

The effect of anhydrous ammoniation on the nutritive value of whole oat grain

A.A. Brand, S.W.P. Cloete and N.M. Kritzinger

Winter Rainfall Region, Elsenburg

Treatment of oat grain by anhydrous ammonia at levels of approximately 0,75; 1,5 and 3,0% was investigated. Diets consisting of untreated oat grain and oat grain ammoniated for 24 hours at 90°C in an AN-STRA-VERTER® -oven at the respective treatment levels were individually fed at an intake level of 45 g/kg W^{0,75} to adult SA Mutton Merino wethers in an *in vivo* digestibility and N balance trial. The crude protein (CP) content of the experimental diets was 10,3; 16,0; 16,8 and 17,3% respectively. The apparent digestibility of dry matter (DM) and organic matter (OM) was significantly ($P \leq 0,01$) increased from 68,1 to 69,6; 72,2 and 76,5% and from 69,1 to 71,4; 73,8 and 78,0% respectively. CP digestibility of untreated oat grain was significantly ($P \leq 0,01$) improved from 72,6 to 78,4% at the 0,75% treatment level. No significant further improvement was obtained at higher ammoniation levels. Ammoniation at a level of 3,0% significantly ($P \leq 0,01$) improved the apparent digestibility of neutral detergent fibre (NDF) and hemicellulose (HC) by 22 and 29,4 percentage units respectively. The slight improvement in the digestibility of acid detergent fibre (ADF) with increased ammoniation levels was not significant. Like CP digestibility, ammoniation significantly ($P \leq 0,05$) affected N balance. Ammoniation levels above 0,75%, however, resulted in no further improvement in the N balance.

S. Afr. J. Anim. Sci. 1985, 15: 43–46

Die behandeling van hawerpitte by anhidriese ammoniakpeile van ongeveer 0,75; 1,5 and 3,0% is ondersoek. Diëte bestaande uit onbehandelde hawerpitte en hawerpitte behandel in 'n AN-STRA-VERTER® -oond vir 24 uur by 90°C by die onderskeie ammoniakpeile is teen 45 g/kg W^{0,75} individueel aan volwasse SA Vleismerino-hamels in 'n *in vivo* verterings- en N-balansstudie gevoer. Die ruproteïen (RP)-inhoud van die proefdiëte was 10,3; 16,0; 16,8 en 17,3% onderskeidelik. Die skynbare verteerbaarhede van droëmateriaal (DM) en organiese materiaal (OM) is betekenisvol ($P \leq 0,01$) verhoog van 68,1 tot 69,6; 72,2 en 76,5% en van 69,1 tot 71,4; 73,8 en 78,0% onderskeidelik. RP-verteerbaarheid is betekenisvol ($P \leq 0,01$) verhoog van 72,6 tot 78,4% by die 0,75% behandelingspeil waarna slegs 'n geringe verdere verhoging by hoër ammonifiseringspeile verkry is. Die skynbare verteerbaarheid van die selwande (SW) en hemicellulose (HS) is betekenisvol ($P \leq 0,01$) verhoog met onderskeidelik 22 en 29,4 persentasie eenhede deur ammonifisering by die 3% behandelingspeil. Die geringe verhoging in die verteerbaarheid van suurbestande vesel (SBV) met ammonifisering was nie betekenisvol nie. Ooreenstemmend met RP-verteerbaarheid is N-balans betekenisvol ($P \leq 0,01$) verbeter met ammonifisering. NH₃-peile bokant 0,75% het egter geen verdere verbetering in N-balans tot gevolg gehad nie.

S.-Afr. Tydskr. Veek. 1985, 15: 43–46

Keywords: Anhydrous ammonia, nutritive value, oat grain, sheep, digestibility, N-retention

A.A. Brand*, S.W.P. Cloete and N.M. Kritzinger
Winter Rainfall Region, Private Bag, Elsenburg, 7607
Republic of South Africa

*To whom correspondence should be addressed

Received 22 November 1984

Introduction

According to Ørskov (1979) some of the main reasons for the physical and chemical processing of grains are to ensure that whole grain does not pass through the gut undigested, destruction of hard indigestible seeds of weeds thus avoiding the uncontrolled spread thereof, and to ensure proper mixing with other food ingredients such as minerals and vitamins. Hale (1973) listed 18 different methods of grain processing to improve its starch digestion in the rumen. However, Ørskov (1976) concluded that the processing of grain for sheep is of limited value while some processing is necessary for cattle which have large reticulo-omasal orifices through which non-processed whole grain can bypass the rumen and appear undigested in the faeces. Ørskov & Greenhalgh (1977) and Ørskov & Macdearmid (1978) demonstrated that cattle consumed and digested sodium hydroxide (NaOH)-treated whole barley grain and physically processed barley grain equally well. Both NaOH-treated and physically processed barley grain were digested better than unprocessed barley grain. Similarly, NaOH-treated oat grain was superior to physically processed oat grain (Ørskov, Solaiman & Macdearmid, 1978). In a digestibility and growth trial Ørskov, Macdearmid, Grubb & Innes (1981) reported a greater rate of carcass gain and improved feed conversion and digestion for lambs receiving NaOH-treated oat grain when compared to untreated oat grain. Apart from handling problems and its corrosive nature, however, NaOH treatment has several other disadvantages such as impairment of the flow characteristics of grains after treatment and increased water consumption by the animals. Treatment of straw with ammonia on the other hand has several nutritional and practical advantages over NaOH treatment (Ørskov, Reid, Holland, Tait & Lee, 1983). Ammoniation of cereal straws increases intake and improves the digestibility of dry matter, organic matter, and crude fibre (Lawlor & O'Shea, 1979; Sundstøl, Coxworth & Mowat, 1978; Horton & Steacy, 1979; Alibes, Munoz & Faci, 1984). Ammoniation improved the digestibility of whole dry and moist barley grain over that of unprocessed and ground barley grain (Laksesvela & Slagsvold, 1980; Laksesvela, 1981) while it also serves as an extremely suitable preserving agent (Bothast, Adams, Hatfield & Lancaster, 1975; Britt & Huber, 1976).

Optimal treatment conditions for ammoniation of whole cereal grains have so far not been investigated. Three percent anhydrous ammonia was used in trials reported by Laksesvela & Slagsvold (1980) and Laksesvela (1981). Anhydrous ammoniation by the AN-STRA-VERTER® technique (thermo-ammoniation) for the upgrading of low quality roughages is

already practised on some South African farms. It is therefore possible that treatment may easily be expanded to cereal grains, especially since ammoniation may beneficially affect digestibility of fibrous grains in particular. The present trial was thus conducted to evaluate the effects of thermo-ammoniation on the digestibility of oat grain and determine optimum levels of ammoniation.

Materials and Methods

Batches of whole oat grain contained in sisal bags, weighing 70 kg each, were subjected to thermo-ammoniation by an AN-STRA-VERTER® at 90°C for 24 hours at anhydrous ammonia levels of approximately 0,75; 1,5 and 3,0% respectively. The treated grain was then stored for 2 months before use.

Twenty adult SA Mutton Merino wethers with a mean livemass of 61,1 ± 4,5 kg were used in the experiment. The animals were stratified according to livemass in four groups of five animals each and within groups randomly allotted to the four treatment diets. These diets consisted of untreated whole oat grain and whole oat grain ammoniated at 0,75; 1,5 and 3,0% respectively. A fifth treatment diet consisting of temperature-treated whole oat grain was included in the trial to examine the effect of the temperature treatment as such during oven ammoniation. Temperature treatment only (fifth treatment diet) had no mentionable effect on any digestibility parameter. These results were therefore omitted.

The animals were individually penned with free access to water. The level of feeding was restricted to 45 g/W^{0,75}/sheep per day since rumen stasis occurred in some of the animals when fed the diets *ad libitum*. The diets were fed twice daily to each individual animal. All refusals were recorded and subtracted from the amount of diet fed. The trial consisted of a 14-day adaptation and a 7-day collection period. During the collection period rumen stasis occurred in two sheep on the diet treated at the ammoniation level of 1,5%, markedly affecting the results obtained from these animals. It was therefore decided to omit these data in the statistical analyses and further discussions. Samples of the experimental diets offered and of the refusals collected were taken daily and combined over the digestion phase of the trial.

Total faeces and urine of individual animals were collected daily. The urine was preserved by the addition of 0,25 ml of 20% H₂SO₄ to the urine collection bottles daily. A 10% aliquot of the daily urine output was kept and pooled on completion of the collection phase. The pooled sample was further subsampled for N analysis.

At the end of the collection period composite faecal samples from each individual wether together with composite samples of the experimental diets were dried to constant mass at 103°C to determine total DM intake and total DM faecal excretion. Composite samples were also dried at 55°C for 48 hours, milled through a 1-mm screen and subsampled in duplicate for chemical analyses. The DM, OM, and CP were determined by the methods of the AOAC (1970). Acid detergent fibre (ADF), neutral detergent fibre (NDF) and hemicellulose (HC) of the feed and faeces were determined by the method of Van Soest (1963) and Van Soest & Wine (1967). The apparent digestibility coefficients of DM, OM, CP, NDF, ADF, and HC as well as the N balance were calculated by standard methods (Schneider & Flatt, 1975). Digestibility and N balance results were analysed in a completely randomized design. Treatment means were tested for significance by the method of Bonferroni (Van Ark, 1981).

Results and Discussion

Anhydrous ammonia treatment resulted in a visual change in colour of the oat grain like ammoniation of roughages. The colour tended to darken with increased NH₃ level.

Results of chemical analysis of the treated and untreated oats are shown in Table 1. The CP content of the ammoniated oat grain increased from 10,3 to 16,0; 16,8 and 17,3% corresponding to ammoniation levels of 0,75; 1,5 and 3,0% respectively. Assuming that all the added ammonia had bounded to the oats and no volatilization had taken place thereafter, the three ammoniated diets would theoretically have CP values of 14,2; 18,0 and 25,8% CP respectively (anhydrous ammonia has a calculated N content of 82%). Thus the calculated increase in the CP value of the 0,75% treated oat grain would therefore be 12,8% above the expected value. This indicates that anhydrous ammonia treatment levels are difficult to control and that extensive care must be taken during ammoniation to ensure correct treatment levels to avoid animal losses owing to over-treatment. It is evident that, like ammoniated roughages (Solaiman, Horn & Owens, 1979), oat grain also seems to have only a limited N-binding capacity. Where the 1,5% ammoniation level raised the CP content by a substantial 6,5 percentage units, an additional 1,5% NH₃ only accounted for the further addition of a mere 0,5 percentage units.

Table 1 Chemical composition (%) of the diets (DM basis except DM)

Component	Diets			
	Untreated	0,75% NH ₃	1,5% NH ₃	3,0% NH ₃
DM	91,2	91,6	91,0	91,7
CP	10,3	16,0	16,8	17,3
NDF	42,4	42,7	42,5	42,6
ADF	17,9	17,6	16,6	16,1
HC	24,5	25,0	26,3	26,5

The ADF content of the diets tended to decrease slightly with increasing level of NH₃ treatment while HC slightly increased. This observation is contrary to the results obtained for ammoniated wheat straw by Oji & Mowat (1979); Solaiman, *et al.* (1979); Horton (1981); Horn, Batchelor, Manor, Streeter & McLaughlin (1983) and Herrera-Saldanha, Church & Kellems (1983) who reported an increase in the ADF content. Although this phenomenon is difficult to explain, it must be borne in mind that the HC content of oat grain and the chemical nature of its fibrous components differs largely from those found in roughages. Contrary to findings that it is mainly the HC in roughages that is solubilized, it appears in the case of oat grain that part of the cellulose fraction is solubilized during anhydrous ammoniation.

The effect of level of ammoniation on the apparent *in vivo* digestibility coefficients of DM, OM, and CP of oats are presented in Table 2. The apparent digestibility of DM and OM was improved by 12,4–12,9%. The results in Table 2 are in agreement with corresponding improvements of 5–8 units in the apparent digestibility of the DM of dry and moistened barley grain as reported by Laksessvela & Slagsvold (1980) and Laksessvela (1981). A smaller increase of 4 units in DM and OM digestibility of oat grain treated with 40 g NaOH/kg oat grain was obtained by Ørskov, *et al.* (1981).

Ammoniation resulted in a significant ($P \leq 0,05$) improve-

Table 2 Apparent digestibility coefficients (%) of different dietary components for sheep fed untreated and ammoniated oat grain

Component	Diet				SE mean
	Untreated	0,75% NH ₃	1,5% NH ₃	3,0% NH ₃	
DM	68,1 ¹	69,6 ¹	72,2 ^{1,2}	76,5 ²	0,66
OM	69,1 ¹	71,4 ¹	73,8 ^{1,2}	78,0 ²	0,63
CP	72,6 ¹	78,4 ^{1,2}	79,0 ^{1,2}	79,4 ²	0,63
NDF	40,2 ¹	45,2 ¹	51,2 ^{1,2}	62,2 ²	1,32
ADF	37,7	39,2	40,8	46,0	1,34
HC	42,2 ¹	50,9 ¹	57,7 ^{1,2}	71,6 ²	1,29

^{1,2,3}— denotes significant differences ($P \leq 0,01$) in the same row.

ment of approximately 8% in the CP digestibility of oat grain (Table 2). Seeing that ammoniation also increased the CP content of oats, these results are not directly comparable. The digestible crude protein (DCP) content of the respective diets was thus calculated. DCP content increased from 7,5% to 12,5, 12,2 and 13,8% on the respective ammoniation levels. Results from the literature indicated that the CP digestibility of ammoniated diets tends to be relatively low when compared to similar untreated diets supplemented to iso-nitrogenous levels (Oji, Mowat & Winch, 1977; Garret, Walker, Kohler & Hart, 1979; Morris & Mowat, 1980; Horton, Nicholson & Christensen, 1982; Cloete & Kritzing, 1984). The present results are not directly comparable with those of the above-mentioned authors as the diets were not presented on an iso-nitrogenous basis. It is nevertheless evident that ammoniation significantly increased CP digestibility and DCP content of the experimental diets. These findings are in general agreement with the majority of published results on low quality roughages (Horton & Steacy, 1979; Bareeba, Ingalls, McKirdy & Sharma, 1983; Cloete & Kritzing, 1984), in spite of a substantially higher CP content of the control diet in the present investigation compared to the literature cited. It is, however, in contrast to results published by Stallcup, Harrison, Kreider & Bayley (1982), who reported a decrease in the CP digestibility of ammoniated coastal Bermuda grass hay when compared to an untreated control diet. The CP levels investigated by the above-mentioned authors correspond to those obtained in the present investigation.

The effects of level of anhydrous ammonia treatment on the apparent digestibility of the NDF, ADF, and HC fractions of the oats are presented in Table 2. The apparent digestibility of HC and NDF was significantly ($P \leq 0,01$) improved with the 3% NH₃ treatment by 22 and 29,5 percentage units respectively (Table 2). The corresponding improvement in the ADF digestibility of 8,2 percentage units, however, was not significant. This improvement in NDF digestibility may be compared to results obtained by Low & Kellaway (1983) who obtained an improvement of 20 percentage units in the NDF digestibility of moistened wheat grain ammoniated by 4% anhydrous ammonia. The present results are also in general agreement with those reported for the ammoniation of low quality roughages (Oji & Mowat, 1979; Morris & Mowat, 1980; Herrera-Saldana, *et al.*, 1983; Cloete & Kritzing, 1984).

As a result of the increased nitrogen content, N intake was significantly ($P < 0,01$) increased in the ammoniated diets (Table 3). Faecal and urinary nitrogen losses were correspondingly increased by approximately 1,1 and 4,4 g N/day respectively. Although results presented in Table 3 indicated

Table 3 Nitrogen balance for sheep fed untreated and ammoniated oat grain

Measurement (gN/day)	Diet			
	Untreated	0,75% NH ₃	1,5% NH ₃	3,0% NH ₃
N intake	14,2 ¹	22,3 ²	22,8 ²	24,2 ²
N excretion				
faeces	3,9 ^a	4,8 ^{a,b}	4,8 ^{a,b}	5,0 ^b
urine	8,8 ¹	11,6 ^{1,2}	12,9 ^{1,2}	13,2 ²
N balance	1,6 ¹	5,9 ²	5,1 ²	6,1 ²

^{a,b,c}— denotes significant differences ($P \leq 0,05$) in the same row.

^{1,2,3}— denotes significant differences ($P \leq 0,01$) in the same row.

that most of the nitrogen obtained through ammoniation was lost in the urine, a positive N balance was obtained on all diets. Anhydrous ammonia significantly ($P \leq 0,01$) increased the N balance of the experimental diets from 1,6 g N/day in the untreated control diet to approximately 5,1 to 6,1 g N/day in the respective ammoniated diets. Ammoniation level, however, did not affect N balance significantly. Dolberg, Saadullah, Haque & Ahmed (1981) and Cloete & Kritzing (1984) also reported significant improvements in the N balance of sheep consuming ammoniated wheat straw when results were compared to untreated wheat straw.

In conclusion, thermo-ammoniation significantly improved the DM, OM, NDF, and HC digestibility of oat grain. Other methods of ammoniation may also be of value in the treatment of grain but optimum treatment conditions should be investigated. CP digestibility and N retention were significantly improved compared to the untreated control, while ammoniation level above 0,75% did not result in further improvements of CP digestibility or N retention. It is thus evident that an ammoniation level of 0,75% is sufficient for optimal nitrogen utilization in oat grain while higher ammoniation levels are associated with further increases in the digestibility of DM, OM, and the various fibre fractions. The ammoniation of fibrous grain may thus be of value for the replacement of expensive concentrates in production rations for sheep. Ammoniation of oat grain may also be of value when fed in cattle diets as it may limit the passage of undigested grain through the digestive tract as was found with NaOH-treated grain (Ørskov, *et al.*, 1978). This aspect, however, needs to be investigated before any conclusions can be made.

Acknowledgements

The authors thank Mr F. Franck and his laboratory staff for their technical assistance and Dr L.P. Vosloo (University of Stellenbosch) for providing the AN-STRA-VERTER® -oven.

References

- ALIBES, X., MUÑOZ, F. & FACI, R., 1984. Anhydrous ammonia-treated cereal straw for animal feeding. Some results from the Mediterranean area. *Anim. Fd Sci. Technol.* 10, 239.
- AOAC, 1970. Official methods of analysis (11th Ed). Association of Official Analytical Chemists, Washington D.C.
- BAREEBA, F.B., INGALLS, J.R., MCKIRDY, J.A. & SHARMA, H.R., 1983. Apparent digestibility and nutritional value of urea or ammonia-solution treated corn silage for lactating Holstein cows and mature sheep. *Can. J. Anim. Sci.* 63, 871.
- BOTHAST, R.J., ADAMS, G.H., HATFIELD, E.E. & LANCASTER, E.B., 1975. Preservation of high moisture corn; microbiological evaluation. *J. Dairy Sci.* 58, 386.
- BRITT, D.G. & HUBER, J.T., 1976. Preservation of and animal

- performance on high moisture corn treated with ammonia or propionic acid. *J. Dairy Sci.* 59, 668.
- CLOETE, S.W.P. & KRITZINGER, N.M., 1984. Urea ammoniation compared to urea supplementation as a method of improving the nutritive value of wheat straw for sheep. *S. Afr. J. Anim. Sci.* 14, 59.
- DOLBERG, F., SAADULLAH, M., HAQUE, M. & AHMED, R., 1981. Storage of urea-treated straw using indigenous material. *World Anim. Rev.* 38, 37.
- GARRETT, W.N., WALKER, H.G., KOHLER, G.O. & HART, M.R., 1979. Response of ruminants to diets containing sodium hydroxide or ammonia treated rice straw. *J. Anim. Sci.* 48, 92.
- HALE, W.H., 1973. Influence of processing on the utilisation of grains (starch) by ruminants. *J. Anim. Sci.* 37, 1075.
- HERRERA-SALDANA, R., CHURCH, D.C. & KELLEMS, R.O., 1983. Effect of ammoniation treatment of wheat straw on *in vitro* and *in vivo* digestibility. *J. Anim. Sci.* 56, 938.
- HORN, G.W., BATCHELOR, D.G., MANOR, G., STREETER, G.L. & McLAUGHLIN, G.L., 1983. Ammoniation of wheat straw and native grass hay during baling of large round bales. *Anim. Fd Sci. Technol.* 8, 35.
- HORTON, G.M.J., 1981. Composition and digestibility of cell wall components in cereal straws after treatment with anhydrous ammonia. *Can. J. Anim. Sci.* 61, 1059.
- HORTON, G.M.J., NICHOLSON, H.H. & CHRISTENSEN, D.A., 1982. Ammonia and sodium hydroxide treatment of wheat straw in diets for fattening steers. *Anim. Fd Sci. Technol.* 7, 1.
- HORTON, G.M.J. & STEACY, G.M., 1979. Effect of anhydrous ammonia treatment on the intake and digestibility of cereal straws by steers. *J. Anim. Sci.* 48, 1239.
- LAKSEVELA, B., 1981. A note on the use of whole, moist barley treated with ammonia as a feed supplement for sheep. *Anim. Prod.* 32, 231.
- LAKSEVELA, B. & SLAGSVOLD, P., 1980. A note on the digestibility in lambs of whole dry barley treated with ammonia. *Anim. Prod.* 30, 417.
- LAWLOR, M.J. & O'SHEA, J., 1979. The effect of ammoniation on the intake and nutritive value of straw. *Anim. Fd Sci. Technol.* 4, 169.
- LOW, SUSAN, G., KELLAWAY, R.C., 1983. The utilization of ammonia-treated whole wheat grain by young steers. *Anim. Prod.* 37, 113.
- MORRIS, P.J. & MOWAT, D.N., 1980. Nutritive value of ground and/or ammoniated corn stover. *Can. J. Anim. Sci.* 60, 327.
- OJI, U.I. & MOWAT, D.N., 1979. Nutritive value of thermo-ammoniated and steam treated maize stover. 1. Intake, digestibility and nitrogen retention. *Anim. Fd Sci. Techn.* 4, 177.
- OJI, U.I., MOWAT, D.N. & WINCH, J.E., 1977. Alkali treatments of corn stover to increase nutritive value. *J. Anim. Sci.* 44, 798.
- ØRSKOV, E.R., 1976. The effect of processing on digestion and utilization of cereals by ruminants. *Proc. Nutr. Soc.* 35, 245.
- ØRSKOV, E.R., 1979. Recent information on processing of grain for ruminants. *Livestock Prod. Sci.* 6, 335.
- ØRSKOV, E.R. & GREENHALGH, J.F.D., 1977. Alkali treatment as a method of processing whole grain for cattle. *J. Agric. Sci., Camb.* 89, 253.
- ØRSKOV, E.R. & MACDEARMID, A., 1978. Utilization of alkali-treated grain by cattle. *Anim. Prod.* 26, 401.
- ØRSKOV, E.R., MACDEARMID, A., GRUBB, D.A. & INNES, G.M., 1981. Utilization of alkali-treated grain. 1. Alkali-treated grain in complete diets for steers and lambs. *Anim. Fd Sci. Technol.* 6, 273.
- ØRSKOV, E.R., REID, G.W., HOLLAND, S.M., TAIT, G.A.G. & LEE, N.H., 1983. The feeding value of ruminants of straw and whole crop barley and oats treated with anhydrous or aqueous ammonia or urea. *Anim. Fd Sci. Technol.* 8, 247.
- ØRSKOV, E.R., SOLAIMAN, H.S. & MACDEARMID, A., 1978. Intake of hay by cattle given supplements of barley subjected to various forms of physical treatment or treatment with alkali. *J. Agric. Sci., Cam.* 90, 611.
- SCHNEIDER, B.H. & FLATT, W.P., 1975. The evaluation of feed through digestibility experiments. University of Georgia press, Athens.
- SUNDSTØL, F., COXWORTH, E. & MOWAT, D.N., 1978. Improving the nutritive value of straw and other low quality roughages. *World Anim. Rev.* 26, 13.
- SOLAIMAN, S.G., HORN, G.W. & OWENS, F.N., 1979. Ammonium hydroxide treatment on wheat straw. *J. Anim. Sci.* 49, 802.
- STALLCUP, O.T., HARRISON, K.L., KREIDER, D.L. & BAYLEY, P., 1982. Ammonia treatment of Bermuda grass hay. *Ark. Farm Res.* 31, 9.
- VAN ARK, H., 1981. Eenvoudige biometrische tegnieke en proefontwerpe met spesiale verwysing na entomologiese navorsing. *Wet. Pamf. Dept. Landb. Vis. Repub. S. Afr.* No. 396.
- VAN SOEST, P.J., 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fibre and lignin. *J. Assoc. Off. Agric. Chem.* 46, 825.
- VAN SOEST, P.J. & WINE, R.H., 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *J. Assoc. Off. Agric. Chem.* 50, 50.