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NUTRITIVE VALUE AND AFLATOXIN CONTAMINATION OF GLUTEN-FREE FLOURS

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Abstract: There is a growing interest in making versatile gluten-free (GF) foods not only for patients, but also for health-conscious individuals who choose to keep a gluten-free diet. We aimed to investigate gluten –free flours from natural sources and from minimal processing through comparative analyses of the nutrition facts from the labels, and the determination of total aflatoxin contamination levels. Of the 49 flour samples, 6 types were from gluten-free cereals, 5 types from pseudo-cereals, and 15 types were oilseed press cake powder. Almond flour, grape skin powder and GF oat bran. Aflatoxin was determined using Neogen Q+ for Aflatoxin procedure after sample extraction with aqueous ethanol solution. We found significant differences regarding the fat, dietary fiber and protein content, the energy content did not differ significantly and was in the range 290-360 kcal/100 g. With the exception of walnut press cake powder, fatty cocoa powder and quinoa flour, all the samples were qualified as safe ingredient as their aflatoxin content did not exceed the official limit. The studied gluten-free flours are safe and nutrient rich materials for gluten-free diet.

Keywords: aflatoxin, oil seed press flour, gluten-free flour, pressing residue, nutrient

1. Introduction

Consumers in Europe and in the USA are becoming more health conscious, seeking alternative dietary options that align with their wellness goals. Gluten free flours have gained popularity for its perceived nutritional benefits (http1, http2). The gluten-free bread category is the primary driver of the GF segment and its market is predicted to grow because to increased consumer awareness of healthy eating options such as natural, organic, and gluten-free products. (http2) For consumers suffering certain form of gluten related diseases, gluten-free diet is the only option, a cornerstone of their treatment (Taraghikhah et al. 2020).

Many GF flours (pseudocereals, oil seed, and their pressing residues) are rich in nutrients, vitamins, and minerals, making them an attractive choice for those seeking the well rounded diet. (http1, Ramachandran et al. 2007, Zhang et al. 2023), therefore these flours are applied to enrich GF bakery products based on starch mixture. The incorporation of pseudo-cereals (i.e., amaranth, quinoa, buckwheat) into GF formulation results in higher antioxidant capacity and total phenolic content compared to the control (Hayta & İşçimen 2019). In animal feeding trial, grape seed meal diminished the harmful effect of aflatoxin B1 (liver oxidative stress and liver histological damage) caused in piglets (Taranu et al. 2023). Aflatoxin is a carcinogenic mycotoxin produced on fields and during post harvest processing and storage, at a minimum 15°C and lower moisture content (aw: 0,84) by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins considered one of the most possible human carcinogens (Spanjer, 2019). The safety of GF flours is crucial for the further processing (Salvatore et al. 2024). Regarding the oil seeds, majority of the mycotoxins in contaminated oil seeds might get partitioned into the seed cake rather than directly into the oil, of that limited information exist (Bhat & Reddy 2017).

We aimed to study the safety and the quality of GF flours based on the total aflatoxin content and the nutritive facts from the products' label, with special interest in oil seed press cake powders (PCPs). The results indicate that a wide range of natural flours and powders with different and rich nutrient content can be applied to prepare healthy and safe gluten free meals.

2. Materials and methods

2.1. Materials: Samples and Reagents

Of the total 49 GF flour samples, 55% were fine powders from oilseed press cakes (PCP), 37% grain flour (GrF) (cereal, pseudo-cereal, legume), and 8% were other grits (OF) (almond flour) and by-products (oat bran, grape skin flour). The oilseed press cake powder samples were (28 pcs): 1 hazelnut flour, 3 brown flaxseed flour, 1 yellow linseed flour, 2 sea buckthorn seed flour, 2 milk thistle seed flour, 2 rosehip seed flour, 4 pumpkin seed flour, 1 walnut press flour, 2 white sesame seed flour, 2 coconut flour, 1 fatty cocoa powder, 1 hemp seed protein powder. The gluten-free grain samples were (17 pcs): 3 brown rice flour, 3 oatmeal flour, 1 white rice flour, 1 corn flour, 1 sorghum flour, 1 millet flour, 1 amaranth flour, 2 buckwheat flour, 2 quinoa grain, 2 chickpea flour. Other meals (4 samples) included 2 almond flour samples, 1 red grapeskin flour and 1 gluten-free oat bran. Oat bran and quinoa samples were ground into fine flour with a GM 300 ball mill (Retsch) in the laboratory. The samples were purchased from local market.

Reagent grade ethanol (VWR) was used in the aflatoxin measurement. The aflatoxin kit was purchased from Neogen Hungary Ltd. The study was achieved in 2023, in Agricultural and Molecular Research and Service Institute, University of Nyíregyháza.

2.2. Procedure for Aflatoxin Quantification

Aflatoxin concentrations were determined using Reveal Q+ for Aflatoxin kit (Product No. 8085) and Raptor integrated Analysis Platform (Neogen Product No. 9680). This method is valid mainly for cereals, pseudo-cereals, some bran types and oilseeds (65% ethanol extraction), and of the oilseed press cake types, only for peanut pellets (75% ethanol extraction), therefore oilseed press cake powders were extracted

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with 75% aqueous ethanol. The method in brief: 10 g of sample was extracted with 50 ml aqueous ethanol (ratio 1:5) by vigorous manual shaking for 3 minutes and then filtered. 100 µl of the extract and 500 µl of diluent was mixed thoroughly mixing pipette. A cartrigde with 2 test strips were placed into the Raptor apparatus. Then 400 µl of the diluted extract was pipetted into the cartrigde and after a 5-minute sample incubation at 30 °C the measured aflatoxin concentrations were displayed on screen in ppb (µg/kg). The method's threshold is 3-100 µg/kg aflatoxin.

2.3. Data collecting from labels

The nutrition information were collected from the products' label, namely energy content (kcal/100 g), total fat (%), saturated fat (%), total carbohydrates (%), sugar (%) protein (%), dietary fiber (%), salt (%).

2.4. Data analysis

The aflatoxin content per sample was obtained from two parallel measurements from the same extraction. The data was analyzed through descriptives, Anova, and multiple comparision by Scheffle at 95% confidence interval for means, using SPSS ver.20 statistical program.

3. Results

3.1. Aflatoxin contamination in gluten-free flours

The aflatoxin levels of Press Cake Powders were grouped by oilseed and analyzed (results not shown in *Tables*). Of the 27 PCP samples, 3 (11%) were non-compliant, found to be unsafe food. The highest level of aflatoxin was found in cocoa powder (average: 29.5 µg/kg) that is 7-fold limit exceed, and in the walnut PCP samples with an average of 5 µg/kg. Of the studied grain flours, a quinoa grain sample's' aflatoxin level (4.7 µg/kg) exceeded the official limit (4 µg/kg). These products were within their best before time and required to be withdrawed or recalled.

Table 1.: Descriptition of GF flours' aflatoxin content (µg/kg) grouped by type.

	N	Mean	Std. dev.	Std. error	Lower bound	Upper bound	Min.	Max.
PCP	54	3.19	5.29	0.72	1,75	4.64	0.57	31.00
GrF	36	2.14	0.64	0.11	1.92	2.36	1.30	4.70
OF	8	1.66	0.42	0.15	1.31	2.01	1.20	2.30
total	98	2.68	3.97	0.40	1.88	3.48	0.57	31.00

PCP: oilseed press cake powder, GrF: gluten-free grain flours, OF: other flours. Source: Authors' own editing based on their own results (2024)

A poppy seed, and a brown linseed PCP samples had an increased aflatoxin level, an average 3.3 and 3.6 μg/kg. Other PCP samples' aflatoxin contaminations were in the range of 0.57 and 3.5 μg/kg, below the limit and were compliant. 11% of the aflatoxin data exceeded 3 μg/kg aflatoxin levels.

Grouping the samples by flour type, those were oil pressing residues, and starchy flours and other flours, we stated the decreasing order of aflatoxin hazard in gluten-free flours is: PCPs > gluten-free grain flours > other flours (almond flour and skin flours), while there is no significant differences between groups (p>0.355, see *Table 1* and *Table 2*.).

Table 2.: ANOVA analysis of GF flours on aflatoxin content (μg/kg) grouped by flour type, p<0,05. Authors' own editing based on their own results (2024)

ANOVA analysis	sum of squares	df	mean square	F (Sig.)
between groups	33.094	2	16.547	1.048 (0.355)
within groups	1499.46	95	15.784	
total	1532.557	97		

3.2. Nutritive value of gluten-free flours based on product label

The energy and nutrient content of the different types of gluten-free flours are shown in *Table 3.* There were no significant differences regarding the level of energy (p=0.114), the saturated fatty acid (p=0.120) and the sugar (p=0.088) of GF flours from different sources. The Anova analysis validated significant difference of the 3-kind flours in all the main nutrient groups: total fat (p=0.001), total carbohydrate (p=0.000), protein (p=0.000) and dietary fiber (p=0.001).

Table 3.: Nutritive Value of Gluten-Free Flours by Type and by Component

	Oil Seed Press Cake Powders	Gluten-Free Grain Flours	Other Flours
N	28	17	2
Energy (kcal/100g)	312.1±57.7a	352.3±18.3a	298.5±87.0a
Total Fat (%)	10.4±4.8b	3.9±2.1a	5.9±1.0a
Saturated Fat (%)	3.0±3.4a	0.7±0.5a	1.3±0.2a
Total Carbohydrate (%)	11.5±14.0a	65.4±9.8c	26.7±35.9b
Sugar (%)	5.4±5.0a	1.6±1.7a	1.8±1.4a
Protein (%)	34.0±15.8b	11.3±3.2a	14.2±4.0a
Dietary Fiber (%)	31.2±13.7b	6.6±4.4a	41.6±41.8b
Salt (%)	0.51±1.14	0.02±0.02	0.18

Other Flours includes solely data of oat bran and grape skin powder. The mean difference is significant at the 0.05 level. The significant difference is marked with different letters within row.
Source: Authors' own editing based on their own results (2024)

The press cake powders from oil pressing are the highest in fat (10%), abundant in protein (34%), and dietary fiber content (31%), but low in total carbohydrates (11%). The oat bran and the grape skin powder (other type flours) have high dietary fiber (41%), low fat content (~6%), while their protein level (14%) is comparable with the cereals one. Among the samples, we found extreme salt content in some cases specifically pumpkin seed PCP (4% salt) and white sesame seed PCP (7,4% salt level).

4. Discussion

4.1. Aflatoxin contamination in gluten-free flours

Based on safety parameters, compliance of food is crucial to be marketed for households or food industry. With the exception of the walnut and cocoa PCPs and quinoa flour, all the studied gluten-free flours or powders can be regarded as safe material. Our results from the inappropriate walnut PCP samples were higher, while hazelnut PCP samples' aflatoxin level was much lower (2.2 µg/kg) contaminated than that Golge et al. (2016) found in whole walnut (2.97 µg/kg) and in full fat hazelnut (13.8 µg/kg). As aflatoxin and ochratoxin-A remains bound in the press cake (e.g. cocoa press cake), a higher level of non-fat cocoa solids tend to be concentration of higher level of such mycotoxins. Furthermore damaged pods, poor fermentation, slow drying, poor storage increases the aflatoxin level in cocoa beans, then the secondary processing technology decreases these contaminants' level (Copetti et al. 2014). We can conclude that, in some cases, the oilseed raw material could have already been contaminated with aflatoxin, and together with inadequate and prolonged storage time may result in a much higher aflatoxin contamination, above the official limit. This emphasizes the need to monitor aflatoxins in PCPs. Through the food chain, all the stakeholders must apply consequently the good manufacturing practices to prevent losses and to market healthy, safe food ingredients.

4.2. Nutritive value of gluten-free flours

Due to the high price of gluten-free product, to the access of increasing variety of novel food ingredient and to health-conscious nutrition, the home-made gluten free baking is popular activity and spreads. Many home-made and healthy gluten free recipes, techniques are shared on internet. In the absence of in depth knowledge of the food industry, it is difficult work to prepare acceptable, tasty gluten free bakery products. The nutrient content of a gluten-free ingredient significantly affect the technological properties of the mixture and the final quality of the baked product. The energy content of the gluten free flours did not differ significantly, that means 326 kcal/100 g. The PCPs are complex, fiber and protein rich materials, with a relatively high poly-unsaturated fatty acid addition potential. This latter component group susceptible to rancidity, therefore the good manufacturing practice has the importance to save them from chemical and microbiological deterioration. Gluten free skin flours: oat bran, grape skin flour (grouped as other flours) are fiber, minerals, bioactive, antioxidant component sources, and comparing with cereals they have moderate protein content, and a relatively low carbohydrate content. The PCPs' technological and biological properties might be enhanced using texturization by extrusion, as reported Bhise et al. (2015) on hulled sunflower press cake's study. With these gluten free powders and flours broad varieties of nutrient rich meals can be prepared, and processed into high value added product. However they as minimally processed food materials also have high value.

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