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Article

Fatty Acid Composition of Gluten-Free Food (Bakery Products) for Celiac People

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Abstract: The aim of this study (first analytical approach) was to obtain data on the concerning to fatty acid composition of gluten-free foods (bakery products) for celiac people. The study included 35 different products (snacks, biscuits, bakery products, pasta, flours, etc.) from several manufacturers. After extraction and esterification, the fatty acid composition content was determined by GC-MS.

Regarding to fatty acids profile, The monounsaturated fatty acids (MUFAs) are the major constitutes (57%) of the fatty acids pool followed by saturated fatty acids (SFAs) (30%) and polyunsaturated fatty acid (13%). Only fifteen, of the thirty-five gluten free samples analyzed, provide adequate energy intake, while in eleven samples, saturated fatty acids take more energy than that recommended by EFSA.

It has emerged that local producers generally use the finest raw materials such as olive oil, etc. compared to the industries which, as has been pointed out, in many cases use palm and palm kernel oils although gluten free commercial products are high added value foods, expensive and intended for a particularly sensitive public.

Moreover, data analyses showed that, although gluten free commercial products are high added value foods, local producers compared to industry products which in many cases use palm and palm kernel oils , generally use finest raw materials such as olive oil.

Keywords: gluten-free foods; fatty acids; GC-MS; celiac

1. Introduction

Celiac disease is a persistent systemic autoimmune disorder caused by an enduring intolerance to gluten proteins in genetically predisposed people and is characterized by an wrong immune response of the T-lymphocytes of the small intestines to gluten peptides [1]. This disease is characterized by chronic inflammation of the intestinal mucosa, atrophy of intestinal villous and several clinical manifestations.

Epidemiological research found that celiac disease is very common and that the prevalence of celiac disease is approximately 1% in the general population [2]. At present, the only available treatment is a lifelong strict gluten-free diet, which leads to reinstatement of the atrophied intestinal villi.

Gluten is a universal term used to designate a mixture of proteins of wheat, barley, rye and derivatives.

The constant increase of celiac populace leads to a higher demand of gluten-free food without wheat, rye, barley or spelt wheat proteins (EU Regulation, 2014) [3]. Among these food, gluten-free bakery products (bread, bread sticks, cookies, etc) have significant advantages related to the high nutritional profile, ready-to-eat, variety of presentations and flavors. In several cases, they may also include seeds, fruit pieces, honey, chocolate, yogurt, etc. Usual ingredients of gluten-free foods are starch and flour of corn, potato, tapioca, rice, etc. In several cases, foods are integrated with vitamins and minerals [4, 5]. Gluten-free foods, generally, have high fats, sugars and minerals contents to

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improve the flavor, consistency and appearance [6], moreover, celiac people tend to offset the limitations of own diet consuming food containing high levels of fat, sugar and calories (snacks and biscuits, etc.). Saturni [6] affirms that celiac patients may show an excessive consumption of total fats and saturated fats. Other researchers confirmed that, the alimentation of adolescent celiac people results hyperproteic and hyperlipidic and contains low amounts of carbohydrates, iron, calcium, fiber, etc. and, in several cases, is the origin of overweight patients [7-9]. Has been established that several gluten free products contain trans fatty acids [9] that may have a negative effect on health (coronary heart disease, obesity, etc.).

Commonly, celiac people need assistance from a dietician that have not knowledge regarding fatty acid composition of gluten free products; for this reason is necessary to know the composition of the foods consumed by individuals with celiac disease and, nowadays, data do not exist. Considering the high number of celiac, these data are urgently required so that the daily dietary fatty acids intakes in the celiac population can be estimated from available food consumption data. While the macro and microelements intake levels for celiac people are documented [10, 11] there are no information available on fatty acids composition. Therefore, the aim of this study was to obtain information on the fatty acid content of several common gluten-free foods (bakery products) for celiac people, in particular, in several products sold out in Italy Italian products (snacks, biscuits, bakery products, pasta, flours, etc.). In this research, the authors have investigated The study included 35 different products for celiac people from several manufacturers. After extraction and esterification, the fatty acid content was determined by GC-MS.

Such as proteins, carbohydrates, and alcohol, lipids are a major energy source for human being. The energy produced of the most common fats in the diet is approximately 9 kilocalories per gram. Lipids and fatty acids are need for many vital functions (structural components of cell membranes, precursors for bioactive molecules, regulators of enzyme activities, regulation of gene expression, etc.), also, promote the absorption of other important dietary components such as vitamins.

2. Materials and Methods

2.1. Quality control and quality assurance

For each analytical batch, several blanks were run up in order to Periodically, blanks were run up and demonstrate that the treatment used for cleaning vessels and flasks is suitable to obtain the quality assurance required in this study. All the analyses of fatty acid in gluten-free food samples were repeated three times and the relative standard deviation results ranged from 0.5 to 10%. The repeatability, calculated as the relative standard deviation (RSD%) of three independent measurements of a standard mix solution at 10 $ng \cdot mL^{-1}$, ranged from 0.3 to 8.0 %. The repeatability of the whole method, calculated as the relative standard deviation (RSD%), for three independent analysis of independent different subsamples portions the same sample, ranged from 0.4 to 8.8%. A blank was run up every six samples. All reported data were blank corrected.

2.2. Samples

Thirty-five samples (Table 1) of gluten-free foods (pasta, biscuits, flours, etc), produced and sold out in Italy were collected from markets and pharmacies in Palermo city (Italy). The selected samples were representative of the Italian market. The data reported in of Table 1 were obtained from the nutrition labels shown on the packs. The total fat content has been verified by us on about 50% of the samples (see § 2.3). Only the bread and breadsticks (samples n° 3, 7) were produced in local (Palermo and neighborhood) bakeries.

In order to obtain a representative sample, 50 g of each different sample were was milled and homogenized by using a food processor (plastic-coated) and then sub-sampled for analysis. Samples were handled immediately or stored in the refrigerator at 4°C until analysis for a period of less than 24 h.

Table 1 – Nutritional characteristics of analyzed gluten free samples and reference food (Rx)

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N°	Sample	Energy (Kcal/100 g)	Total fat (g/100 g)	Saturated fatty acids (g/100 g)	Carbohydrates (g/100 g)	Proteins (g/100 g)	Sals (g/100 g)
			Flours				
1	Rice flour	365	1.3	0.29	80.15	6.67	0.10
2	Red teff flour	380	1.4	0.27	-	-	-
3	Flour for bread	352	1.2	0.3	79.6	5.0	0.01
R_{flour0}	Flour 0 with gluten	363	1.5	0.27	73.8	11.5	-
$R_{flour00}$	Flour 00 with gluten	366	1.5	0.30	76.2	9.7	
R_{rice}	Rice flour	366	1.4	0.39	80.1	5.9	
R_{corn}	Corn flour	375	1.4	0.17	82.8	5.6	
			Bread				
4	Handcrafted bread	360	3.7	0.90	-	-	-
R_{breada}	Bread with gluten	271	3.5	0.86	50	8.8	
R_{breadb}	White Bread with gluten	238	2.1	0.63	43.9	10.6	
			Breadsticks				
5	Breadsticks a	425	9.0	1.8	75.0	2.4	1.9
6	Breadsticks b	416	8.5	3.2	79.9	4.0	2.7
7	Breadsticks c	462	17.2	2.54	73.57	0.99	1.6
8	Breadsticks d	446	14.6	2.9	75.0	2.4	1.9
9	Breadsticks with olive oil	431	12.0	3.5	79.0	1.30	2.12
10	Breadsticks with rosemary	460	16.0	5.13	76.32	2.04	2.80
11	Mini breadsticks	427	12.8	1.7	74.9	1.4	1.2
			Crackers				
12	Buckwheat crackers	369	3.6	0.6	72.0	9.1	2.0
13	Crackers with rosemary	446	12.0	7.1	79.0	3.3	1.3
14	Rice Crackers	454	13.8	1.3	78.5	2.8	1.9
15	Turmeric crackers	455	17.0	1.8	66.0	7.6	2.1
$R_{crackers}$	Crackers with gluten	421	8.9	2.0	74.3	9.5	-
16	Corn and quinoa crackers	422	9.8	0.9	79.5	1.8	1.7
			Snack				
17	Apricot snack	620	7.5	0.99	71.0	2.50	0.23
18	Crispy sheets	436	12.0	1.5	78.5	2.0	1.86
22	Rice and corn flakes	382	2.0	0.4	83.0	6.4	0.3
			Biscuits				
19	Chocolate biscuits	453	14.0	7.70	77.0	4.0	0.55
20	Biscuits d	422	18.4	2.3	66.3	4.6	0.3
21	Biscuits f	451	15.0	7.8	72.0	5.4	0.4
23	Lemon biscuits	435	11.0	1.5	78.0	4.6	0.9

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24	Seven cereal biscuits	456	16.5	9.1	72.0	3.5	3.0
25	Biscuits o	456	13.0	4.6	83.0	2.0	0.57
26	Chocolate wafer	505	25.0	16.0	62.1	6.4	0.25
R_w	Wafer with gluten	433	14.2	4.2	72.4	6.6	-
29	Biscuits n	439	14.0	4.2	69.4	7.0	0.7
30	Tumeric biscuits	441	16.0	1.8	78.0	2.3	0.33
31	Chocolate biscuits	425	11.7	2.7	73.6	5.1	0.8
33	Buckwheat biscuits	444	14.0	8.9	72.0	4.2	0.85
34	Biscuits c	372	19.9	2.5	52.8	4.4	0.07
35	Crunchy cereal biscuits	472	18.0	8.0	70.0	5.3	0.75
	Muffin and cakes						
27	Easter cake	410	18.0	14.0	-	-	-
32	Quinoa cake	407	17.0	2.5	55.0	7.3	0.50
28	Chocolate muffin	441	23.0	8.6	52.0	3.9	0.5
R_m	English muffin with gluten	223	2	0.29	44.8	8.7	-

2.3. Total fats quantification

The samples (5-10 g) were extracted by refluxing for 2-3 hours with ethyl ether, in a Soxhlet apparatus. The solvent was eliminated in a rotating evaporator and the residue dried at 70-80°C for 30 min was weighted.

2.4. Fats extraction and preparation of methyl esters (FAMEs)

A portion of the homogenized sample (about 10 g) was extracted with methanol:chloroform mixture according to Folch method [12]. The lipid extract was converted into fatty acid methyl esters (FAME) by treatment with 0.01 M sodium hydroxide in methanol at 60–65 °C, for 30 min at room temperature, followed by collection of the FAME dissolved in hexane (Analytical grade from Sigma Aldrich, Italy).

2.5. GC-MS analysis

The analysis of standard (FAMEs) and samples was carried out using an Gas Chromatography (Agilent Technologies 7890 B GC System) coupled with a Mass Spectrometry (Agilent Technologies 7000 C GC/MS Quad.) using He 5.5 type as carrier gas (flow 3ml/min). 1µl of the sample was injected. A Capillary column (30 m x 0,25 mm i.d. x 0,25 µm film thickness) coated with 5% phenyl-methylpolysiloxane stationary phase (DB-5 Agilent) was employed. The injector with splitless system was set at the 250 °C. Oven temperature was programmed at the beginning to 40 °C, increased to 250 °C with a 2 °C/min increment (hold time 15 min) and finally reached 270°C with a 10 °C/min increment. The septum flow to split vent was 3 ml/min and purge flow to splint vent was 15 ml/min, GC transfer line was set at 295 °C and source temperature at 200 °C. Using the above instrumental conditions, the different fatty acids methyl esters were clearly identifiable in the chromatograms, in detail particular, C18:1cis (R_t = 81.884 min), C18:1 trans (R_t = 82.183 min), C18:2 cis (R_t = 81.484 min) and C18:2 trans (R_t = 81.984 min) resulted well separated.

QP-GC/MS worked in Electron Ionization mode (EI 70 eV) and carried out in full-scan acquisition (with quantification based on the TIC) as well as in SIM mode. All mass spectra were acquired over the m/z range 50-550 except during SIM. Three replicates were injected for each sample.

The data acquisition and processing were carried out using Mass Hunter Workstation Agilent Technologies Agilent software. Peaks of fatty acids were identified by comparison with those of standards Supelco™ Component FAME Mix 37 and confirmed using the NIST mass spectral

database. No internal standard have been used. The confirmation of the structural and geometric isomers of fatty acids was carried out using Mix FAMEs C4:0 - C24:1 and FAMEs Mix C20:1 - C20:5.

In Figures 1 and 2, as examples, are shown the chromatograms of a standard fatty acids methyl esters mix and the a wafer sample.

Figure 1. Chromatograms of fatty acid methyl esters standard mix.

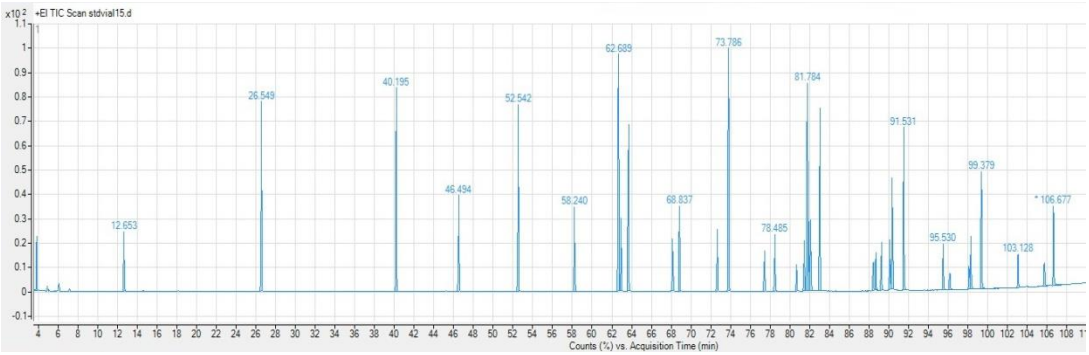
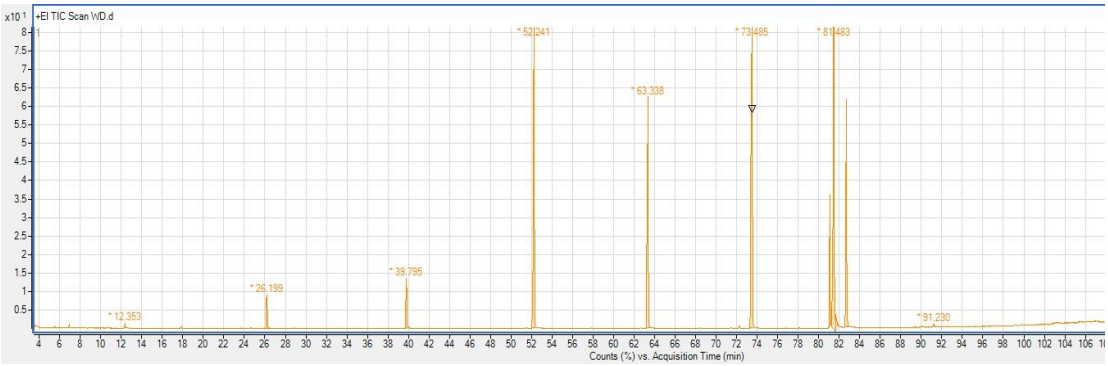


Figure 2. Chromatograms of the wafer sample.



Quantification of the fatty acids methyl ester profiles was done considering the relative areas of peaks, expressed as the relative percentage of the individual area of each one relative to total area of compounds in the chromatogram.

3. Results

In the present study a total of 35 gluten free food samples were analyzed from 2014 to 2017 to assess their nutritional characteristics with regard to the quantification of fatty acids. Overall, 37 fatty acids were detected (Table 2). Fatty acid compositions of gluten free food are summarized in Table 2. The percentages of single fatty acids indicated in the text (Table 2) are referred to the total fat content in the sample and not to the whole food, while the total fat content (Table 1) refers to the food in the conditions in which it is consumed (g/100 g food). Data are shown only if the FA was present at > 0.01 % of total.

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Table 2. Fatty acids composition (% on total fats) of gluten free foods.

Fatty acid	Samples																																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	mean
C4:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.22	0.01	0.01	0.01	0.01	1.29	0.21	0.01	0.08	0.01	0.01	0.01	0.01	0.01	0.4	0.01	0.01	0.071
C6:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.54	0.01	0.06	0.3	0.01	2.25	0.24	0.1	1.3	0.01	0.01	0.01	0.01	0.01	0.6	0.01	0.01	0.162
C8:0	0.01	0.01	0.01	0.01	0.01	0.04	0.01	0.04	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.42	0.01	0.8	1.84	0.01	3.06	0.23	1.7	0.87	0.01	0.01	0.01	0.01	0.01	0.94	0.01	0.01	0.291
C10:0	0.01	0.01	0.01	0.01	0.01	0.05	0.01	0.03	0.01	0.01	0.05	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.63	0.01	0.82	1.65	0.01	0.65	0.67	2.32	1.5	0.03	0.01	0.04	0.01	0.01	2.5	0.01	0.01	0.321
C11:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.06	0.01	0.04	0.01	0.01	0.14	0.01	0.02	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.017
C12:0	0.01	0.01	0.01	0.01	0.16	0.39	0.01	0.08	0.01	0.01	0.2	0.01	0.21	0.01	0.01	0.02	0.01	0.01	0.88	0.01	10.1	16.9	0.01	1.89	0.96	20.6	2.8	0.67	0.01	0.01	0.01	0.01	3.5	0.01	0.04	1.70
C13:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.1	0.01	0.01	0.01	0.01	0.45	0.04	0.04	0.09	0.01	0.01	0.01	0.01	0.01	0.11	0.01	0.01	0.03
C14:1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.22	0.01	0.01	0.01	0.01	4.0	0.49	0.01	0.62	0.01	0.01	0.01	0.01	0.06	1.1	0.01	0.01	0.20
C14:0	0.18	0.01	17.7	1.2	0.01	1.3	0.01	0.32	0.1	0.01	0.79	0.05	1.3	0.0	0.03	0.05	0.09	0.17	3.3	0.2	4.6	10.6	0.05	8.11	3.15	12.6	9.3	0.26	0.01	0.16	0.06	0.12	11.0	0.04	0.64	2.50
C15:1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C15:0	0.01	0.01	0.01	0.01	0.01	0.1	0.01	0.01	0.01	0.01	0.05	0.03	0.11	0.01	0.01	0.0	0.01	0.02	0.64	0.05	0.17	0.01	0.01	0.7	0.6	0.08	0.76	0.16	0.01	0.02	0.01	0.03	1.44	0.01	0.03	0.15
C16:1	0.01	0.01	0.01	0.01	1.3	0.6	0.01	7.4	1.23	0.01	1.1	1.7	0.53	0.01	0.01	0.0	0.11	0.58	1.16	0.01	1.2	0.01	0.29	1.03	1.02	0.26	0.98	2.87	0.01	0.5	0.43	0.71	1.77	0.38	0.01	0.77
C16:0	15.4	13.6	0.01	17.6	11.5	33.3	4.5	22.8	11.9	34.3	7.4	18.6	51.1	2.8	4.9	2.2	9.7	10.9	29.4	10.1	33.3	24.0	6.1	28.0	16.0	22.0	34.6	33.4	**1.3**	4.1	8.9	12.1	28.9	4.8	41.0	17.4
C17:1	0.01	0.01	0.01	0.01	0.23	0.14	0.01	0.74	0.13	0.01	0.2	0.01	0.2	0.01	0.07	0.01	0.01	0.17	0.01	0.19	0.15	0.01	0.03	0.01	0.48	0.01	0.01	0.01	0.01	0.09	0.03	0.05	0.01	0.08	0.01	0.09
C17:0	0.01	0.01	0.01	0.01	0.15	0.19	0.01	0.51	0.12	0.01	0.12	0.01	0.31	0.01	0.03	0.02	0.01	0.13	0.32	0.08	0.33	0.01	0.03	0.19	0.28	0.2	0.31	1.12	0.02	0.06	0.06	0.06	0.7	0.04	0.05	0.158
C18:3 w6	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C18:2n6 cis	38.0	40.7	29.4	4.5	3.9	5.1	7.9	22.8	1.6	6.0	4.1	26.8	3.9	1.9	10.6	8.4	13.5	12.5	3.1	11.4	7.1	10.5	47.2	3.2	2.1	5.75	3.58	6.9	0.92	4.2	40.9	47.0	6.55	7.84	14.7	13.3
C18:1n9 cis	42.9	40.7	52.9	70.5	74.5	55.4	82.0	22.8	78.7	56.3	79.2	48.9	37.3	92.3	79.9	83.5	71.8	67.4	42.2	72.0	35.3	26.2	42.7	33.8	67.4	22.7	28.0	37.4	96.5	80.2	43.9	34.3	25.1	79.3	37.6	55.5
C18:2 n6 tr	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C18:1 n9 tr	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C18:0	3.0	4.8	0.01	6.2	5.9	2.3	4.6	16.7	4.2	3.3	1.4	3.2	3.9	3.1	2.0	1.4	4.0	5.4	16.7	4.9	4.2	8.0	2.8	9.2	4.9	12	14.2	8.7	0.23	1.4	2.5	3.7	12.2	2.6	5.8	5.41
C20:4 n6	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.21	0.27	0.01	0.01	0.01	0.01	0.01	0.01		0.02
C20:5 n3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C20:3n3+n6	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.42	0.01	0.01	0.1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.024
C20:2 n6	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C20:1	0.01	0.01	0.01	0.01	0.97	0.47	0.01	2.09	0.7	0.01	0.01	0.36	0.01	0.01	0.49	0.01	0.01	0.73	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	1.6	0.01	1.7	0.49	0.08	0.01	0.85	0.01	0.307
C20:0	0.59	0.18	0.01	0.01	0.98	0.66	0.29	2.9	0.91	0.01	1.9	0.4	0.99	0.01	0.59	0.96	0.41	0.88	0.25	0.43	1.0	0.01	0.22	1.1	0.45	0.24	0.39	4.6	0.45	1.7	1.2	0.11	0.19	0.96	0.17	0.746
C21:0	0.01	0.01	0.01	0.01	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.06	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.013
C22:6	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.03	0.01	0.07	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.013
C22:2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C22:1n9	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C22:0	0.01	0.01	0.01	0.01	0.51	0.27	0.73	0.58	0.36	0.01	2.8	0.01	0.07	0.01	1.1	2.9	0.31	0.81	0.16	0.62	0.34	0.01	0.49	0.42	0.9	0.18	0.09	0.9	0.65	4.8	1.4	0.24	2.9	2.9	0.01	0.787
C23	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.12	0.01	0.07	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.04	0.01	0.01	0.02	0.7	0.01	0.07	0.01	0.16	0.01	0.01	0.01	0.043
C24:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.17	0.01	0.01	0.8	0.01	0.2	0.01	0.34	0.52	0.07	0.28	0.01	0.01	0.01	0.01	0.11	0.12	0.3	0.01	0.06	0.55	0.01	1.01	0.2	1.24	0.01	0.6	0.01	0.193
C24:1	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

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Fatty acids The identified and quantified fatty acids were grouped into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Figure 3).

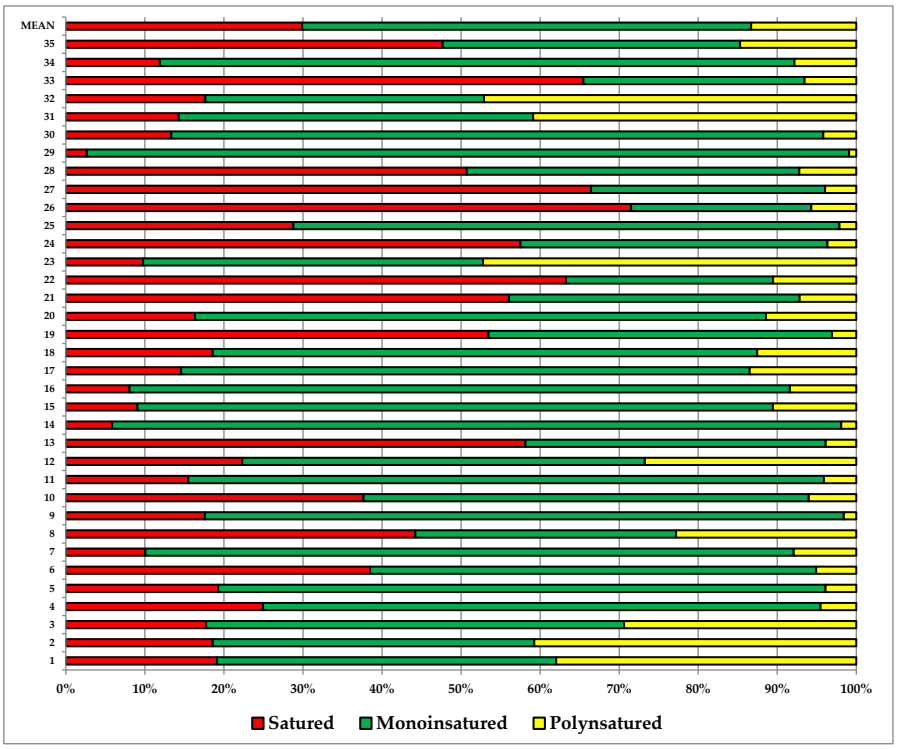

Figure 3. Saturated, mono and polynsatured fatty acids in gluten free samples.

In the analyzed samples, the mean percentage of the total lipids was 12.6% The highest contents of fats were found in the samples n°26 (Wafer biscuit) (25%) and n°28 (Muffin) (23%) while the lowest in the sample n°3 (Flour mix for bread) (1.2%).

Saturated fatty acids were detected in all analyzed gluten free food and the percentages ranged from 2.8 to 72%. The highest contents were found in the samples n°26 (Wafer biscuit) (72%), n°27 (Easter cake) (66%) and in the rice, corn and red fruit flakes sample (n°22) (63%), while the lowest (2.8%) in a Biscuit sample (n°29).

Palmitic acid (C16:0), the most prevalent SFA in the human diet, was noticed measured as the major component and high amounts of this fatty acid were detected in all samples. The highest content (51%) was found by us in the sample n°13 (Rosemary crackers) while the lowest (1.3%) in the sample n°29 (Biscuits n).

In the analyzed gluten free samples, monounsaturated fatty acids ranged from 23 to 96%. The highest content was found in the sample n°29 (Biscuits n), while the lowest in the Wafer biscuits (sample n°26). Oleic acid is the quantitatively most important representative fatty acid in the studied samples in the diet and in tissue lipids, in fact, in several samples, monounsaturated fatty acids are constituted of oleic acid, in the samples n° 26 (Wafer) and n°29 (Biscuits n) respectively ranged from 23% to 97%.

In the analyzed gluten free food samples, other monounsaturated fatty acids, such as palmitoleic and eicosenoic, are present in lower amounts, in particular, they ranged from 0.11% (n°17 Apricot snacks) to 7.4% (n° 8 Mini breadsticks d) and from 0.08% (n°32 Nougat) to 2.1% (n° 8 Mini breadsticks) respectively.

The primary n-6 polyunsaturated fatty acids detected in our samples were linoleic acid, eicosatrienoic and arachidonic acids. Linoleic acid in gluten free food analyzed by us ranged from 0.92 (n° 29 Biscuits n) to 47.2% (n° 23 Lemon biscuits).

Arachidonic acid in gluten free analyzed food was found only in the Easter cake (n°27) and muffin (n°28) samples at very low percentages (0.21 and 0.27% respectively).

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The only n-3 polyunsaturated fatty acid detected (at trace levels) in our samples was the docosahexaenoic acid. Traces of omega 3 were found only in three gluten free samples (n°24, 25, 27), while omega 6 are contained in all samples with percentages ranging from 1.2 % to 48%.

Odd-chain fatty acids have been identified in 29 gluten free samples with a mean value of 0.76% and the percentages ranged from 0.02% (n°16 corn and chinoa crackers) to 1.8% (n°28 chocolate muffin). Trans fatty acids were absent in all the analyzed gluten free food.

4. Discussion

Adequate quantity of fat in the diet is indispensable for health. In addition to their contribution to energy needs, quantity of dietary fat must be sufficient to furnish the essential fatty acids and in the absorption of fat-soluble dietary components such as some vitamins. The minimum quantities to ensure human health varies throughout a person's life and among individuals. For example, adequate intake of dietary fat is particularly important prior to and during pregnancy and lactation.

With regard to the total fat content in food, the National Institute of Health [13] proposes RDA values only (31 and 30 g/d) for children between 0 and 6 months and 6 and 12 months. This RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a group. Food and Agriculture Organization of the United Nations (Fao) [14] provides recommended intakes of nutrients and *safe levels of intake*, which apply to groups of persons and not to individuals (Table 3). In particular In detail, they pertain to healthy, not diseased people. Based on present knowledge, the reported values are designed to recommend intakes of fat that will maintain health, prevent deficiency diseases and allow adequate stores in normal circumstances. The data for children are for amounts of fat that allow proper growth, and those for women of child-bearing age take into account their special needs, including those of pregnancy and lactation.

Sex and age group	Weight (kg)	Energy (Kcal)	*Fat (g)	Quantity of gluten-free food to take the requirement amount of fat
		Children		
6-12 months	8.5	950	-	-
1-3 years	11.5	1350	23-32	182
3-5 years	15.5	1600	27-62	214
5-7 years	19	1820	30-71	238
7-10 years	25	1900	32-74	254
		Boys		
10-12 years	32.5	2120	35-82	278
12-14 years	41	2250	38-88	302
14-16 years	52.5	2650	44-103	349
16-18 years	61.5	2770	46-108	365
		Girls		
10-12 years	33.5	1905	32-74	254
12-14 years	42	1955	33-76	262
14-16 years	49.5	2030	34-79	270
16-18 years	52.5	2060	34-80	270
		Men active		

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18-60 years	63	2895	48-113	381
> 60 years	63	2020	34-79	270
		Women active		
Not pregnancy or lactating	55	2210	37-86	294
Pregnancy	55	2410	40-94	317
Lactating	55	2710	45-105	357
> 60 years	55	1835	31-71	246

*Fat requirements were calculated at the recommended range of 15 to 35 percent of average energy requirements

Table 3 - Average individual energy requirements and safe levels of intake for fat

Considering the mean of fat content in gluten-free foods analyzed by us in the present study (12.6%), to take intake the request amount suggested by the FAO (Table 3), it would be essential to consume from 182 to 365 grams of gluten free food.

The total fat content of gluten-free flours is similar to that of traditional flours (flour 0, flour 00, rise flour, corn flour, etc) (R_{flour0}, $R_{flour00}$, R_{rice}, R_{corn},) [15,16] (Tables 1 and 2).

The gluten-free bread handcrafted produced in Palermo (sample n° 4), in terms of total fat content (3.7%), is similar to those reported in the literature ($R_{bread\ a}$, $R_{bread\ b}$) (2.1-3.5%) [15,16] but, compared to industrially produced bread, contains higher percentage of oleic acid, (20%) and (71%) respectively. compared to industrially produced bread, On another hand, sample n ° 4 also contains a lower content of linoleic acid (4.5%) (C18:2) (Figure 4). Oleic acid is considered to be responsible for lowering the LDL-cholesterol levels in the blood. Moreover, oleic acid present in foods has preventive effects on several chronic diseases (cardiovascular diseases, cancer or age related cognitive decline). Like other fatty acids, monounsaturated ones are almost completely absorbed from the intestine and are oxidized (for energy production), converted into other fatty acids, or incorporated into tissue lipids.

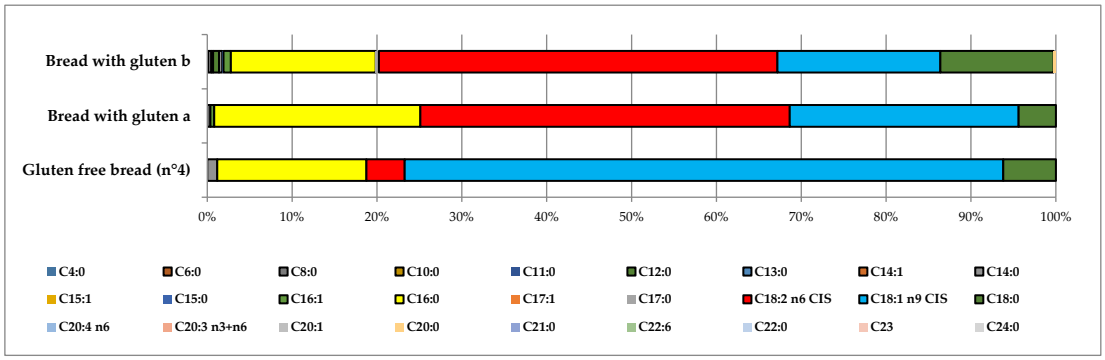

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Figure 4. Comparison between fatty acids distribution of gluten free bread and of two bread containing gluten.

The sample n°13 (Rosemary crackers) differs from all the others gluten free samples by the greater percentage (51%) of palmitic acid (Figure 5). Although, it has been produced by a multinational company, it can probably be assumed that, palm or kernel oil has been used.

Significant Several relevant differences have been highlighted in the composition of gluten-free Wafers (sample n°26), in detail particular, the total fat content (25%) is greater than that of traditional wafers (Table 1) (R_w) [15,16]. A similar situation was observed for the Muffin sample (sample n° 28). Furthermore, in gluten-free wafers, dodecanoic and myristic acids are 21% and 13% respectively,

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significantly higher than those of the other foods studied. These high percentages can be attributed to the use of palm kernel oil, a product very available on the market and of low commercial value.

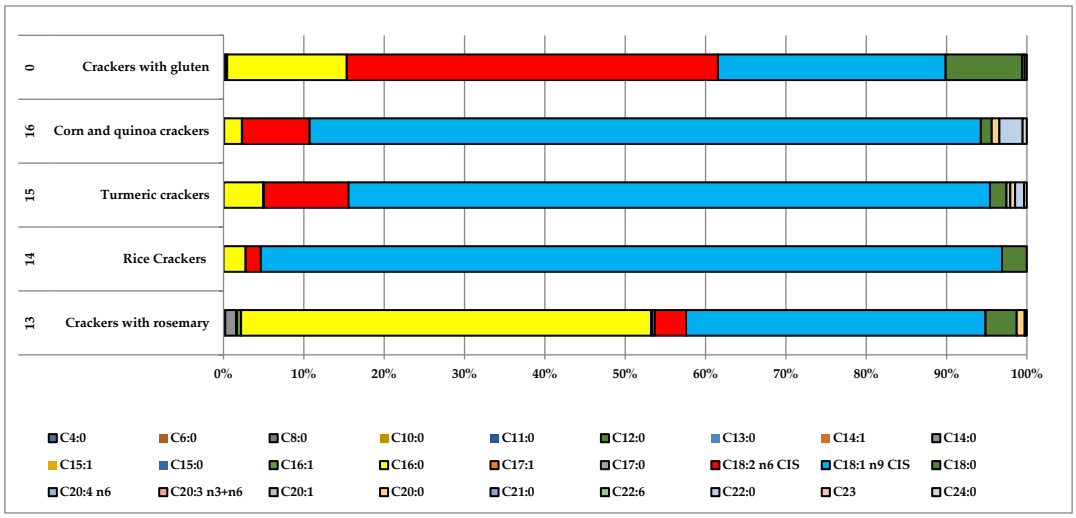

Figure 5. Comparison between the fatty acids distribution in the gluten free crackers and with gluten ones.

Arachidonic and eicosapentaenoic acids (the later not found in the analyzed samples) can be further transformed to eicosanoids, a group of biologically active components including prostaglandins, prostacyclins and leukotrienes, which are very important in the regulation of blood pressure, renal function, blood coagulation, inflammatory and immunological reactions and other functions in tissues [17].

Omega-3 fatty acids (α-linolenic acid) have anti-inflammatory properties and therefore may be useful in the management of inflammatory and autoimmune diseases [18]. The scientific results concerning the benefits deriving from the intake of omega-3 rich foods are discordant; a 2013 study states that in patients with multiple cardiovascular risk factors, daily treatment with n-3 fatty acids did not reduce cardiovascular mortality and morbidity [19]. In the Western diet, the omega-6/omega 3 fatty acid ratio has been determined to range from 15/1 to 16.7/1 [19,20].

Simopoulos [20,21] indicates that people evolved on a diet with a ratio of omega-6/omega 3 essential fatty acids of ~1 whereas in Western diets the ratio is 15/1 to 16.7/1. An high omega-6/omega 3 ratio, as in our case and in the Western diets, could promote the pathogenesis of many diseases, including cardiovascular disease, cancer, osteoporosis, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 polyunsaturated fatty exert suppressive effects.

The absence of trans fatty acids in gluten free analyzed samples implies that, on used fat, bacterial transformation of unsaturated fatty acids (in the rumen of ruminant animals), industrial hydrogenation, deodorization of unsaturated vegetable oils, heating of oils at temperatures higher 220°C [17], were not carried out.

The EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) [17] affirms that precise quantities of fatty acids intake cannot be indicated, however it is known that, a fat intake lower < E 35% (as energy) produces a reduced energy intake and therefore low weight reduction and/or prevention of weight increase. Based on practical considerations (e.g. current levels of intake, achievable dietary patterns), the EFSA Panel concludes that there are not sufficient available data to define a Lower Threshold Intake (LTI) or Tolerable Upper Intake Level (UL) for total fat intake, but only a Reference Intake range can be established. In European countries, no overt signs of deficiencies nor undesirable effects on blood lipids have been observed for intakes of total fats lower E 20 % while intakes > E 35 % may be compatible with both good health and normal body weight depending on

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dietary patterns and the level of physical activity. EFSA establish to set for adults a lower bound of the Reference Intake range of 20 E% and an upper bound of 35 E%. Energy intakes from total fatty acids of the analyzed samples were calculated as reported in equation 1.

$$E\ (\%) = (L_{total} \cdot 9 \cdot 100)/CAL \qquad (1)$$

where L_{total} represents the fat content (g/100) determined by us in gluten-free food and CAL (Kcal) the energy supply of the food sample (obtained from the nutritional data provided in the package) respectively. Energy intake levels for celiac people were estimated for total lipids and for saturated fatty acids (Figure 4) consuming daily amount of 100 g day-1 of gluten-free food.

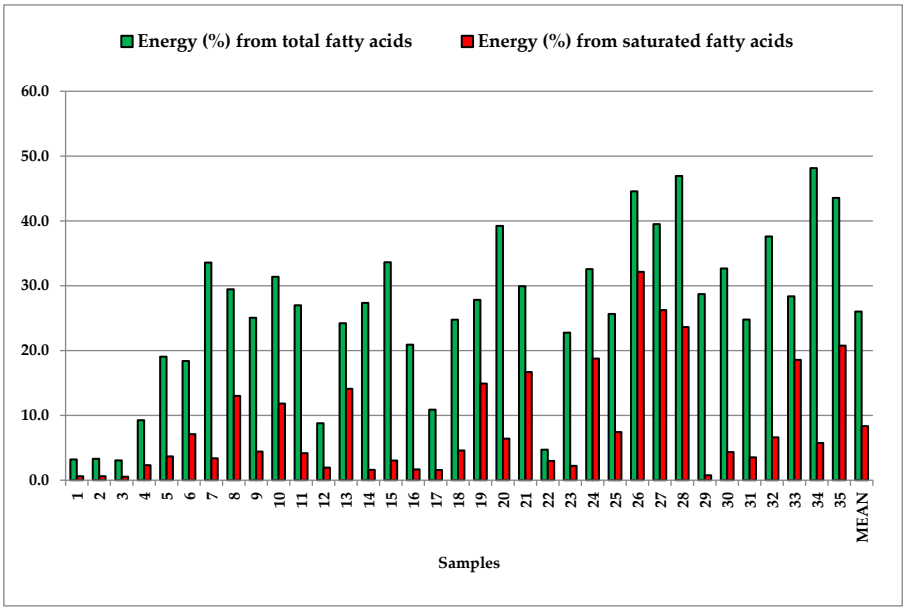

Figure 6. Energy (%) from lipids eating 100 g of gluten free samples.

In our case (Figure 6), total lipids supply energy from 3.1 (n°3, flour mix for bread) to 48 % of energy (n°34, biscuits), while saturated fatty acids from 0.55 (n°3, flour mix for bread) to 32% (n°26 Chocolate wafers). The results revealed that, meanly, monounsaturated fatty acids (MUFAs) constituted the majority (57%) of the fatty acids pool followed by saturated fatty acids (SFAs) (30%) and polyunsaturated fatty acid (13%).

Saturated fatty acids of analyzed samples supply energy from 0.55% (n°3, mix flour) to 32% of energy (n°26, chocolate wafers). Based on EFSA [17] considerations, intakes of saturated fatty acids should provide no more than 10% of energy. Only fifteen, of the thirty-five gluten free samples analyzed, provide adequate energy intake, while in eleven samples, saturated fatty acids take more energy than that recommended by EFSA.

Linoleic acid is essential in the diet because cannot be synthesized by humans and a its deficiency results in unpleasant clinical symptoms, including a scaly rash and reduced growth, also is the precursor to arachidonic acid, which is the substrate for eicosanoid production in tissues. An Acceptable Macronutrient Distribution Ranges for linoleic acid is 0.6 -1.2 percent of energy [17]. In our case, the energy obtained from the consumption of linoleic acid, present in gluten-free foods, varies from 0.92 (sample n° 29 biscuits) to 47% (sample n° 32 Quinoa cake). The last percentage corresponds to the highest linoleic acid intakes from foods (no gluten free) consumed by people in the United States and Canada [17]. High intakes of linoleic acid may constitute a protection against coronary heart diseases.

From a nutrition point of view, MUFAs have received growing attention because of their diverse effects on human health. Recent researches tends to indicate more beneficial effects, in particular, on reducing risk of cardiovascular diseases and other inflammation related diseases [22].

5. Conclusions

This paper is the first analytical approach to the study of 37 fatty acids in 35 different gluten-free foods for celiac people produced in Italy. Only the GC-MS technique was used to investigate the fatty acids composition. One of the advantages about the use of GC-MS for this characterization is the high sensitivity that improved the limits of quantification levels for analita that are presents at low levels in some samples. The data indicate a considerable variability between samples with respect to single fatty acid percentages, which could be due to the proportion of different ingredients used in the food compositions.

From the analytical data, we can conclude that only about the 40% of samples taken in account in this study, provide adequate energy intake, and in several samples, saturated fatty acids take more energy than that recommended by EFSA.

Fortunately, from the nutritional point of view, celiac patients also feed themselves with foods of vegetable or animal nature (fresh) that compensate for any imbalances resulting from the use of bakery products of industrial origin.

It has emerged that local producers generally use the finest raw materials (olive oil, etc.) compared to the industries which, as has been pointed out, in many cases use palm and palm kernel oils although gluten free commercial products are high added value foods, expensive and intended for a particularly sensitive public..

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