Nigerian J. Anim. Sci. 2019, 21 (1): 135-142

Effects of two different processing methods on chemical composition and dry matter losses of *Hibiscus sabdariffa L.* (Roselle) seeds

Ibrahim, T.A., Jamilu, M., Turang, S., Abdu, S. B., Hassan, M.R., Jinadu, L.A. and Ishiyaku, Y. M.

Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria
[2]NAPRI/ABU, Zaria, Nigeria.

Corresponding Author: taibrahim@abu.edu.ng,

Target Audience: Nutritionists, Feed manufacturers

Abstract

This study was carried out to evaluate effects of processing methods on chemical compositions and dry matter losses of Hibiscus sabdariffa L. seeds. Two processing methods (cooking and sprouting) with each having three different durations were adopted. There were seven treatments with unprocessed H. sabdariffa (control) and three cooking duration methods (30min, 60min and 90min) and three sprouting duration methods (3days, 6days and 9days) with each treatment having three replicates in a Completely Randomized Design (CRD). Results indicated that there was a significant ($p < 0.05$) differences observed for the mean values in all the parameters except dry matter. The dry matter losses of the processed seeds increased ($p < 0.05$) in sprouting and cooking with time, There was an ($p < 0.05$) increase in crude proteins and ash contents for the two processing methods but a ($p < 0.05$) decrease was observed for ether extract and fiber fractions. In addition, a ($p < 0.05$) decrease was observed for phytate contents in both processing methods. However, a ($p < 0.05$) decrease was observed for tannin content in cooking, whereas sprouting showed a significant increase in tannin levels. The cooking ($p < 0.05$) declined the oxalate content in H. sabdariffa seeds, and sprouting on a contrary had no effect on it. There was an ($p < 0.05$) increase in Phosphorus and Iron concentrations but a ($p < 0.05$) decrease was observed for magnesium and potassium concentrations as compared to the unprocessed seeds. It could be concluded that the two processing methods improved the nutritional quality of H. sabdariffa seeds, with some amount of losses in dry matter.

Key words: Cooking, Sprouting, Chemical compositions, Mineral contents, Anti-nutrients, Dry matter losses

Description of Problem

Hibiscus sabdariffa plant has been found to grow well on a wide range of tropical soil conditions, and on relatively infertile soil [1]. It is popularly called "Yakuwa" in Hausa and belongs to the family of Malvaceae and is a popular vegetable in Indonesia, India, West Africa and many tropical regions [2, 3]. The vegetable is widely grown in the North-Eastern and middle belt regions of Nigeria [4]. It is widely cultivated for its pleasant red color calyx, used in making a local drink (Sobo) and wine.

The seeds were reported to have high content of oil and protein. [10] reported that seeds contained 25.20% CP while [5] reported a value of 23.46% CP. It was also considered to be potentially rich in amino acids such as Valine, Lysine, Tryptophan and Isoleucine [10] and fatty acids such as Linoleic, Oleic, Palmitic and Stearic acids. Also, it contains inorganic elements such as Potassium, Sodium

and Magnesium [18]. The seeds are usually available in abundance, but they are highly underutilized despite all these good potentials to be used as feed material.

The major factors militating against the utilization of *H. sabdariffa* seeds as an alternative feed source for livestock might be linked to presence of some anti-nutritional factors such as Tannin, Phytate, and Trypsin inhibitor activity [6, 5]. However, various processing methods have been adopted to reduce its anti-nutrients [5, 7, 9, 26]. Cooking and sprouting have been found to be effective in reducing anti-nutrients, and these could destroy up to eighty percent (80%) in both methods [5, 7]. Sprouting is a well-known processing technique for high fodder yield and least water consumption [26]. Sprouts fodder production requires only about 2-3% of that water used under field conditions to produce the same amount of fodder [9].

However, there are dry matter losses in feed which differ in amount with the type of feed ingredients (leaves, stems, seeds, nature of seeds coats etc) and processing methods adopted. The amounts of losses in dry matter for some seeds associated with sprouting methods have been widely reported [14, 12, 15, 22]. The chemical composition and dry matter losses in feedstuffs resulting from cooking in comparison with sprouting has created a paucity of information. On this basis, this study was designed to evaluate effects of cooking and sprouting under different durations on chemical compositions and dry matter losses of *H. sabdariffa* seeds.

Materials and Methods

Processing of *H. sabdariffa* seeds

Cooking method: A pot was filled with 600 ml of water and boiled to 100^{o}C using a calibrated electric cooker. Three portions (200g each) of well sun dried *H. sabdariffa* seeds were poured into a boiling water (1:3w/v) and allowed to boil for 30, 60 and 90 minutes, respectively with each of the three boiling duration carried out in triplicate in a Complete Randomized Design. The samples were scooped out and oven dried at 60^{o}C for 24 hours and percentage dry matter losses were determined. It was then grounded using laboratory hammer mill fitted with 1.5 mm sieve and then stored in a labeled polythene bags until required for laboratory analysis.

Sprouting method: Three equal portions (200g each) of well sun dried *H. sabdariffa* seeds were soaked in clean water (1:3w/v) for 8 hrs. Sprouting was carried out by spreading each of the soaked seeds into porous plastic pots with sheet of paper underneath and kept at 25^{o}C for 3days, 6 days and 9 days of germinations period respectively with each of the three sprouting duration carried out in triplicate in a Complete Randomized Design . The seeds were kept moistened throughout sprouting by spraying with 500 ml of clean water twice daily at 8:00am and 4:00pm. At the end of every sprouting period, the sprouts were oven dried at 60^{o}C for 24 hours and percentage dry matter losses were determined. The dried sprouts were crushed, milled using laboratory hammer mill fitted with 1.5 mm mesh sieve and then stored in a labeled polythene bags, until required for laboratory analysis.

$$\% \text{ DM Loss} = \frac{W_1 - W_2}{W_1} \times 100$$

Where; W_1= Weight of seeds before processing

W_2= Weight of seeds/sprouts after processing

Determination of Chemical compositions

The proximate analysis of the samples was conducted according to standard methods [11]. Nitrogen was determined by the micro Kjeldahl method with Tecator Product apparatus (Kjeltec^{TM} 2100), while crude protein was calculated by multiplying Nitrogen content × 6.25. The Soxhlet extraction

procedure was used for determination of crude fat (ether extract) using electromantle ME. The ash content was determined by combustion of the dried material in a muffle furnace at 600^{o}C for 8 hrs. The crude fibre fractions such as neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using Tecator Line (FT 122 Fibertec^{TM}) according to the method described by [28]. The concentration of phytic acid was determined according to [24]. A standard curve of ferric nitrate was plotted. Phytate phosphorus was calculated from the standard curve assuming a 4:6 Fe to P molar ratio. The concentration of total tannins content in the samples was assessed colorimetrically as described in [11], whereby tannic acid was used as a reference standard. The total oxalates concentration was determined by calcium oxalate precipitation (titrimetric method) of [20]; the method involved titration of acidic aqueous extracts of the sample with a standard solution of potassium permanganate. Determination of some mineral concentration (Calcium, Phosphorus, Potassium, Iron and Magnesium) was carried out using atomic absorption spectrophotometry in wet feed samples digested by concentrated nitric and perchloric acids, using PG instrument AA500 model.

Statistical analysis

All data collected during the experiment were subjected to statistical analysis using the general linear models (GLM) procedure of SAS version 9.13 [21] in a Complete Randomized Design at 5% level of significance and means were compared using Duncan multiple range test [16].

Results and Discussion

Effects of cooking and sprouting under different durations on the chemical composition of *H. sabdariffa* seeds

Table 1 shows the results of the chemical compositions of *H. sabdariffa* seeds under different durations for the two processing methods. The 60mins cooking of *H. sabdariffa* seeds had the least significant ($p < 0.05$) mean value for DM (93.00%) compared to all other treatments. Sprouting for 9 days had the highest ($p < 0.05$) DM loss (33.97%) while cooking for 30mins recorded the lowest DM loss (8.10%). The unprocessed *H. sabdariffa* seeds had significant lower CP value (25.18%) as compared to cooking and sprouting methods. The CP value for sprouting was observed to increase with increase in sprouting duration, and sprouting for 9days had the highest significant value (28.54%) for crude fibre than all other treatments. The unprocessed *H. sabdariffa* seeds recorded the higher ($p < 0.05$) value (15.18%) for ether extract than all other treatments while sprouting for 6days had the least significant value (8.20%). For ash content, 3days sprouting (12.68%) and 60min cooking (12.32%) were statistically similar and significantly higher ($p < 0.05$) than all other treatments. The NFE for 90mins cooking of *H. sabdariffa* seeds had significantly higher value, while 9days sprouting recorded the least significant value.

The losses in DM might be due to leaching of materials and oxidation of substances resulting from the increased metabolic activities of sprouting seeds as reported by [14]. [15] reported DM losses ranging from 9.4% to 18% with sprouting cereals from 5 to 7 days at 21^{o}C in hydroponically fodder production. The increase in percentage crude protein in sprouting is however, due to DM loss giving an apparent increase in CP as observed by [14]. Also, [27] reported an increase in CP of sprouted grains resulting from the conversion of starch and lipids into usable forms, which was also in tandem with the work of [22] who observed the effect of sprouting time (from 0-96 hours) on chemical composition of Mungbean. [13] assumed that the increase was due to synthesis of enzyme proteins by

germinating seed or a compositional change following the degradation of other constituents. The decrease in ether extract for cooking could be attributed to leaching of materials, while for sprouting, might be due to oxidation of lipids resulting from the increased metabolic activities of seeds as reported by [14]. This result is also in line with the work of [12] and [15] who reported significant losses in lipid content during canola sprouting. The decrease in fat content of seed could be attributed to total solid loss during soaking prior to germination [23] or use of fat as an energy source in sprouting process [18]. [22] reported significant increase in ash content during sprouting in mungbean seed and [18] on mungbean, pea and lentil seed. The decrease in crude fat and carbohydrate contents during sprouting might have led to the apparent increase observed in ash and other chemical components. Active utilization of minerals usually occurs in sprouting after 4days [19].

Effect of cooking and sprouting under different durations on % change on some anti-nutrients of *H. sabdariffa* seeds

Table 2 shows the effects of cooking and sprouting as processing methods on anti nutritional factors of *H. sabdariffa* seeds as compared to the unprocessed seeds. There was significant ($p < 0.05$) reduction in phytic acids for both cooked and sprouted *H. sabdariffa* seeds as compared to the unprocessed (0.17%) *H. sabdariffa* seeds. Cooking of *H. sabdariffa* seeds significantly reduced the tannin levels while sprouting however, increased it with the 9days sprouting (3.27%) recording statistically higher ($P<0.05$) tannin values. The oxalate value for unprocessed *H. sabdariffa* seeds (1.46%) was found to be statistically similar to the sprouts but significantly higher ($P<0.05$) than the cooked *H.sabdariffa* seeds. However, cooking method decreased the oxalate values significantly ($P<0.05$) from 60min to 90min.

Sprouting and cooking have been found effective in the reduction of phytic acids [14, 5, 22]. The reduction in tannin and oxalate for cooking agrees with the work of [5] while the increase in tannin for sprouted *H. sabdariffa* seeds agrees with [14] who reported that sprouting did not decrease the tannin content of grains but rather favours the formation of complexes between testa tannins and endosperm proteins. In addition, [25] observed that cooking decreased significantly the tannin content in the *H. sabdariffa* seeds while sprouting and soaking did not. Heat degradation, leaching out effects, change in chemical activity and formation of insoluble complexes could be the factors responsible for tannin reduction in *H. sabdariffa* seeds by cooking [8, 25].

Effects of cooking and sprouting under different durations on some mineral concentrations (g/L) of *H. sabdariffa* seeds.

The mineral concentrations of *H. sabdariffa* seeds as affected by cooking and sprouting durations are presented in Table 3. The calcium level of sprouted *H. sabdariffa* seeds were found to be significantly higher ($p < 0.05$) than 30min (0.88g/L) and 60min (0.93g/L) cooking but statistically similar to 90min cooked (0.97g/L) and unprocessed (1.10g/L) seeds. For Phosphorus, 6days sprouting had significantly higher ($p < 0.05$) values (10.31g/L) than the cooked, sprouting for 3days (5.83g/L), 9days (4.22g/L) and the unprocessed (4.30g/L) *H. sabdariffa* seeds. Sprouting and cooking had statistically similar values for Magnesium content and were significantly lower ($p < 0.05$) than the unprocessed (0.56g/L) *H. sabdariffa* seeds. For Iron Concentration, 60min cooking (3.06g/L) and 9days sprouting (2.96g/L) were statistically similar and significantly higher ($p < 0.05$) than all other treatments while the values of Potassium for unprocessed *H. sabdariffa* seeds (11.98g/l) was significantly higher ($p < 0.05$) than both cooking and

sprouting methods. The calcium levels increased linearly as duration of cooking advanced, though, lower than unprocessed seeds. Sprouting from 3-9days had no effect on the level of Calcium compared to the unprocessed *H. sabdariffa* seeds. The results showed that cooking and sprouting reduced concentrations of Magnesium and Potassium in *H. sabdariffa* seeds but had increasing effects on Phosphorus and Iron. This reduction in certain mineral levels might be due to leaching, heat degradation and active utilization from physiological activity of the seeds. This was similar to observation by [5] who reported reduction in certain minerals like Magnesium, Calcium, Potassium, and Zinc in cooked *H. sabdariffa* seeds at different durations (24-72hrs). The decrease in Potassium content for sprouts with time was in accordance with the earlier report of [17] who made similar observation with sprouted barley grains.

Conclusion and Applications

1. The cooking and sprouting as methods of processing employed in this study were found to apparently increase the crude protein and crude mineral contents of *H. sabdariffa* seeds, but decreased the ether extract and fibre contents of *H. sabdariffa* seeds.
2. The cooking and sprouting methods increased the dry matter losses of *H. sabdariffa* seeds with increase in duration.
3. Sprouting apparently increased the tannin contents of *H. sabdariffa* seeds with increase in sprouting duration while cooking reduced it.
4. The cooking and sprouting methods increased the Phosphorus and Iron contents of *H. sabdariffa* seeds with increase in duration, the Magnesium and Potassium contents on the contrary were decreased. However, while no effect was observed on Calcium level with increase in sprouting time; a decrease was noted for cooking method which is unaffected by cooking duration.

Table 1. Effects of cooking and sprouting under different durations on dry matter losses and chemical compositions of *H. sabdariffa* seeds.

	US	Cooking (mins)			Sprouting (days)			
Parameters		30	60	90	3	6	9	SEM
Dry matter	94.67	94.47	94.00	93.82	94.17	94.44	94.37	0.25
%DM loss	0.00^{f}	8.10^{e}	11.38^{d}	17.01^{b}	11.92^{d}	14.00^{c}	33.97^{a}	0.53
Crude protein	25.18^{d}	31.68^{c}	34.66^{a}	31.59^{c}	30.92^{c}	31.00^{c}	33.27^{b}	0.67
Crude fibre	27.26^{bc}	25.71^{d}	24.30^{e}	26.75^{c}	27.97^{b}	24.85^{e}	28.54^{a}	0.42
Ether extract	15.18^{a}	8.98^{c}	9.68^{b}	9.53^{b}	8.97^{c}	8.20^{d}	9.53^{b}	0.24
Ash	9.18^{e}	11.74^{bc}	12.32^{ab}	11.35^{c}	12.68^{a}	11.99^{b}	9.94^{d}	0.21
NFE	21.40^{c}	21.94^{c}	20.38^{c}	25.99^{a}	24.10^{b}	24.01^{b}	18.98^{d}	0.90
ADF	35.98^{a}	28.96^{b}	29.36^{b}	29.06^{b}	20.33^{d}	22.22^{c}	23.00^{c}	0.44
NDF	62.89^{a}	50.21^{d}	52.26^{c}	55.52^{b}	47.49^{f}	49.06^{e}	55.35^{b}	0.47
Hemicellulose	11.59^{a}	8.06^{cd}	9.12^{b}	6.90^{f}	8.31^{c}	7.39^{e}	7.89^{d}	0.19
Processing cost (₦/kg)	0.00	10.00	16.00	21.00	9.00	16.00	23.00	-

^{a,b,c,d,e,f}:means with different superscripts on the same row are significantly different ($p < 0.05$), SEM=Standard Error of Mean, US= Unprocessed Seeds, NFE=Nitrogen Free Extract, ADF=Acid Detergent Fiber, NDF=Neutral Detergent Fiber.

Table 2. Effects of cooking and sprouting under different durations on % change on some anti-nutritional factors of *H. sabdariffa* seeds.

	US	Cooking (min)			Sprouting (days)			
Parameters (%)		30	60	90	3	6	9	SEM
Phytic acid	0.17^{a}	0.14^{b}	0.12^{b}	0.12^{b}	0.13^{b}	0.14^{b}	0.12^{b}	0.01
%	-	17.65	29.41	29.41	23.53	17.65	29.41	
Tannin	2.40^{d}	2.20^{e}	2.10^{e}	2.11^{e}	2.88^{b}	2.64^{c}	3.27^{a}	0.11
%	-	8.33	12.50	12.08	+20.00	+10.00	+36.25	
Oxalate	1.46^{a}	1.31^{b}	1.24^{b}	1.13^{c}	1.42^{a}	1.43^{a}	1.41^{a}	0.10
%	-	10.27	15.07	22.60	2.74	2.05	3.42	

^{abcde}=Means with different superscript on the same row are significantly different ($p < 0.05$), SEM=Standard Error of Mean, US= Unprocessed Seeds.

Table 3. Effects of cooking and sprouting under different durations on some mineral concentrations of *H. sabdariffa* seeds

	US	Cooking (min)			Sprouting (days)			
Parameters (g/l)		30	60	90	3	6	9	SEM
Calcium	1.10^{abc}	0.88^{d}	0.93^{cd}	0.97^{bcd}	1.15^{ab}	1.31^{a}	1.21^{ab}	0.10
Phosphorus	4.30^{f}	9.87^{b}	6.01^{d}	6.37^{c}	5.83^{e}	10.31^{a}	4.22^{f}	0.14
Magnesium	0.56^{a}	0.40^{bc}	0.40^{bc}	0.39^{c}	0.41^{bc}	0.42^{b}	0.40^{bc}	0.01
Iron	0.36^{c}	1.42^{b}	3.06^{a}	0.88^{b}	1.23^{b}	1.08^{b}	2.96^{a}	0.19
Potassium	11.98^{a}	10.00^{c}	10.17^{c}	10.85^{b}	10.63^{b}	8.74^{d}	7.41^{e}	0.15

^{abcdef}=Means with different superscript on the same row are significantly different ($p < 0.05$), SEM=Standard Error of Mean, US= Unprocessed Seeds.

References

1. Adanlawo, I. G. and Ajibade, V. A. (2006). Nutritive values of two varieties of *Hibiscuss sabdariffa* Seed calyxes soaked in wood ash. *Pakistan Journal of Nutrition* 5(6): 555-557.
2. Tindall, H. D. (1986). Vegetable in the Tropics. Macmillan Education Ltd., Houndmills, Basingstoke, Pp: 256.
3. Fbabatunde, F. E. (2003). Intercrop productivity of *Hibiscuss sabdariffa* in Nigeria. *African Crop Science Journal* 11: 43-47.
4. Akanya, H.O., Oyeleke, S. B., Jigm, A. A. and Lawal, F. F. (1997). Analysis of sorrel drink Zoborodo. *Nigeria Journal of Biochemistry* 12: 77-81.
5. Abdu, S.B., Adegoke, T. F., Abdulrashid, M., Duru, S., Yashim, S. M. and Jokthan G. E. (2008). Effect of cooking duration on chemical composition of Roselle *(Hibiscus sabdariffa)* seed. In: Bawa, G.S., Akpa, G.N., Jokthan, G.E., Kabir, M. and Abdu, S.B. (Eds) *13th Proc, Of the Animal science association of Nigerian (ASAN)*, at A.B.U. Zaria 15th September 2008. Pp 521 -523.

6. Abu-Tarboursh, H. M. and Basher Ahmed, S. A. (1996). Studies on Karkade (*Hibiscus subdariffa*) Protease inhibitors, Phytate, in vitro protein digestibility and gossypol content. *Journal of food chemistry* 56: 15-19.
7. Adamu, M. (2008). Effects of germination on the chemical composition and nutritive value of *Hibiscus sabdariffa* seeds. Undergraduate Thesis. Department of Animal Science, Ahmadu Bello University. Pp:30.
8. Alonso, R.., Aguirre, A. and Marzo, F. (2000). Effects of extrusion and traditional processing methods on antinutrients and *in vitro* digestibility of protein and starch in faba and kidney beans. *Food Chemistry*, 68: 159-165.
9. Al-Karaki, G. N. (2010). Hydroponic green fodder alternative method for saving water in dry areas. *Proceedings of the "Second Agricultural Meeting on Sustainable Improvement of Agricultural and Animal Production and Saving Water Use.*
10. Al-wandawi, H., Aisackly, K. and Abdulrahman, M. (1984). *Hibiscuss sabdariffa* seeds: A new protein source. *Journal of Agriculture and Food Chemistry*, 32: 510-512.
11. AOAC. (2005). Official methods of Analysis, 15th ed. Association of Official Analytical Chemistry, Washington, D. C.
12. Badshah, A., Zeb, A. and Sattar, A. (1991). Effect of soaking, germination and autoclaving on selected nutrients of rapeseed. *Pakistan Journal Science of Indian Research*, 34: 446-448.
13. Bau, H. C., Villaume, J. N. and Mejean, L. (1997). Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soya bean (*Glycine max*) seeds. *Journal of Science of Food and Agriculture*, 73(1): 1-9.
14. Chavan, J. and Kadam, S. S. (1989). Nutritional improvement of cereals by sprouting. *Critical Reviews in Food Science and Nutrition*, 28(5): 401-437.
15. Chung, T. Y, Nwokolo, E. N. and Sim, J. S. (1998). Compositional and digestibility changes in sprouted barley and canola seeds. *Plant Foods for Human Nutrition*, 39: 267-278.
16. Duncan, D.B. (1955). Multiple range and Multiple F-tests. *Biometrics*, 11: 1-42.
17. Dung, D. D., Godwin, R. and Nolan, I. V. (2010). Barley Grain and Sprouted Barley. *Journal of Animal and Veterinary Advances*, 9: 2485-2492.
18. El-Adawy, T. A. and Khalil, A. H. (1994). Characteristics of *Hibiscuss sabdariffa* seeds as a new source of Proteins and Lipids. *Journal of Agriculture Food and Chemistry*, 42: 1896-1900.
19. Morgan, J., Hunter, R. R. and O'Haire, R. (1992). Limiting factors in hydroponic barley grass production. In: *8th proceedings of the International congress on soil less culture*, pp: 241-261.
20. Oke, L. O. (1966). Composition of some Nigeria leafy vegetables. *Journal of Animal Dietetic Association*, 53: 130-132.
21. SAS Institute (2002). SAS/START. User's guide. 6.03 Edition. Inc. Cary, NC.U.S.A.
22. Syed, A. S., Aurang Z., Tariq M., Nadia N., Sayed J. A., Muhammad S., Abdul, A. and Asim, M. (2011). Effects of sprouting time on biochemical and nutritional qualities

of Mungbean varieties. *African Journal of Agricultural Research*, 6(22): 5091-5098.

23. Wang, N., Lewis, M. J., Brennan, J. G. and Westby, A. (1997). Effect of processing methods on nutrients and antinutrional factors in cowpea. *Food Chemistry*, 58: 59-68.
24. Wheeler, E. I. and Ferrel, R. E. (1971). A method for phytic acid determination in wheat and wheat fractions. *Cereal Chemistry*, 48: 312-320.
25. Yagoub Abu, El Gasim, A. and Mohammed, A. M (2008). Furundu, a Meat Substitute from Fermented Roselle (*Hibiscus sabdariffa* L.) Seed: Investigation on Amino Acids Composition, Protein Fractions, Minerals Content and HCl-Extractability and Microbial Growth. *Pakistan Journal of Nutrition*, 7(2): 352-358.
26. Fazaeli, H., Golmohammadi, H. A., Tabatabayee, S. N. and Asghari-Tabrizi, M. (2012). Productivity and nutritive value of barley green fodder yield in hydroponic system. *World Application Science*, 16: 531-539.
27. Tudor, G., T. Darcy, P. Smith and F. Shallcross. (2003). The intake and live weight change of drought master steers fed hydroponically grown, young sprouted barley fodder (Autograss). Department of Agriculture Western Australia.
28. Van Soest, P. J. and Wine, R. H. (1967). Use of Detergents in the Analysis of Fibrous Feeds. IV. Determination of Plant Cell-Wall Constituents. Journal of the Association of Official Analytical Chemists, 50: 50–55.