Mol. Nutr. Food Res. 2006, *50*, 799 – 804 DOI 10.1002/mnfr.200500249

Study on fluorescence of Maillard reaction compounds in breakfast cereals

Cristina Delgado-Andrade, José A. Rufián-Henares and Francisco J. Morales

Consejo Superior de Investigaciones Científicas, Instituto del Frío, Madrid, Spain

During the advanced stage of the Maillard reaction (MR) in food processing and cooking, Amadori rearrangement products undergo dehydration and fission and fluorescent substances are formed. Free and total (free + linked to the protein backbone) fluorescence (FIC) due to Maillard compounds in 60 commercial breakfast cereals was evaluated. Pronase was used for efficient release of linked fluorescent Maillard compounds from the protein backbone. Results were correlated with some heat-induced markers of the extent of the MR or sugar caramelisation during cereal processing, such as hydroxymethylfurfural, furfural, glucosilisomaltol and furosine. The effect of sample composition (dietary-fibre added, protein, *etc.*) on levels of FIC, expressed as fluorescence intensity (FI) *per* milligram of sample, is discussed. FIC is significantly correlated to the protein content of the sample and fluorescent Maillard compounds are mainly linked to the protein backbone. The ratio of total-FIC to free-FIC was 10.4-fold for corn-based, wheat-based and multicereal-based breakfast cereals but significantly higher in rice-based samples. Addition of dietary fibre or honey increased the FIC values. Data support the usefulness of FIC measurement as an unspecific heat-induced marker in breakfast cereals.

Keywords: Breakfast cereals / FIC / Fluorescence / Maillard reaction

Received: December 1, 2005; revised: February 14, 2006; accepted: February 19, 2006

1 Introduction

Breakfast cereals have become an important source of energy in human nutrition (Montaner, J., El consumo de cereales en el desayuno, on CONSUMER-website, http://www.consumaseguridad.com/web/es/sociedad_y_consumo/2004/05/25/12507.php, 2004). Breakfast cereals are high in carbohydrate, some are high in dietary fibre and many contain appreciable amounts of vitamins and minerals [1]. The usual manufacturing process of these foods in the industry is extrusion, followed by drying and toasting steps, which gives the pleasant flavour and colour for this kind of foodstuffs [2].

The main chemical reactions involved during the manufacture of breakfast cereals are essentially the Maillard reaction (MR) and caramelisation, and both depend on the type of reagent, temperature, water activity and pH. MR occurs between the free amino group of lysine and/or other amino acids and the carbonyl groups of reducing sugars such as glucose and maltose [3, 4].

Traditionally, the unspecific procedures to monitor the advanced stage of the MR in food processing have been the measurement of colour production at 420 nm [5] or by using spectrophotometric tristimulus colour measurement [6, 7]. In the last few decades, the determination of fluorescence has been proposed as an effective procedure to assess the extent of the MR. The Amadori rearrangement product undergoes dehydration and fission and yields colourless reductones as well as fluorescent substances are formed [8]. From the literature, fluorescence measurement is frequently used in MR studies at physiological conditions in relation to glycosylation of proteins in the human body, the AGEs formation, and AGEs relates pathologies as well [9].

The measurement of fluorescence related to Maillard compounds (FIC) in milk and milk-resembling systems has been studied in detail by Morales *et al.* [10]. Later, determination of fluorescence and front-face fluorescence has also been used as an indicator of nutritional damage in heat-treated milk, breakfast cereals, cooked salmon and roasted soy [11]. It must be remarked that, within the food matrix, the total pool of fluorescent Maillard compounds is formed by those that are free in the media and those linked-to-proteins fraction. Both free and total FIC index can be determined

Correspondence: Dr. Francisco J. Morales, Instituto del Frío, CSIC, José Antonio Novais 10, Madrid 28040, Spain
E-mail: fjmorales@if.csic.es
Fax: +34-91-549-36-27

Abbreviations: FI, fluorescence intensity; **GIM,** glucosilisomaltol; **HMF,** hydroxymethylfurfural; **MR,** Maillard reaction

by different analytical procedures, and then the linked-to-protein fraction can be estimated by subtraction [12].

The aim of this paper was to determine free and total (free + linked-to-protein backbone) fluorescence related to Maillard compounds (FIC) in commercial breakfast cereals. Levels of free and total FIC, expressed *per* milligram of sample, were correlated with other well-established heat-induced markers of the extent of the MR or sugar caramelisation during cereal processing.

2 Materials and methods

2.1 Chemicals

All chemicals used were of analytical grade and were obtained from Merck (Darmstadt, Germany), unless mentioned otherwise.

2.2 Samples

Sixty commercial breakfast cereals manufactured in Europe were purchased from supermarkets. The samples were randomly named using a letter followed by a number. The total content of each package was mixed and powdered in a grinder, homogenised, fractionated and stored in polyethylene containers under vacuum at 4°C until analysed. Average levels of 7.8 ± 9.3% and 8.0 ± 2.7% were obtained for fibre and protein.

2.3 Protein determination

Samples (0.800–1.000 g) were analysed by the AOAC 992.15 [13] procedure for total protein content by heating to 1050°C in a LECO model FP-2000 protein/nitrogen analyser (Leco Instruments, Madrid, Spain) calibrated with EDTA (Dumas method). The nitrogen-to-protein conversion factors used were 5.70, 5.95 or 6.25 for wheat, rice and the other cereals, respectively. Results were expressed as grams of protein per 100 g of product.

2.4 Measurement of free fluorescence intermediary compounds

Free FIC was determined according to Morales and Van Boekel [12] with some minor modifications. Briefly, 500 mg of the sample was suspended in 5 mL of deionised water in a 10 mL centrifuge tube. The tube was shaken vigorously for 1 min and clarified with 0.25 mL each of Carrez I (potassium ferrocyanide, 15% w/v) and Carrez II (zinc acetate 30% w/v) solutions. The resulting mixture was centrifuged at 4500 × *g* for 10 min at 4°C. The supernatant was collected in a 10 mL volumetric flask and two further extractions were performed using 2 mL of deionised water. The supernatants were mixed and the volume was made up to 10 mL with deionised water. Solutions (pH 6.0 approx.) were filtered (0.45 μm) and adequately diluted (at least 1/16) to prevent quenching effects. Diluted solutions (final pH 6.0–6.5) were measured at an excitation wavelength of 347 nm and emission wavelength of 415 nm. The linearity of fluorescence response was checked with a quinine sulphate solution of 1 μg/mL dissolved in 0.1 mol/L H_2SO_4. Unless mentioned otherwise, FIC values are expressed as fluorescence intensity (FI) *per* milligram of sample, but could also be converted into FI *per* milligram of protein, as appropriate. A fluorescence spectrophotometer (SMF-25, Kontron Instruments, Milan, Italy) was used for the determination of fluorescence. Quarzglass cuvettes (QS-1.000 Suprasil, Hellma, DE) with light path of 1 cm were used. At least, an average of two independent readings was recorded.

2.5 Measurement of total fluorescence intermediary compounds

Total FIC (free + linked-to-protein backbone) was determined according to Morales and Van Boekel [12], by enzymatic hydrolysis using pronase E. Briefly, 100 mg of the sample was digested with 3 mL of a 0.375 mg/mL pronase E solution (1500 U/mL in 1 M sodium-borate solution, pH 8.2) in a stoppered test tube for 36 h at 40°C in a water bath under shaking. After cooling, the solution was centrifuged at 4500 × *g* for 10 min at 4°C. The supernatant was filtered (0.45 μm) and adequately diluted (at least 1/50) to prevent quenching effects whereas pH was about 7.5. Afterwards, the measurement of the fluorescence was done setting the same conditions as for free FIC. Results were expressed as FI *per* milligram of sample, but also could be converted into FI *per* milligram of protein.

2.6 Measurement of hydroxymethylfurfural (HMF), furfural and glucosilisomaltol (GIM)

Furanic compounds and GIM were analysed by RP LC as described elsewhere [14].

2.7 Measurement of furosine

Furosine was analysed by CZE as described elsewhere [15].

2.8 Statistical analysis

To make a representative statistical analysis, comparisons were made between samples with different types of cereal

(corn, wheat, rice or mixture). Samples were also compared according to the existence of cocoa powder or honey in its composition, the percentage of dietary fibre, percentage of total protein content, final physical form of the product (flaked or puffed) and the group of consumers (adults or children) to whom products are focused.

Statistical significance of data was tested by one-way analysis of the variance (ANOVA) (Table 1), followed by Duncan test to compare means that showed significant variation ($p < 0.05$). Analyses were performed using Statgraphics Plus, version 5.1, 2001 (Statistical Graphics, Rockville, MD, USA).

3 Results and discussion

Free and total FIC were analysed in 60 breakfast cereals from 12 different manufacturers. Levels ranged from 30 to 697 FI/mg sample and from 234 to 2408 FI/mg sample for free and total FIC, respectively. As expected, values of free FIC were significantly lower than the corresponding total FIC values, since total FIC comprises free fluorescence compounds in the food matrix as well as that from the linked-to-protein backbone (Fig. 1). Results agree with the stated previously by Morales and Van Boekel [12] for milk-resembling systems. Fluorescent MR compounds in breakfast cereals behave as described for milk-based systems, whereas protein-linked fluorescent structures are quantitatively more relevant in the assessment of the overall fluorescence. The ratio of total to free FIC was 10.4 ± 4.12 (mean ± SD), but the ratio was significantly higher in the group of rice-based breakfast cereals (Fig.1). The number of rice-based breakfast cereals analysed were limited ($n = 3$) and therefore the result is not representative enough.

In contrast, a statistically significant correlation was found between the free and total FIC values ($r = 0.8409$; $p = 0.0000$) (Fig. 2) for breakfast cereals. Results suggest that free FIC is representative enough to evaluate the presence of fluorescent Maillard compounds in breakfast cereals, although total FIC determination offers some additional information especially for high-protein breakfast cereals, such as wheat-based. It cannot be concluded whether free fluorescent structures are directly derived from protein-linked ones or not, with the data obtained, but the relationship between the pool of fluorescent substances accounting for free-FIC and total-FIC is significant. In addition, a statistically significant relationship between total FIC and protein content was found. It is clear that the contribution of linked-to-protein fluorescence compound to the total fluorescence within this food matrix ($r = 0.5761$; $p < 0.01$) is important. Since protein is plausible to be the main source of fluorescent Maillard compounds generation,

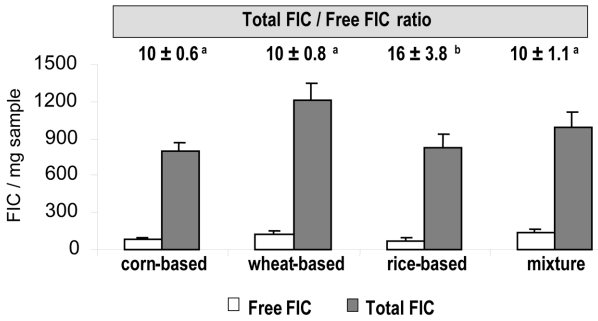

Figure 1. Free and total FIC (FI/mg sample) content in different groups of breakfast cereals depending on the type of grain. Free FIC/total FIC ratio for each group is also displayed. Different letters indicate significant differences (one-way ANOVA and Duncan test $p < 0.05$).

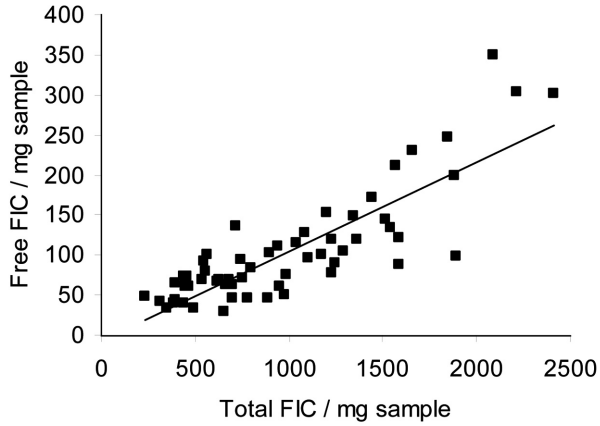

Figure 2. Relationship between free and total FIC in commercial breakfast cereals.

FIC values are also expressed according to the protein content of the sample (Table1).

Commercial breakfast cereals are mainly classified according to the type of flour used in the formulation. Four classes were identified; corn, wheat, rice and multicereal where at least two types of cereals are used. Distribution of samples are representative of the type of breakfast cereals available to consumers. Corn-based breakfast cereals showed a mean value of 82 ± 9 and 798 ± 75 FI/mg sample for free and total FIC respectively, keeping the total to free-FIC ratio at 10.3 within this group. A statistically significant correlation between protein content and total FIC value (FI/mg sample) was found for this group ($r = 0.5537$; $p < 0.05$). Wheat-based breakfast cereals showed free and total FIC values (FI/mg sample) substantially higher as compared with corn or rice-based groups (Table 1), probably due to the high level of protein in the wheat-based breakfast cereals (8.99%). The lowest FIC values were recorded in the rice-based breakfast cereal group, probably due to the lowest

Table 1. Statistical treatment for free and total FIC present in breakfast cereals grouped according the type of cereal, possible consumers, dietary fibre, protein content, presence of honey or cocoa powder and final physical form of the product (factor)

Factor	Free FIC[a)]		Total FIC[a)]		*N*[b)]
	FI/mg sample	FI/mg protein	FI/mg sample	FI/mg protein	
Total					
Average	114 ± 13	1439 ± 130	1005 ± 69	12 745 ± 729	60
Median	86	1279	892	12 980	
Minimum	30	332	234	4 148	
Maximum	697	6043	2408	30 921	
1st quartile	61	789	560	7 907	
3rd quartile	126	1857	1355	16 444	
Type of cereal					
Corn	82 ± 9	1330 ± 145	798 ± 75	12 706 ± 984	16
Wheat	129 ± 18	1419 ± 171	1205 ± 138	13 479 ± 1434	21
Rice	64 ± 26	1223 ± 499	826 ± 114	15 771 ± 2541	3
Mixture	131 ± 33	1580 ± 327	989 ± 124	11 544 ± 1340	20
Consumers					
Adult population	185 ± 44	1608 ± 385	1530 ± 133	13 199 ± 1224	15
Infant population	90 ± 8	1383 ± 120	830 ± 62	12 593 ± 889	45
Dietary fibre content					
>5%	207 ± 48	1883 ± 402	1497 ± 178	13 715 ± 1377	13
<5%	88 ± 8	1317 ± 121	869 ± 60	12 477 ± 853	47
Protein content					
>7.5%	148 ± 26	1461 ± 224	1315 ± 101	13 126 ± 973	
<7.5%	86 ± 10	1421 ± 153	752 ± 69	12 433 ± 1072	27
					33
Honey					
Presence	144 ± 26	1842 ± 317	1276 ± 178	16 222 ± 1896	13
Absence	106 ± 15	1328 ± 139	930 ± 70	11 783 ± 721	47
Cocoa powder					
Presence	74 ± 6	1127 ± 105	659 ± 61	10 001 ± 1010	18
Absence	131 ± 18	1573 ± 177	1154 ± 85	13 921 ± 895	42
Physical form					
Flaked	93 ± 13	1300 ± 156	869 ± 91	11 979 ± 1060	21
Puffed	125 ± 19	1514 ± 182	1079 ± 93	13 157 ± 970	39

a) Values represent mean ± SD.
b) Number of samples.

protein percentage present in them (5.32%). Most of the samples were manufactured from a mixture of different types of flour, being a heterogeneous group. The multicereal-based group also maintained a relationship protein content-total FIC value ($r = 0.5064$; $p < 0.05$).

Levels of FIC were correlated with well-established markers of the heat-treatment applied. Statistically significant relationships were manifested between free and total FIC and furosine, HMF, GIM and furfural. For HMF, statistically significant correlations were found with free FIC ($r = 0.8805$; $p < 0.01$) and total FIC ($r = 0.9622$; $p < 0.01$). Relationships were also observed with furfural ($r = 0.6948$ and $r = 0.6805$, for free and total FIC respectively, both $p < 0.05$). However, only the total FIC value was correlated with GIM ($r = 0.5894$; $p < 0.05$) which is in agreement with the fact that GIM comes specifically from the MR [16]. As a consequence of the high protein level in the wheat-based group, a significant relationship was found between furosine content and FIC values, especially in the case of free FIC ($r = 0.5737$; $p < 0.01$), indicating that free fluorescence compounds could be related to blocked lysine.

Commercial samples were also compared, according to the presence of cocoa powder or honey in the composition, to their percentage of fibre or protein, to the consumers on whom they are focused (adults or children) and the final physical form in which they were marketed.

Two homogeneous groups of breakfast cereals were identified according to the fibre content. Cereals usually have dietary fibre contents of about 3–4%, but when enriched

with wheat-bran, dietary fibre increases up to 10 – 30%. Statistically significant differences ($p < 0.05$) in the free and total-FIC values between both groups were observed (Table 1). This could be explained by the fact that fibre enrichment of breakfast cereal also includes the increase of the protein fraction, which increases the rate of the MR. No significant increase in free or total FIC was measured in unprocessed wheat bran.

Concerning the target consumers, a statistically significant increase was found in the free and total FIC content of breakfast cereals destined to the adult population compared to those for children (Table 1). This is explainable on the basis of the fact that breakfast cereals focused to adult consumers had higher protein content (11.86%) than those for the infant population (6.66%) and also taking into account that the former had higher dietary fibre content usually enriched with wheat-bran because of the healthy orientation of this type of products. In accordance with these findings, the presence of statistically significant higher FIC values in samples with protein content over 7.5% did not come as a surprise in the light of the correlation established between protein percentage and fluorescence formation. The limit at 7.5% in protein content was established according to the normal distribution of data in two homogeneous groups.

Other classifications were done in order to determine the influence of different ingredients added during breakfast cereal manufacture such as honey. A statistically significant higher FIC content was found in those cereals with honey added (Table 1). A possible explanation could be the previous presence of some fluorescent compounds in this ingredient before adding to the breakfast cereal, since although honey has an important content of sugars and free amino acids such as glutamine [17], it is used in the sugar coat applied after toasting so that it does not influence FIC formation within the breakfast cereal.

Finally, differences in FIC values were also studied according to the final step of the industrial processing conducted to obtain flakes or puffed cereals (Table 1). When presented as flakes, cereals are usually manufactured by the extrusion process called pellet-to-flaking extrusion cooking (PFEC), consisting of boiling at 80 – 95°C (16 – 20% humidity) followed by drying and toasting steps [18]. However, puffed cereals are usually manufactured by direct expansion extrusion-cooking (DEEC process) which includes boiling of the mixture at 140 – 180°C for 22 – 26% humidity and subsequent drying and toasting. Thus, because of the higher moisture content of puffed breakfast cereals, a stronger drying and tempering step must be performed in order to equilibrate humidity to approximately 10% to improve shelf stability [19]. Then, it could be plausible that the higher thermal input during the DEEC process could give rise to higher FIC values than those observed in breakfast cereals manufactured by the PFEC process. However, values tended to increase in puffed cereals but did not reach statistical significance. These results suggest that formation of fluorescent Maillard compounds are enhanced during processing of puffed breakfast cereals.

4 Concluding remarks

The analytical procedure described is effective to estimate the overall fluorescence due to the presence of fluorescent intermediary compounds formed during the time of the MR. Formation of fluorescent MR compounds are somewhat related to the type of formulation and processing conditions applied for breakfast cereals since a wide range of FIC was measured. Data of the present study support the hypothesis that determination of FIC becomes an unspecific but adequate tool to assess the extent of thermal processing in ready-to-eat cereals, since statistically significant correlations were found between FIC values and other heat-induced markers frequently used. Furthermore, it offers a complete vision of both MR and caramelisation process within the food matrix. As expected, the higher the protein level present in the breakfast cereal, the higher the total FIC value, which indicate the direct participation of proteins in the formation of the fluorescent compound derived from the MR.

The addition of different ingredients is an important factor accounting for the overall fluorescence. Hence, dietary fibre addition increases the FIC levels, probably due to the higher protein content of this ingredient, especially when it is added as wheat bran. Addition of honey also induced an increment of FIC values.

These observations are a staring point to take into account the measurement of FIC as an unspecific heating index in commercial breakfast cereals. Another study has been initiated to analyse real marketed samples and stronger relationships among the variables studied are expected in studies conducted under controlled sample formulation and equivalent heating conditions. More detailed studies should be conducted using model systems in order to gain knowledge about the application of this tool to suitable parameters that control heat damage during breakfast cereals manufacture.

This research was supported by two postdoctoral grants from Consejeria de Educacion y Ciencia (Junta de Andalucia) and by the Spanish Ministry of Science and Technology under project AGL2005-1735/ALI.

5 References

[1] Bertais, S., Luque, M. L. P., Preziosi, P., Fieux, B., *et al.*, *Ann. Nutr. Metab.* 2000, *44*, 249 – 55.

[2] Huang, W. N., *Cereal Foods World* 1998, *43*, 641 – 643.

[3] Camire, M. E., Camire, A., Krumbar, K., *Crit. Rev. Food Sci. Nutr.* 1999, *29*, 35 – 57.

[4] Friedman, M., *J. Agric. Food Chem.* 1996, *44*, 631 – 653.

[5] Palombo, R., Gertler, A., Saugy, I., *J. Food Sci.* 1984, *49*, 1609 – 1613.

[6] Pagliarini, E., Vernile, M., Peri, C., *J. Food Sci.* 1990, *55*, 1766 – 1767.

[7] Rampilli, M., Andreini, R., *Ital. J. Food Sci.* 1992, *4*, 285 – 291.

[8] Baisier, W. M., Labuza, T. P., *J. Agric. Food Chem*. 1992, *40*, 707 – 713.

[9] Sell, D. R., Monnier, V. M., *J. Biol. Chem*. 1989, *264*, 597 – 602.

[10] Morales, F. J., Romero, C., Jiménez-Pérez, S., *Food Chem.* 1996, *57*, 423 – 428.

[11] Birlouez-Aragón, I., Leclere, J., Quedraogo, C. L., Birlouez, E., Grongnet, J. F., *Nahrung/Food* 2001, *45*, 201 – 205.

[12] Morales, F. J., Van Boekel, J. S., *Int. Dairy J.* 1997, *7*, 675 – 683.

[13] Association of Official Analytical Chemists, in: Patricia Cuniff (Ed.), *Association of Official Analytical Chemists*, 16th Edn., Washington DC 1995, pp. 3 – 6.

[14] Rufián-Henares, J. A., Delgado-Andrade, C., Morales, F. J., *J. AOAC Int.* 2006, *89*, 161 – 165.

[15] Delgado-Andrade, C., Rufián-Henares, J. A., Morales, F. J., *Eur. Food Res. Technol.* 2005, *221*, 707 – 711.

[16] Resmini, P., Pellegrino, L., Pagani, M. A., De Noni, I., *Ital. J. Food Sci.* 1993, *4*, 341 – 353.

[17] Hermosiín, I., Chicón, R. M., Cabezudo, M. D., *Food Chem.* 2003, *83*, 263 – 268.

[18] Guy, R., *Extrusión de los alimentos. Tecnología y aplicaciones*, Acribia, Zaragoza, Spain 2002, pp. 131 – 160.

[19] Eastman, J., Orthoefer, F., Solorio, S., *Cereal Foods World* 2000, *46*, 468 – 471.

