

Analysis of risk and consumers' awareness regarding the gluten content in meat products on the example of frankfurter type sausages

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RESEARCH ARTICLE

Abstract

The long life gluten-free diet is the most effective method of preventing symptoms in celiac disease. Thus, awareness of 160 patients suffering from the disorder was examined, as a crucial parameter for their health-safety. The adherence to the gluten-free diet did not depend on their education level. 88.1% of patients followed the diet strictly, 94.4% controlled the products labels and 38.1% were afraid to consume unknown food. Over 80% of respondents expected gluten in meat products. Therefore, the presence of gluten in the frankfurter sausages not labelled either as containing gluten or as gluten-free was controlled. The analysed product was classified as gluten-free (14.3 mg/kg), despite the use of food additives of wheat origin during production. Thus, gluten content control and proper products labelling give benefits both to the producers (widening consumers' group) and to the celiac patients (diversity of diet composition).

Keywords: celiac disease, consumer's concerns, ELISA, gluten-free sausages, producer's concerns

1. Introduction

Celiac disease (CD) is a chronic systemic autoimmune disorder inducing enteropathy of the small intestine, occurring in genetically predisposed individuals. CD can be life-threatening, it increases the risk of malignancy and lymphoma, osteoporosis and anaemia compared to the general population (Cizkova and Cervinkova, 2015; Kochhar *et al.*, 2016; Lambert and Ficken, 2016). Recent epidemiological studies show that a prevalence of the CD increases, and reaches the level of up to 1:100 individuals in many countries (West *et al.*, 2014). Moreover, gluten proteins cause also other diseases: allergy (wheat allergy), autoimmune diseases (dermatitis herpetiformis, and gluten ataxia), as well as a possible immune-mediated disease (gluten sensitivity) (Cizkova and Cervinkova, 2015).

Simultaneously, the only effective method of prevention of the CD symptoms is a life-long gluten-free diet (GFD) (Kohout, 2014). This diet is very restrictive and sometimes

difficult to balance. A variety of products obtained from gluten-free cereals have a small nutritional value. In case of low nutritional knowledge such a diet may be monotonous and lead to malnutrition (Bascuñán *et al.*, 2017; Scanlon and Murray, 2011).

Thus, many patients suffer from deficiency of important food ingredients. Gluten exclusion from the food consumed results in a limited supply of plant proteins. Homogenised meat products could significantly diversify their diet with valuable and easily digestible protein. Meat is also an important source of heme-iron. Thus, a decreased amount of meat products in the GFD may affect other symptoms associated with CD, such as iron deficiency anaemia (Cizkova and Cervinkova, 2015).

It should not be a surprise that both the sick individuals and food producers are increasingly interested in extending the list of food products offered to consumers with CD. Even if these patients are generally aware of their dietetic

problems, it is natural that both in periods of well-being and when fatigued with the restrictive nutrition model, they are looking for some new types of food. Social isolation affects the adherence to GFD; it was proved that children adherence to the diet is worse when they participate in parties or other events compared to when they are at school or home (Bacigalupe and Plocha, 2015; MacCulloch and Rashid, 2014).

The enrichment of the GFD with different meat products may increase the protein and iron supply while diversifying the food consumed. Thus, the aim of the study was to estimate the patients' awareness and to confront it with the results of analyses conducted on the frankfurter-type sausages produced in the manufacturer's assumption but not labelled as a gluten-free product. These sausages were selected as tested material, because this type of meat product has a high consumer acceptance, and is an example of often consumed, finely minced meat products.

2. Materials and methods

Questionnaire survey

Individuals participating in the survey

All patients (n=160) >18 years of age with CD confirmed by a physician, living in Poland (Wielkopolska region) were surveyed using a personal questionnaire in November 2017. Women accounted for 85.6% of the respondents (Table 1). To investigate the impact of education level on adherence to GFD and other answers to survey questions, respondents were divided into two groups: with high (HEL; people with bachelor`s or master`s degrees) and lower (LEL) education level. Most respondents (53.1%) were aged 26-45 years.

Interview questionnaire

The questionnaire was created in the Department of Gastronomy Science and Functional Foods, Poznań University of Life Sciences. It was pretested individually by 19 celiac patients diagnosed within 4 years of the survey conducted in presence of the investigator. The survey took

the form of an interview questionnaire with patients waiting for a medical appointment in the Gastroenterology Clinic. The questionnaire was anonymous and voluntary, did not interfere with the patient's other duties, ensured freedom of expression and comfort. The interviewer explained all the doubts without delay.

Even if the questionnaire included some additional questions, mainly regarding problems of medical care, only those significant for the presented work have been discussed here.

Food testing

Meat samples

Gluten content in sausage stuffing was analysed in the samples obtained from the three stages of the frankfurters production, i.e. 1 = minced meat; 2 = minced meat with different functional additives; 3 = heat-treated, ready-to-eat sausages (without casing). The material was obtained from industrial production of a Polish company. The producer also provided food additives for the analysis (soy protein isolates: Pro-Fam 648 and Kawiks, fine crystal glucose, and wheat fibre Vitacel WF 600R). The presence of gluten in the final product was not declared. Composition of material provided for analysis, according to the label of bulk container, is presented in Table 2.

Table 2. Composition of the studied product, declared on the label of bulk container.

Product composition	%
Mechanically deboned meat	0
Protein	12
Fat	2
Water	60
Food additives:	
Soy protein	1.5
Glucose	1
Wheat fibre	1

Table 1. Characteristic of respondents taking part in the research (n=160).

		Patients with higher education level	Patients with lower education level	Both groups together
Number		80	80	160
Sex [%]	Women	87.5	83.8	85.6
	Men	12.5	16.2	14.4
Age [%]	18-25	21.3	43.8	32.5
	26-45	66.2	40.0	53.1
	46-65	12.5	16.2	14.4

Sample preparation

Two grams of meat samples obtained from the manufacturer were extracted with 10 ml of cocktail solution (CS) (based on 2-mercaptoethanol and guanidine hydrochloride, patent WO 02/092633 A1) Obtained extracts were analysed with ELISA procedure.

ELISA test

Sandwich immunoassay RIDASCREEN® Gliadin (R-Biopharm, Darmstadt, Germany) ELISA kit based on R5-Mendez method was used for the detection of gliadin content in the obtained samples.

The concentration of gliadin in the analysed samples was calculated from the equation derived from the standard curve, designated on the basis of used standard absorbance. Then, the result was doubled in order to express the concentration of gluten, and recalculated for mg/kg (if necessary).

Statistical analysis

A chi-squared test ($P < 0.05$) was used (Statistica 10.0) for the examination of the influence of education level on respondents' awareness.

Meat samples were collected five times and tested in triplicates. One-way analysis of variance and post-hoc student's tests for the significance level $P < 0.05$ were carried out using Statistica 10.0.

3. Results and discussion

In the questionnaire, adherence to dietary regimens was defined as a discrete variable i.e. strict ('less than one serving of gluten per week'), partial, and non-adherent. Both groups (HEL and LEL patients) have shown strict adherence, independently of education level, to GFD at over than 87% (Table 3). Hall *et al.* (2013) have been reported that compliance to the GFD ranges between 36 and 96% and it is associated with a variety of demographic, psychological and clinical factors. Knowledge of the disease and gluten content of food, as well as the time elapsed from the diagnosis of CD to acceptance of the need to control the safety of food, may be also essential factors for dietary compliance.

According to the chi-square test the influence of education level on compliance with GFD was not found ($P = 0.59$). It is noteworthy that a very low percentage of respondents did not comply with GFD (no one from the HEL and 1.2% from LEL groups). The data are consistent with information published by Hall *et al.* (2009). No correlations between compliance with GFD and educational status were observed either by Cassellas *et al.* (2006) or Leffler *et al.* (2008).

As the literature data show, GFD significantly improves health-related quality of life in adults with coeliac disease (Arias-Gastelum *et al.*, 2018; Burger *et al.*, 2017).

Therefore we asked people who did not comply fully or partially with GFD about the main reasons for possible exceptions to the GFD rules (Figure 1). The vast majority (96.3%) claimed that the mainspring is, as it is usually noted in the literature (Arias-Gastelum *et al.*, 2018), a lack of access to proven gluten-free products. A minor group of respondents indicated the desire to eat the products, and other reasons. The significance of the impact of education level on the choice of selected answers is presented over the columns in Figure 1. Respondents with LEL more often than with HEL chose gluten products because of the desire to eat them ($P = 0.00$) or other reasons ($P = 0.00$), e.g. 'someone persuaded me', 'I did not know that there is gluten in it' or 'I cannot justify it'. However, the overall impact of education on all respondents' answers was estimated as significant ($P = 0.00$). Certainly, a question arises whether it could be the consequence of the attitude of the respondents, who are tired of maintaining compliance with GFD in everyday life. Adherence to dietary recommendations is seen as a nuisance and perhaps not all deal with it (Leffler *et al.*, 2008). On the other hand, access to gluten-free products is really difficult. According to Rajpoot *et al.* (2015) and Arias-Gastelum *et al.* (2018) the most common barrier to adherence was non-availability of GF products. Furthermore, many processed foods are contaminated with gluten, staple GF products are

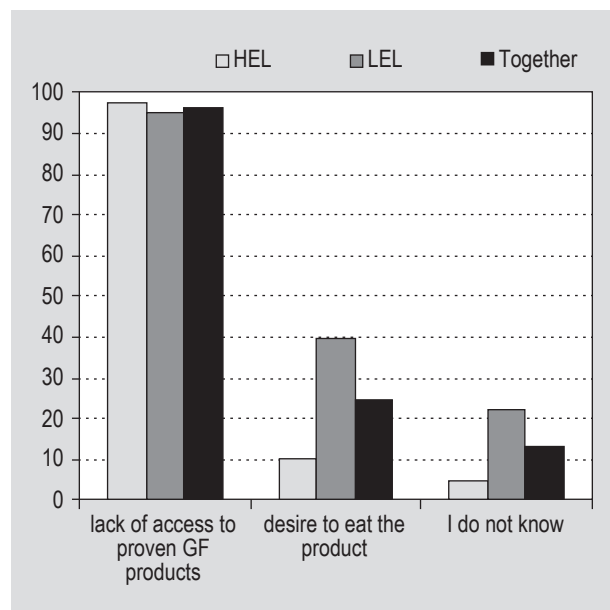


Figure 1. The most common reasons for deviations from gluten-free diet (GFD) declared by respondents (for 100% of adopted individuals who fully or partially do not follow a GFD). HEL = consumers with high education level, LEL = consumers with lower education level.

not widely available and the price of such foods is often high (Arias-Gastelum *et al.*, 2018; Ciacci *et al.*, 2015).

As MacCulloch and Rashid (2014) reported, good adherence is most strongly related to outcomes, because the cost of GF products was a substantial obstacle. The GFD negatively affected family finances for 70% of the participants of their study. Also, according to Welstead (2015), social or financial burdens along with inadvertent gluten ingestion, or cross-contamination, present obstacles to maintaining a GFD.

Label reading was also defined as a discrete variable. As it has been presented in Table 3, all respondents, independently of education level ($P=0.73$), declared to read labels: always (94.4%) or usually (5.6%). This shows patients' responsible approach to their illness and indicates consumers' awareness of the fact that the key to following a GFD is to be a good label reader. It has been reported that an average person with CD spends an extra 10-20 hours per month checking food labels for gluten content (Pietzak, 2005). Thus, food producers should be aware of how important it is to label products precisely as containing gluten or gluten-free.

In the European Union, you can observe an increase in the overall availability of gluten-free products. There are also favourable changes of the rules regarding declaration of the presence of allergens (including gluten) on packaged and unpackaged products (EC, 2011). Nevertheless, the offer of safe gluten-free products is still quite limited, and their price is still high (Arias-Gastelum *et al.*, 2018).

The respondents were also asked about any adverse reactions after consuming products that, according to the label, should not contain gluten. It turned out that almost one in seven of them experienced such a situation (20% of respondents with HEL and 11.3% with LEL). However, 13.7% of patients with HEL and 21.3% with LEL did not know if the symptoms observed were caused by the intake of hidden gluten. The impact of education on the type of answers was not statistically significant ($P=0.2$). According to the recommendation, all patients should avoid any possible sources of known and hidden gluten in their diet. Although there is a wide variation in the sensitivity among patients, the daily limit of gluten is probably around 10 mg, because such a dose of gluten is unlikely to cause significant histological abnormalities (Catassi *et al.*, 2007; Rajpoot and Makharia, 2013).

Therefore, especially in the case of patients strictly adhering to the diet, gluten present in the products resulting from contamination in the food production, not declared on the label, is particularly dangerous. It should be noted here that declaration of unintentional cross contact with gluten and other allergens is still optional, according to the still valid legislation (European Commission, 2011). Stating that food 'may contain traces of ...' depends on the producer's will. Based on our unpublished experience, we can confirm the presence of gluten in raw materials in which its presence was not expected. This concerned mainly mixes for baking gluten-free bread and corn flour, in which the content exceeded even the limit for gluten proteins (20 mg/kg). However, bread baked from these raw materials, due to the

Table 3. Respondents' answers to selected questions from the questionnaire and results of chi-square independence test (P). NS = not significant at the $\alpha=0.05$.

Question		% of answers			Chi-square independence test	
		Patients with higher education level	Patients with lower education level	Both groups together	P-value	Significance
How do you follow the GFD?	strictly	87.5	88.8	88.1	0.59	NS
	partially	12.5	10.0	11.3		
	I do not follow	0.0	1.2	0.6		
How often do you read the labels of products bought?	always	93.8	95	94.4	0.73	NS
	usually	6.2	5	5.6		
	rarely	0	0	0		
	never	0	0	0		
Do you observe adverse reactions after consuming products which (according to the declaration on the label) do not contain gluten?	yes	20	11.3	15.6	0.20	NS
	no	66.3	67.4	66.9		
	I do not know	13.7	21.3	17.5		
Are you afraid to consume new products?	yes	33.7	42.5	38.1	0.25	NS
	no	66.3	57.5	61.9		

addition of water, yeast and oil, remained gluten-free. The contamination resulted from grain milling processes. It is a proof that gluten risk management needs to be integrated with the overall food safety management approach. It should also be fully supported by a system framework that includes elements of good manufacturing practices (GMP) and HACCP. Cross contamination should be minimised by segregation, traffic control (raw material, packaging and employees), use of separate processing lines and equipment, a validated cleaning program and effective control of work in progress.

Investigations confirm that CD and associated adherence to a proper diet affect the nutritional status, as well as social interactions and the quality of life (Arias-Gastelum *et al.*, 2018; Bacigalupe and Plocha, 2015). One of the elements of life quality is sense of security. Therefore, the questionnaire included a question on the concerns of respondents associated with the consumption of new products (Table 3). More than 38% of respondents were afraid to consume new, unknown food products and education did not influence the type of answers significantly ($P=0.25$). It is understandable, but on the other hand – disturbing, because of the need to ensure a well-balanced diet for people suffering from CD. A necessary component of a valuable diet is its diversity. In the case of patients with CD, the range of products allowed in their diet is quite limited, and fears can narrow this choice even further.

An interesting question was: in which products did the respondents expect the presence of gluten (Figure 2). Participants could select responses given in the questionnaire, as well as add their own. Most of their indications concerned the breadcrumbs (>93%) and meat products (>73%). Gluten content in beer (>65%) was less obvious for the respondents, in spite of it being produced from gluten raw materials. Fewer than 30% of respondents were concerned about the presence of gluten in cheese. Thus, it can be expected that some of them consume cheese without controlling labels, while analogs and processed cheese may contain cereal proteins in their composition (Black and Orfila, 2011). The presence of gluten protein in these cheese is more common (data not published, presented as educational information for a celiac person) than in cottage cheese which was not expected as a safe product by 34% of the respondents. In these cases, the influence of the education level on respondents' suspicions regarding the presence of gluten in the products was not shown. The only exception were meat products; more respondents with higher education level assumed the possibility of the presence of gluten in them ($P=0.03$). The overall impact of education on all respondents' answers was estimated as not significant ($P=0.79$).

In fact, gluten raw materials are often introduced into meat products; however, it must be declared on the label. The

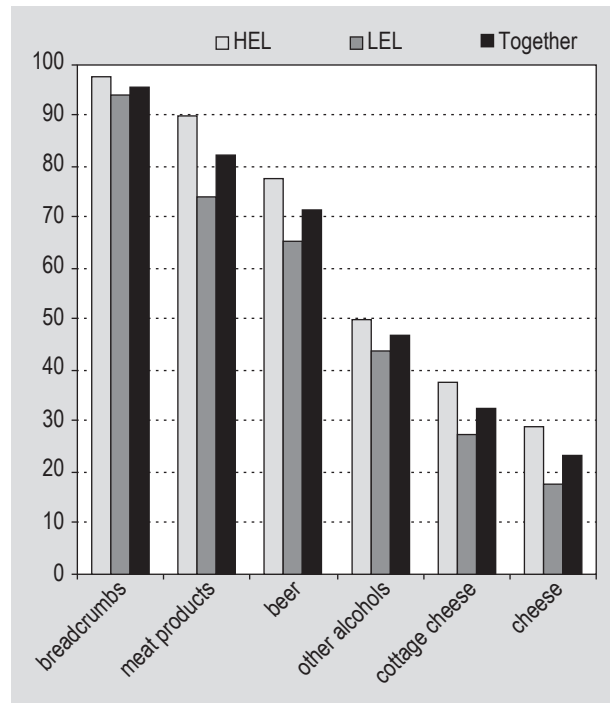


Figure 2. In what products are you expecting the presence of gluten? (respondent's answers). HEL = consumers with high education level, LEL = consumers with lower education level.

same rule should apply to possible cross-contamination that may occur during processing; however, currently this is not obligatory.

Consumers do not need to know when and why gluten is added to meat products, but they should be assured that if the ingredient is not declared on the label, it is not present in the product. Only in this way confidence in food producers can be built. As our earlier research shows, it does not work in some cases.

The presence of meat products in the diet of people suffering from CD is especially important because of anaemia, which often concerns them. According to Rashtak and Murray (2009), due to deficiencies of important nutrients, especially iron and folates, anaemia occurs in many individuals with CD. Patients with CD should be screened for nutritional deficiencies, such as iron deficiency anaemia both during diagnosis and the annual check-up (Wild *et al.*, 2010).

The easiest way to prevent iron deficiency is to increase the content of meat and meat products in the diet. Fresh meat is free of gluten protein, of course. However, during manufacturing, it may be contaminated (consciously or not) with those plant proteins.

It was observed on the market that consumption of frankfurter sausages is on the increase and the statistical Pole eats them once a week, on average (Przybyłowicz *et*

al., 2012). On the other hand, the variety of additives used for their production can introduce gluten contamination. Gluten improves viscoelastic properties, sappiness, colour stability, solidity, and water-retention capacity of the product, decreases cooking loss. Thus, it positively influences structural and sensory characteristics of meat-products (Řezáčová-Lukášková *et al.*, 2014). Thus, the presence of gluten in sausages may result from producers' conscious or unconscious conduct. As it was said above, according to the legislation (European Commission, 2011), a food producer has to label the intentional addition of gluten to the product. Producers may, but do not have to, declare unintentional, possible introduction of gluten into the product, quite often resulting from cross-contamination.

At first, gluten content declaration in randomly selected points of sale was checked. The gluten presence or sign 'gluten free' was not declared for any frankfurter sausages intended for sale. The product of one producer who produced sausages on a specially disinfected technological line, but with addition of food additives obtained from wheat raw materials, was controlled. The producer was aware of problems related to the presence of gluten and allergenic cereal proteins in food. The producer was sure that his product was free of gluten and delivered raw materials (including additives) and ready-to-eat sausages for the experiment.

Generally, only two analytical methods are recommended in the European Union for gluten determination in food products: ELISA method and rtPCR analysis (CEN,

2009a,b). The results of gluten content analysis by ELISA method in meat products and raw materials used for sausages production analysis are presented in Table 4 and 5. Based on the presented results, it may be suggested that these tested products were fortunately safe for patients suffering from CD, even if they contained some additives produced from wheat.

The European Community Directives 41/2009 and 1169/2011 (EC, 2011; EC 2009) regulate rules on the labelling related to the absence of gluten in food. They set out the conditions under which foods may be labelled as 'gluten-free' (less than 20 mg/kg) or 'very-low gluten' (less than 100 mg/kg). This statement must also be applied to non-pre-packed foods, such as frankfurter sausages. The contamination of the examined sausages was so low that they could be labelled as 'gluten-free'. The content of gluten in the tested sample was lower compared to that with 3 wheat grains in 1 kg of the product.

Keeping in mind the aforementioned daily limit of consumed gluten (10 mg) (Catassi *et al.*, 2007), the gluten content in the product was recalculated for the average consumed portion. The average weight of frankfurter sausages eaten in Poland is between 30–100 g. Thus, if one sausage is consumed weekly (Przybyłowicz *et al.*, 2012), it can be concluded that even celiac patients could introduce the studied sausages into their diet. For their safety, the acceptable daily intake (ADI) of the proposed product could also be included on the label. This ADI could be calculated following long-term product testing by the manufacturer,

Table 4. The content of gluten [mg/kg] detected in the examined sausages on three producing stages.¹

Analysed sample	Minced meat	Minced meat with functional additives	Ready to eat sausage stuffings
Gluten content in the sample [mg/kg]	4.7±0.0a	13.5±0.2b	14.3±0.1c
Gluten content in the average consumed portion [mg/100 g]	0.47±0.0a	1.4±0.0b	1.4±0.0b

¹ Values denoted by different letters in rows of the table differ statistically significantly at the significance level $\alpha=0.05$.

Table 5. The content of gluten [mg/kg] detected in the examined food additives.¹

Analysed sample	Soy protein isolate 1	Soy protein isolate 2	Glucose	Fibre
Gluten content in the additives	0±0.0a	0.1±0.0b	3.7±0.1c	7.8±0.0d
Gluten amount in the sausage with the maximum allowed dose of the additives – theoretically calculated	0	0.002	0.056	0.012
SUM	0.07			

¹ Values denoted by different letters in rows of the table differ statistically significantly at the significance level $\alpha=0.05$.

and taking into account the average consumed portion of the product by the statistical consumer. It could then be possible to introduce the following information on the label: 'consumption of two frankfurters a day is safe for a patient suffering from CD'. This information would facilitate a consumer's choice. It would also increase the demand for a given product.

We could also observe (Table 4) that the content of gluten protein increased statistically ($P < 0.05$) during the processing of raw materials (a higher content of those proteins was found in the ready-to-eat stuffing than in minced meats). The question is whether the rapidly increased content of gluten (almost three times) resulted from the operations carried out during production (i.e. contamination of the production line) or from the additives used for the sausages production.

Thus, it was necessary to analyse these functional additives used during the production. Sausages, typical for the European market, depending on the type and purpose contain 0.2 to 2% soy protein (in the form of isolates or concentrates), glucose or glucose syrup (0.1 to 1.5%), and various kinds of fibre (0.2-1.5%) (Fernández-López *et al.*, 2008). Many producers are not concerned about the possible gluten contamination of the proposed additives, because it is used in small amounts during food processing. Gluten may be introduced into the additives unintentionally, therefore it is not declared on the label of the additive. The producers are not even aware of the method and raw materials used to obtain them. The problem is that wheat starch is often used as a raw material for glucose, and wheat straw for fibre preparations. Even strong processing of these raw materials may not be sufficient to ensure the safety of people with CD. Such high processing, as was used for the production of glucose, and application of material for fibre production, did not eliminate completely gliadin from the analysed additives (Table 5). Obviously, the amounts of additives used in the ready-to-eat sausages were not significant for the gluten content in the intended-to-eating product. The maximum amount of gluten which could be added, according to the European standards, together with the examined additives to the product, was theoretically calculated as 0.07 mg/kg (Table 5).

However, the manufacturer should always remember about the risk of introducing gluten into the final product with food additives, regardless of how small the amount can be. It should be strictly controlled, and information about the usage of additives should always be labelled.

It was also observed that heat treatment did not significantly influence the gluten content ($P > 0.05$) – it did not 'flow out' of the sausage while cooking, and it is also important information for the patients (Table 4).

We may suggest that the increased gluten content during the sausages production ($P < 0.05$) was caused mainly by contamination of the production line. Most often, contamination of the production line comes from crossing the lines, from an improperly cleaned production line, or from the behaviour of the staff (e.g. consumption of meals in their work uniforms). After proper education and scrupulous compliance to the rules, it should not be difficult to eliminate that problem. We must remember that food for special medical purposes is quite often more expensive, thus some investment may still be profitable for food producers.

Therefore, we should make a strong effort to educate manufacturers, including meat product producers. They should understand how important it is to have a very responsible approach to the issue of gluten control in products. In this way we can build customer's confidence and extend the customer group. Since maintaining appropriate procedures (disinfection of the line, employees' education) and selection of raw materials allowed production of sausages that can be offered to people suffering from celiac disease, it is worth increasing the meat products offer and expanding the market of potential recipients. Undoubtedly, this will involve some cost (related to the cost of gluten content analysis and cost of purchase of proven quality raw materials), but it seems that the result could translate into an increased turnover. Simultaneously, consumers with the celiac disease obtain the possibility to diversify their diet, and thanks to proper labelling, the possibility of a safe diet composition.

4. Conclusions

The interview questionnaire conducted with celiac respondents did not indicate the influence of the education level on their dietary awareness. The patients surveyed showed great responsibility in the approach to their illness: they confirmed their strict adherence to GFD, declared to be reading labels and were afraid to consume unknown food products. Although they carefully and honestly complied with doctors' recommendations, almost 20% of them did not expect gluten presence in meat products.

Even if sausages exclusion from the GFD is often recommended, and it is known that some producers use gluten raw materials in meat processing, we proved that some frankfurter sausages could be classified as 'gluten-free' (containing less than 20 mg/kg of gluten), because the percentage of functional additives in the final product is always small. The responsibility for proper labelling of food is a duty of food producers, thus they should also be informed about the purity of used food additives. Moreover, even if the calculated amount of gluten introduced with them into the sausages was regarded as

safe, the producer must be conscious of the problem of trace cross-contamination of the product.

Thus, if food producers will remember about the purity of the line and properly train their staff, they have a chance to produce a certified gluten-free product and gain new customers. Widening the range of safe meat products is important for consumers who follow the restrictive GFD. Because almost all of them follow the diet strictly and control the labels, a label properly prepared for them should contain both the gluten content and the acceptable daily intake of the proposed product. It helps the celiac patients to compose a safe diet and to build confidence in food producers.

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