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ENDOGENOUS FORMALDEHYDE CONTENT IN TYPICAL ITALIAN CHEESES



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INTRODUCTION

Commission Regulations EU 05.06.2014 n. 605^[1] and UE 23/3/2015 n. 491^[2] recently updated the classification of formaldehyde (FA) as carcinogen 1B – H 350 and the International Agency for Research on Cancer (IARC) classified FA as “carcinogen”.

FA, even if not used as antibacterial agent and preservative in food processing, occurs also naturally in free and bound forms with protein residues yielding methylol groups, Schiff bases, methylene bridges, and other adducts. According to various authors, only free FA is of toxicological interest^[3, 4] and is measured as a potential adulterant. FA data content naturally occurring in milk (27-164 µg/kg), and detected by HPLC, are reported^[5] and EFSA estimated a tolerable daily exposure at 100 mg/kg food per day^[6]. This note provides a first contribution to knowledge of free FA endogenous data for some typical Italian cheeses distributed on the national market.

MATERIALS AND METHODS

Twenty samples of cheese commercially available in Italian market are analyzed. Analytical evaluation of free FA was realized in our work using H₂O as extractant, following studies mentioned by Storey et al.^[7]. High performance liquid chromatography- diode array detector (HPLC-DAD) was adopted, and standard addition method was adopted. Each water extract from all samples was spiked with various aliquots of FA standard solution, realizing the response curve for FA-DNPH.

Operative Conditions

Column: ZORBAX Eclipse PAH – 5.0 µm, 4.6 mm x 150 mm; Flow: 1 mL/min; λ = 360 nm; mobile phase: A = H₂O ; B = CH₃CN; isocr. 50% B x 5 min., incr. to 100% B in 3 min.

Sample Preparation

Six aliquots of 7g each of grated cheese are added to six aliquots of 20 mL deionized H₂O, mixed for 60 min. and then centrifuged for 3 min. (2000 rpm). All solutions are filtered with a 0.45 µm filter and 10 mL of each solution are collected in vial adding 0.5 mL of derivatizing agent (DNPH). The derivatizing solution was prepared dissolving 428.7 mg of DNPH in CH₃CN and diluting to 100 mL vol. Five vials receive respectively 10, 25, 50, 100, and 200 µL, of standard formaldehyde at conc. 37.7 mg/L while the reference solution doesn't receive FA addition. All the resulting solution are vortexed for 1 min. and placed in the dark for 60 min. at 20-25 °C for the reaction to be completed. The content of each vials is filtered through Finisterre C18 SPE cartridge previously conditioned with CH₃CN (6 mL) and H₂O (2 mL).

Each solution is eluted with 2 mL + 2 mL CH₃CN and collecting the eluate in 5 mL flask.

The solutions are filled to 5 mL and are then ready for HPLC-DAD analysis (λ = 360 nm). The sample injection volume was set at 20 µL.

Figure 1. Data produced for Parmigiano Reggiano (sample 13).

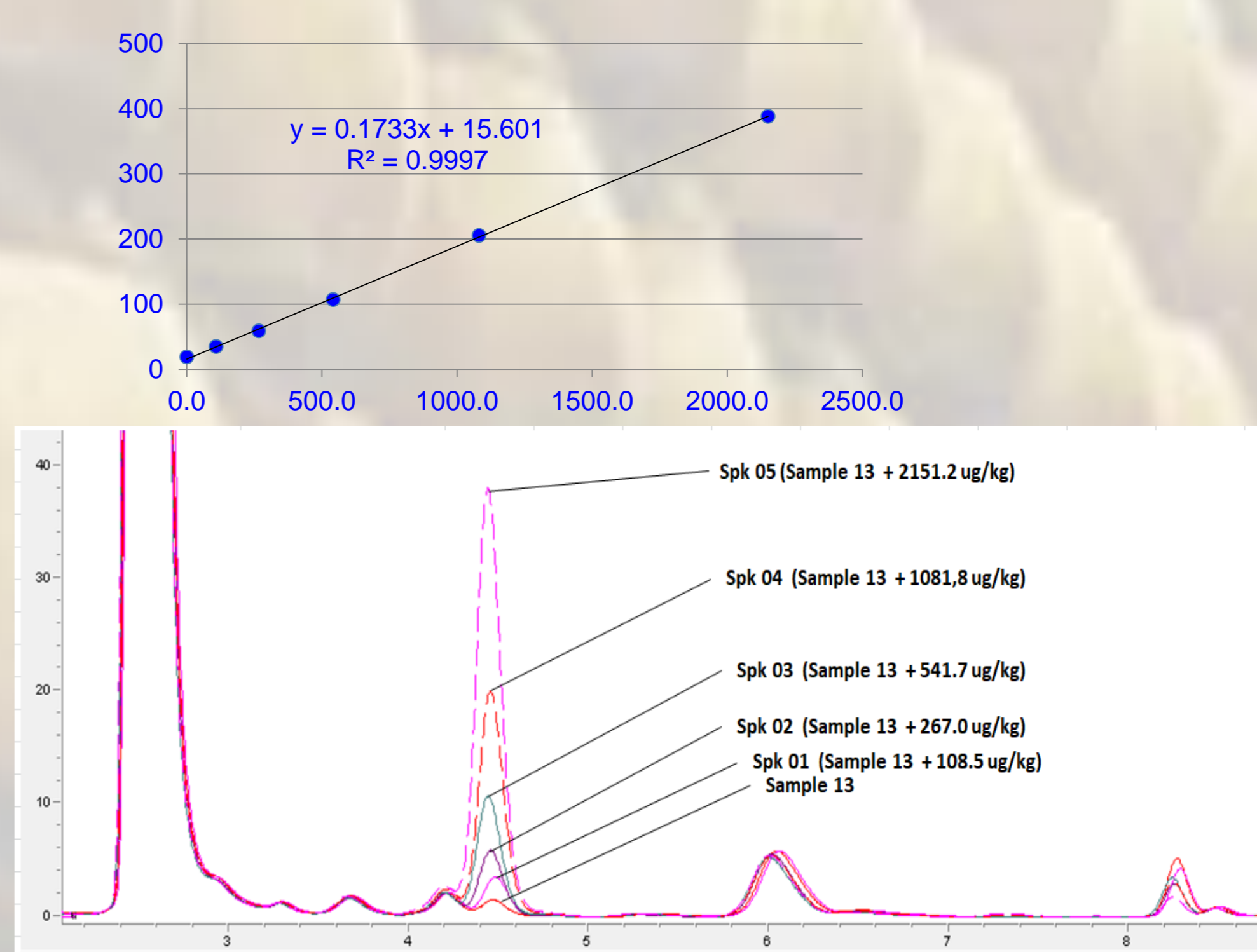


Table 1. Free endogenous FA content in various cheese samples available on the Italian market.

n.	sample	brand	CH ₂ O (µg/kg)
1.	Grana Padano	Virglio	95.7
2.	Parmigiano Reggiano	Virglio	63.1
3.	Provolone	Auricchio	59.3
4.	Trentingrana	Trentingrana	113.5
5.	Parmigiano Reggiano	Parmareggio	102.0
6.	Parmigiano Reggiano	Biraghi	97.3
7.	Grana Padano	Agriform	88.6
8.	Prix Cheese	Coop Svizzera Basilea	59.0
9.	Sbrinz Cheese	Coop Svizzera Basilea	75.9
10.	Parmigiano Reggiano	Granduca	91.6
11.	Grana Padano *	Esselunga	115.0
12.	Parmigiano Reggiano *	Parmareggio	76.0
13.	Parmigiano Reggiano * ***	Parmareggio	90.0
14.	Parmigiano Reggiano *	Carrefour	54.4
15.	Gran Biraghi *	Biraghi	94.7
16.	Grana Padano *	Gran Soresina	81.8
17.	Grana Padano * **	Ferrari	100.1
18.	Grana Padano	Caseificio Europeo	81.6
19.	Grana Padano	Solo Bontà Sfiziose	79.6
20.	Provolone	Simply	94.3

(*) milled; (**) 20 months old; (***) 30 months old

RESULTS AND DISCUSSION

Figure 1 shows the data produced for sample 13.

The coefficient of determination (R²) for all curves produced by standard addition method and corresponding to all samples analyzed is included in the range 0.9966 to 0.9999 with a standard deviation (σ) 0.0011. All RSD values are < 2% and the free endogenous FA for all samples are evaluable to be < 250 µg/kg, whatever the recovery value. In all samples the free endogenous FA shows values are very lower than EFSA tolerable daily exposure.

The results are reported in Table 1 and the Figure 2 summarized the distribution of free endogenous FA in samples considered.

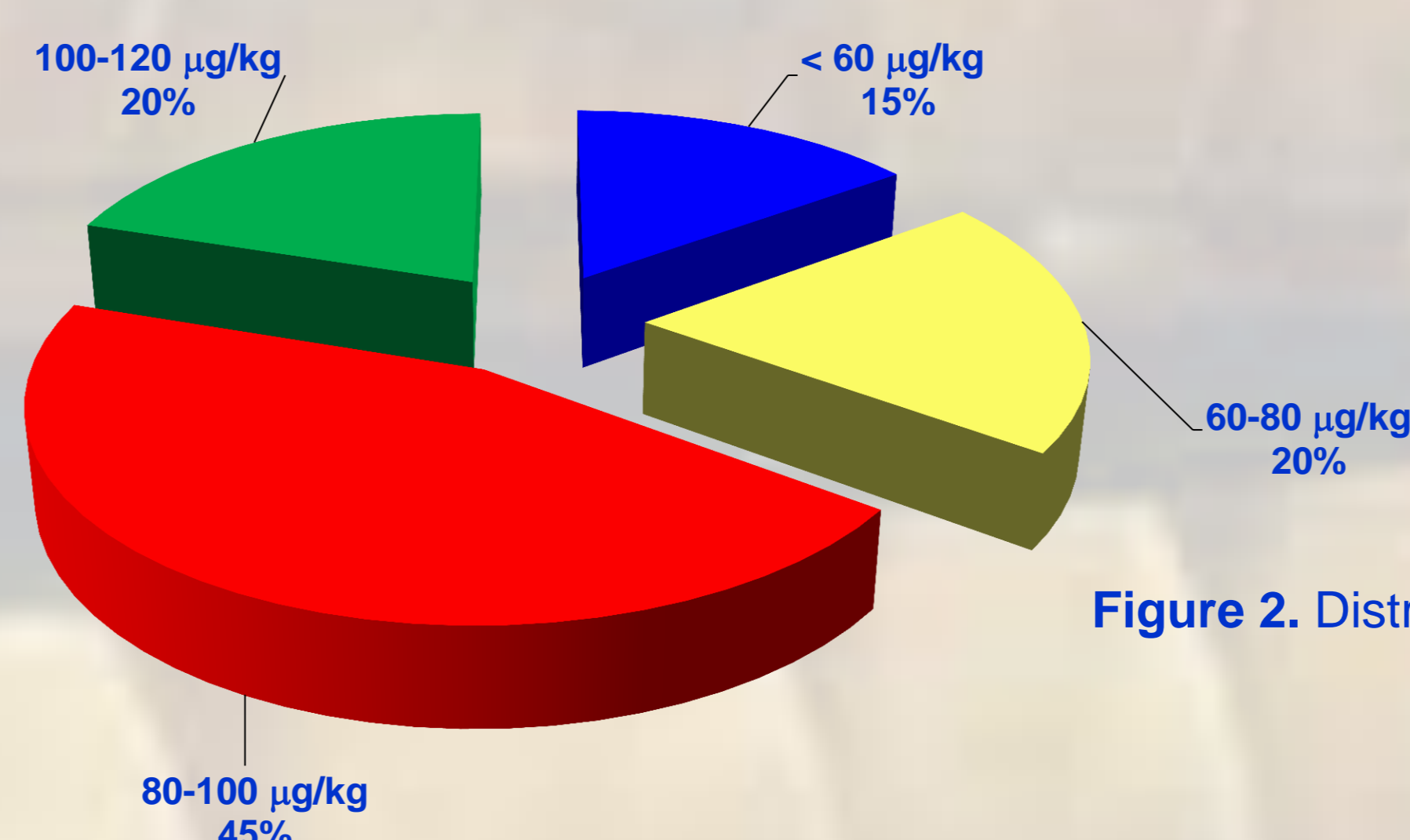


Figure 2. Distribution of free endogenous FA values deduced from Table 1.

REFERENCES

[1] Commission Regulation (EU) No 605/2014 of 5 June 2014; [2] Commission Regulation (EU) No 491/2015 of 23 March 2015; [3] Tai-Sheng Yeh et al., J. Food Drug Anal. **2013**, 190-197; [4] Bechmann I.E., J. Lebensm. Wiss. Technol., **1998**, 449-453; [5] Kaminski J. et al., J. AOAC Int., **1993**, 1010-1013; [6] EFSA, **2014**, EFSA J., 12, 3561; [7] Storey M.J. et al., 2016, Food Add. & Contam., **32**, 657-664.