Post-Harvest Content of Free Titratable Acids in the Grain of Proso Millet Varieties (*Panicum milliaceum* L.), and Changes during Grain Processing and Storage

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Abstract: A triennial evaluation of the content of titratable acids (TA) in grain was carried out on 12 varieties of proso millet (*Panicum miliaceum* L.). The model variety, Mironovskoe 94, harvested in 2004, was used for the monitoring of sensory changes in TA content, in relationship to different storage conditions and the application of different grain processing methods. The Czech state norm (CSN 56 0512-9 1995) Determination of Titratable Acids, with a slight modification, was used as the standard method. The TA contents in dry matter varied between 39 and 78 mmol/kg of grain dry matter, and significant differences between varieties and experimental years were found. The method of proso millet processing had a key role in the final sensitivity to rancidity. The storage conditions had a much lower influence on TA content than the processing technology. The clearly detected sensory changes found in scoured grains corresponded with TA contents within the range of 90 to106 mmol/kg. The monitoring of the TA content can be considered as a suitable tool for the prediction of rancidity processes in millet grains.

Keywords: *Panicum miliaceum* L.; proso millet; rancidity; storage conditions; titratable acids

The rapid development of organic farming in the Czech Republic and other East European countries, as well as the increasing demands for healthy nutrition, have supported an increased interest in the planting and consuming of minor crops over the last ten years. This mainly relates to the increase in the consumption of: spelt wheat (*Triticum spelta* L.), common buckwheat (*Fagopyrum esculentum* Moench), amaranth (*Amaranthus* sp.), and also proso millet (*Panicum milliaceum* L.). The millet grains are characterized by higher nutritional quality and digestibility, in comparison with primary cereals such as wheat, rice, oats, etc. (Fujino *et al.* 1996; Serna-Saldivar *et al.* 2000). Petr *et al.* (2003) mentioned the tolerance of millet in patients suffering from celiac disease. The inclination of some consumers to use these products was also been brought about by their distrust of additives in common food products. Thus, the opportunity to prepare their own food from intact, or only mechanically processed grain, is very attractive for them (Hansen & Madeleine 1996).

The uses of proso millet in the household can be various. The most common use of scoured millet grain is as a side dish for meat, vegetable, and mushroom dishes. It is suitable for soup, dumplings, and dessert preparations (Ang *et al.* 1999). However, the final products of proso millet were often characterized by impaired organoleptic properties (a bitter flavour), which are connected with the higher fat content in the grains, and the possibility of fat degradation (rancidity of the lipids) during unsuitable storage. Campbell *et al.* (1991) reported that the shelf life of proso millet

Table 1. Weather conditions during proso millet cultivation at Crop Research Institute, Prague

Year	Month					Vegetation period*	Year average
	V	VI	VII	VIII	IX		
Average temperatures (°C)							
2001	15.0	14.7	18.2	18.7	12.1	15.7	8.7
2002	15.4	17.6	18.6	19.5	13.2	16.9	9.5
2003	15.8	20.4	19.5	21.4	14.7	18.4	9.4
1953–2000	13.1	16.3	17.8	17.6	13.6	15.7	8.2
Sum of precipitations (mm)							
2001	44.7	70.4	102.0	93.8	87.9	398.8	635.3
2002	8.5	53.4	93.2	147.3	55.6	358.0	687.7
2003	72.0	28.2	73.8	30.6	28.2	232.8	346.6
1953–2000	60.7	65.3	69.8	62.4	34.7	292.9	477.3

*Vegetation period = from 1st May to 30th September

flour is very limited, and rancidity symptoms occur within two weeks. It is caused by the high hydrolytic activity of enzymes (lipases) and lipid oxidation in the proso millet grain (Kaced *et al.* 1984; Lorenz & Hwang 1986; Dendy 1995). According to Ekstrand *et al.* (1993), rancidity processes usually start with enzymatic hydrolysis, and then the oxidation of free fatty acids (FFA) continues it. To increase proso millet's shelf life, Seitz *et al.* (1993) recommends physical protection methods such as microwave heating, extrudation, or the precooking of whole grains before milling.

The content of FFA and the evaluation of its peroxide number are reliable indicators of fat rancidification according to Hansen and Madeleine (1996). The present Czech norm (CSN 56 0512-9 1995) only evaluates the organoleptic changes in traditional wheat and rye flours, according to the assessment of titratable acid (TA) contents in a water extract of flour. The AACC method (02-02A: Fat Acidity – Rapid Method for Small Grains) mentions the procedure of fat acidity evaluation on a wider assortment of small grain cereals, but without concrete species recommendations. Thus, the official norms and quality parameters of fresh flour or scoured grains are not straightforward for proso millet or other minor crops. Minimal information has been published about the natural value of TA contents in proso millet grain, as well as the effects on this parameter by the variety or external factors.

The objective of this study was to evaluate the significance of variety and external factors on the post-harvest content of TA in different proso millet varieties. A second aim was to determine changes in the TA content in millet grain under different storage conditions and with the use of different grain processing methods.

MATERIALS AND METHODS

Twelve selected proso millet varieties (Volnoe – RUS, Kinelskoe Skorospeloe – RUS, Mironovskoe 94 – RUS, Saratovskoe 3 – RUS, Orlovskoe 7 – RUS, Lipetskoe 19 – RUS, Kazansk 61 – RUS, Kharkov 25 – RUS, Veselopodolianskoe 367 – RUS, Raoluoga – RUS, Sirocket 5 – AUT, and Fertodi 2 – HUN) were cultivated for three years (2001–2003) in the experimental fields of Crop Research Institute, Prague. The randomized block method, in three repetitions with a plot area of 4 m^2 was used. The weather conditions during the experimental cultivation of millet varieties were recorded (see Table 1).

The content of titratable acids (TA mmol/l) in the grain was determined in these twelve proso millet varieties each year, using the standard method (CSN 56 0512-9 1995 Determination of Titratable Acids), which was slightly modified. TA content was determined pursuant to the amount of 0.1M NaOH required to achieve the equivalence point (pH 9.5) in the distilled water extract of millet flour. The pH value of the equivalence point was indicated by a pH-meter on the basis of repeated blank experiments with 100 ml distilled water titrated by 0.1M NaOH and phenolphthalein as the indicator.

Flavour changes (sensory test) and changes of TA content were examined in four different grain processing variants and three storage conditions of the Mironovskoe 94 variety, harvested in 2004. This variety was cultivated in the same locality and by using an identical randomized block method as the previous millet varieties.

Processing variants: (1) whole grain, (2) scoured grain (groats), (3) ground grain from whole grains, (4) ground grain from scoured grain.

Storage conditions: Climatic chamber – relative humidity 65%, temperature 35°C.

Normal lab conditions – relative humidity 45–55%, temperature about 20°C.

Cooling box – relative humidity 30%, temperature 6°C.

Evaluations of the FFA content and sensory tests were carried out at weekly intervals over 14 weeks. Five grams of ground grain from all tested storage variants were used for an additional sensory test. Each sample was mixed with boiling water and the rancidity (bitterness) level of the sample was tested after 15 min. The significant flavour changes were subjectively detected by 3 persons.

Table 2. Average content of titratable acids (mmol/kg of dry matter) in 12 millet varieties, over three years; *F* values from ANOVA and results of multiple comparisons using Tukey HSD test ($P < 0.05$).

Source of variation	*F*-values of the ANOVA
Variety	43.64**
Year	377.45**
Variety × year	16.19**
Variety/year	mean value and standard deviation
Raoluoga	50.79 ± 6.49^{a}
Mironovskoe 94	52.85 ± 10.82^{ab}
Kinelskoe Skorospeloe	53.26 ± 8.81^{ab}
Saratovskoe 3	54.46 ± 6.44^{bc}
Fertodi 2	55.12 ± 3.98^{bc}
Kharkov 25	57.71 ± 4.50^{bc}
Volnoe	60.19 ± 7.58^{cd}
Kazansk 61	60.83 ± 4.81^{cd}
Sirocket 5	62.37 ± 6.54^{def}
Veselopodolianskoe 367	62.66 ± 7.87^{def}
Orlovskoe 7	63.94 ± 8.00^{fg}
Lipetskoe 19	65.15 ± 13.59^{fg}
2001	50.01 ± 5.86^{a}
2002	62.64 ± 7.47^{b}
2003	62.18 ± 5.79^{b}

Significantly different samples do not contain any common letter; $^{**}P \leq 0.01$

RESULTS AND DISCUSSION

An analysis of variance (Table 2) showed the significance of the effects of variety and year on the TA content, and the significance of the interaction between these factors. According to this analysis, the highest contribution to total variation was the experimental year (46%), followed by variety (30%) and year by variety interaction (22%).

Table 2 also shows differences between individual varieties and years. The range of TA contents among the tested varieties and years was high, and varied from 39.18 mmol/kg in the variety Mironovskoe 94 in 2001 to 78.35 mmol/kg in the variety Lipetskoe 1' in 2002. The average value of TA content of millet varieties harvested in the year 2001 was significantly lower than in the other two years. The triennial evaluation of average content of TA enabled us to find two groups of millet varieties with significantly lower and higher TA content, respectively. The significantly lower TA contents were found in the varieties Raoluoga (50.79 mmol per kg), Mironovskoe 94 (52.85 mmol/kg),and Kinelskoe Skorospeloe (53.26 mmol/kg). The group with significantly higher values of the TA content included the varieties Volnoe (60.19 mmol per kg), Kazansk 61 (60.83 mmol/kg), Lipetskoe 19 (62.37 mmol/kg), Sirocket 5 (62.66 mmol/kg), Orlovskoe 7 (63.94 mmol/kg), and Veselopodolianskoe 367 (65.15 mmol/kg).

There is neither enough evidence within the available literature on the TA contents in proso millet varieties nor of the impacts of year or means of cultivation. Millet flour showed about 20 to 30 mmol/kg higher TA content in comparison with the declared TA content in standard wheat

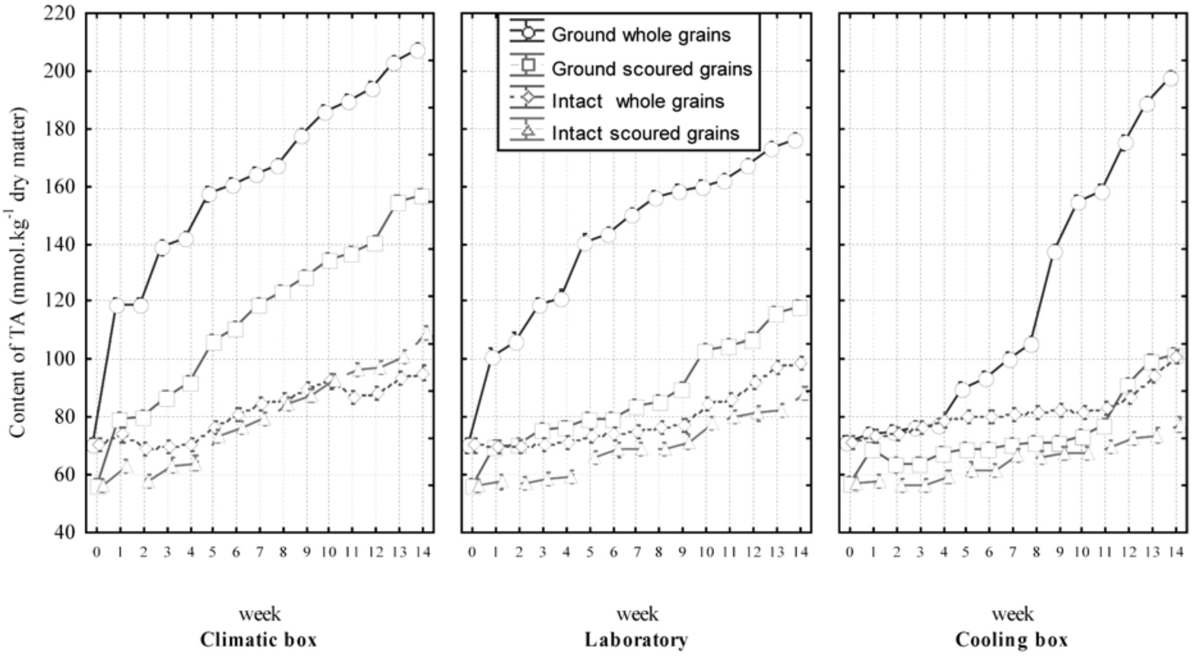

Figure 1. The time changes in the contents of titratable acids of millet grain, in comparison with different methods of processing and storage

flour published in the CSN 56 0512-9 (1995). This is probably caused by a higher fat content in the millet grain, influencing higher free TA contents. Moudrý *et al.* (2005) evaluated the content of fat in similar collections of proso millet over three years. They confirmed the factor of year to be a significant parameter. Warmer and drier weather conditions significantly increased the fat content. Baltensperger (1996) published that 20–30 days before the end of physiological maturity, the plant actively accumulates dry matter, particularly in the grains. In this context, the higher TA content detected in years 2002 and 2003 also corresponded to a significantly higher average temperature in these periods (Table 1). On the other hand, the sum of precipitation did not seem to be a decisive factor for the variability of TA contents.

The statistical evaluation of TA content changes during storage produced the following results. All three evaluated factors: storage period, means of processing, and means of storage (inclusive of all interactions) had a statistically significant effect on the variability of TA contents. The means of processing factor had the highest percentage

Table 3. The results of a three-way analysis of variance of the content of titratable acids in millet grain

Source of variation	df	*F*-value	Percentage ratio of total variation (%)
1. storage period	14	2 977.70**	25.06
2. processing variant	3	28 691.25**	51.11
3. way of storage	2	6 157.49**	7.34
1 × 2	42	338.50**	8.46
1 × 3	28	73.83**	1.23
2 × 3	6	1 074.75**	3.84
1 × 2 × 3	84	59.89**	2.99

**$P < 0.01$; df – degree of freedom

Table 4. The time to first detection of sensorial changes in differently processed and stored millet grain (in weeks); and the corresponding TA (titratable acids) content (in mmol/kg of d.m.)

Way of storing/way of grain processing		Ground whole grain	Ground scoured grain	Intact whole grain	Intact scoured grain
Climatic chamber	time to detected sensorial changes	9	5	–	10
	TA content	178	106	–	92.5
Laboratory	time to detected sensorial changes	9	9	–	14
	TA content	158	90	–	91
Cooling box	time to detected sensorial changes	9	12	–	–
	TA content	137	91	–	–

influence (51.11%) on changes of TA content. A lower influence was found for the storage period factor (25.06%). The significant factor means of storage showed the lowest effect (7.34%) on the TA content (Table 3).

The highest level of TA was detected in all processing categories which were stored in the unfavourable conditions of the climatic chamber. Among the processing variants, the highest TA level was detected in ground grain, prepared from intact whole grain (Figure 1). This significant increase was already evident during the second week of storage in the case of the climatic box and laboratory conditions. The increase in TA content of ground whole grain was a bit slower in the cooling box. Nevertheless, the final content of TA (in week 14) was comparable to the value of TA found under the conditions of the climatic chamber and laboratory. Thus, these results confirmed that it is not possible to store the whole grain flour of millet, even in a refrigerator, for a longer period of time.

The long term storage of intact whole grains and intact scoured grains were characterized by a slower TA content increase, which mostly did not exceed 100 mmol/kg. The intact scoured grain showed the lowest value of TA during storage in both laboratory and cooling box conditions. In the case of ground scoured grains, the TA increase was higher in the climatic chamber. Under the conditions of the laboratory and cooling box, the TA level of ground scoured grains was similar to the TA increase of intact scoured and intact whole grains (Figure 1).

It was easy to detect sensory changes (bitter flavour symptoms) in millet scoured grains in both processed categories (intact or ground scoured grain) and in all storage conditions. The first significant flavour changes corresponded, in all tested conditions, with a concentration of TA within the range 90–106 mmol/kg. The flavour changes during the period tested were evidently influenced by storage conditions. The most rapid flavour changes occurred in the unfavourable conditions of the climatic chamber, with high humidity and temperature (the 5th week – ground scoured grains; the 10th week – intact scoured grain – Table 4).

Sensory evaluations of ground whole grains were much more complicated. The natural flavour of these samples was slightly bitter, which is caused by the presence of bitter substances located in the outer layers of millet (Chow 2007). The increase in bitterness was not so significant during storage as it was in scoured grains, and the significant flavour changes were detected on a higher level of TA content (137–178 mmol/kg) than in the case of scoured grains. Significant flavour changes of the intact whole grain were not reliably detected.

We do not know of any other official references to proso millet rancidity. Nevertheless, it is probably possible to find principles of rancidity similar to those which were found in oats or other cereals. Ekstrand *et al.* (1993) reports that rancidity, and the development of rancid flavour, varies in different kinds of oat products. The lipase activity is not distributed in the whole grain, but is confined to the aleuron layer and embryonic tissues, where lipids are metabolised. The processing (milling) of grains leads to tissue damage and contacts between enzymes and the fat substrate increases. A similar opinion was reported by Malekian *et al.* (2000). These conclusions are also applicable for our results on the tested varieties of millet.

Higher flavour stability was observed in the case of intact whole and intact scoured grains. It is possible to anticipate a similar enzyme (lipases) distribution to that in other cereals. In the case of scoured grains, the outer layers, aleuron layer, and a large part of the germ were removed; significantly eliminating the potential risk of rancidity. The significant decrease of fat in processed scoured grain (about 50%) was also confirmed by Dendy (2001). Rancidity results (TA content) of ground scoured grains were comparable to the results of intact whole grains, and were significantly lower in comparison with ground whole grain. Thus, the character of a millet product is the crucial main factor for the storage conditions and the final shelf life of the product.

To conclude, it is important to emphasize that the monitoring of the TA content is a suitable tool for the prediction of rancidity processes in millet grains. In spite of genetic (varietal) and year effects on the value of TA content in millet grains, the key role in the final sensitivity to rancidification is the nature of the final grain processing.

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References

Ang C.Y.W, Liu K., Huang Y-W. (1999): Asian Food: Science and Technology. Technomic Publishing Company, Lancester.

Baltensperger D.D. (1996): Foxtail and Proso Millet. In: Janick J. (ed.): Progress in New Crops. ASHS Press, Alexandria, 182–190.

Campbell J., Hauser M., Hill S. (1991): Nutritional characteristic of organic, freshly stone-ground, sourdough and conventional breads. EAP Publication 35. Available at http://eap.agrenv.mcgill.ca/Publications/EAP35.htm (accessed April 4, 2009)

Chow K.C. (2007): Fatty Acids in Foods and their Health Implications. 3rd Ed., Series: Food Science and Technology, University of Kentucky, Lexington.

Dendy D.A.V. (1995): Sorghum and Millets. Chemistry and Technology. American Association of Cereal Chemists, St. Paul.

Dendy D.A.V. (2001): Sorghum and the millets. In: Dendy D.A.V., Dobraszczyk B.J. (eds): Sorghum and Millets: Chemistry and Technology. American Association of Cereal Chemists, St. Paul, 11–27.

Ekstrand B., Gangby I., Akesson G., Stöllman U., Lingnert H., Dahls S. (1993): Lipase activity and development of rancidity in oats and oat products related to heat treatment during processing. Journal of Cereal Science, **17**: 247–254.

Fujino Y., Kuwata J., Mano Y., Ohnishi M. (1996): Other grain components. In: Henry R.J., Kettlewell P.S. (eds): Cereal Grain Quality. Chapman & Hall, London, 289–320.

Hansen L., Madaleine S.R. (1996): Sensory acceptability is inversely related to development of fat rancidity in bread made from stored flour. Journal of the American Dietetic Association, **96**: 792–793.

Kaced I., Hoseney R.C., Varriano-Marston E. (1984): Factors affecting rancidity in ground pearl millet (*Pennisetum americanum* L.). Cereal Chemistry, **61**: 187–192.

Lorenz K., Hwang Y.S. (1986): Lipids in proso millet flours and brans. Cereal Chemistry, **63**: 387–390.

Malekian F., Rao R.M., PrinyawiwatkulW., Marshall W.E., Windhauser M., Ahmedna M. (2000): Lipase and Lipoxigenase Activity, Functionality, and Nutrient Losses in Rice Bran During Storage. LSU AgCenter Research & Extension, Bulletin No. 870, Baton Rouge.

Moudrý J., Kalinová J., Petr J., Michalová A. (2005): Buckwheat and Proso Millet in Czech. UZPI. Praha. (in Czech)

Petr J., Michalík I., Tláskalová-Hogenová H., Capouchová I., Faměra O., Urminská D., Tučková L., Knoblochová H. (2003): Extension of the spectra of plant products for the diet in celiac disease. Czech Journal of Food Science, **21**: 59–70.

Seitz L.M., Wright R.L, Waniska R.D., Rooney L.W. (1993): Contribution of 2 acetyl-1-pyrroline to odors from wetted ground pearl millet. Journal of Agriculture Food Chemistry, **41**: 955–958.

Serna-Saldivar S., McDonoug C.M., Rooney L.W. (2000): Millets. In: Lorenz K.L., Kulp K. (eds): Handbook of Cereal Science and Technology. Marcel Dekker, New York, 177–202.