD, DM digestibility; NDFD; NDF digestibility.

Table 12. Correlation between DM content and water activity of fresh wheat crop and silage

Cultivar	Forage	Type	DM (g/kg)	a_w	a_w range	DM (g/kg) range	Correlation coefficient
BH	Fresh crop	FD	316±5.5	0.972	0.947 – 0.972	316 – 442	-0.83 (*P*<0.16)
		FW	385±3.6	0.969			
		MD	406±5.3	0.965			
		MW	442±3.6	0.947			
	Silage	FD	287±4.0	0.978±0.004	0.951 – 0.982	287 – 441	-0.60 (*P*<0.03)
		FW	362±7.0	0.954±0.004			
		MD	398±6.0	0.964±0.01			
		MW	427±8.0	0.960±0.002			
Galil	Fresh crop	FD	222±2.0	0.992±0.003	0.946 – 0.994	220 – 443	-0.94 (*P*<0.0001)
		FW	379±3.0	0.963±0.04			
		MD	323±2.0	0.983±0.002			
		MW	441±3.0	0.952±0.006			
	Silage	FD	201±3.0	0.964±0.002	0.935 – 0.966	198 – 440	-0.97 (*P*<0.0001)
		FW	379±16.0	0.947±0.001			
		MD	301±3.0	0.953±0.001			
		MW	438±2.0	0.933±0.004			

DM, dry matter; a_w; water activity, FD; flowering direct-cut, FW; flowering wilted; MD; milk direct-cut; MW; milk wilted.

is that changes differed in their direction and magnitude among the various experiments or between the control and inoculated silages for various measured parameters. Table 12 summarizes the relationship between DM content and water activity of fresh crop and silage from two different cultivars of wheat. The table also includes the actual values on DM and water activity for each type and maturity stages within fresh crop or silage for both cultivars. In cultivar Galil water activity has higher negative correlation with DM content of both silage (P<0.0001) and fresh crop (P<0.0001). In BH cultivar, water activity has negative correlation with DM content of silage (P<0.03) but negative correlation between water activity and DM content of fresh crop is non-significant (P<0.16).

3. Sorghum and corn silage:

Tables 13 and 14 summarize the changes and losses in fresh sorghum crop during ensiling and the effect of spraying aqueous corn silage extract on the sorghum silages and 7 days of their aerobic exposure. During ensiling, the pH decreased, WSC content decreased, and lactic acid and ethanol concentration increased. The silage showed increase in ash and NDF content and reduced DMD. During aerobic exposure, silages were very stable, with no or slight change in silage composition although silage started heating after 100 hours of aerobic exposure (Fig. 6) and had higher numbers of yeasts and molds on day 7 of aerobic exposure. In case of sorghum silages sprayed with aqueous extract of corn silages, although, silages did not change pH, they showed a higher CO_2 production and had higher DM losses than the non-sprayed silages, leading enhanced aerobic deterioration due to sparying.

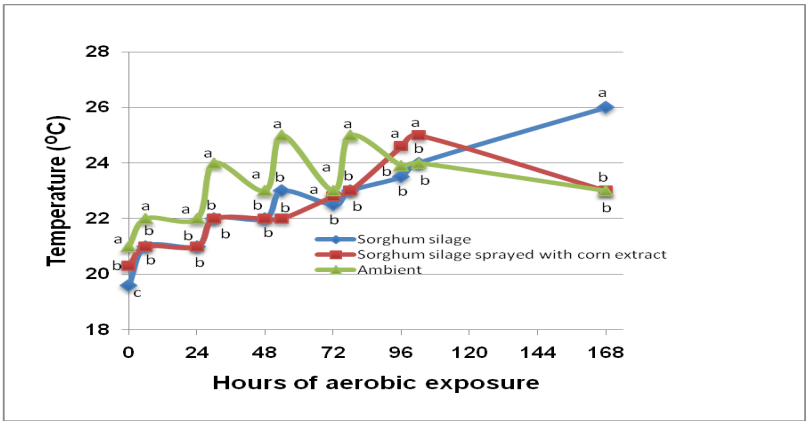

Fig. 6. Changes in temperature during aerobic exposure of sorghum silage and sorghum silage sprayed with corn extract. Within sampling hours, means followed by different letters are significantly different (P<0.05).

Table 13. Changes in chemical and microbiological composition during ensiling and aerobic exposure of sorghum silage and sorghum silage sprayed with corn silage extract.

Chemical parameters are in g/kg DM and microbiological data are in log CFU/g DM. (means±s.d.)

Day	pH	LAB	Yeasts	Molds	Ash	WSC	Lactic acid	Ethanol	Acetic acid
Fresh crop	5.64^{a}	3.9^{b}	5.6^{e}	5.7a	62.6^{b}	170.6^{a}	-	-	-
Silage	3.8±0.0^{bc}	5.6±0.2^{a}	5.6±0.3^{e}	1.5±1.3b c	81.6±2.2^{a}	47.2±11.1^{b}	33.8±6.6^{b}	45.2±8.4^{a}	7.1±1.9^{ab}
AE2	3.9±0.0^{bc}	-	5.5±0.2^{e}	NFc	-	-	-	-	-
AE3	3.8±0.0^{c}	-	5.7±0.2^{de}	0.7±1.3b c	-	-	-	-	-
AE4	3.8±0.0^{bc}	-	6.3±0.1^{cd}	NFc	82.5±1.7^{a}	48.5±19.3^{b}	33.2±2.5^{b}	28.9±4.9^{b}	5.8±0.4^{b}
AE7	3.9±0.0^{bc}	-	6.8±0.4^{bc}	2.9±0.1b	81.6±1.1^{a}	48.5±8.0^{b}	49.8±2.1^{a}	37.3±8.5^{a}	8.0±0.7^{ab}
Sprayed AE4	3.9±0.0^{bc}	-	7.7±0.2^{a}	2.7±0.3b	84.4±1.4^{a}	25.5±3.9^{bc}	36.6±2.4^{b}	32.4±7.5^{a}	6.2±0.9^{b}
Sprayed AE7	3.9±0.1^{b}	-	7.2±0.3^{ab}	1.1±2.0b c	83.8±3.1^{a}	13.6±3.7^{b}	35.9±0.9^{b}	47.5±8.8^{a}	10.0±0.8^{a}

Within each column, means followed by different letters are significantly different (P<0.05). LAB, lactobacilli; WSC, water-soluble carbohydrates; NF, not found; AE, aerobic exposure. Propionic and butyric acids in the silage were 0.34±0.8 g/kg while during aerobic exposure their were below detectable levels. The water activity of the silage was 0.955±0.002. The water activities of non-sprayed and sprayed silages during day 4 of aerobic exposure were found to be 0.955±0.01 and 0.959±0.004 respectively.

Table 14. Losses during ensiling and aerobic exposure of sorghum and sorghum silage sprayed with corn silage extract (means±s.d.)

Day	CO_2 (g/kg DM)	DM (g/kg)	% DM loss	NDF (g/kg DM)	DMD (g/kg DM)	NDFD (g/kg DM)
Fresh crop	-	335±2.3^{a}	-	495.2±6.2^{b}	710.5±12.0^{a}	525.3±17.4^{a}
Fresh silage	-	305.6±5.7^{c}	11.0±1.7^{a}	550.5±20.8^{a}	658.6±24.0^{b}	517.4±13.3^{ab}
AE2	0.4±0.3^{c}	322.5±6.8^{ab}	0.0^{b}	-	-	-
AE3	0.9±0.7^{c}	327.4±2.0^{ab}	0.0^{b}	-	-	-
AE4	5.3±0.2^{bc}	320.6±4.5^{b}	0.0^{b}	538.6±12.7^{ab}	648.3±7.2^{b}	483.6±7.4^{b}
AE7	7.4±0.8^{abc}	324.2±6.9^{ab}	0.0^{b}	532.4±15.2^{ab}	663.9±14.2^{b}	503.3±15.6^{ab}
Sprayed AE4	13.6±7.9^{ab}	293.6±4.3^{c}	5.4±1.3^{b}	543.5±33.9^{a}	660.3±3.0^{b}	518.6±5.8^{ab}
Sprayed AE7	15.6±0.7^{a}	294.1±1.6^{c}	3.9±0.5^{b}	550.4±18.9^{a}	650.4±7.7^{b}	496.0±8.0^{ab}

Within each column, means followed by different letters are significantly different (P<0.05).
DMD, dry matter digestibility; NDFD, NDF digestibility; AE, aerobic exposure.
No propionic and butyric acids were found in any of the sorghum silage samples. Within column % DM loss, negative values obtained for day AE2 to AE7 are considered as zero.
The water activity of the silage was 0.955±0.002 whereas; non-sprayed and sprayed silages during day 4 of aerobic exposure had 0.955±0.01 and 0.959±0.004 respectively.

Tables 15 and 16 summarize the changes and losses in fresh corn crop during ensiling and the effect of spraying aqueous sorghum silage extract on silage and 7 days of their aerobic exposure. During ensiling, pH decreased and lactic acid concentration increased. During 4 and 7 days of aerobic exposure, silages showed increased pH, decreased lactic and acetic acid and increased CO_2 production along with higher numbers of yeasts and molds and rise in temperature (Fig. 7). During 7 day of aerobic exposure, silage had higher DM losses and reduced DMD. Unexpectantly, in case of corn silages sprayed with aqueous extract of sorghum silages, silages increased pH, decreased lactic and acetic acid, had lower DMD and higher DM losses and yeasts and molds population along with highest temperature at 72 hours of aerobic exposure (Fig. 7). Sprayed silages at 7 days of aerobic exposure were most unstable indicated by the highest pH and ash content and the highest DM losses and CO_2 production. These silages also had the highest NDF content and lowest DMD along with highest numbers of yeasts and molds. We also calculated the polyphenol content of both sorghum and corn silage extracts, as potential antioxidant constituents which might have enhanced aerobic stability and they were found to be 28.1 and 13.5 mg/ ml respectively.

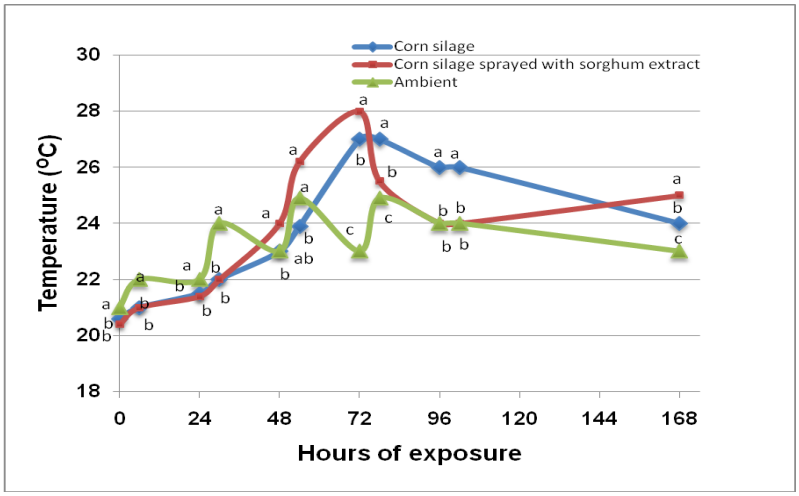

Fig. 7. Changes in temperature during aerobic exposure of corn silage and corn silage sprayed with sorghum silage extract. Within sampling hours, means followed by different letters are significantly different (P<0.05).

Table 15. Changes in chemical and microbiological composition during ensiling and aerobic exposure of corn silage and corn silage sprayed with sorghum extract.

Chemical parameters are in g/kg DM and microbiological data are in log CFU/g DM (means±s.d.).

Day	pH	LAB	Yeasts	Molds	TC	Ash	WSC	Lactic acid	Acetic acid
Fresh crop	6.0^{b}	5.4	6.9^{ab}	6.5ab	-	49.7^{b}	73.6^{a}	-	-
Fresh silage	3.6±0.0^{d}	3.1±2.8	3.5±3.1^{b}	1.2±2.1c	-	51.4±1.7^{b}	33.2±14.1^{ab}	41.9±6.9^{a}	7.2±0.6^{a}
AE2	3.6±0.0^{d}	-	8.5±0.2^{a}	NFc	-	-	-	-	-
AE3	3.7±0.0^{d}	-	8.8±0.1^{a}	NFc	-	-	-	-	-
AE4	4.1±0.2^{c}	-	9.3±0.1^{a}	4.8±1.3b	-	52.4±0.9^{b}	24.1±8.0^{b}	19.1±0.9^{b}	0.6±1.1^{b}
AE7	5.9±0.2^{b}	-	7.4±3.4^{ab}	8.3±0.7a	9.7±0.4	53.9±1.7^{b}	22.2±2.9^{b}	5.0±1.7^{c}	0.0^{b}
Sprayed AE4	5.8±0.2^{b}	-	9.6±0.2^{a}	6.2±0.6a b	-	54.4±1.4^{b}	22.2±6.9^{b}	4.1±1.9^{c}	0.0^{b}
Sprayed AE7	7.0±0.0^{a}	-	9.7±0.2^{a}	8.7±0.1a	9.9±0.2	62.3±2.6^{a}	24.1±41.7^{b}	2.8±1.8^{c}	1.0±1.7^{b}

Within each column means followed by different letters are significantly different (P<0.05).
No propionic and butyric acids were found in any of the corn silage samples.
LAB, lactobacilli; TC, total counts; WSC, water-soluble carbohydrates; NF; not found; AE, aerobic exposure.
In fresh silage, ethanol was 1.9±1.6 g/kg DM, while for rest of silages, it was not detected.
The water activity of the silage was 0.979±0.002. The water activities of non-sprayed and sprayed silages during day 4 of aerobic exposure were found to be 0.986±0.004 and 0.994±0.004 respectively.

Table 16. Losses during ensiling and aerobic exposure of corn silage and corn silage sprayed with sorghum silages (means±s.d.)

Day	CO_2 (g/kg DM)	DM (g/kg)	% DM loss	NDF (g/kg DM)	DMD (g/kg DM)	NDFD (g/kg DM)
Fresh crop	-	321±10.1^{ab}	-	442.4±32.0^{b}	723.8±0.7^{a}	481.5±2.1
Fresh silage	-	319.4±8.7^{ab}	0.9±2.7^{bcd}	413.5±13.7^{b}	706.9±9.8^{ab}	419.6±14.7
AE2	4.6±0.7^{d}	323.0±6.8^{ab}	0.0^{cd}	-	-	-
AE3	14.3±3.4^{cd}	326.7±2.7^{a}	0.0^{d}	-	-	-
AE4	38.3±23.0^{bc}	326.8±1.4^{a}	0.0^{cd}	444.3±30.9^{b}	693.0±10.2^{bc}	440.8±51.9
AE7	47.7±2.8^{b}	312.6±7.2^{ab}	4.4±2.5^{bc}	452.2±24.0^{ab}	685.0±11.2^{cd}	441.4±31.4
Sprayed AE4	40.0±8.2^{bc}	305.8±0.9^{b}	6.0±0.4^{b}	457.2±19.9^{ab}	686.8±8.1^{cd}	461.3±26.6
Sprayed AE7	101±13.7^{a}	274.2±8.2^{c}	16.8±2.4^{a}	503.5±25.9^{a}	673.3±10.6^{d}	480.4±26.3

Within each column means followed by different letters are significantly different (P<0.05)
DMD, dry matter digestibility; NDFD, NDF digestibility; AE, aerobic exposure. No propionic and butyric acids were found in any of the corn silage samples.
TC, total counts; WSC, water-soluble carbohydrates; NF, not found.
Within column % DM loss, negative values obtained for day 2 to 4 is considered as zero.
The water activity of the silage was 0.979±0.002. The water activities of non-sprayed and sprayed silages during day 4 of aerobic exposure were found to be 0.986±0.004 and 0.994±0.004 respectively.

V. DISCUSSION

Aerobic stability is a term that indicates the length of time that silage remains cool and does not spoil after it is exposed to air. In this context, there may be few major aspects of managing the problem of aerobic deterioration. First can be the lowering the degree of aerobic exposure which controls the growth of the aerobic spoilage microorganisms (Pahlow and Muck, 2009). One might think that proper fermentation of silage by appropriate management of ensiling process such as quick filling, good compaction, faster sealing, and right choice of silos with less porosity etc. may ensure the aerobic stability silage. Although, good silage fermentation helps to slow the growth of initiators of aerobic deterioration, it does not prevent their growth during aerobic exposure (Pahlow and Muck, 2009).

Second aspect of managing silage aerobic deterioration would be the harvesting of ensiling crops at right DM content and stage of maturity along with other treatments such as wilting and use of suitable additives. The current study focuses on the second option with the hypothesis that whole-wheat crop harvested at different stages of maturity and ensiled directly after harvest (DC) or wilted to different DM contents exhibit different magnitude of aerobic deterioration. In the silage aerobic stability test, how long the silages could be exposed to air before spoiling is an important question. In the current experiments, we followed changes and losses of various wheat, corn, and sorghum silages during aerobic exposure, where, the aerobic stability test lasted for 7 days, long enough time, which would enable to observe differences between various types of silages.

1. Wheat Silage:

In the experiment, we used two wheat cultivars BH and Galil at two different stages of maturity: flowering and milk stage. After harvesting, crops were ensiled directly (DC) or subjected to wilting to study these effects on ensiling characteristics and on aerobic stability parameters. The DM contents of DC silages were higher in cultivar BH as compared to Galil, whereas similar DM contents were obtained upon wilting in both cultivars (Tables: 5 and 10). During harvesting of cultivar BH, there was a drought condition but crops from cultivar Galil were harvested 1 day after rainfall. In addition, crops from cultivar Galil were inoculated with homofermentative LAB *(Lactobacillus plantarum)* to understand its effect on ensiling and aerobic parameters, and to learn its interaction with other factor such as stage of maturity and wilting. In

both cultivars BH and Galil, flowering DC, flowering wilted, milk DC and milk wilted are the types of crops used in the experiments. Thus, wheat served as a very good model to explore the relationship between silage composition and aerobic stability by harvesting crop at different stage of maturity, by either ensiled as DC or wilted and having different ferementation profiles, which can affect on time on extent of aerobic deterioration of silages.

In cultivar BH, within each silage type, slightly lower WSC concentration in milk stage could be due to the conversion of WSC to starch during kernel formation. As the milk stage progresses, the developing endosperm starts as a milkey fluid that increases in soilds which could reduce the overall concentration of WSC. Regardless of maturity stage, wilting tended to decrease WSC of the fresh crop, because sucrose is catabolised to carbon dioxide in wilting grass, and fructans and total soluble fructose residues decrease almost continuously throughtout the wilting period probably because of the activities of plant enzymes (McDonald et al., 1991). However, in cultivar Galil, wilting increased WSC in milk stage which maybe due to photosynthesis, as realtively short period of wilting (8 hours) along with sufficient moisture content resulted sugar assimilation in wilted biomass. In general, wilting reduced ensiling DM losses except for milk silages from cultivar BH where ensiling losses were similar.

In cultivar BH, in the milk stage, both DC and wilted silages, although decreased pH but it was unusually high as compared to silages at flowering stage. Within type, wilted silages had significantly higher pH (4.9) as compared to DC silages (4.7) (Table. 5). Higher pH obtained in milk stages may be due to poor compaction resulting from higher DM content of silages. In general, only wilted silages (except for milk DC silages of cultivar BH) did not decrease WSC during ensiling which could be due to the release of WSC as a result of hemiceullulose hydrolysis during ensiling (Morrison, 1979). pH dependent activity of hemicelluose hydrolyzing enzymes might brought such a results as higher DM content and lower water activity do not support the LAB activity, resulting no immdiate decrease in pH which favors the activity of plant enzymes involved in hemicllulose hydrolysis. In cultivar BH, flowering stages had higher ensiling losses as compared with milk stage, which is similar with the previous findings by Chen and Weinberg (2009). Whereas, in cultivar Galil, DC silages from both maturity stages had higher ensiling as compared with

respective wilted silages. Ashbell and Weinberg (1992) mentioned the high losses in horizontal bunker silos associated with decreased DM content, increased pH and ash content and high counts of molds. Although, Kung (2005) mentioned that drier silages are more susceptible to aerobic spoilage due to a restricted fermentation and lower acid end products in drier silages, results have shown the better aerobic stability of silages with advancing maturity. Lower availability of WSC at later stage of maturity could be one of the reasons that restricts the growth of yeasts and molds. In addition, if there is lack of adequate water activity in a harvested crop, even yeasts are unable to grow and the dry forage is stable (Kung, 2005).

As compared with regular silage, baled silage is generally baled at a higher DM content, stored at a lower bulk density, has a thinner plastic barrier protecting it from oxygen and has 6-8 times the surface area in contact with the plastic film. McEniry et al. (2007) showed that baled silage was less stable on exposure to air on day 98 as compared to precision-chopped silage with a faster time to onset of heating, a higher maximum temperature rise and higher accumulated temperature during both 120 and 192 h aerobiosis.

Results in this study clearly present the trend and magnitude of aerobic stability in two cultivars of wheat that were at different level of DM contents, both harvested at flowering and milk stage of maturity, and ensiled as DC or wilted. In addition, effects of homofermentative inoculants, both separately and interactively, with other crop factors were also observed in cultivar Galil. A summary on aerobic stability in two different wheat cultivars is presented in table 17.

Regardless of difference in DM content and cultivar, flowering DC were the most stable silages upon aerobic exposure as compared to other types. In cultivar BH, during 4 and 7 days of aerobic exposure, the interaction of maturity stage and silage type were significant ($p<0.01$) for pH and ash content and were insignificant for WSC content, lactic and acetic acid concentration, CO_2 production and DM losses (Table. 5). In cultivar Galil, The interactions of maturity stage and silage were non-significant for pH, ash content, WSC level, lactic and volatile fatty acids, NDF content and DMD and highly significant ($P<0.01$) for NDFD (Table. 11).

Table 17. A summary on aerobic stability of wheat silages cultivar BH and Galil, and effect of homofermentative inoculation on aerobic stability

Cultivar	Type	DM content (g/ kg)	Aerobic stability	Inoculation
BH	F DC	287.0	Stable	-
	F W	362.2	Stable	-
	M DC	398.4	• Stable but showed higher CO_2 production on day 7th of AE	-
	M W	427.0	• Stable but showed slightly higher CO_2 production and higher numbers of yeasts and molds on day 7th of AE	-
Galil	F DC	199.2	• Stable	• Resulted aerobic deterioration during 4 and 7 days of AE
	F W	378.8	• Unstable during 7 day of AE	• Inoculation did not result further deterioration.
	M DC	299.2	• Stable till day 4 of AE but already spoiled on 7 day of AE	• Slightly enhanced aerobic deterioration
	M W	437.3	• Had higher amount of CO_2 and higher number of yeasts on day 4 and further rapidly spoiled on day 7 of AE	• Inoculation enhanced deterioration on day 4 but did not have major effects on 7th day of AE

DM, dry matter; BH, bet hashita; F, flowering; M, milk; DC, direct-cut; W, wilted; AE, aerobic exposure.

In general, during aerobic exposure, NDF content increased and DMD decreased due to loss of soluble constituents, which is in agereement with previous results reported by Sanderson (1993). Results show that silages at milk stage were slightly unstable upon aerobic exposure as compared to flowering stage in both wheat cultivars (Tables. 5 and 10). This result is consistent with the previous results by Chen and Weinberg (2009), as the silage at the flowering stage was more aerobically stable than at the milk stage. Ashbell et al. (1984) also reported that wheat silages at the milk stage were less stable during aerobic exposure, as compared with the dough stage. Silages at flowering stage counted lower number of yeasts as compared to milk stage. The reason for the higher aerobic deterioration in milk stage could be explained by silage composition. Silages at the flowering stage had lower DM content and with higher concentration of VFAs, mainly butyric acid, which has antifungal properties (Moon, 1983), could be a reason for its aerobic stability. Regardless of similar DM content (362 and 378 g/kg DM for cultivars BH and Galil, respectively), flowering wilted silages only from cultivar Galil were unstable. The probable reason behind it

could be silage composition. Flowering wilted silages from cultivar Galil had higher level of WSC and numbers of yeasts, lack of propionic and butyric acids and lower acetic acid concentration as compared with silages from cultivar BH. Cultivar factor may be the one to bring the changes in silage composition, along with differences in climate during growth and harvest.

In general, wilting enhanced the aerobic deterioration of silages except for flowering wilted silages from cultivar BH. In milk silages from cultivar BH, wilting enhanced aerobic deterioration with slightly higher amount of CO_2 production and higher numbers of yeasts and molds mainly on day 7 of aerobic exposure (Tables. 1-5). Within the milk stage, wilted silages had lower concentration of butyric acid and had higher numbers of yeasts and molds and show a higher degee of aerobic deterioration as compared to milk DC silages (Table. 5). In cultivar Galil, both for flowering and milk stage silages, wilting enhanced aerobic deterioration. For inoculated silages, wilting showed its effect only for milk silages with increased deterioration during day 4.

Within same maturity stage and silage type, difference in DM contents in two cultivars brought changes in silage composition and aerobic stability. Milk DC silages from cultivar BH (398.4 g/kg DM) were stable but showed slightly higher CO_2 production on the 7^{th} day of aerobic exposure without changing other parameters (Tables. 3-4). Milk DC silages from cultivar Galil (299.2 g/kg DM) were stable until day 4 but spoiled during 7^{th} day of aerobic exposure showing higher CO_2 production and DM losses (Tables. 8-9). The difference in silage aerobic stability may be explained by silage composition. Milk DC silages from cultivar Galil had higher number of yeasts and had no propionic and butyric acids but had acetic acid whereas, cultivar BH had almost lack of yeasts and molds and had propionic and butyric acids. Difference in DM content, although did not show much difference in water activity values, may also be a reason to bring such difference in aerobic stability. However, in future, comparison of milk DC silages from these two cultivars with similar DM content would show whether cultivar is the factor that gives a different response to the aerobic stability test. For cultivar BH, milk wilted silages were stable until day 4 and had slightly higher amount of CO_2 and higher numbers of yeasts and molds on day 7 (Tables. 3-4). Control milk wilted silages from cultivar Galil had singificant level of CO_2 and yeast population was high during day 4 and rapidly spoiled on day 7 (Tables.

8-9). DM contents were similar in both cultivars, but had different silage composition *i.e.* silages from cultivar BH had acetic, butyric and little propionic acids whereas cultivar Galil had only little acetic acid, in addition to difference in other ensiling parameters. These results suggest that cultivar factor may be the one to give different degree of aerobic deterioration in these silages.

For the pooled results of day 4 and 7 during aerobic exposure, the interactions of silage type and inoculation were signicant ($P<0.01$) for pH, lactic acid and CO_2 production (Table 11). The interactions of maturity stage, silage type and inoculation were signicant ($P<0.05$) for pH and WSC content (Table. 11). In general, inoculation of homofermentative microorganisms enhanced the aerobic deterioration of silages, although inoculation had improved the ensiling parameters. The inoculants used substrates only for lactic acid production, lowering the VFAs content in silages, which indeed play a vital role in inhibiting the spoilage microorganisms during aerobic exposure. These results are similar with the previous results reported by Weinberg (1993), who showed a faster aerobic deterioration by homofermentative inoculation in wheat and sorghum silages at milk stage of maturity. Cai et al. (1999) also showed that silages treated with *Lactobacillus plantarum* FG 10 did not inhibit yeasts growth and increased aerobic deterioration of silages. Weinberg et al. (1999) and Weinberg (2002) also showed a higher aerobic deterioration in wheat silages from milk stage when inoculated with homofermentative LAB (*Lactobacillus plantarum*). Filya et al. (2004) also showed aerobic instability of wheat silages from early dough stage (indicated by higher CO_2 level and numbers of yeasts, and molds) inoculated with *Lactobacillus plantarum,* during 5th day of aerobic exposure.

The factors behind the enhancement in aerobic instability of silages inoculated with homofermentative LAB are high levels of residual sugars, higher lactic acid production, and low level or lack of protective VFA (Weinberg et al., 1993). In inoculated silages, less sugar is utilized to produce lactic acid because of efficient homolactic fermentation. Then residual WSC content and higher lactic acid serve as substrate of yeasts and molds along with lack of antimycotic VFAs. In silages from milk stage, for both DC and wilted silages, inoculation resulted an early aerobic deterioration and more intensive spoilage as compared with non-inoculated silages. The temperature profiles of the silages agree with this observation. In spite of adverse effect of inoculation on silage aerobic stability, inoculation did not further deteriorate

flowering wilted silages during aerobic exposure. Inoculation also did not have major effects on ensiling parameters in these silages with no efficient homolactic fermentation, which could be reason behind the no further aerobic deterioration of these silages with inoculation. DM content or water activity value could not explain this results as milk-wilted silages with lower water activity value and higher DM content as compared to flowering wilted were further deteriorated by inoculation. Results show that during aerobic exposure, DC silages are sucesptible to aerobic spoilage following homofermentative inoculation regardless of maturity stages, showing changes in silage composition.

Thus, maturity stages, silage type and inoculation, either separately or interactively, affect the magnitude of silage aerobic stability by changing silage composition. We performed stepwise regression analysis between CO_2 produced during days 4 and 7 of aerobic exposure and DM content, acetic and butyric acids, WSC concentration of silages and following regression models were obtained:

CO_2 (day 4) = 36.0 – 3.5 acetic acid (P<0.0001)

CO_2 (day 7) = 96.0 – 8.8 acetic acid (P<0.0001)

DM content, butyric acid, and WSC content could not meet the siginificance level to be entered into the above models.

Here we studied the effect of homofermentative inoculants along with other crop factors; Weinberg and Muck (1996) suggested the use of heterofermentative LAB as silage starters from the aerobic stability perspective. Yeasts and molds are inhibited by short chain fatty acid (mainly acetic acid) resulting from heterolactic fermentation then aerobic stability of silage can be improved. Therefore, in future, study on effect of heterolactic inoculants along with other crop factors would widen our knowledge on interaction of the inoculants with other crop factors to affect aerobic stability of silages.

Although this study included wheat with a wide range of DM content, between 200 and 437 g/kg, the water activity values of the fresh crops and silages were between 0.99 and 0.93, respectively, which enables the activity of many microorganisms. Although previous researchers agree on potential effects of water activity on composition and aerobic stability of silages, to the best of our knowlede, only Greenhill (1964) included water activity measurements in a silage experiment.

Greenhill (1964) showed models of ryegrass and alfalfa with variable moisture contents, which correspond to 100-500 g/kg DM, obtained water activity values of 0.995-0.958, respectively; hence, small differences in water activity values reflect large differences in DM content and the availability of moisture in various crops for microbial activity with impact on the ensiling fermermentation. In the current study, high negative correlation coefficients were obtained between DM content and water activity of both fresh crops as well as silages.

Decision on when to harvest the wheat crops during ensiling depends upon different parameters such as DM yield, DM losses, digestibility, and aerobic stability. In an entire silage making process, DM losses include the losses starting from harvesting through out ensiling and feed out. During harvesting, wilting, ensiling, and aerobic exposure, there is a loss of DM either in fresh crops and/or in silages. However, this study mainly focused on ensiling DM losses and DM losses during aerobic exposure. Losses should be evaluated by using the product of DM content in silages and their digestibility. In our study, although, flowering DC silages were stable during aerobic exposure, they exhibited higher ensiling DM losses. Therefore, harvesting crops at flowering stage with relatively higher DM content may reduce ensiling DM losses with better aerobic stability.

2. Sorghum and corn silage:

Results show that both sorghum and corn silages decreased pH and WSC level, and increased lactic acid concentration during ensiling (Tables. 13-15). During aerobic exposure, sorghum silages were quite stable with no change in pH, little CO_2 production, and zero DM losses (Tables. 13-14). On the other hand, corn silages (which were made from half milk-line stage) were aerobically deteriorated during 4 and 7 days of aerobic exposure, indicated by increased pH and CO_2 production, increased DM losses along with higher numbers of yeasts and molds (Tables. 15-16) and rise in temperature (Fig. 9). Previous studies indicated that corn silages are very susceptible to aerobic deterioration whereas sorghum and legume silages are stable upon aerobic exposure (Weinberg et al., 2002). This agrees with Filya et al. (2004) who observed a lower (although, non-significant) CO_2 production (20.4 g/kg DM) with lower number of yeasts and molds in un-treated (control) sorghum silage (76 g/kg WSC) as compared with control corn silage (25.6 g/kg DM CO_2 and 22 g/kg DM WSC).

Filya (2004) studied the aerobic stability of whole crop maize silages harvested at four different stages of maturity. He showed that the maize silage of the early dent stage (211 g/kg DM) was susceptible to aerobic exposure (indicated by higher pH and CO_2 production, and lower acetic acid) as compared with silages at one-third milk-line (282 g/kg), two-thirds milk-line (358 g/kg) and black-line (420 g/kg) stages. Hu et al. (2009) studied the effects of bacterial inoculants on corn silages at two different DM contents. In untreated (control) silages, silages at normal maturity (32.7 % DM and 1.22 % WSC) were less stable as compared with silages at later maturity (39.8 % DM and 0.89 % WSC). Previous researches have tried to find ways for improving aerobic stability of corn silages. For example, Weinberg et al. (2002) improved the aerobic stability of corn silages by inculating with heterofermentative LAB (*Lactobacillus buchneri*). Filya et al. (2004) showed an improvement in aerobic stability of corn silages by inoculating with *Propionibacterium acidipropionici*. Silages inoculated with *P. acidipropionici* had higher levels of propionic and acetic acids than control silages.

In general, crops with a high starch and sugar concentration are prone to aerobic spoilage as yeasts use sugars as energy sources during fermentation (Weinberg et al., 1993). However, sorghum silages, in spite of higher WSC concentration, are more stable to aerobic exposure than the corn silages and the reason behind it is still not clear. When we compared silage compositon between corn and sorghum silage, the major parameters, which are likely to affect aerobic stability, were similar. For example, in both silages, lactic (P<0.06), acetic (P<0.93), propionic (P<0.34) and butyric acids (P<0.34) were not significantly different. These results suggest that other factors might affect aerobic stability of sorghum and corn silages.

Probably, there might be some intrinsic compounds in sorghum, which may affect aerobic stability. Sorghum is unique among major cereals because some cultivars contain polymeric polyphenol compounds. Previous studies show that some cultivars of sorghum contain polyphenol as condensed tannins (Jansman, 1993). All sorghums contain phenols, most contain flavonoids, and some contain phenolic acids (Waniska, 2000). Polyphenols are known to serve as natural antioxidants and therefore, we decided to find out whether they play a role in aerobic stability. Antifungal properties of sorghum polyphenols have also been reported, showing improved mold resistence, and decreased fungal attack and mycotoxin problems in

sorghum crop (Waniska, 2000). Therefore, we hypothesized that spraying of sorghum silage extract on corn silage could improve the aerobic stability corn silage and on the other hand spraying of corn silage extract on sorghum silage could initiate or enhace the aerobic deterioration of sorghum silage. Results showed that extract of sorghum silage had more polyphenol level (28.1 mg/ ml) as compared with corn silage (13.5 mg/ ml) (*P<0.0001)*, which could be a factor behind the better aerobic stability of sorghum silage. We tested these hypotheses by mutual spraying of aqueous extracts of corn and sorghum silages on each other.

Not surprisingly, when sorghum silages were sprayed with aqueous extract of corn silages and subjected to aerobic exposure, the sprayed silages showed higher CO_2 production, higher DM losses, and incresed yeasts and molds population without increment in pH (Tables. 13-14). Since yeasts and molds can tolerate water activity as low as 0.8, the additional moisture brought about by spraying probably did not affect these populations. For both sprayed and non-sprayed silages, water activities during day 4 of aerobic exposure were 0.959±0.004 and 0.955±0.01, respectively, which is favorable range for yeasts and molds for their growth (Marriott, 2004).

Unexpectantly, in case of corn silages, spraying of aqueous extract of sorghum silages further increased aerobic deterioration with higher CO_2 production, increased pH, and with higher number of yeasts and molds (Tables. 15-16). Water activities during 4 day of aerobic exposure for sprayed and non-sprayed silages were 0.994±0.004 and 0.986±0.004. In addition, in order to know whether sprayed extract changed microbiological composition in sprayed silages, we calculated the total counts of bacteria for sprayed and non-sprayed corn silages for day 7 and these values were similar for both silages (Table. 15). Another parallel study which was done in our laboratory also showed that spraying of aqueous extract of sorghum silages on corn silages enhanced the aerobic deterioration of corn silages (Yildiz 2009, personal communication). These results clearly showed that spraying of sorghum silage extract cannot improve the aerobic stability of corn silage. In this study, we prepared aqueous extracts as a ratio of 1:4 (50 gm of silages: 200 ml distilled water) and cross-sprayed at the rate of 6.7% (133 ml extract solution in 2 kg of silages). We enumerated the total counts in sprayed and non-sprayed corn silages during 7 day of aerobic exposure, which were found to be similar (9.7 and 9.9 CFU/ g DM bacteria for non-sprayed and sprayed silages, repectively).

In our study, we prepared aqeuous extract of sorghum silages and spryaed on corn silages, which added polyphenol 1.9 mg/ g of corn silages, which was not a substantial addition. Other extraction methods such as extraction on organic solvents e.g. on methanol, acid butanol etc. may exhibit efficient extraction of polyphenols and then may show an effectiveness of spraying of sorghum silage extract in improving aerobic stability of corn silages. Within polyphenols, antinutritional effects of tannins such as diminished protein digestibility have been identified therefore more emphasis on extraction other polyphenolic compounds such as phenolic acids would avoid such an antinutritional effect of tannin as a result of sparying extracted solution. In addition, comparison of aerobic stability in sorghum silages from two different types: white and brown sorghum would show the role of polyphenols in aerobic stability because brown sorghum plants contain more polyphenols (Waniska, 2000). In addition, aerobic exposure of sorghum silages harvested at different stages of maturity may show different magnitude of aerobic deterioration as polyphenol contents in sorghum may vary with maturity stages. In addition, antifungal proteins also have been identified in sorghum (Joshi et al., 1998) which may be reponsible behind the aerobic stability of sorghum silages. Therefore, further study is needed to understand the aerobic stability behind sorghum silage and to exploit such property for other aerobically susceptible silages as the aerobic instability is one of the major problems in many commercial silages that results in higher DM losses and then a depression in nutritive value of silages.

VI. NOVELTY OF THE RESEARCH

In our study, showing the effects of different intrinsic (crop) factors *i. e.* stage of maturity and type, and homofermentative inoculation, and their interaction was the major novelty in wheat silages. This study showed that these factors affected interactively silage composition and hence exhibited different responses to aerobic exposure of silages. A relationship has been established between silage composition and aerobic stability by taking CO_2 (as an indicator of aerobic deterioration) and other factors, which are most likely to affect the aerobic deterioration, mainly VFAs. In corn and sorghum silages experiment, we tried to explore what would be the factors responsible for aerobic stability of sorghum silages. We sprayed sorghum silages extracts on corn silages with an assumption that polyphenol compounds are responsible for the aerobic stability of sorghum silages. Further studies are warranted

in sorghum silages: compounds itself *i. e.* either polyphenols or antifungal proteins, method of extraction *i. e.* in organic solvents such as methanol, acid butanol etc., and method of application. These studies can ultimately widen our knowledge in future to understand the mechanism behind aerobic stability of sorghum silages and to exploit such property for improvement in aerobic stability of other suceptible silages like corn. In our study, we also measured water activity for both fresh crops and silages and established correlation between DM content water activity values, as water activity is one of the important parameters for growth and multiplication of many microorganisms included with those found in silages.

VII. CONCLUSION

In this experiment, we studied aerobic the stability of wheat silages of two cultivars, harvested at two different stages of maturity *i. e.* flowering and milk stage, ensiled as DC or wilted, and inoculated with homofermentative LAB (*L. plantarum*). In addition, we evaluated the aerobic stability of corn and sorghum silages alone or cross-sprayed with aqueous extract of each silage to another. Silages made from wheat harvested at milk stage of maturity were overall less stable as compared with those made from the flowering wheat. Silages of cultivar BH were more stable during aerobic exposure than those of cultivar Galil. Within cultivars and maturity stages, wilting showed a higher degree of aerobic deterioration as compared with DC silages. Silages with higher DM content tended to remain stable during aerobic exposure. Inoculation with homofermentative LAB enhanced the aerobic deterioration of silages. In general, silages with higher concentration of VFAs had improved aerobic stability whereas higher level of residual WSC supported aerobic deterioration of silages.

During aerobic exposure, sorghum silages were very stable with no change in pH, little CO_2 production, and zero DM losses, whereas corn silages were aerobically deteriorated. Differences in ensiling parameters between sorhgum and corn silages were too small to explain the differences in aerobic stability between them. We hypothesized that sorghum silages, which are by far more aerobically stable than corn silages contains some intrinsic factor, which contributes to this characteristic. Spraying sorghum and corn silages with the opposite aqueous extracts enhanced aerobic deterioration. Polyphenol content, which was higher in aqueous extract of

sorghum silages, could not explain this result. Further research is needed in order to explore the factor behind the aerobic stability of sorghum silages for its potential exploitation to improve aerobic stability of suceptible silages, which is still a serious problem in most silage farms.

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תקציר

הכנת תחמיץ מבוססת על תסיסה של חיידקי חומצת חלב בגידולי מספוא בתנאים אנאירוביים. תחמיצים נחשפים לאוויר בזמן ההכנה, השימור וההאבסה. באופן מעשי קשה למנוע את החשיפה של תחמיצים לאוויר. בזמן חשיפת תחמיצים לאוויר, פעילות מיקרוביאלית אירובית גורמת להפסדים בערכים התזונתיים של התחמיץ. בנוסף לגורמים סביבתיים, עמידות תחמיצים בעת חשיפה לאוויר תלויה בהרכב התחמיץ ובשימוש בתוספים. מטרת המחקר הנוכחי הייתה לקבוע שינויים והפסדים אירוביים של תחמיצי חיטה שהוכנו משני זנים (בית השיטה וגליל) ואשר נקצרו בשלבי פריחה וחלב, הוחמצו ישירות או אחרי הקמלה וטופלו בזן מסחרי של חיידק הומו-לאקטי (*Lactobacillus plantarum*). בנוסף, נבחנה השפעת הרכב התחמיצים על עמידותם האירובית. מטרה נוספת הייתה לקבוע הפסדים בזמן חשיפה לאוויר של תחמיצי תירס וסורגום ולבחון האם אחד משני התחמיצים מכיל גורם שמייצב את התחמיץ בעת חשיפה לאוויר, ע"י ריסוס הדדי של מיצוי מימי של התחמיצים. הנחות המחקר היו ששלב ההבשלה של החיטה בעת הקציר, הקמלה, והרכב כימי של התחמיצים (תכולת חומר יבש, סוכרים מסיסים במים, חומצת חלב וחומצות שומן נדיפות [חש"ן]) פועלים בנפרד או בהשפעת גומלין על היציבות האירובית של התחמיצים. כמו כן הנחנו שתחמיצי הסורגום שיציבים בחשיפה לאוויר מכילים גורם מייצב שניתן להפיקו ולנצלו על מנת לייצב סוגי תחמיצים רגישים העת חשיפה לאוויר.

תחמיצים הוכנו בצנצנות אטומות בנפח 1.5 ליטר אשר אוחסנו ל-4-6 חודשים בטמפרטורת החדר (26±2 ^{0}C). בתום תקופת האחסון הצנצנות נפתחו והתחמיצים עברו מבחן חשיפה לאוויר במשך 7 ימים. במהלך המבחן נבדקו פרמטרים כימיים (pH, שארית סוכרים מסיסים במים, חומצת חלב וחש"ן, ויצירת פחמן דו-חמצני [פד"ח], נעכלות חומר יבש ודופן התא), שינויי טמפרטורה, מספרי שמרים ועובשים וכן התרשמות חושית (מראה וריח) אשר שמשו כמדדים לשינויים שחלים בעת חשיפת התחמיצים לאוויר.

הפסדי ההחמצה בתחמיצים שהוכנו מזן החיטה בית השיטה משלב הפריחה בקציר ישיר (287 ג'/ק"ג ח"י) ואחרי הקמלה (362 ג'/ק"ג ח"י) היו 11.5 ו-8.0% ח"י , בהתאמה. בתחמיצים מהבשלת חלב בקציר ישיר (398 ג'/ק"ג ח"י) ואחרי הקמלה (427 ג'/ק"ג ח"י) הפסדי ההחמצה היו רק 3.7 ו-4.7% ח"י, בהתאמה. התחמיצים משלב הפריחה ומהבשלת חלב מקציר ישיר היו יציבים במבחן החשיפה לאוויר בעוד שהתחמיצים המוקמלים מהבשלת החלב נטו להתקלקל אחרי 7 ימי חשיפה לאוויר והיו בהם אוכלוסיות יותר גדולות של שמרים ועובשים ויצירה מוגברת של פד"ח.

הפסדי ההחמצה בתחמיצים שהוכנו מזן החיטה גליל משלב הפריחה בקציר ישיר (199 ג'/ק"ג ח"י) ביו 11.8% ח"י אך הם היו יציבים במבחן החשיפה לאוויר. הטיפול בחיידק גרם לקלקול אירובי ניכר עם יצירת פד"ח של 149.6 ג'/ק"ג ח"י, הפסדי ח"י 12.7% והתחממות ל- 32 מ"צ לאחר 4 ימי חשיפה לאוויר. התחמיצים המוקמלים משלב הפריחה התקלקלו במהלך 7 ימי מבחן החשיפה לאוויר ותרבית החיידקים לא שינתה את המצב. בתחמיצים מהבשלת החלב בקציר ישיר (299 ג'/ק"ג ח"י) הפסדי ההחמצה היו 8.9% והם התקלקלו בעת החשיפה לאוויר עם יצירת פד"ח של 67.2 ג'/ק"ג ח"י, הפסדי ח"י 8.8% והתחממות ל- 31 מ"צ לאחר 7 ימי חשיפה לאוויר. התרבית קלקלה עוד יותר את התחמיצים

האלה במבחן החשיפה לאוויר עם 82.3 ג'/ק"ג ח"י פד"ח. בתחמיצים מוקמלים מהבשלת החלב (437 ג'/ק"ג ח"י) הפסדי ההחמצה היו 1.7% והם התקלקלו כבר כעבור 4 ימי חשיפה לאוויר עם יצירת פד"ח של 34.0 ג'/ק"ג ח"י, הפסדי ח"י 4.5%); לאחר 7 ימי חשיפה לאוויר כמות הפד"ח שנוצרה הייתה 133.2 ג'/ק"ג ח"י והפסדי ח"י 12.9%. התרבית קלקלה עוד יותר את התחמיצים האלה במבחן החשיפה לאוויר. המסקנות הן שבתחמיצי חיטה הזן, שלב ההבשלה בקציר, הקמלה, ותכולת ח"י משפיעים על מהלך תסיסת ההחמצה, הרכב התחמיץ ויציבותו האירובית. מבחן רגרסיה STEPWISE הצביע על רמות חומצת חומץ כגורם מובהק שמשפיע על יציבות התחמיצים בעת חשיפה לאוויר.

לתחמיצי סורגום (306 ג'/ק"ג ח"י) היו הפסדי החמצה של 11.0% ח"י בעוד שבתחמיצי תירס (319 ג'/ק"ג ח"י) הפסדים אלה הסתכמו ב- 0.9% בלבד. תחמיצי סורגום היו יותר יציבים במבחן החשיפה לאוויר (7.4 ג'/ק"ג ח"י פד"ח לאחר 7 ימים) אך תחמיצי התירס התקלקלו כבר ביום הרביעי לחשיפה (38.3 ג'/ק"ג ח"י פד"ח), וביום השביעי נמדדו 47.7 ג'/ק"ג ח"י פד"ח. המיצויים המימיים של תחמיצי התירס והסורגום הכילו 13.5 ו- 28.1 מ"ג/ג' פולי-פנולים , בהתאמה. כצפוי, ריסוס תחמיצי סורגום במיצוי מתחמיצי התירס גרם לקלקול אירובי של תחמיצי הסורגום עם 15.6 ג'/ק"ג ח"י פד"ח והפסדי ח"י 3.9% ביום השביעי לחשיפה לאוויר. שלא כצפוי, ריסוס תחמיצי תירס במיצוי מתחמיצי הסורגום לא שיפר את היציבות האירובית של תחמיצי התירס אלא אף גרם לקלקול מוגבר שלהם עם 101.0 ג'/ק"ג ח"י פד"ח ביום השביעי לחשיפה לאוויר. למרות שתחמיצי הסורגום היו יציבים בעת חשיפה לאוויר והמיצוי המימי שלהם הכיל ריכוזים יותר גבוהים של פולי-פנולים, הריסוס של תחמיצי תירס במיצוי המימי של תחמיצי הסורגום לא שיפר את היציבות האירובית של תחמיצי התירס. דרוש מחקר נוסף כדי לגלות את הגורם או המרכיב שקיים בתחמיצי הסורגום אשר תורם ליציבותם האירובית, ולפתח שיטה הולמת לניצול מרכיב זה על מנת לשפר את היציבות האירובית של תחמיצים רגישים כדוגמת תחמיצי תירס.

מילות מפתח: תחמיצי חיטה, תירס, סורגום, הפסדי חומר יבש, יציבות אירובית.