Changes in available methionine and tryptophan contents during cereal flake production

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Abstract: The changes in available methionine and tryptophan contents during industrial production of wheat, rye, barley and oat flakes have been investigated. The contents of available methionine and tryptophan were lowered significantly ($p = 0.05$) under conditions of technological processing. The total decrease in available methionine ranged from 13 to 26% and tryptophan from 10 to 20%. Hydrothermal treatment (120 °C, 60 min, 1.99×10^5 Pa) of whole cereal grain had a considerable impact on the damage to available methionine, but the highest decrease in tryptophan was determined after the flaking process (70 °C, 0.3 mm gap). The total decrease in these amino acids correlates significantly ($p = 0.001$) and positively with values for lipid oxidation products, expressed on a total lipid basis, in cereal flakes as well as in untreated cereal grains. Available methionine and tryptophan contents in proteins of cereal flakes equalled or exceeded requirements for adults recommended by the FAO 1985 reference pattern.

Keywords: cereal flakes; available methionine; tryptophan

INTRODUCTION

The content and bioavailability of essential amino acids can be changed during food processing, and the extent and nature of modification depend on the conditions of the technological process.[1,2] Thermal and hydrothermal treatments applied to cereal flake production can stimulate the interactions of essential amino acids with other components.[3–5] Oxidative degradation of amino acids, particularly methionine and tryptophan, connected with the oxidation of lipids has been the subject of many investigations.[6–11] Since cereal lipids are characterised by relatively large amounts of polyenoic fatty acids that are very susceptible to oxidation by lypoxigenase and non-enzymatic catalysts,[12,13] it is important from a nutritional viewpoint to understand the changes in essential amino acids in whole grain cereal-based products treated at elevated temperature.

In this paper the changes in available methionine and tryptophan contents during industrial flake production of various cereals have been investigated.

MATERIALS AND METHODS

The investigation was carried out on two series of commercial wheat, rye, barley and oat grains during industrial flake production (manufacturing plant ‘VITA’, Skender Vakuf, Bosnia and Herzegovina). The apparatus for production of flakes comprised a steaming unit and a crushing roller unit. The same technological processing was used in both production series for all raw materials except oat grain. Cereal grains used for the procedure were cleaned, selected and de-husked, then treated with live steam in a steam conveyor at 120 °C for 60 min, with continuous mixing at a pressure of about 1.99×10^5 Pa. Oat grains were previously heat-treated (drying at 100 °C), then fragmented in production series 2 but not in production series 1. The flaking step was done on rollers (0.3 mm gap) heated to 70 °C. Flakes were separated from tiny particles in an aspirator, then cooled in an elevator at room temperature. The moisture content of flakes ranged from 100 to 110 g kg^{-1}. The samples from the two production series of wheat, rye, barley and oat flakes were gathered for analysis in particular phases of technological processing (from raw material to final product).

Total nitrogen content was determined by the Kjeldahl method.[14] Standard deviation and confidence limits at a level of 95% probability were established.[15] The nitrogen-to-protein conversion factor used was 5.7 for wheat and 6.25 for other cereals.[16] Available methionine was determined by the McCarthy and Sullivan method after papain hydrolysis of samples.[17] Tryptophan was determined using spectrofluorometry of barytic hydrolysates.[18] Available methionine and tryptophan contents were calculated according to the linear regression equation (estimated by the method of least squares). For each result the confidence limits at a level of 95%

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Table 1. Total nitrogen, available methionine and tryptophan contents during industrial production of wheat flakes

Phase of processing	*Nitrogen (g kg^{-1} dry weight)*[a] *Series 1*	*Series 2*	*Available methionine (g kg^{-1} crude protein)*[b] *Series 1*	*Series 2*	*Tryptophan (g kg^{-1} crude protein)*[b] *Series 1*	*Series 2*
Wheat grain						
Cleaned	21.88 ± 0.24	23.86 ± 0.26	18.12 ± 0.35	17.33 ± 0.34	12.33 ± 0.29	10.65 ± 0.25
Hydrothermally treated	21.86 ± 0.24	23.50 ± 0.26	16.24 ± 0.32*	15.47 ± 0.30*	11.38 ± 0.27*	9.91 ± 0.24*
Wheat flakes						
Under roller[c]	—	23.61 ± 0.26	—	15.47 ± 0.30	—	9.53 ± 0.23
After separation[c]	—	23.25 ± 0.26	—	15.78 ± 0.31	—	9.56 ± 0.23
After cooling	21.27 ± 0.23(**)	23.08 ± 0.25(**)	15.41 ± 0.30*(**)	15.13 ± 0.30*(**)	11.19 ± 0.26(**)	9.25 ± 0.22(**)

[a] $\overline{X} \pm S_{\overline{x}} \cdot t$, $t_{0.05\,(n-1)} = 2.048$.
[b] $\overline{X} \pm SE_{\overline{x}} \cdot t$, $t_{0.05\,(n-1)} = 2.045$.
[c] Samples were not gathered for analysis from series 1.
* Significantly ($p = 0.05$) lower in relation to previous processing phase.
(**) Significantly ($p = 0.05$) lower in relation to untreated grain.

probability were established.[15] Polyenoic fatty acids and lipid oxidation products were established spectrophotometrically after extraction of samples with butan-1-ol-saturated water.[19] Absorbance units at wavelengths of 274 and 244 nm corresponding to 0.1 mg lipids were used for the quantification of polyenoic fatty acids and lipid oxidation products respectively. All analyses were made in triplicate.

RESULTS AND DISCUSSION

The contents of total nitrogen, available methionine and tryptophan in untreated grains, hydrothermally as well thermally treated grains and final products of the various cereals are shown in Tables 1–4.

The total nitrogen content in dry matter of whole untreated grain of the various cereals for flake production ranged within literature values.[20–24] During technological production of flakes the changes in total nitrogen content, with a tendency to decrease, were minimal. Taking into account confidence limits, there were no significant changes ($p = 0.05$) in total nitrogen content in particular processing phases compared with the corresponding previous phase for

Table 2. Total nitrogen, available methionine and tryptophan contents during industrial production of rye flakes

Phase of processing	*Nitrogen (g kg^{-1} dry weight)*[a] *Series 1*	*Series 2*	*Available methionine (g kg^{-1} crude protein)*[b] *Series 1*	*Series 2*	*Tryptophan (g kg^{-1} crude protein)*[b] *Series 1*	*Series 2*
Rye grain						
Cleaned	18.73 ± 0.21	18.69 ± 0.21	19.81 ± 0.39	18.45 ± 0.36	8.13 ± 0.19	6.06 ± 0.14
Hydrothermally treated	18.59 ± 0.21	18.45 ± 0.20	14.83 ± 0.29*	14.10 ± 0.28*	7.65 ± 0.18*	5.79 ± 0.13*
Rye flakes						
After cooling	18.22 ± 0.20(**)	18.19 ± 0.20(**)	14.76 ± 0.29(**)	13.61 ± 0.27(**)	6.17 ± 0.15*(**)	5.10 ± 0.12*(**)

[a] $\overline{X} \pm S_{\overline{x}} \cdot t$, $t_{0.05\,(n-1)} = 2.048$.
[b] $\overline{X} \pm SE_{\overline{x}} \cdot t$, $t_{0.05\,(n-1)} = 2.045$.
* Significantly ($p = 0.05$) lower in relation to previous processing phase.
(**) Significantly ($p = 0.05$) lower in relation to untreated grain.

Table 3. Total nitrogen, available methionine and tryptophan contents during industrial production of barley flakes

Phase of processing	*Nitrogen (g kg^{-1} dry weight)*[a] *Series 1*	*Series 2*	*Available methionine (g kg^{-1} crude protein)*[b] *Series 1*	*Series 2*	*Tryptophan (g kg^{-1} crude protein)*[b] *Series 1*	*Series 2*
Barley grain						
Cleaned	17.44 ± 0.19	18.55 ± 0.21	19.28 ± 0.38	19.26 ± 0.38	8.47 ± 0.20	9.52 ± 0.23
Hydrothermally treated	17.45 ± 0.19	18.34 ± 0.20	15.43 ± 0.30*	15.51 ± 0.30*	7.91 ± 0.19*	8.84 ± 0.21*
Barley flakes						
After cooling	17.24 ± 0.19	18.13 ± 0.20(**)	15.27 ± 0.30(**)	14.94 ± 0.29(**)	7.18 ± 0.17*(**)	7.78 ± 0.18*(**)

[a] $\overline{X} \pm S_{\overline{x}} \cdot t$, $t_{0.05\,(n-1)} = 2.048$.
[b] $\overline{X} \pm SE_{\overline{x}} \cdot t$, $t_{0.05\,(n-1)} = 2.045$.
* Significantly ($p = 0.05$) lower in relation to previous processing phase.
(**) Significantly ($p = 0.05$) lower in relation to untreated grain.

Table 4. Total nitrogen, available methionine and tryptophan contents during industrial production of oat flakes

Phase of processing	Nitrogen ($g\,kg^{-1}$ dry weight)[a]		Available methionine ($g\,kg^{-1}$ crude protein)[b]		Tryptophan ($g\,kg^{-1}$ crude protein)[b]	
	Series 1	Series 2	Series 1	Series 2	Series 1	Series 2
Oat grain						
Cleaned	25.95 ± 0.29	26.35 ± 0.29	15.22 ± 0.30	14.38 ± 0.28	9.87 ± 0.23	9.78 ± 0.23
Thermally treated	25.72 ± 0.28	26.28 ± 0.29	14.34 ± 0.28*	13.65 ± 0.27*	9.64 ± 0.23	9.54 ± 0.23
Fragmented[c]	—	26.31 ± 0.29	—	13.20 ± 0.26	—	9.34 ± 0.22
Hydrothermally treated	25.63 ± 0.28	25.89 ± 0.29	13.99 ± 0.27	12.90 ± 0.25	8.80 ± 0.21*	9.12 ± 0.22
Oat flakes						
After cooling	25.53 ± 0.28	26.00 ± 0.29	13.46 ± 0.26(**)	12.54 ± 0.25(**)	8.76 ± 0.21(**)	8.85 ± 0.21(**)

[a] $\overline{X} \pm S_{\overline{x}} \cdot t$, $t_{0.05\,(n-1)} = 2.048$.
[b] $\overline{X} \pm SE_{\overline{x}} \cdot t$, $t_{0.05\,(n-1)} = 2.045$.
[c] Not fragmented in series 1.
* Significantly ($p = 0.05$) lower in relation to previous processing phase.
(**) Significantly ($p = 0.05$) lower in relation to untreated grain.

all cereals. However, a significantly lower nitrogen content was established in wheat and rye flakes in relation to untreated grains, the decrease amounting on average to 3.0 and 2.7% respectively.[25]

The content of available methionine in proteins of untreated grain of the various cereals ranged from 14.80 to 19.27 $g\,kg^{-1}$, slightly higher than literature data for particular cereals.[20–22,24,26,27] The relation among the various cereals concerning the available methionine content in untreated grain proteins was inverse to their interrelationship with regard to the total nitrogen content expressed on a dry matter basis.

The amount of available methionine in crude proteins was lowered significantly ($p = 0.05$) during flake production, and the total decrease in relation to untreated grain ranged on average from 13% in oat flakes to 26% in rye flakes. The total decrease for wheat flakes amounted on average to 14% and for barley flakes to 22%. The rate of available methionine loss varied with particular phases of technological processing, and significant differences between particular cereals in the same processing phase were also determined. Under conditions of hydrothermal treatment of grain the available methionine content decreased significantly in both production series of wheat, rye and barley, the decrease in relation to the previous phase amounting on average to 11, 25 and 20% respectively. For oat grain, no significant changes were determined after this step. Thermal treatment applied to oats resulted in a significant loss of available methionine, the average decrease amounting to 6%. The flaking process did not significantly affect the available methionine content in proteins of the various cereal flakes.

Proteins of untreated cereal grain contained from 6.06 to 12.33 $g\,kg^{-1}$ tryptophan and the values were somewhat lower than literature data for particular cereals.[20,22,26,28,29] The tryptophan content decreased significantly ($p = 0.05$) during production of all cereal flakes, the total decrease in relation to untreated grain amounting on average to 11 and 10% in wheat and oats respectively. Considerable damage to tryptophan was found for rye and barley, amounting on average to 20 and 17% respectively. The tryptophan decrease due to hydrothermal treatment was significant, whereas it did not significantly differ among the various cereal grains, ranging from 6 to 8%. However, the flaking process adds to differences between cereals in the total decrease in tryptophan content. A significant decrease in tryptophan after the flaking phase in relation to the corresponding previous processing phase was found for rye and barley, amounting to 16 and 11% respectively. The changes in this amino acid content due to flaking were non-significant in wheat and oat proteins. Thermal treatment of oat grain did not affect significantly the tryptophan content. The relation among the various cereals with regard to the extent of total damage to tryptophan during flake production was equal to their interrelationship regarding the extent of total damage to available methionine.

Based on the results obtained, it can be concluded that technological parameters during cereal flake production determined the damage to available methionine and tryptophan connected with the instability of lipids. The content of total lipids and polyenoic fatty acids expressed on a grain dry matter basis was higher for wheat and oats. However, the content of polyenoic fatty acids expressed per unit of total lipids was higher for rye and barley. A greater decrease in available methionine and tryptophan was found with these cereals. In addition, the correlation between the values for polyenoic fatty acids and lipid oxidation products was positive and highly significant ($p = 0.001$) for untreated grain of the various cereals as well as for the final product (Table 5). The relative decrease in available methionine and tryptophan correlated positively and highly significantly ($p = 0.001$) with the values of lipid oxidation products in cereal flakes. It should be mentioned that the initial values of lipid oxidation products in untreated cereal grains also contributed to the total decrease in available methionine and tryptophan in cereal flakes; the correlation between these parameters was also positive and significant (Table 5). The results are in

Table 5. Correlation of relative decrease in available methionine and tryptophan in cereal flakes with polyenoic fatty acids and oxidation products of lipids

	Correlation coefficient (r)[a]			
	Total decrease in available methionine (%)	*Total decrease in tryptophan (%)*	*Oxidation products ($A_{244}mg^{-1}$ lipids) of untreated cereal grains*	*Oxidation products ($A_{244}mg^{-1}$ lipids) of cereal flakes*
Untreated cereal grains				
Polyenoic fatty acids ($A_{274}mg^{-1}$ lipids)	0.783***	0.651**	0.995***	
Oxidation products ($A_{244}mg^{-1}$ lipids)	0.790***	0.619*		
Cereal flakes				
Polyenoic fatty acids ($A_{274}mg^{-1}$ lipids)	0.874***	0.692**		0.950***
Oxidation products ($A_{244}mg^{-1}$ lipids)	0.900***	0.853***		

[a] $r_{0.05\,(n-2)}=0.497$, $r_{0.01\,(n-2)}=0.623$, $r_{0.001\,(n-2)}=0.742$.

Table 6. Ratios for available methionine and tryptophan in proteins of cereal flakes based on FAO 1985 amino acid scoring pattern

	Children			
	2 years old	*2–5 years old*	*10–12 years old*	*Adults*
Ratio for available methionine (%)				
Wheat flakes	36	61	70	90
Rye flakes	34	57	65	83
Barley flakes	36	60	69	89
Oat flakes	31	52	59	77
Ratio for tryptophan (%)				
Wheat flakes	60	93	114	204
Rye flakes	33	51	63	113
Barley flakes	44	68	83	150
Oat flakes	52	80	98	176

accordance with many investigations which proved high susceptibility of methionine and tryptophan connected with the instability of lipids at elevated temperatures[6,8,30–32] and with the presence of oxidising agents, especially lipid peroxides.[31,33,34]

Available methionine and tryptophan contents in proteins of cereal flakes equalled or exceeded requirements for adults recommended by the FAO reference pattern[35] as a standard for general human requirements (Table 6). A relatively high ratio for tryptophan in flake proteins, particularly wheat and oat flakes, is important because of the metabolic role of tryptophan as a niacin precursor.

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