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Ensiling Whole Wheat at Various Maturation Stages: Changes in Nutritive Ingredients during Maturation and Ensiling and upon Aerobic Exposure

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Whole wheat plants were ensiled at four maturation stages, shooting, flowering, milk, and dough ripeness, in order to study changes in nutritive ingredients. Amino acids and microflora were determined in the fresh material and following ensilage and aerobic exposure. In fresh material dry matter content ranged from 20.1 to 40.4%, crude protein 13.6 to 6.3%, crude fiber 24.0 to 36.5%, water-soluble carbohydrates 13.3 to 28.1%, and in vitro digestibility of organic matter 76.7 to 63.0%, according to maturation stage. All stages yielded good silage. The most marked changes in nutritive ingredients and yield were between the flowering and milk ripeness stages. The highest water-soluble carbohydrate concentration (28.1%) was found at the milk stage. Crude protein decreased during maturation, but maximal crude fiber was recorded at the milk stage. Lactic acid in silage ranged between 2.0 and 6.5% of dry matter. All the silages were stable to aerobic exposure. Amino acid concentration decreased during maturation, parallel with the crude protein. Arginine was found at highest concentrations in the fresh material, where most of the amino acids were in the bound form. However, most of the amino acids were in the free form in the silage and after aerobic exposure. Glutamine and asparagine were detected in the free form only. The results suggest that the milk ripeness stage was most suitable for wheat ensiling.

Wheat is grown widely for cereal production, although in many situations there is value in using the whole plant for fodder as silage. This is especially true when a second crop is possible in the same year. Small grain cereals, including wheat, will normally produce up to twice as much for the same area when harvested as silage, as compared with the same total digestible nutrient crop harvested for grain (Lawes and Jones, 1971). Although wheat is not yet utilized commonly as a fodder crop, the whole plant harvested for silage has the advantage of flexibility in harvesting at different times and maturation stages according to need. The whole wheat plant and other cereal crops for silage have been studied by several investigators. Voelker (1977) reported on the advantages of ensiling a combination of barley, wheat, and oats at the boot and dough stages. Oltjen and Bolsen (1978) concluded that wheat and barley silages are excellent for growing cattle. Baxter and Montgomery (1980) increased the intake of wheat silage and alfalfa fed in combination with corn silage. Lactic acid bacteria were found to be active during fermentation of wheat silage (Moon et al., 1981). Baxter et al. (1978) investigated the addition of formic acid to wheat silage for growing heifers and found that wheat silage was an excellent forage and that formic acid did not produce a beneficial effect at the dough stage. [We have recently studied changes in amino acid composition of wheat and silage and shown that ammonia and free amino acids increased in silage (Ashbell et al., 1983)]. However, extensive studies of the composition of wheat plants and silage at different physiological stages have not been reported.

The periods between different maturation stages for wheat are relatively short (in comparison with those of corn and sorghum). Therefore, in order to determine the optimum harvest time for silage, it is important to know how

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the maturation stage affects the ensiling process.

The objective of this study was to determine the changes in nutritional content at different maturation stages and also the influence of postsilage aerobic exposure.

MATERIALS AND METHODS

Preparation of Silage and Fermentation Conditions. Wheat plants, var. "Kranich", a winter wheat with a low to medium protein level, were harvested at four stages of maturation: shooting, flowering, milk, and dough ripeness (Feekes scale: 5, 10.5.2, 11.1, and 11.2, respectively). Tests were conducted on 100-m^2 plots for each maturation stage in 2 successive years. Plots differed from year to year and the topographical conditions and agrotechnical treatments in the fields were as uniform as possible. The material was hand-harvested from each plot and weighed to determine the yield. The material was chopped and ensiled in special 1.5-L glass jars (J. Weck, GmbH u. Co., Wher-Öflingen, West Germany) generally used for conservation of food products by fermentation. The jars were held in a room at a constant temperature of 30 °C for 90 days. Aerobic exposure (AE) was carried out at 30 °C for 7 days according to the methods developed by Woolford et al. (1977). Representative samples for wet analysis were taken at all stages and stored at −30 °C until analyzed. Drying was done by the freeze-drying method (Typ.-Epsilon, Martin Christ, 3360 Ostenrode am Harz, West Germany).

Chemical Analyses. *Dry matter* (DM) was determined by the toluene distillation method with correction for volatile fatty acids (Dewar and McDonald, 1961).

Ethanol, lactic acid, and volatile fatty acid (*VFA*) concentrations were determined by gas chromatography (Theune, 1978).

Crude fiber (CF) was determined according to the AOAC method (AOAC, 1980).

Protein. Total nitrogen (TN) was determined by the Kjeldahl method and crude protein (CP) was calculated by multiplying TN by 6.25.

Ammonia N was determined by using an ammonia electrode (Orione Research Inc., Cambridge, MA, USA). With this method 10 g of silage was mixed with 90 mL of distilled water for 3 min and then filtered on Whatman No. 1 filter paper; 20-mL aliquots of the filtrate were used for analysis. A calibration curve was obtained by using a standard concentration of ammonium chloride.

Amino Acids. Free amino acids (AA) were extracted after homogenizing 100–200-mg samples in 80 mL of a cold mixture of acetone and water (7:3 v/v) and analyzed directly after filtering. The bound AA were analyzed following hydrolysis (6 M HCl, 24 h, 120 °C) of the material remaining on the filter paper. The AA were determined from freeze-dried material by using an amino acid analyzer (Biotronic Wissenschitliche Geräte GmbH, Model L.C. 6000). Total nitrogen analysis was also carried out on the solution prepared for the AA analysis to verify recovery.

Water-soluble carbohydrates (*WSC*) were determined as described by Bommer (1959).

The ash content was determined by heating in an oven to 600 °C for 2 h.

The ether extract was determined by the Soxhlet method (AOAC, 1980).

The buffering capacity was determined according to McDonald and Henderson (1962).

The in vitro organic matter digestibility (IVOMD) was determined according to Tilley and Terry (1963).

Microflora Determination. Silage microflora were determined quantitatively from serial dilutions of culture on selective media for lactic acid bacteria (Rogosa agar), yeasts and molds (Martins medium), proteolytic bacteria (calcium caseinate agar), and coliform bacteria (Crystal Violet bile salts–lactose agar). Duplicates of two samples were examined in each case.

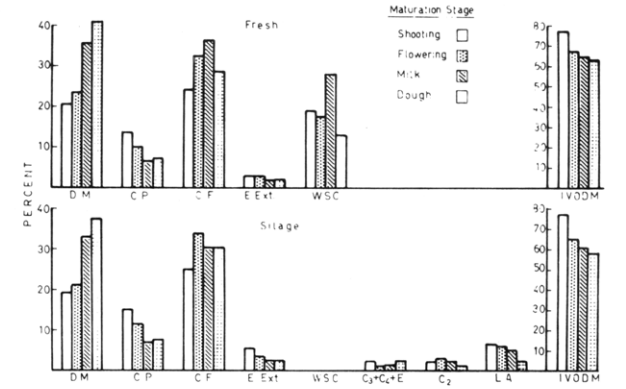

Figure 1. Nutritional ingredients of whole wheat plants at four maturation stages, for fresh material and after ensiling. DM = dry matter. CP = crude protein. CF = crude fiber. E. Ext. = ether extract. WSC = water-soluble carbohydrates. E = ethanol. LA = lactic acid. C_2 = acetic acid. C_3 = propionic acid. C_4 = butyric acid. IVODM = in vitro organic dry matter.

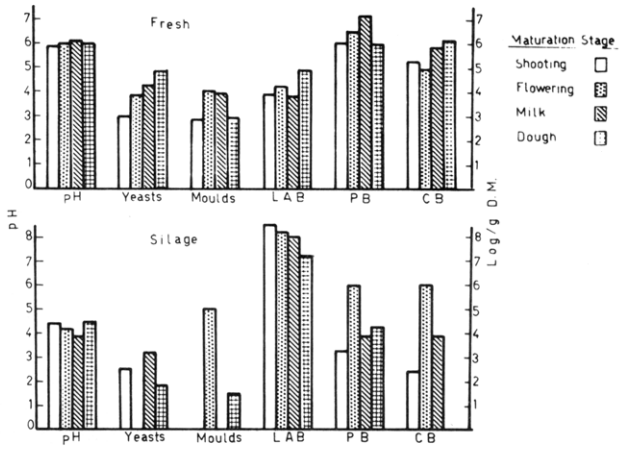

Figure 2. Microbial count and pH of whole wheat plants at four maturation stages for fresh material and after ensiling. LAB = lactic acid bacteria. PB = proteolytic bacteria. CB = coliform bacteria.

RESULTS

The nutritional ingredients of the whole wheat plant in four maturation stages are given in Figure 1; the pH and microflora count are given in Figure 2. The most marked changes were found between the flowering and milk ripeness stages. These consist of an increase of about 50% in DM and 60% in WSC together with decreases of 30–40% in the CP ether extract and in the buffering capacity.

At the dough ripeness stage the WSC decreased further in comparison with the milk ripeness stage and reached the lowest level of all the stages investigated. From the shooting to milk stage there was an increase in CF followed by a reduction at the dough ripeness stages. The CP showed a decrease during maturation, although at the milk and dough ripeness stages similar amounts were recorded. No significant trend in bacterial load was noted; however, yeast counts increased with maturation stage. Yields of whole wheat plant per 100 m^2 are given in Figure 3. At dough ripeness, the yields of all ingredients were at their maximum except for WSC, which was about half the level obtained at milk ripeness but was higher than at the first

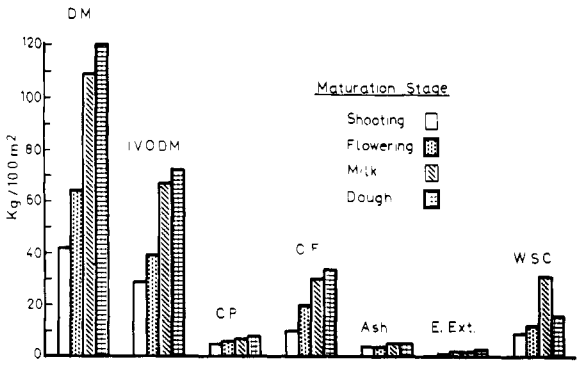

Figure 3. Yield of nutritive ingredients of fresh wheat at four maturation stages (the yields were based on 100-m^2 plots). CP = crude protein. CF = crude fiber. E. Ext. = ether extract. WSC = water-soluble carbohydrates. DM = dry matter. IVODM = in vitro organic dry matter.

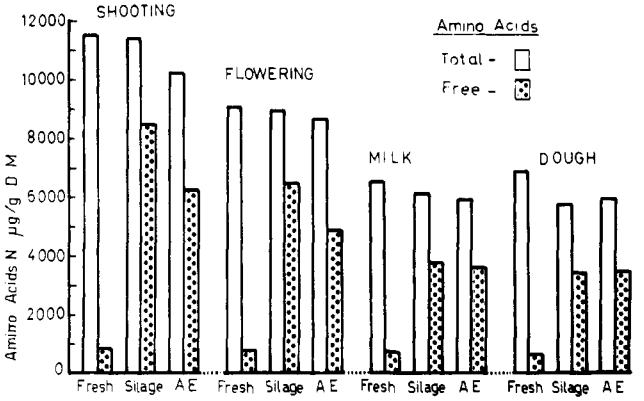

Figure 4. Total and free amino acids in whole wheat plants at four maturation stages in fresh wheat material, silage, and aerated silage. AE = aerobic exposure.

two stages. Here, too, the most marked increase in yield occurred at milk ripeness.

Total and free AA contents determined in the fresh wheat plant in the silage and in aerated silage at the four physiological stages are given in Figure 4. In fresh material most of the AA were in bound form except for glutamine and asparagine, which were detected in free form only.

Changes in ingredients and microflora counts of the different maturation stages during AE are shown in Figure 5. The silage was stable during AE, and the DM, CP, and ash contents following AE were similar to those in the silage. The lowest pH and ammonia N in silage and AE were recorded in the milk ripeness stage, while the highest lactic acid concentration and lactic acid bacteria count in both silage and AE were found in the shooting stage. The highest counts of proteolytic and coliform bacteria in silage and AE were noted in the flowering stage.

In the silage most of the AA were in the free form, decreasing progressively from 73% in the shooting stage to 72% in the flowering, 62% in the milk stage, and 60% in the dough ripeness stage. Amino acid nitrogen of whole wheat at various maturation stages is given in Table I. In the AE most of the AA were also in the free form, but the range was limited to between 56 and 61% of the TAA (total amino acids). The changes in the AA at the ensiling and AE are shown in Figure 6. The relative concentration of AA in the fresh material changed during the ensiling and AE process. Thus, alanine had the highest concentration in the silage (average 10.2%) and, together with lysine, leucine, glycine, proline, valine, and aspartic acid, constituted over 50% of the TAA. However, in the AE,

Table I. Amino Acid Nitrogen of Whole Wheat at Various Maturation Stages (Fresh and Wilted)[a]

source	Arg	Lys	Glu	Ala	Leu	Gly	Asp	Val	Pro	Ser	His	Thr	Ile	Phe	Tyr	γ-amino-butyric acid	Gln	Met	Asn	Orn
shooting fresh	8.90	5.60	4.10	4.10	3.60	3.20	3.20	3.50	3.40	2.30	2.60	2.40	2.20	2.10	1.10	0.80	0.30	0.50	0.30	0.10
SD	0.49	0.07	0.01	0.09	0.01	0.01	0.04	0.32	1.70	0.22	0.08	0.12	0.04	0.07	0.05	0.11	0.16	0.01	0.14	0.01
% in free form	2.30	2.60	0.80	18.00	3.50	2.90	3.50	5.20	17.60	8.10	3.50	6.00	4.00	3.70	4.80	72.10	100.00	1.90	100.00	26.10
flowering fresh	8.00	6.00	4.60	4.80	3.90	3.80	3.80	3.30	2.90	2.70	2.90	2.50	2.70	2.20	1.00	0.70	0.90	0.50	0.40	0.20
SD	0.09	0.15	0.03	0.13	0.01	0.12	0.05	0.04	0.30	0.12	0.31	0.01	0.50	0.08	0.08	0.04	0.20	0.07	0.12	0.03
% in free form	1.90	2.80	5.00	18.70	3.40	2.70	4.50	5.00	8.50	12.00	4.00	7.20	3.70	3.30	4.50	100.00	100.00	1.80	100.00	42.90
milk ripeness	8.90	5.90	6.30	5.50	5.50	4.70	4.20	3.70	2.90	3.30	2.70	2.70	2.40	2.20	1.00	1.80	1.10	0.30	0.60	0.30
SD	0.01	0.04	0.50	0.01	1.00	0.14	0.08	0.07	0.87	0.03	0.16	0.02	0.08	0.14	0.06	0.37	0.18	0.02	0.29	0.17
% in free form	3.10	4.60	5.50	19.40	5.20	6.50	4.70	6.90	11.30	18.10	6.80	10.10	6.20	5.20	7.50	51.30	100.00	10.50	100.00	48.10
dough ripeness	7.80	4.50	9.10	4.60	4.10	4.20	3.60	3.40	4.00	3.20	2.60	2.30	2.20	2.10	0.90	0.90	0.90	0.30	0.20	0.10
SD	0.31	0.09	0.37	0.14	0.13	0.14	0.09	0.17	1.21	0.16	0.14	0.07	0.08	0.01	0.01	0.05	0.09	0.06	0.03	0.07
% in free form	3.20	4.30	4.00	18.60	4.00	5.10	5.90	5.00	5.70	10.90	5.10	8.00	5.10	4.30	7.40	51.60	100.00	4.10	100.00	53.20
shooting wilted	8.50	5.50	3.70	4.00	3.60	3.10	3.30	3.00	4.30	2.50	2.70	2.50	2.30	2.20	1.10	0.90	1.30	0.50	1.40	0.20
SD	0.27	0.14	0.23	0.38	0.19	0.21	0.22	0.28	0.64	0.33	0.13	0.20	0.11	0.03	0.03	0.26	0.12	0.01	0.41	0.04
% in free form	6.60	5.70	2.30	20.60	12.00	4.80	10.10	16.50	41.10	21.90	15.10	17.70	15.40	16.10	17.80	83.20	100.00	5.30	100.00	30.40
flowering wilted	7.80	5.70	4.00	4.30	3.90	3.40	3.60	3.80	3.40	2.70	2.60	2.50	2.30	4.50	1.00	0.90	2.30	0.40	1.50	0.10
SD	1.09	0.48	0.40	0.47	0.40	0.40	0.37	0.29	0.34	0.24	0.24	0.25	0.22	2.16	0.01	0.06	0.16	0.02	0.81	0.03
% in free form	7.80	6.20	5.30	10.10	13.50	3.90	10.70	17.60	40.70	25.50	19.20	20.30	17.70	16.40	16.80	100.00	100.00	16.70	100.00	42.10

[a] Results are given as percent of the nitrogen. The free form is given as percent of the total amino acid.

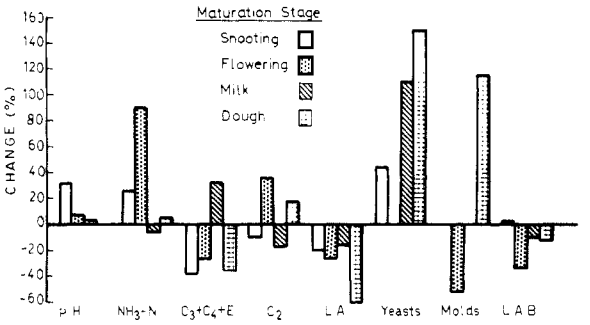

Figure 5. Changes in nutrient ingredients and microfloral counts in silage after aeration of whole wheat plants at four maturation stages. C_2 = acetic acid. C_3 = propionic acid. C_4 = butyric acid. LA = lactic acid. LAB = lactic acid bacteria.

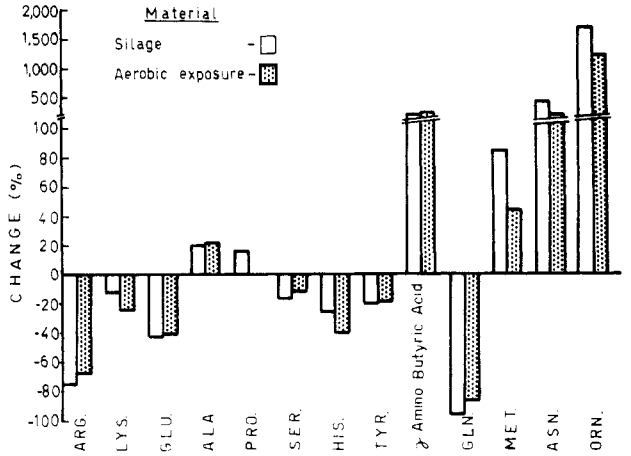

Figure 6. Changes in amino acid after ensiling and aerobic exposure of whole wheat plants. (results expressed as means of four maturation stages; changes less than ±10% are not shown).

lysine was the most abundant (average 8.4%) and, with leucine, glycine, alanine, valine, proline, and aspartic acid, comprised over 50% of the TAA. The AA that decreased the most during fermentation and AE were glutamine, arginine, and glutamic acid, while ornithine, asparagine, γ-aminobutyric acid, methionine, and alanine increased. In the AE, AA concentrations were similar to those of the silage except for the increase in arginine and the decrease in asparagine, ornithine, and methionine.

DISCUSSION

The whole wheat plant can provide good forage for ensiling (Baxter et al., 1978; Baxter and Montgomery, 1980; Oltjen and Bolsen, 1978; Voelker, 1977), the quality being dependent upon the stage of maturation at the time of harvest, as shown here. Flexibility of harvesting and ensiling at different stages of maturation has advantages in unstable weather, especially when double cropping is being practiced and time is limited. Moreover, it enables more efficient use of equipment.

It should be noted that the best yield of fresh plants was obtained at the dough ripeness stage. This finding is similar to those of Oltjen and Bolsen (1978) and of Lawes and Jones (1971). The levels of WSC and DM in the fresh material are very important factors in determining the extent of fermentation during ensiling. In low DM silage, extensive fermentation must take place before sufficient acidity to preserve the material is obtained (McDonald et al., 1968). The most important changes in ingredients in the plant take place after flowering. Especially marked is the increase in DM and WSC concentrations. The latter decreases later, while the DM continues with a limited increase. The WSC influences silage quality by determining the pH level. Milk ripeness seems to be the most promising stage for ensiling, since it produces a silage with the lowest pH and ammonia N, a relatively high lactic acid content, and a low proteolytic bacteria count. Results similar to the above were obtained for barley by MacGregor and Edwards (1968).

According to McDonald et al. (1960), the amount of ammonia N in fresh herbage is usually less than 1% of the total nitrogen. In this study it ranged between 0.6% at the shooting stage and 1.5% in the dough stage. These levels of ammonia N may be due to the putrefaction of the dead lower leaves, which increases during maturation. Wilson and Tilley (1973) found that herbage protein does not vary greatly with plant species and that protein preparations of different herbage were of very similar amino acid composition. In all samples arginine occurred in the highest concentration and, with lysine, aspartic acid, glutamic acid, alanine, leucine, and glycine, accounted for 63% of the TAA nitrogen.

Similar results were obtained in this study. The reduction in concentration of AA with maturation paralleled the decrease in CP; however, methinine, tyrosine, lysine, and arginine showed the greatest decrease, while glutamine and glutamic acid actually increased. The relative proportion of the wheat grain in plant increases with maturation and glutamic acid is the predominant AA in the grain (Pomeranz, 1971).

The deterioration due to exposure to air was very small at all stages; this relative stability in air was also reported by Woolford et al. (1982) and may be due to the relatively high butyric acid content.

Registry No. Lactic acid, 50-21-5; acetic acid, 64-19-7; propionic acid, 79-09-4; butyric acid, 107-92-6.

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