CROATICA CHEMICA ACTA
CCACAA **81** (1) 131–137 (2008)

ISSN-0011-1643
CCA-3222
Original Scientific Paper

Accumulation of Amadori and Maillard Products in Wheat Seeds Aged under Different Storage Conditions

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RECEIVED MARCH 30, 2007; REVISED JULY 27, 2007; ACCEPTED AUGUST 2, 2007

Occurrence of protein modification by Amadori and Maillard reactions in wheat seeds during ageing at different temperatures and relative humidities (RH) has been investigated over 12 month period. Spectrofluorimetric determination of Maillard products of partially purified proteins extracted from aged seeds revealed increase of protein fluorescence at 325/425 nm (excitation/emission wavelength). The highest increase of Maillard products was observed for seeds aged at 40 °C, RH = 45 %, followed by storage at 25 °C, RH = 45 % and storage at warehouse conditions (10–30 °C, RH = 40–70 %), while seeds kept at 4 °C, RH = 40 % did not show significant accumulation of Maillard products. Similar pattern of accumulation were observed for Amadori products, except for seeds aged at 40 °C, RH = 45 %, where products decreased after 270 days of storage. Obtained data indicate that a wheat seed ageing during storage is associated with protein modification by Amadori and Maillard reactions. However, the contribution of these reactions varies under different storage conditions.

Keywords
advanced glycosylation-end products
Amadori products
Maillard products
seed ageing
wheat

INTRODUCTION

Maillard reactions are a series of complex chemical reactions that generally follow four-step process: (a) the non-enzymatic condensation of a reducing sugar with free amino group of proteins or nucleic acids to form glycosylamine, (b) the rearrangement of the glycosylamine to Amadori products, (c) subsequent degradation and dehydration of Amadori products into amino or carbonyl intermediates, and (d) the reaction of carbonyl intermediates with other amino groups followed with subsequent rearrangement to advanced glycosylation end-products (AGE).[1–4] Their role in the ageing process of human and animal system has been reviewed extensively in medical literature,[5–8] whereas there are only few reports concerning Maillard reactions during seed ageing. Wettlaufer and Leopold observed accumulation of Maillard product in soybean axes during the accelerated ageing conditions,[9] while Sun and Leopold established correlation between the accumulation of Maillard products and loss of soybean seed viability under long-term storage conditions.[10] During ageing of mung been seeds (*Vigna Radiata*) accumulation of lipid peroxidation products, glucose, Maillard and Amadori products in seed axes was observed, and correlation established between Amadori and lipid peroxidation products accumulation, as well as Maillard products and glucose.[11,12] Accelerated ageing of wheat seeds is associated with loss of viability, accumulation of glucose and

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loss of phospholipids in embryo.[13] Such changes within wheat seeds indicate possible occurrence of non-enzymatic glycosylation, but data on Amadori and Maillard reactions during ageing are lacking.

In addition to their physiological role in seed biology, Maillard products plays important role in food quality. Increase of Maillard products could positively affect the food flavor, texture and aroma, but can also cause loss of food nutritional value and amino acid bioavailability.[14] At the same time, some of the products are antioxidants, antimutagens, as well as antimicrobials.[15,16] Considering their ubiquitous occurrence and duality of their nature, importance of surveying these products in food and raw-materials becomes apparent.

Since data on Amadori and Maillard reactions in wheat seeds during ageing are especially deficient, in the present study, we have investigated occurrence of protein modification by Amadori and Maillard reactions in wheat seeds during ageing at different storage conditions.

EXPERIMENTAL

Three winter wheat varieties from 2005 harvest, Divana, Žitarka and Srpanjka, were generously supplied by the Agricultural Institute Osijek. Nitroblue tetrazolium chloride was from Eurobio (France), and HEPES [*N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid)] from Sigma (USA) while all other chemicals were of *pro analysis* purity and were purchased from Kemika, Zagreb, Croatia.

Seed Storage

Seeds of examined wheat varieties, containing 13.6 % of moisture, were divided in batches (4 ageing conditions x 12 months; 1 kg of seed per batch), packed in paper bags, bags sealed, and storage of wheat seeds performed at four different conditions of environmental temperature and relative humidity (RH): (i) 4 °C, RH = 40 %; (ii) 25 °C, RH = 45 %; (iii) 40 °C, RH = 45 %, (iv) warehouse conditions dependent of the climate environmental conditions, varying from 10 to 30 °C and RH = 40–70 %. Storage at above defined conditions was carried out in a refrigerator (i), in a storage box placed in conditioned warehouse (ii), in thermostatic incubator Heraeus (Heraeus, Germany) (iii), and on a shelf positioned 20 cm from the floor of unconditioned warehouse (iv). Relative humidity of 40 or 45 % during storage was adjusted with saturated solutions of calcium chloride or potassium nitrite.[17] Samples were taken monthly over a period of 12 months.

Protein Extraction

One gram of wheat grains were disintegrated in mortar with pestle using liquid nitrogen, suspended in 5 cm^3 of 0.05 mol dm^{-3} phosphate buffer pH = 7.2, and extracted under inert atmosphere of nitrogen for 30 minutes at 4 °C with vortexing 30 seconds each 5 minutes. Extracts were clarified by centrifugation (5000 *g*, 5 min, 4 °C) and 0.5 cm^3 of 100 g dm^{-3} streptomycin sulphate dissolved in 0.05 mol dm^{-3} HEPES pH = 7.2 was added to supernatant for nucleic acids precipitation. After vortexing and centrifuging (15000 *g*, 15 min, 4 °C) another 0.5 cm^3 of streptomycin sulphate was added and suspension centrifuged again. Seed proteins in supernatant were precipitated with ammonium sulphate (0.55 g per 1 cm^3) for 30 minutes at 4 °C with vortexing 30 seconds each 5 minutes, and after centrifugation (15000 *g*, 15 min, 4 °C) re-dissolved in 3 cm^3 of 0.05 mol dm^{-3} phosphate buffer pH = 7.2. Protein solution was further purified using PD-10 columns (Amersham Biosciences, Sweden) to exclude substances with molecular mass less than 5000 Da. Procedure of protein partial purification minimized the interference of non-protein substances and stabilized protein fluorescence readings, as previously reported.[10–12] Partially purified seed protein extracts were used for measurement of Amadori and Maillard products.

Measurement of Amadori and Maillard Products

The content of Amadori products in extracts of seed proteins was determined using nitroblue tetrazolium method according to Wettlaufer and Leopold.[9] One cm^3 of 0.0005 mol dm^{-3} nitroblue tetrazolium chloride dissolved in 0.1 mol dm^{-3} sodium carbonate solution of pH = 10.3 was added to 0.1 cm^3 of partially purified seed protein extract (0.3 g dm^{-3}) and incubated at 40 °C in water bath. Absorbance at 550 nm was read after 10 and 20 minutes. The increase in absorbance (ΔA_{550nm}) was used as a measure of Amadori products. The content of Maillard products was determined using protein fluorescent method. Extracted seed proteins (0.3–0.4 g dm^{-3}) were scanned with excitation wavelength from 270–400 nm and emission wavelength from 320–500 nm using Cary Eclipse spectrofluorimeter (Varian, USA). To omit any possible variations in protein and Maillard products (*e.g.* AGE) content among independent extractions, the content of Maillard products was expressed using FAST index.[18] FAST index was calculated by formula: $\text{FAST} = (\text{Fluorescence}_{\text{AGE}} / \text{Fluorescence}_{285\text{ nm} / 340\text{ nm}}) \cdot 100$.

Statistical Analysis

Spearman rank correlation between temporal changes of levels of Amadori and Maillard products in wheat seeds was calculated using statistical software Statistica (Stat Soft). Differences between varieties in the content of Maillard products were examined using Mann-Whitney U-test, which was performed with the above mentioned statistical software.

RESULTS AND DISCUSSION

Amadori Products Accumulation during Ageing of Wheat Seeds

Non-enzymatic glycosylation of wheat seed proteins would lead to formation of glycosylamine, which rearrange to Amadori products. Amadori products accumulation during ageing of wheat seeds was similar for all three wheat varieties, and dependent on storage conditions, as shown

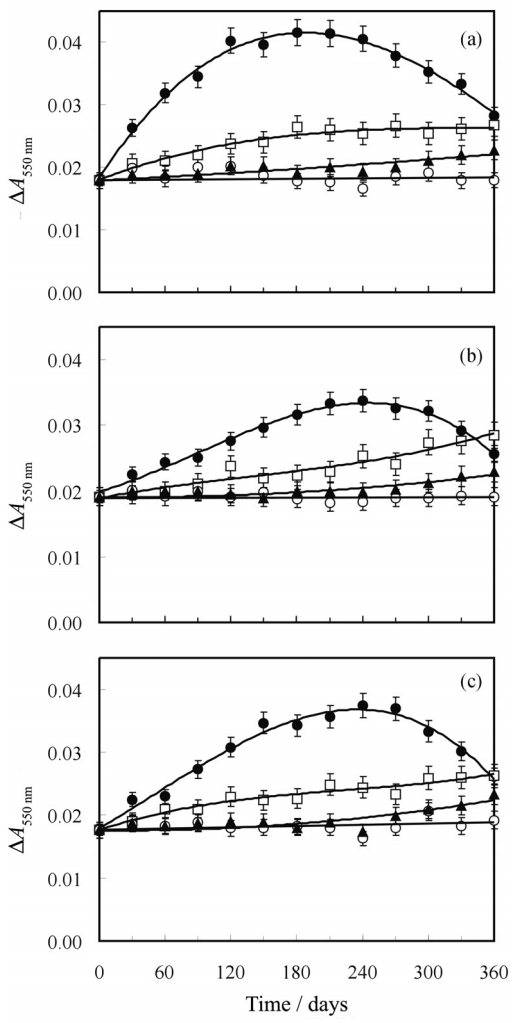

Figure 1. The absorbance of Amadori products (ΔA_{550nm}) in seeds of examined wheat varieties during ageing under different storage conditions. Varieties: (a) Divana, (b) Žitarka, (c) Srpanjka. Ageing conditions: 40 °C, RH = 45 % (●), 25 °C, RH = 45 % (□), 4 °C, RH 40 % (○), and 10–30 °C, RH = 40–70 % (▲). Bars present standard error of the mean calculated from three independent repetitions.

in Figure 1. Seeds kept at higher storage temperature (40 °C, RH = 45 %) were showing different pattern of Amadori products accumulation, than seeds kept at lower temperatures.

Seeds aged at 40 °C, RH = 45 % accumulated Amadori products with increase of 80–150 % during first 150 days of storage, maintaining the same level of accumulation till 270 days of storage, after which the content of products start to decline. Continuous accumulation of Amadori products during the whole storage period was observed for seeds aged at 25 °C, RH = 45 %, which contained 50 % more products at the end of storage period. Slight increase (20–30 %) was observed for seeds aged at warehouse conditions, but this increase started at 270 days and continued to 360 days of storage. In case of seeds kept at 4 °C, RH = 40 % no significant increase in Amadori products over 360 day period (less than 5 %) could be observed.

The results demonstrate occurrence and accumulation of Amadori products during ageing of wheat seeds, and their dependence on storage conditions, consistent with previous findings for soybean and mung bean seeds.[9–12] Significant accumulation of Amadori products followed by decline in wheat seeds aged at 40 °C, RH = 45 % has been previously reported for soybean seeds artificially aged at 45 °C, RH = 100 %,[9] and mung been seeds aged at 33 °C.[11] Amadori products "increase-decrease" curve for wheat seeds was apparently more flattened than curves observed for soybean and mung bean seeds,[9,11] which is probably related to the whole wheat seed examination instead of seeds axes. Several factors may have affected detection of non-enzymatic glycosylation of proteins: (a) protein susceptibility to non-enzymatic glycosylation due to low amount of target amino acids,[19] which was reflected as lower Amadori products increase (80–150 %), and (b) detection of non-enzymatic glycosylation of proteins not just in embryo, which is the first part of the seed exposed to ageing changes,[20] but in other parts of the seed which are affected by ageing at later stage, resulting in a maintained level of products from 150 till 270 days. Although curve shapes differ among soybean, mung bean and wheat seeds aged at high temperatures (higher that 30 °C), attributable to application of different ageing conditions, moisture content within seeds, as well as part of a grain examined, it seems that at higher temperatures the increase-decrease pattern of Amadori products accumulation could be expected for other seeds as well. Continuous increase of Amadori products in wheat seeds aged at 24 °C, RH = 45 %, was similar to results published for soybean seeds aged at 30 °C, RH = 75 %,[9] and mung been seeds aged at 33 °C,[12] and differences in maximal increase, 50 % for wheat seeds compared to 100–400 % for mung bean seed axes, could be explained, as mentioned above, by applied ageing conditions, as well as part of a grain examined. Delay in increase for wheat seeds aged at warehouse conditions could be attributed to low warehouse temperatures (not exceeding 20 °C) during winter and spring, while the increase between days 270–360 is most likely connected to higher warehouse temperatures caused by summer months of the year. Lack of changes in Amadori product content for wheat seeds aged at 4 °C, RH = 40 % was expected due to low storage temperature that: (a) is unfavorable for non-enzymatic glycosylation reactions in model solution, and (b) causes that seed cytoplasm exists in a glassy state which prevents molecular mobility necessary for sugar-protein interactions.[9,21–23]

Maillard Products Accumulation during Ageing of Wheat Seeds

Formation of Amadori product during wheat seeds ageing should lead to further rearrangement of Maillard re-

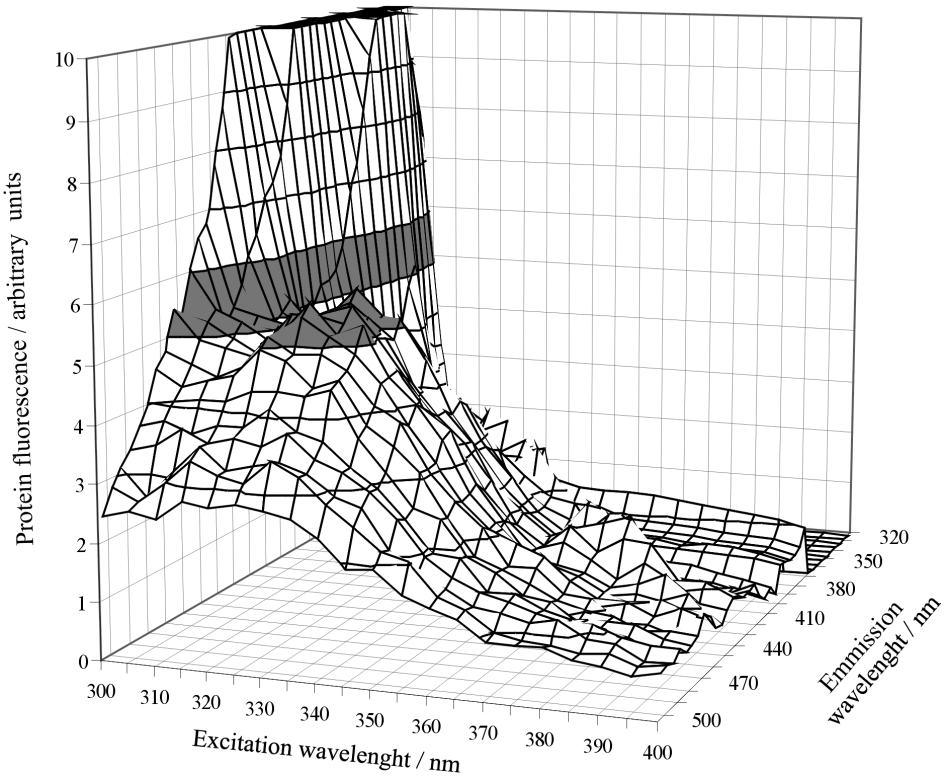

Figure 2. Three-dimensional fluorescence spectra of proteins extracted from aged wheat seeds. The aqueous solution of protein was scanned with excitation wavelength from 270–400 nm and fluorescence intensity was recorded at emission wavelength from 320–500 nm. Peak of protein fluorescence at 325/425 nm (excitation/emission) present a measure for the content of Maillard products (area in grey).

action intermediates to UV-active fluorescent compounds – Advanced Glycosylation End (AGE) products. In order to find the best wavelengths of excitation and emission for determination of AGE in wheat seeds, fluorescence of partially purified proteins in seeds extracts were scanned with excitation wavelength from 270–400 nm and emission wavelength from 320–500 nm. Three-dimensional fluorescence spectra revealed two main fluorescence peaks, one significantly stronger at 285/340 nm and one weaker at 325/425 nm (excitation/emission wavelength). Since the nature of fluorescence peak centered at 285/340 nm is well known and attributed to the presence of certain amino acids in a protein,[18,24] fluorescence peak centered at excitation wavelength of 325 nm, and emission wavelength at 425 nm, shown in Figure 2, was due to presence of AGE-products in wheat seed proteins. During seed ageing the fluorescence of this peak increased, and fluorescence intensity at 325/425 nm was used as a measure of Maillard products. Similar fluorescence peak has been found for mung been seeds, where Murthy and Sun recorded fluorescence intensity peak at 350/420 nm which increased during ageing.[11]

Maillard products (AGE) accumulation correlated well with storage conditions, but this accumulation was slightly varied among varieties (Figure 3). Wheat seeds aged at 40 °C, RH = 45 % and 25 °C, RH = 45 % continuously accumulated Maillard products during whole the storage period, and at the end of storage, products increase was 72–80 % for seeds aged at 40 °C, RH = 45 %, and 15–40 % for seeds kept at 25 °C, RH = 45 %. A slight increase in Maillard products (6–25 %) was observed for seeds aged at warehouse conditions, but this increase only started at 270 and continued to 360 days, similar to the trend observed for Amadori products. Expectedly, seeds kept at 4 °C, RH = 40 % did not show any significant increase (less than 5 %) in Maillard products. Maillard products accumulation differed between varieties during ageing of seeds at 25 °C, RH = 45 % and warehouse conditions, which was more pronounced in the case of variety Divana showing at least 50 % lower total increase ($p < 0.01$). The finding suggests that AGE accumulation during ageing of wheat seeds could be dependent on wheat variety, which is probably related to differences among varieties in Maillard product precursor content, such as amount and type of available reducing sugars, availability of target amino acid side chains, as well as content of AGE natural inhibitors – endogenous reducing compounds such as glutathione, cysteine side chains and ascorbate.

Continuous accumulation of advanced glycosylation end-products during wheat seeds ageing at higher temperatures (higher than 20 °C) is consistent with previous

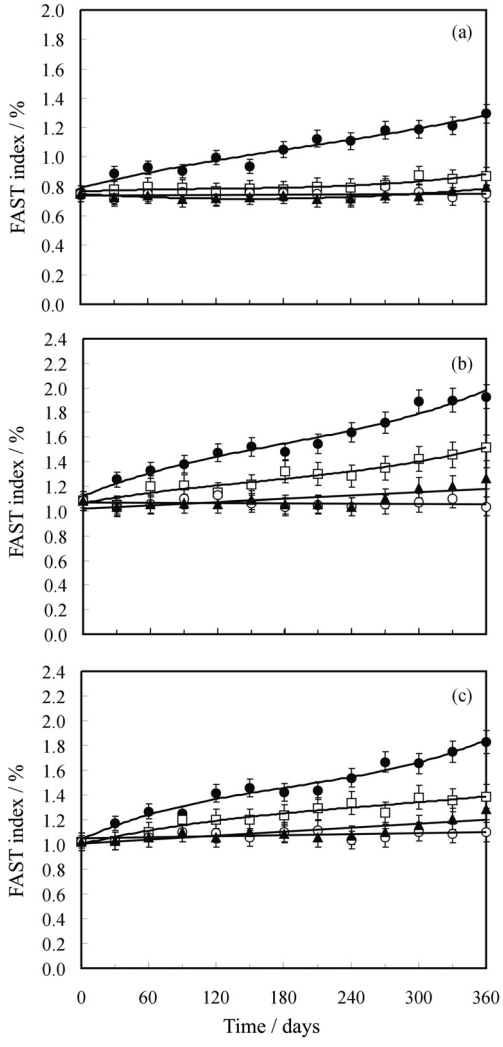

Figure 3. The FAST index of Maillard products in seeds of examined wheat varieties during ageing under different storage conditions. Varieties: (a) Divana, (b) Žitarka, (c) Srpanjka. Ageing conditions: 40 °C, RH = 45 % (●), 25 °C, RH = 45 % (□), 4 °C, RH 40 % (○), and 10–30 °C, RH = 40–70 % (▲). Bars present standard error of the mean calculated from three independent repetitions.

findings for soybean seeds,[10–12] although the increase intensity of wheat protein fluorescence was much lower, probably as a result of the whole seed examination instead of seed axes. Start of AGE increase at day 270 in wheat seeds aged at warehouse conditions matched the observed start of Amadori products increase, while lack of changes in Maillard products during wheat seeds ageing at 4 °C, RH = 40 % was expected, since no Amadori products increase could be detected at this storage temperature.

Maillard product increase matched the Amadori product increase during the wheat seeds ageing, but when rates of Amadori and Maillard products accumulation were compared, significant correlation ($r > 0.57$; $p < 0.05$) could only be found for wheat seeds aged at 25 °C, RH = 45 %. The variety Divana showed the lowest correlation ($r = 0.57$; $p < 0.05$), compared to Žitarka ($r = 0.86$; $p < 0.05$) and Srpanjka ($r = 0.97$; $p < 0.05$), which is probably related to the observed differences between varieties in Maillard product accumulation. The variety's distinguishing traits are the gluten strength, implying numerous disulphide bridges between cysteine residues,[25] and reduced glutathione content,[26,27] which might explain its greater resistance to AGE formation. Cysteine and reduced glutathione are potent inhibitors of AGE reactions.[28]

AGE levels in wheat seeds aged at 40 °C, RH = 45 %, 4 °C, RH = 40 % and warehouse conditions were only weakly correlated to the level of Amadori products ($r < 0.27$; $p < 0.05$) probably due to the Amadori products decrease after the day 270 in wheat seeds kept at 40 °C, RH = 45 %, an unchanging level of AGE and Amadori products in seeds kept at 4 °C, RH = 40 %, and a short accumulation time (started at day 270) in seeds kept at warehouse conditions. Similar lack of significant correlation between Amadori and Maillard products has been previously reported for mung been seeds aged at 33 °C, as well glucose/protein model system kept at 33 °C.[11]

The present significant correlation between AGE and Amadori products in wheat seeds aged at 25 °C, RH = 45 % indicates a more straightforward rearrangement of Maillard products intermediates to AGE during one year ageing, and similar velocities of formation of Amadori product and AGE, at applied ageing conditions. However, since the increase of Amadori products at the end of storage period for wheat seeds aged at 25 °C, RH = 45 % was only about one third to one half of maximal increase observed for wheat seeds aged at 40 °C, RH = 45 %, it may be expected that prolonged storage would result in weakened correlation due to lower levels of Amadori products caused by depletion of available target amino acid side chains or reducing sugars, as well as Amadori rearrangement to AGE.

Results indicate occurrence of non-enzymatic glycosylation of wheat seed proteins during ageing which can be detected as accumulation of Amadori and Maillard advanced glycosylation end product in seeds (Figure 1, Figure 3). Formation and accumulation of Amadori and AGE products in wheat seeds is affected by ageing conditions. At low storage temperature these products were not formed, nor accumulated within wheat seeds, while at storage temperatures exceeding 20 °C products accumulate significantly. Prerequisite for Amadori product accumulation is the exsistence of reducing sugars.[9] Since reducing sugars are present within wheat seeds in small amounts, [29–31] and more sugars could be formed during ageing,[13,32,33] accumulation of Amadori products in wheat seeds is something to be expected. Decrease of Amadori products beyond day 240 observed at 40 °C (Figure 1) indicates their decomposition. Since Amadori rearrangement is an irreversible reaction, observed decomposition of Amadori products is probably due to (a)

inability of the system to produce more products, and (b) further rearrangement of Amadori products in Maillard reaction intermediates. It is possible that Amadori products within wheat seeds accumulate to chemical equilibrium so further formation is disfavored until some of them rearrange to Mailllard reaction intermediates. This would explain their maintained level from day 180–240, but their decrease is clearly affected by inability of the system to form more Amadori products. The most probable cause for this would be depletion of reducing sugars or available lysine residues, but which one of these two are depleted remains to be clarified. Further rearrangement of Amadori products to Maillard intermediates and consequently to AGE was indicated by the increase in protein fluorescence of partially purified proteins extracted from aged wheat seeds (Figure 2, Figure 3). AGE accumulation within seeds during ageing additionally showed striking dependence on wheat variety examined. Such dependence probably results from variety differences in amount of available reducing sugars and lysine residues, as well as amount of endogenous AGE inhibitors.

CONCLUSION

Wheat seed ageing during storage is associated with protein modification by Amadori and Maillard reactions and extent of these reactions varies under different storage conditions. Accumulation of advanced glycosylation end-products in wheat seeds during ageing may also be highly dependent on wheat variety due to varietal differences in Maillard products precursors and endogenous AGE inhibitor content.

REFERENCES

1. E. R. Stadman, *J. Gerontol. Biol. Sci.* **43** (1988) B112–B120.
2. V. M. Monnier, *J. Gerontol. Biol. Sci.* **45** (1990) B105–B111.
3. A. J. Furth, *Anal. Biochem.* **174** (1988) 347–360.
4. S. R. Thorpe and J. W. Baynes, *Amino Acids* **25** (2003) 275–281.
5. R. Ramasamy, S. J. Yannuci, S. S. D. Yan, K. Herold, S. F. Yan, and A. M. Schmidt, *Glycobiology* **15** (2005) 16R–28R.
6. R. Singh, A. Barden, T. Mori, and L. Bellin, *Diabetologia* **44** (2001) 129–146.
7. W. L. Dills, *Am. J. Clin. Nutr.* **58** (1993) 779S–787S.
8. E. B. Frye, T. P. Degenhardt, S. R. Thorpe, and J. W. Baynes, *J. Biol. Chem.* **273** (1998) 18714–18719.
9. S. H. Wettlaufer and A. C. Leopold, *Plant Physiol.* **97** (1991) 165–169.
10. W. Q. Sun and A. C. Leopold, *Physiol. Plant.* **94** (1995) 94–104.
11. U. M. N. Murthy and W. Q. Sun, *J. Exp. Bot.* **51** (2000) 1221–1228.
12. U. M. N. Murthy, P. P. Kumar, and W. Q. Sun, *J. Exp. Bot.* **54** (2003) 1057–1067.
13. M. B. McDonald, *Seed Sci. Technol.* **27** (1999) 177–237.
14. A. G. Miller and J. A. Gerrard, *Prog. Food Biopolym. Res.* **1** (2005) 69–86.
15. K. G. Lee and T. Shibamoto, *Food Res. Int.* **18** (2002) 151–175.
16. X. Li and S. C. Ricke, *J. Food Sci.* **67** (2002) 2126–2128.
17. L. O. Copeland and M. B. McDonald, *Principles of seed science and technology*, Kluwer Academic Publisher Group, Dordrecht, The Netherlands, 1999, pp. 199–202.
18. J. Leclére and I. Biroluez-Aragon, *J. Agric. Food Chem.* **49** (2001) 4682–4687.
19. K. R. Preston and J. E. Kruger, *Mobilization of Monocot Protein Reserves During Germination,* in: M. J. Dalling (Ed.), *Plant Proteolytic Enzymes,* Vol. 2, CRC Press, Inc., Boca Raton, Florida, 1986, pp. 1–18.
20. G. Das and S. Sen-Mandi, *Seed Sci. Technolog.* **20** (1992) 367–373.
21. I. Bernal-Lugo and A. C. Leopold, *J. Exp. Bot.* **49** (1998) 1455–1461.
22. C. Walters, *Seed Sci. Res.* **8** (1998) 223–244.
23. A. C. Leopold, W. Q. Sun, and I. Bernal-Lugo, *Seed Sci. Res.* **4** (1994) 267–274.
24. D. J. Holme and H. Peck, *Analytical Biochemistry,* Addison Wesley Longman Limited, Essex, 1998, pp. 386–388.
25. R. Lásztity, *The Chemistry of Cereal Proteins*, CRC Press, Inc., Boca Raton, Florida, 1996, pp. 19–139.
26. X. Chen and J. D. Schofield, *J. Agric. Food Chem.* **43** (1995) 2362–2368.
27. J. D. Schofield and X. Chen, *J. Cereal Sci.* **21** (1995) 127–136.
28. T. D. Lehman and B. J. Ortwerth, *Biochim. Biophys. Acta* **1535** (2001) 110–119.
29. T. M. Kuo, J. F. VanMiddlesworth, and W. J. Wolf, *J. Agric. Food Chem.* **36** (1988) 32–36.
30. M. Black, F. Corbineau, M. Grzeshik, P. Guy, and D. Côme, *J. Exp. Bot.* **47** (1996) 161–169.
31. A. Lehner, C. Bailly, B. Flechel, P. Poels, D. Côme, and F. Corbineau, *J. Cereal Sci.* **43** (2006) 175–182.
32. L. Petruzzelli, *Ann. Bot.* **58** (1986) 259–265.
33. L. Petruzzelli and G. Taranto, *Ann. Bot.* **66** (1990) 375–378.

SAŽETAK

Nakupljanje Amadorijevih i Maillardovih produkata u zrnu pšenice tijekom starenja pri različitim uvjetima skladištenja

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Modifikacija proteina Amadorijevim i Maillardovim reakcijama tijekom starenja zrna pšenice pri različitim temperaturama i relativnim vlažnostima (RH, engl. *relative humidity*) ispitivana je tijekom dvanaestomjesečnog razdoblja. Spektrofluorimetrijsko određivanje Maillardovih produkata djelomično pročišćenih proteina ekstrahiranih iz starenih zrna pokazalo je povećanje fluorescencije proteina pri 325/425 nm (valna duljina ekscitacije/emisije). Najviši porast Maillardovih produkata uočen je za zrna ostarjela pri 40 °C, RH = 45 %, potom zrna ostarjela pri 25 °C, RH = 45 %, te zrna čuvana pri skladišnim uvjetima (10–30 °C, RH = 40–70 %), dok zrna skladištena pri 4 °C, RH = 40 % nisu pokazala značajno povišenje Maillardovih produkata tijekom dvanaest mjeseci skladištenja. Sličan profil nakupljanja uočen je za Amadorijeve produkte, izuzev u slučaju zrna ostarjelih pri 40 °C, RH = 45 %, kod kojih je uočen pad nakon 270 dana starenja. Na osnovi dobivenih rezultata može se zaključiti da je starenje zrna pšenice povezano s modifikacijom proteina Amadorijevim i Maillardovim reakcijama, ali je udio produkata ovih reakcija ovisan o uvjetima skladištenja.