Research Article

Maillard reaction indicators in diets usually consumed by adolescent population

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Contents of some indicators of the Maillard reaction (MR) in two diets adjusted to the adolescent requirements are compared: the A-diet, usually consumed by the adolescent population containing their preferred foods cooked by the culinary techniques more frequently chosen; and the B-diet, with the same foods, except those industrially processed and with high content of Maillard reaction products (MRP), cooked in softer processes to minimise the MR. Aliquots of a lunch-dinner (LD) and breakfast-afternoon (BA) snack pools separately from both diets were ground and lyophilised. Fluorescence associated to MRP, CIELAB colour parameters, furosine, hydroxymethylfurfural (HMF), carboxymethyllysine (CML) and sugar contents were investigated in the samples. Significant losses of lightness were manifested in A samples compared to the B ones. Fluorescence intensity was significantly higher in the LD and BA samples from the A-diet. Furosine measured in the A-diet was similar to that found in the B-diet. However, HMF content was significantly higher in the A-diet, as well as CML. Data showed significant higher levels of advanced MRP in the usual diet. Thus, MRP intake associated to alimentary pattern in the adolescence population might be studied in order to assess the health implications of the MRP consumption and its possible synergic effect with endogenous advanced glycation endproducts.

Keywords: Adolescence / Carboxymethyllysine / Diet / Fluorescence / Furosine

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1 Introduction

Over the past few decades, food habits among adolescents have changed significantly. The tendency of eating away from home, particularly at fast-food restaurants, has increased. The frequency of snacking has risen as well and according to several studies the in-between-meals provide about one fifth of the total energy intake but only about one sixth of the nutrient requirements [1, 2].

Certainly, before consuming, most of the foods must be processed to some extent to guarantee adequate microbiological security, enzymatic inactivation, destruction of toxic substances, preservation and also to enhance the development of aromas, colours and flavours, and also the palatability. On the basis of the necessity of food processing, it is important to analyse the appearance of some new substances during those treatments, their nutritive consequences and biological effects after their consumption.

Processing, together with the food's particular composition, makes possible the development of the Maillard reaction (MR) and the formation of browning products, responsible for the improvement of food palatability [3]. The final products of the reaction are high-molecular-weight coloured compounds, melanoidins, with different physical–chemical properties [4]. Formation of these products depends directly on the temperature and time of processing; and is greatly heightened by long exposure to high heat [5]. These conditions can especially affect fast foods, since they are usually prepared by processes such as frying, roasting, grilling, baking and even reheated before being consumed. Thus, the Maillard reaction products (MRP) content in foods is related not only to their composition, but also to the method and conditions of preparation, as well as with reheating [6]. It can be understood that MRP contents, also known as advanced glycation endproducts (AGEs,) have

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Abbreviations: BA, breakfast-afternoon; **CML,** carboxymethyllysine; **FI,** fluorescence intensity; **HMF,** hydroxymethylfurfural; **MR,** Maillard reaction; **MRP,** Maillard reaction product; **LD,** lunch–dinner; **OPA,** ortho-phtalaldehyde

increased vastly in the last 50 years in the Western diet [7], especially in adolescent diets. As Amorim [8] pointed out, snacks and fast-food consumption is less often in Mediterranean countries than in the north of Europe or in the USA, but the dietetic pattern of our adolescents has lost some of the most relevant values of the Mediterranean diet. In Spain, new alimentary preferences of this population are changing toward less healthy dietetic habits [9].

MRP consumption is associated to some positive biological actions, as antioxidant activity [10], chemopreventive activity [11] or antimutagenic actions, *etc.* [12]. But simultaneously MRP are known to induce negative consequences, among them are antinutritional properties, mainly related to the protein damage, and also to some alterations of vitamins [13] and availability of some minerals [14]. Moreover, mutagenic and carcinogenic actions have been also associated with MRP [12].

Therefore, it is important to characterise and quantify the presence of certain MRP in the diet to strike a balance between their positive and negative effects. There are some data concerning the presence of MRP, most of them about the earlier products of the reaction, in foods like milk [15], bread [16], coffee [17], roasted chicken [18], *etc.* However, there is a lack of information about the presence of the MRP in a whole usual diet.

The purpose of this study was to quantify some indicators of the MR in a diet similar to that usually consumed by adolescents and to verify the influence of the cooking procedures and the selection of foods on the MRP final dietary content. Both factors, the food election and its processing, could be modulated to obtain suitable rates of these compounds in the diet.

2 Materials and methods

2.1 Chemicals

All the chemicals used were of analytical grade and were obtained from Merck (Darmstadt, Germany), unless mentioned otherwise. HPLC-grade ACN was obtained from Lab-Scan (Dublin, Ireland). Methanol, potasium ferrocianide and zinc acetate were purchased from Panreac (Barcelona, Spain). Furosine was obtained from Neosystem Laboratories (Strasbourg, France). Sodium borohydride, ortho-phtalaldehyde (OPA) and 2-mercaptoethanol were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Diets

The diets included in this study were designed and prepared by taking into account:

(i) A dietary questionnaire and an eating-habits survey realised to a sample of healthy male subjects recruited from a group of students aged 11 – 14 years [19]. The dietary questionnaires were a 24-h diet recall and two-day weighted dietary records including a weekend day. Subjects were also asked about the type of preparation of foods, *i.e.*, roasted, fried, toasted, *etc.* In the eating habits survey, children were asked among a list of 32 items about what foods they usually ate and how often, which were their favourite foods and those they liked less, in order to establish a hypothetical usual menu. The survey included foods frequently found in adolescent's diet, like pizzas, hamburgers, precooked foods, *etc.* Each method assesses different dietary components; the 24-h recall and diaries assess specific dietary content, whereas food frequencies usually determine dietary pattern or habits [20].

(ii) Data from the generational pattern about frequency of consumption for male Spanish adolescents ranging from 11 to 14 years [21].

(iii) Data of daily food intake among scholars of the Andalusian population from epidemiologic studies [22].

This information was used to prepare a diet compounded by 7-day menus including the foods most frequently consumed, prepared in the most usual culinary techniques. This diet, representing that usually consumed by adolescent population, was named A-diet.

Simultaneously a second diet was elaborated, called B-diet, including basically the same foods, but with different food processing, whenever possible, to minimise the development of the MR. Moreover, some foods containing high levels of MRP, as chocolate [23], were eliminated. Almost 45% of the dishes included in the menus were exactly the same in both diets and the rest of them were formed by the same foods but prepared by means of culinary techniques which are less promoters of the MR, *i.e.* fried chicken and boiled chicken, fried potatoes and boiled potatoes. Therefore, although adolescents preferred the A-diet, the B-diet was equally realistic and formed from typical dishes of our cooking prepared by following traditional recipes, except that bread was consumed without crust and the absence of chocolate or cocoa powder.

Both diets did not significantly differ in their energy and nutrient content; the overall values of basic parameters are included in Table 1. Amounts of foods employed to prepare dishes of both menus, as well as some culinary techniques used in each case are described in the additional appendix.

Table 1. Overall daily content of energy and basic nutrients in the A and B-diets

Parameter	Quantity
Proteins (g)	88.0
Carbohydrates (g)	316.9
Fat (g)	100.6
Fibre (g)	25.1
Cholesterol (mg)	311.4
Energy (kcal)	2525
% energy from protein	14.0
% energy from carbohydrates	49.9
% energy from fat	36.1

Table 2. LD 7-day menu of the different diets

Diet[a]		First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day
A	Lunch	Empanadillas[b] with salad Legumes (lentils) Chocolate yoghurt Bread	Gratin macaroni with béchamel sauce Bananas Bread	Spanish omelette with ham Meatballs (veal) with vegetables Chocolate custard Bread	Legumes (chickpeas) Chocolate yoghurt Bread	Salad (lettuce, tomatoes…) Paella Apple Bread	Fried chicken and fried potatoes Salad (lettuce, tomatoes…) Pears Bread	Tropical salad Spanish omelette Rice with milk and cinnamon Bread
	Dinner	Consommé with noodles Breaded fish Pears Bread	Sauté vegetables Griddle loin of pork and fried potatoes Torrija[c] Bread	Pizza Bananas Bread	Soup of pasta and chicken Hamburger with fried potatoes Bananas Bread	Vegetables cream with croutons Breaded hake fish-fingers Chocolate custard Pan	Fish croquettes Caramel custard Bread	Breaded fish and rice Oranges Bread
B	Lunch	Russian salad with tuna Legumes (lentils) Strawberry yoghurt Bread without crust	Spaghetti with tomato sauce, cheese and ham Bananas Bread without crust	Boiled potatoes, boiled eggs and ham Baked meat (veal) with vegetables Custard Bread without crust	Soup of pasta and chicken Sausages with mashed potatoes Bananas Bread without crust	Salad (lettuce, tomatoes…) Stewed rice Apple Bread without crust	Boiled chicken and potatoes Salad (lettuce, tomatoes…) Pears Bread without crust	Tropical salad Tuna-filled eggs Rice with milk Bread without crust
	Dinner	Consommé with noodles Baked fish with boiled potatoes Pears Bread without crust	Vegetable stew Baked loin of pork and boiled potatoes Syrup peach Bread without crust	Pasta with tomatoes and cheese Bananas Bread without crust	Legumes (chickpeas) Strawberry yoghurt Bread without crust	Vegetables cream Fish pudding Custard Bread without crust	Baked fish Custard Bread without crust	Fish with cream and rice Oranges Bread without crust

a) Diets: A, diet similar to those usually consumed by adolescent population; B, diet containing the same foods as in the A-diet, but with softer processing wherever possible.
b) Small tuna-filled breaded pasties.
c) Fried bread with milk, sugar and cinnamon.

Lunch–dinner (LD) meals for the 7-day menu were prepared in a local catering, always under the strict control of the investigators (Table 2). Breakfast–afternoon (BA) snack was prepared in the laboratory as follows: A-diet, whole milk with cocoa powder and breakfast cereals in the morning and milk shake and sandwich (pâté, boiled ham or mortadella) or buns in the afternoon; B-diet, whole milk and butter and bread in the morning and whole milk and sandwich without crust (pâté, mortadella or boiled jam) in the afternoon. Dishes of each menu were prepared twice and mixed.

On each diet, the edible portion (the part of the food that one can eat: in some foods, there is an inedible portion that has to be removed, *e.g.*, chicken or fish bones or fruit skin) of LD for each day (prepared in duplicate) were removed, weighed and homogenised with a hand blender (Taurus, vital CM, Spain).

Aliquots of the 7 day from both diets were well mixed and lyophilised separately; these samples are hereafter referred to as LD-A or LD-B, respectively for A or B-diet. In the same manner, BA snack from both diets (also in duplicate) were mixed and lyophilised; samples were named BA-A and BA-B. Four aliquots of all the mentioned samples were used in the analysis of different indicators, unless mentioned otherwise.

2.3 Measurement of colour

The determination of colour of different samples was carried out using a HunterLab D25-9 optical sensor (Hunter Associates Laboratory, Reston, Virginia, USA) according to the CIE Lab scale [24, 25]. The system provides the values of three colour components; L^* (black–white component, luminosity), and the chromaticness coordinates, a^* (+red to –green component) and b^* (+yellow to –blue component) [26]. Samples were placed into a 5-cm diameter glass Petri dish. The sample was illuminated with D65-artificial daylight (10° standard angle) according to

conditions provided by the manufacturer. The E index is calculated from the equation: $E = (L^{*2} + a^{*2} + b^{*2})^{1/2}$ and Chroma value according to the next equation: $C = (a^{*2} + b^{*2})^{1/2}$. Solid colours are named according to Kelly and Judd [27]. Each colour value reported was the mean of two determinations at 22 – 24°C.

2.4 Measurement of fluorescence

Fluorescence associated with MRP was determined according to Morales *et al.* [28]. Briefly, 100 mg of each sample was weighed and mixed with 1 mL of 20% w/v trichloroacetic acid. The solution was stirred and, after 15 min at room temperature, centrifuged at $14\,000 \times g$ for 10 min. Supernatants were filtered (0.45 μ acetate filters) and diluted to the concentration of 5 mg sample/mL to prevent quenching effects. The final solutions 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. This solution was assigned 100% of fluorescence intensity (FI) and the results were expressed as percentage of fluorescence with respect to the quinine sulphate solution. A fluorescence spectrophotometer (SMF-25, Kontron Instruments, Milan, Italy) was used for the determination of fluorescence. Quartzglass cuvettes (QS-1.000 Suprasil, Hellma, DE) with light path of 1 cm were used. The average of two readings was recorded.

2.5 HPLC determination of furosine

Furosine determination was performed following the methods described by Delgado *et al.* [29] with some modifications. Briefly, 40 mg of the sample was hydrolysed with 3 mL of 7.95 M HCl at 110°C for 23 h in a Pyrex screw-cap vial with PTFE-faced septa. Hydrolysis tubes were sealed under nitrogen. The hydrolysates were aerated and cooled at room temperature and subsequently centrifuged at $14\,000 \times g$ for 10 min. A 0.5-mL portion of the supernant was applied to a Sep-pak C_{18} cartridge (Millipore), prewetted with 5 mL of methanol and 10 mL of deionised water and was then eluted with 3 mL of 3 M HCl. The dried sample was dissolved in 1 mL of a mixture of water, ACN and formic acid (95 : 5 : 0.2). A degassed mobile phase was prepared with 5 mM sodium heptane sulphonate including 20% of ACN and 0.2% of formic acid. An Extrasyl-ODS2 analytical column (25 cm × 0.40 cm, 5-μm particle size, Tecknokroma, Barcelona, Spain) was used at 32°C. The elution was isocratic and flow rate was 1.0 mL/min. The injection volume was 20 μL and detection at 280 nm. Furosine was quantified by the external standard method. Calibration curve was built from a stock solution (1.2 mg/mL of furosine) in the range of 42.8 – 1.2 mg/L.

2.6 HPLC determination of hydroxymethylfurfural (HMF)

HMF determination was based on Rufián-Henares *et al.* [30]. Sample (100 mg) was suspended in 1 mL of 5% ACN and clarified with 0.125 mL each of Carrez I (15% w/w potasium ferrocianide) and Carrez II (30% w/w zinc acetate) solutions. The resulting mixture was centrifuged at $14\,000 \times g$ for 10 min. The supernatants were then filtered (0.45 μm acetate filters; 13 mm, MSI, Westboro, MA) to analyse HMF content. A degassed mobile phase of water/ACN (95 : 5) was prepared and the flow was 1 mL/min. The same analytical column described above was also used at 32°C. The injection volume was 20 μL and detection at 280 nm was selected. HMF was quantified by the external standard method within the range of 2 – 100 μM.

2.7 HPLC determination of sugars

Lactose, glucose, galactose and fructose were analysed by ion-exchange HPLC, following the procedure as described by Morales *et al.* [28]. Briefly, meal samples (60 mg) were deproteinised with 1.7 mL of 0.5 M perchloric acid solution and held for 1 h at 4°C. Then, centrifuged at $14\,000 \times g$ for 5 min and the supernants were filtered through a 0.45 μm acetate filter. Twenty microliters of the sample was injected into an ION-300 polymeric resin column (300 mm × 7.8 mm, Interaction-Lab, San Jose, CA) at 50°C. A sulphuric acid solution (4.0 mM) was used as eluent at 0.4 mL/min. Sugars were recorded with a refractive index detector (Erma, Tokyo, Japan) and they were quantified by the external standard method within the range of 0.25 – 1% w/v.

2.8 HPLC determination of carboxymethyllysine (CML)

Basically, CML was determined by rp-HPLC with precolumn derivatisation with OPA, as described by Drusch *et al.* [31] with some minor modifications for the chromatographic procedure. Hydrolysate (20 μL) was diluted with 580 μL of 0.4 M sodium borate buffer (pH 9.5). Derivatisation was performed automatically by an autosampler. OPA reagent (50 μL) was added to the sample vial and mixed. After 180 s of reaction, derivatised hydrolysate was injected (10 μL) onto an rp-HPLC analytical column (Tracer-Excell ODS-2, 5 μm, 25 cm × 0.4 cm, Tecknokroma) at 32°C. Elution buffers were: (A) 15 mM sodium phosphate buffer pH 7.2/ACN (83 : 17, v/v) and (B) ACN. The binary gradient was linear from 0 to 30%B in 10 min and stand for 3 min at 60%B. Then the gradient was set back to 0%B within 5 min. The OPA-derivatives were detected fluorimetrically at 340 nm excitation and 455 nm emission wavelengths. Peak identification was confirmed by retention time and standard addition. Quantification was performed

by an external standard, kindly provided by Professor Susan Thorpe (University of South Carolina, Columbia, USA).

2.9 HPLC equipment

The HPLC system consisted of an MD-420 pump, an MD-465 autosampler, an MD-432 UV-visible detector and a DT-450/MT v.3.90 computing integrator connected to a PC, all from Kontron Instruments.

2.10 Protein content

Protein was analysed in a LECO model FP-2000 (Leco Instruments, Madrid, Spain) by following the AOAC 992.15 procedure [32].

2.11 Statistical data analysis

All data were statistically tested by one-way analysis of variance (ANOVA), followed by Duncan test to compare means that showed significant variation ($P < 0.05$). The comparisons were carried out within each parameter between the four samples elaborated (LD-A, LD-B, BA-A and BA-B), to analyse not only the formation of a determined MRP from different types of cooking but also the influence of the different compositions of a meal (referring to LD or BA snack mixtures) in the formation of MRP. The simultaneous inclusion of all data in the ANOVA analysis let us know which meal (LD or BA mixture) made a higher contribution to the daily intake of MRP. Statgraphics Plus, version 5.1, 2001 was used to support the statistical analysis.

3 Results and discussion

3.1 Colour analysis

The colour of foods is the result of coloured natural products associated with raw material and/or coloured compounds generated as a result of processing [33]. Thus, diets included in the present assay had a colour due to their composition and to the cooking procedure of each one. In this sense, it could be useful to express browning as the changes in the visual colour of the diets. The Hunter a^*-value measures redness or greenness and the Hunter b^*-value measures yellowness or blueness. The more positive values of a^* in the A meals compared with the corresponding B ones (Table 3) indicate the progress of the colour to the red zone, in accordance with the higher presence of MRP, although lipid oxidation could also be partly responsible for this observation. In view of the decreased b^*-value for the BA-A sample, this parameter might also be affected by oxidation, since data suggested the predominance of blue, when a higher portion of yellow component was expected in this more processed meal (Table 3). The luminosity (L^*), is the black–white component of the sample. Luminosity significantly decreased in the more severely cooked meals (LD-A and BA-A *vs.* LD-B and BA-B, respectively) (Table 3). Even LD-B luminosity was lower than BA-B value, showing that LD-B was darker, and so more processed, than BA-B snack.

Some equations have been developed to relate these colour parameters provided by a tristimulus colorimeter. One of the most common is the E index, calculated as $E = (L^{*2} + a^{*2} + b^{*2})^{1/2}$ [34]. This parameter is mainly influenced by the brightness of the colour and describes the proximity or fairness between two samples in the colour space [35]. Comparing E values from LD-B *versus* LD-A and BA-B *versus* BA-A a decreased in the index was manifested (Table 3), confirming the loss of lightness likely due to the more severe cooking treatment of meals and the formation of brown pigments in the advance of the MR.

There is another equation useful enough in the analysis of CIELAB-parameters, a more specific formula so-called Chroma value (C) calculated as $C = (a^{*2} + b^{*2})^{1/2}$. C indicates the degree of saturation, purity or intensity of visual colour [36]. The decrease in the Chroma value denotes the introduction of the blue colour in the LD-A and BA-A meals (Table 3), as it has been described above for the b^* values.

Table 3. Colorimetric parameters of meals[a)]

Meals[b)]	L^*	a^*	b^*	E[c)]	C[d)]
LD-A	71.71 ± 0.11[a)]	−1.46 ± 0.12 [a,c)]	23.46 ± 0.20[a)]	75.46 ± 0.05[a)]	23.51 ± 0.19[a)]
LD-B	75.29 ± 0.02[b)]	−1.70 ± 0.03[a)]	24.55 ± 0.12[b)]	79.21 ± 0.05[b)]	24.60 ± 0.11[b)]
BA-A	65.17 ± 0.22[c)]	4.74 ± 0.09[b)]	19.02 ± 0.09[c)]	68.05 ± 0.20[c)]	19.60 ± 0.04[c)]
BA-B	77.57 ± 0.04[d)]	−1.38 ± 0.05[c]	23.18 ± 0.14[a)]	80.97 ± 0.08[d)]	23.22 ± 0.14[a)]

a) Values represent mean ± SD ($n = 4$). Different lower-case letters indicate significant differences (one-way ANOVA and Duncan test, $P < 0.05$) for each studied value within the same column, *e.g.* no difference between values with the same lower case letter.
b) LD-A, 1-wk pool of LD from the A-diet; LD-B, 1-wk pool of LD from the B-diet; BA-A, 1-wk pool of BA snack from the A-diet; BA-B, 1-wk pool of BA snack from the B-diet.
c) $E = (L^{*2} + a^{*2} + b^{*2})^{1/2}$.
d) $C = (a^{*2} + b^{*2})^{1/2}$.

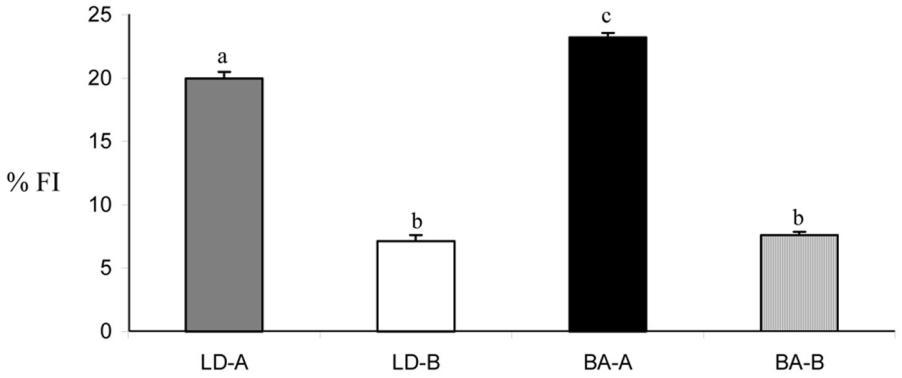

Figure 1. Percentage of FI in different meals (1 μg/mL quinine sulphate solution assigned as 100%). Values represent mean ± SD ($n = 4$). Different letters indicate significant differences (one-way ANOVA and Duncan test). LD-A, 1-wk pool of LD from the A-diet; LD-B, 1-wk pool of LD from the B-diet; BA-A, 1-wk pool of BA snack from the A-diet; BA-B, 1-wk pool of BA snack from the B-diet.

Table 4. Furosine, HMF and CML content in meals[a)]

Meals[b)]	*Furosine*[c)]	CML[c)]	HMF (mg/kg)
LD-A	40.67 ± 0.79[a)]	15.70 ± 0.71[a)]	1.77 ± 0.06[a)]
LD-B	22.63 ± 2.44[b)]	5.30 ± 0.57[b)]	nd
BA-A	45.64 ± 1.31[a)]	15.80 ± 1.56[a)]	8.38 ± 0.01[b)]
BA-B	63.49 ± 3.15[c)]	10.50 ± 1.70[c)]	2.66 ± 0.04[c)]

a) Values represent mean ± SD ($n = 4$). Different lower-case letters indicate significant differences (one way Anova and Duncan Test, $P < 0.05$) for each studied value within the same column, *e.g.*, no difference between values with the same lower case letter.
b) LD-A, 1-wk pool of LD from the A-diet; LD-B, 1-wk pool of LD from the B-diet; BA-A, 1-wk pool of BA snack from the A-diet; BA-B, 1-wk pool of BA snack from the B-diet.
c) mg/100 g of protein.
nd (not detected): lower than 0.25 μg/kg.

Table 5. Lactose, galactose, glucose, fructose and protein content in meals

Meals[a)]	Protein[b)]	Lactose (mg/ 100 g)	*Galactose* (mg/ 100 g)	*Glucose* (mg/ 100 g)	Fructose (mg/ 100 g)
LD-A	15.40	nd	nd	31.68	15.13
LD-B	24.60	nd	nd	20.96	nd
BA-A	14.69	41.38	nd	42.11	42.45
BA-B	15.12	97.41	nd	101.49	106.28

a) LD-A, 1-wk pool of LD from the A-diet; LD-B, 1-wk pool of LD from the B-diet; BA-A, 1-wk pool of BA snack from the A-diet; BA-B, 1-wk pool of BA snack from the B-diet.
b) % Protein (determined by the Kjeldahl method). Different data did not suppose different intakes between diets since values were just the same as expressed in wet matter.
nd: lower than 6 mg/100 g.

3.2 Fluorescence assay

LD and BA snack from B meals reached the lowest and similar values for overall fluorescence associated to MR (Fig. 1). However, LD-A and BA-A had the higher ones, as it was expected due to the promotion of the MR in these meals. The presence of cereals and bread including the crust in the BA-A sample led to a significant increase in the%FI compared with the rest of the samples. Fluorescent compounds are formed before the formation of browning pigments takes place, and subsequently progress with them [37]. This issue has been deeply studied by Baisier and Labuza [38] in model systems of amines/sugars, who demonstrated that the fluorescence accumulation was due to the interaction between reactive reducing compounds and amines, by an irreversible reaction. It has also been described that isomerisation and destruction of some sugars can contribute somewhat to the development of fluorescence and the route of formation could be different depending on the existence and type of amino acids present [15]. Thus, Jing and Kitts [39] found that the source of sugar composing the MRPs influenced the fluorescent and browning patterns in a sugar-casein model system. In this sense, probably the highest value of%FI observed in the BA-A meal could be related to a higher participation of sugars of the A-diet in the MR (*i.e.* from cocoa powder and breakfast cereals). In fact, glucose and fructose decreased in BA-A meal (Table 5).

No literature data are available with which to compare our results because most of the analyses have been carried out in model systems, in isolate foods or in living-tissues, but not in a complex meal matrix. Given that results of FI in this study show clear differences, which can explain the importance of the cooking procedure of foods in the rate of MRP formation, we support the utility of the fluorescence measurement as a fast heat-induced index to evaluate the heat damage in processed food [15, 40].

3.3 Furosine, HMF and CML analyses

Furosine formation [ε-*N*-(furoylmethyl)-L-lysine] is related to the early stage of the MR, as an indicator of losses in

available lysine [12]. Furosine amino acid is formed during acid hydrolysis of the Amadori compound (fructosyl-lysine, lactulosyl-lysine and maltulosyl-lysine) produced by the reaction of ε-amino groups of lysine with glucose, lactose and maltose [41] and by the loss of amino groups (mainly lysine) [42]. Results from furosine determination in LD meals were significantly higher in the A-diet with respect to the B one (Table 4). It seems logical since foods belonging to LD-A meal suffered more severe cooking processes than those from LD-B. However, furosine levels in the BA snack resulted significantly higher in the B- than in the A-diet. This fact could seem inconsistent with the result expected if the hypothesis of a more severe heat treatment is applied as previously mentioned. But furosine assesses the extent of the thermal damage at moderate processing conditions where MR is mainly stopped at the early stage. However, at more severe heating conditions or higher amounts of reactants, Amadori products are significantly degraded and subsequently a reduction in furosine levels is expected. On the other hand, data make sense if furosine value in BA snack is analysed together with HMF and CML results (Table 4). HMF is mainly formed through 1,2-enolisation of Amadori compounds under acid conditions [27] and also due to the acid-catalysed degradation of some sugars [43]. Since HMF is an index of the intermediary, and so for more advanced stages of the MR, the decrease in furosine levels in BA-A meal could be explained by the significantly higher formation of HMF. This would indicate the participation (degradation) of the Amadori compound in that route during the progress of the reaction. According to this, some authors have found high furosine levels in a powder infant formula compared with an in-bottle-sterilised one. They also attributed these results to the intense heat treatment undergone by the in-bottle-sterilised formula, where the further degradation of the Amadori compounds, also the precursors of furosine, led to the formation of typical products of the later stages of the MR [44]. Results of Rada-Mendoza *et al.* [45] regarding the formation of HMF and furosine during the storage of jams and fruit-based infant foods also supported our data, since they attributed, the decreases in furosine and increases in HMF levels to severe processing conditions during the manufacture or elevated temperature of storage. On the other hand, the visible differences between furosine and HMF levels of LD *versus* BA snack meals show the different compositions and processing of both meals. The type of components, which will act as reactants, and the cooking procedures will mark the extent of the reaction and the kind of products formed out of it.

In contrast to furosine, CML may be formed during autoxidation of carbohydrates, 3-deoxyglucosone and ascorbate, from Schiff base and Amadori adducts by autoxidation of fatty acids and amino acids, and from phosphorylated intermediates [46]. Hence, CML is also an index of glycosidative, lipoxidative and oxidative reactions taking place in foods as well as biological systems. Higher differences were observed between LD meals probably due to the participation of oxidised reaction products from a lipidic fraction of the meal, since frying and toasting processes were expected to be more drastic in LD-A samples. But differences, whereas significant, were reduced in the BA set of meals.

3.4 Determination of sugars

Reducing sugars are important promoters of the MR, as well as intermediary compounds from the sugar degradation reactions, such as carbonyl compounds. Furosine, HMF and CML levels are directly correlated to both the levels of reducing sugars and the thermal treatment applied in many food commodities; furthermore, an extrapolation to a complex diet is difficult since many factors are taking part. Results are included in Table 5. It manifested a marked decrease in lactose, glucose and fructose contents in the BA-A meal compared with BA-B. This fact could be explained by the degradation of sugars due to the manufacture process of some of the foods included in the BA snack of the A meal, like breakfast cereals. The findings of the highest HMF values in this sample support this hypothesis, since this route has been pointed out to be important in the formation of the compound [43]. On the other hand, drastic reduction in the reducing sugar content between BA-A and BA-B is an indirect indicator of the extent of the MR in BA-A. It is expected that Amadori products are degraded more rapidly at these conditions and subsequently lower furosine levels were recorded.

No direct justification can be made to understand the hight levels of glucose and fructose measured in the LD-A meal with respect to the LD-B meal but the little differences in the foods of both diet, aimed to have an adequate final content of MRP in each of them, and therefore the content of sugars in them. Perhaps, the rupture of polysaccharides favoured by heating during the food processing could also support the increase of these sugars, in the same way as it is well known by the cleavage of sucrose at temperatures up to 150°C [47].

4 Concluding remarks

In summary, values of furosine, calculated by taking into account the contribution of each meal to the total daily food intake, were 42.25 and 37.15 mg/100 g protein for the A- and B-diets, respectively. Since according to Erbersdobler [48], the rate of transformation of Amadori compounds into furosine under acid hydrolysis is nearly 36%, the content of these products were 117. 35 and 103.18 mg/100 g protein for both diets, respectively, not very different values in spite of the different culinary processes selected to prepare foods. According to Vlassara [7], these data suggest

that any diet, although varied and equilibrated, supposes an MRP intake even using soft culinary techniques, especially of earlier MRP. However, the culinary techniques employed and the foods selected to constitute a diet allow the modulation of the MRP content. The cooking procedures used in the studied dishes, especially preferred by adolescent population, showed to be promoters of more advanced MRP, as expected, and so HMF content in the whole A-diet was significantly higher than in the B-diet (3.87 *vs.* 0.95 mg/kg sample). It must be taken into account that, although still waiting for *in vivo* realistic studies of HMF availability and toxicity, in the last few decades a powerful cytotoxic effect and tumourigenic activity has been attributed to this compound [49 – 51].

On the other hand, CML content was significantly increased in the A-diet with respect to the B diet (15.72 *vs*. 6.62 mg/100 g protein, respectively). Advances in the study of AGEs' implications on health have showed the contribution of compounds such as CML, pentosidine or pyrraline in the progress of degenerative diseases, such as diabetes mellitus and uraemia [52] or osteoporosis [53].

Therefore, besides the well-known and important health problems (obesity, hypertension, cardiovascular disorders, *etc*.) associated with the consumption of imbalanced diets with high fast-food intake, common in the new food pattern of our adolescent population, it is necessary to consider if an excessive MRP consumption, especially advanced MRP, could suppose new long-term implications for human health.

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6 Appendix

Diet A

Day 1	
Lunch	Dinner
Empanadillas: small tuna-filled breaded pasties, Findus (Findus, Barcelona, Spain): 150 g; olive oil 10 g. Salad: lettuce 30 g; tomatoes 30 g; onion 8.5 g; olive oil 6 g; sardines in olive oil 25 g Legumes (lentils): onion 8.5 g; olive oil 10 g; potatoes 90 g; lentils 50 g; tomato purée 50 g Chocolate yogurt: 100 g	Consommé with noodles: chicken broth 150 g; noodles 15 g Breaded fish: hake 130 g; eggs 20 g; flour 5 g; olive oil 15 g Pears: 125 g
Day 2	
Lunch	Dinner
Gratin macaroni with béchamel sauce: macaroni 80 g; flour 10 g; butter 6 g; milk 30 g; veal meat 30 g; olive oil 7; onion 20 g Bananas: 120 g	Sautéed vegetables: cauliflower 40 g; green beans 91 g; peas 20 g; carrots 41 g; onion 20 g; artichoke 18 g; olive oil 10 g Griddle loin of pork and fried potatoes: pork loin 130 g; potatoes 120 g; olive oil 20 g Torrija: bread (30 g) with milk (50 mL), sugar (20 g) and cinnamon, fried in olive oil (10 g)
Day 3	
Lunch	Dinner
Spanish omelette with ham: potatoes 120 g; eggs 50 g; onion 20 g; olive oil 10 g; ham 30 g Meatballs (veal) with vegetables: veal 150 g; eggs 20 g; bread 10 g; flour 10 g; peas 30 g; carrots 30 g; green beans 46; tomatoes 30 g; olive oil 10 g Chocolate custard: 130 g	Pizza: pizza with tomato, cheese and ham 300 g Bananas: 130 g
Day 4	
Lunch	Dinner
Legumes (chickpeas): chickpeas 50 g; chicken 21 g; pork fat 10 g; potatoes 45 g; green beans 18.2 g; pumpkin 14 g Chocolate yogurt: 100 g	Pasta and chicken soup: pasta 30 g; egg 20 g; chicken 21 g; ham 5 g Hamburger with fried potatoes: hamburger 120 g; potatoes 120 g; olive oil 15 g Bananas: 100 g
Day 5	
Lunch	Dinner
Salad: lettuce 30 g; tomatoes 30 g; onion 8.5 g; olive oil 6 g; sardines in olive oil 25 g Paella: rice 100 g; chicken 51 g; prawns 15 g; pimientos 25 g; tomato 13 g; onion 17 g; garlic 5 g; olive oil 8 g Apple: 150 g	Vegetables cream with croutons: olive oil 10 g; zucchini 25 g; carrots 50 g; leek 15 g; celery 5 g; potatoes 75 g; bread 20 g Breaded hake fish fingers Pescanova (Pescanova, Pontevedra, Spain): 200 g; olive oil 15 g
Day 6	
Lunch	Dinner
Fried chicken and fried potatoes: chicken 200 g; olive oil 20 g; potatoes 100 g Salad: lettuce 30 g; tomatoes 30 g; onion 8.5 g; olive oil 6 g; sardines in olive oil 25 g Pears: 100 g	Fish croquettes Pescanova: 200 g; olive oil 10 g Caramel custard: 120 g
Day 7	
Lunch	Dinner
Tropical salad: lettuce 30 g; endives 15 g; pineapple 17 g; peach 26 g; corn 5 g; beetroot 87 g; carrots 50 g; olive oil 10 g Spanish omelet: potatoes 50 g; eggs 50 g; olive oil 15 g Rice with milk and cinnamon Danone (Danone, Barcelona, Spain): 130 g	Breaded fish and rice: swordfish 125 g; eggs 20 g; flour 5 g; olive oil 15 g; rice 50 g Oranges: 150 g

Diet B

	Lunch	Dinner
Day 1	Russian salad with tuna: potatoes 150 g; mayonnaise 50 g; tuna in olive oil 40 g; carrots 41 g; peas 25 g; olives 15 g Legumes (lentils): onion 8.5 g; olive oil 10 g; potatoes 90 g; lentils 50 g; tomato purée 50 g Strawberry yogurt: 125 g	Consommé with noodles: chicken broth 150 g; noodles 15 g Baked fish with boiled potatoes: hake 130 g; potatoes 150 g; olive oil 15 g Pears: 125 g
Day 2	Spaghetti with tomato sauce, cheese and ham: spaghetti 80 g; tomato sauce 100 g; cheese 15 g; ham 30 g Bananas: 120 g	Vegetable Stew: cauliflower 40 g; green beans 91 g; peas 20 g; carrots 41 g; onion 20 g; artichoke 18 g; olive oil 10 g Baked loin of pork and boiled potatoes: loin of pork 130 g; potatoes 120 g; olive oil 20 g Syrup peach: 120 g
Day 3	Boiled potatoes, boiled eggs and ham: potatoes 120 g; eggs 50 g; onion 20 g; olive oil 10 g; ham 30 g Baked meat (veal) with vegetables: veal 150 g; eggs 20 g; peas 30 g; carrots 30 g; green beans 46; tomatoes 50 g; olive oil 12 g Custard: 130 g	Pasta with tomato and cheese: pasta 80 g; tomato purée 100 g; cheese 20 g Bananas: 130 g
Day 4	Legumes (chickpeas): chickpeas 50 g; chicken 21 g; pork fat 10 g; potatoes 45 g; green beans 18.2 g; pumpkin 14 g Strawberry yogurt: 125 g	Pasta and chicken soup: pasta 30 g; egg 20 g; chicken 21 g; ham 5 g Sausages with mashed potatoes: sausages 120 g; potatoes 120 g; olive oil 12 g Bananas: 100 g
Day 5	Salad: lettuce 30 g; tomatoes 30 g; onion 8.5 g; olive oil 6 g; sardines in olive oil 25 g Stewed rice: rice 100 g; chicken 51 g; prawns 50 g; squid 40 g; mussels 50 g; red peppers 25 g; tomato 13 g; onion 17 g; garlic 5 g; olive oil 10 g Apple: 150 g	Vegetables cream: olive oil 10 g; olive oil 15 g; zucchini 25 g; carrots 50 g; leek 15 g; celery 5 g; potatoes 75 g Fish pudding: milk 100 g; hake 100 g; eggs 50 g; olive oil 10 g
Day 6	Boiled chicken and boiled potatoes: chicken 200 g; olive oil 20 g; potatoes 100 g Salad: lettuce 30 g; tomatoes 30 g; onion 8.5 g; olive oil 6 g; sardines in olive oil 25 g Pears: 100 g	Baked fish: sole 200 g; olive oil 20 g Custard: 120 g
Day 7	Tropical salad: lettuce 30 g; endives 15 g; pineapple 17 g; peach 26 g; corn 5 g; beetroot 87 g; carrots 50 g; olive oil 10 g Tuna filled eggs: eggs 80 g; tuna 30 g; mayonnaise 60 g Rice with milk Danone (Danone, Spain): 130 g	Fish with cream and rice: swordfish 125 g; cream 30 g; olive oil 5 g; rice 50 g Oranges: 150 g

Bread and bread without crust (30 g) were included in all meals of diets A and B, respectively.
Frying process was carried out in olive oil at 180°C.
Baking was carried out at 180°C.
The diet composition presented here corresponds to the complete foods, *e.g.*, 200 g of chicken is really 130 g of edible portion.

