ASSESSMENT OF MILK ALLERGENS INTO FOODSTUFFS

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Abstract. *Allergens represent specific substances, such as certain proteins, that are able to give an immune reply very dangerous in the case of the sensitive individual. Food allergens are normally harmless for the non-allergic individuals; but for some people these allergies result in severe symptoms, reasons for which these substances have engendered food safety issues.*
Allergens represent biological hazards for animals and people as well. It is obvious necessary to detect the presence of allergens in agri-food raw materials, food products and fodder for their entire monitoring of the food and feed chain, also taking into account the major financial losses generated by contamination. Food producers must protect allergic people using clear labelling for their food products. As regards the food allergens, in the EU legislation there are no limits specified in the legislation, only the mention of their likely presence on the labels is compulsory.
The paper shows the incidence of certain compounds with potential allergen activity in foodstuffs containing milk by using the S-ELISA Veratox method. We tested milk allergens from a few types of food matrix containing undeclared milk or its derivatives (wafers with cream, instant coffee, sauces). The results indicated different degrees of contamination with potentially allergenic proteins depending on the food matrix type.

Keywords: *food allergens, food safety, milk protein, ELISA, label*

Introduction

1. Food allergens

Allergens are specific substances, such as pollen, food or drugs, which introduced into the body through the digestive, air etc.., produce pathological manifestations caused by hypersensitivity reactions of the immune system. Generally speaking, the substances with allergenic potential in food are certain proteins or glycoproteins [1]. In fact, foods contain many proteins, but only a small fraction is allergens.
Although the structural properties of proteins that cause allergic reactions have not been characterized completely, known food allergens in general have molecular weights between 10 and 70 kDa, and are stable molecules in the sense that they are resistant to processing, cooking, and digestion. In the case of allergic individuals, these substances stimulate the immune response by inducing the production of allergen-specific IgE. [2]
Experience shows that the immune response can be initiated by eating a food, which contains traces of that allergen. The amount of allergen that can generate immune response varies from one allergic individual to another one.
It seems that food matrix can influence the responses to individual proteins; consequently, the food matrix must be taken into account when developing models for allergenic potential assessment [3]. Moreover, any type of food is

potentially allergenic, but the majority of food allergies are caused by a small group of foods, such as: cows' milk, ground peanuts and tree nuts (almonds type, pistachio etc.), wheat, vegetables, eggs, fish and other seafood, soybean and other ingredients that contain protein derived from these food groups (over 90% of allergic reactions are induced by array of protein compounds of these types of foods). Over 170 foodstuffs have been identified as having potentially allergenic compounds in composition, fruits included (strawberries, plums, etc.), sesame seeds, sunflower seeds, poppy seeds, mollusks, peas, lentils, beans, with its different varieties.

Besides protein allergens, foods may contain also haptens, which are substances (small molecules), different from the proteins that may become allergens only when attached to a large carrier such as a protein. [4]

World's statistics figures show that approximately 4% of adults and 8% of the children are allergic to different foods.

The adverse effects induced by food allergens, even when they are ingested in very small amounts, range from rash and tingle to anaphylactic shock and in severe cases to death. For this reason, assessment of food allergens became part of risk assessment in the framework of food safety management system.

Since 2005, according to EU Directive 2003/89/EG of 10 November 2003, in the EU countries all food products must comply with the changed labelling directive. In that way, the allergen ingredients must be declared on food labels, since they can induce allergic reactions. A list of 12 allergens, namely gluten containing grains (e.g. rye, wheat, barley), shellfish, eggs, fish, peanuts, soy, milk, nuts, celery, mustard, sesame seed, sulphur dioxide and sulphites in concentrations over 10 mg SO2/kg, must be compulsory mentioned on the food label. [5]

Laws regarding the labelling of food allergens in the U.S. came into force in January 2006, as following the regulations of the Act on Food Labelling and Consumer Protection (FALCPA) of 2004. In this sense, it requires that food manufacturers use in their plants such processes that reduce or even eliminate cross-contact among the non-allergenic foods and food allergens. The U.S. Federal Food, Drug, and Cosmetic Act was amended and the document imposes new requirements for the labelling of foods containing major food allergens. [6, 7]

Therefore, it is necessary to test compounds potentially allergenic food for any of the following purposes:

→ It is necessary for their identification and assessment; food manufacturers are required to protect allergic people through a clear labelling of their products, with the complete list of the food ingredients;

→ It is a measure that an ingredient potentially allergen, which is not mentioned on the foodstuff's label should not accidentally have entered into product, saying it does not contain any;

→ It is a tool for identification of the cross contamination sources among allergens and non-allergic products during food processing.

2. Milk Allergens

Milk allergy is food allergy, which has as main symptoms gastrointestinal, dermatological and respiratory ones. These can be skin rash, hives, vomiting, and gastric distress such as diarrhoea, constipation, stomach pain or flatulence, or other clinical disorders. The symptoms may occur within a few minutes after exposure to immediate reactions, or after hours or even after several days in delayed reactions. Although the symptoms and treatment are similar to milk allergy and

milk protein intolerance, is not the same disease.

In general terms, milk allergy is one of the most popular food allergies. Moreover, Høst (2002) expressed that milk allergy is the most common food allergy in early childhood, and it affects approximately 2…3% of infants in developed countries. The prognosis of cow's milk allergy is with a remission rate of around 85-90% of the affected children once they surpass 3 years of age, but there is the risk to develop adverse reactions to other foods, later in childhood. [8]

Cow's milk has 3.2% protein composition, consisting of:

→ 80% caseins (αS1-, αS2-, β- and κ-) (main part of coagulum/ curd),

→ 20% lactoserum (β-lactoglobulin, α-lactalbumin, bovine serum albumin) (main whey protein).

Milk basic proteins provide nutritionally important benefits if used as a food ingredient for dairy products, as well as in other foods. Sometimes, milk or its derivates may be present like food contaminant of raw materials or certain processed foodstuffs.

Regarding the cow's milk allergenicity, there are studies on milk proteins identified as allergens and their epitopes were characterized. The results indicated that most milk proteins, even proteins present in low concentrations, are potential allergens, but no particular structure or function is associated with milk allergenicity [9]. In fact, molecules of bovine milk proteins have numerous epitopes, which are located in hydrophobic parts of the molecules where they are inaccessible for IgE antibodies in the native conformation of the proteins but become available after digestion. [10]

As examples of allergenic compounds we can mention □-S1-S2-caseins, □□□□-lactoglobulin, α-lactalbumin, immunoglobulin, and lactoferrin in milk. Referring to the milk allergens, □-lactoglobulin reactivity is lower, but through the heat treatment of milk, it interacts with lactose, which makes its reactivity increase. Allergenic factors generally occur in the early stages of heating, when premelanoidins are formed, compounds involved in these phenomena.

It is considered that the casein products are responsible for allergies in adulthood, while β-lactoglobulin is mainly responsible for children's allergies to milk.

Other authors, based on molecular characteristics and expected exposure of milk basic proteins, expresses opinions according to which protein components in milk are unlikely to present any increased risk of allergy for milk allergic subjects or of cross-reactivity for other allergic subjects. [11]

However, food products containing milk proteins (caseins, lactoglobulins etc.) need to be labelled as containing milk as a caution to warn milk allergic subjects of the potential risk of allergic reactions. In that way the food labelling directives in the US and the European Union are clear, as long as it has not been demonstrated that milk proteins are free from milk allergens.

In conclusion, it is recommended to evaluate the presence of caseins and β-lactoglobulin in various foods with the aim to prevent food safety issues and for a suitable labelling of foodstuffs.

Materials and methods

The purpose of this study was to survey the natural occurrence of the milk allergens in food products declared as not containing milk or milk derivates.

Different food matrices were tested, namely cream wafer, instant coffee, and dressings/ sauces. For this study 17 samples were assessed.

The testing of presence of milk allergens was performed by using the Neogen Veratox kits (Quantitative Milk Allergen Test Kits, code 8470). The allergens test is

based on the sandwich ELISA (S-ELISA) technique. [12]

The milk residues are extracted from samples by using a buffered salt solution (PBS) through stirring into a water bath at preset temperature of 60^0C. The extracted milk residues thus obtained are collected and then added into the capture antibody wells and incubate. The unlinked residues are then washed and the linked ones are treated in a second stage with a detector antibody linked to the specific enzyme. The detector antibody is bound to the residue already linked by capture antibody. After a second washing step, the specific substrate is added and the substrate reacts with the bound enzyme conjugate to produce coloured reaction. Then the reagent stops ass the colour reaction is added and the test wells with standards and samples are analyzed. The results are read by using a microwell reader with a filter at 650 nm as optical density, in equipment Stat Fax. type 321 Plus, from Awareness Technology Inc., SUA. Based on the optical densities curves, milk allergens concentration is calculated using a software Neogen type log/logit.

The equipment and apparatus required to perform these experiments is the following: analytical balance, accurately weighing 0.0001 g; reader Veratox State Neogen Fax 303 Plus, 650 nm filter, mill for grinding solid samples; UNIMAX orbital shaker 1010, equipped with thermostatic chamber (1000 Heidolph incubator) or water bath and thermostatic mixing at 60 ˚ C ± 1 ˚ C; timer.

The performance characteristics for Neogen Veratox kits (Quantitative Milk Allergen Test Kits, cod 8470) are the following:

- Range of quantitation 2.5 to 25 ppm
- Standard deviation (STD) – 0.1
- Uncertainty (Ua) – 8.3
- Repeatability, r – 0.1
- Relative standard deviation (STD) rel, % - 0.3.

Criterion for acceptance/ rejection of the results is the following: curve correlation coefficient r ≥ -0.980. If the curve correlation coefficient is r <-0.980, the analysis is repeated.

The general procedure to determine allergens in milk is briefly shown in figure 1.

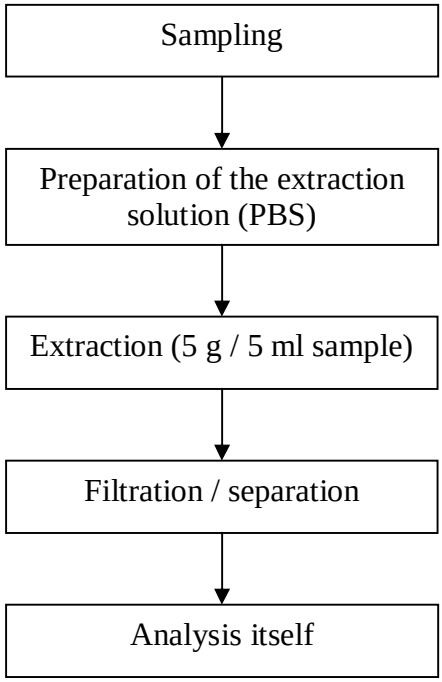

Figure 1. Procedure for the determination of milk allergens

The reagents used in the kits contained specific solutions (standard solutions of different concentrations, extraction solutions, wash buffers, substrate solutions, conjugate solutions, solutions to stop), powdered concentrated solvent extraction of PBS 10 mM, concentrated washing buffer PBS-Tween, extraction additive, deionised or distilled water.

Whole milk powder NIST, code RM 8435 was used as reference material. But the samples used for determination of the food allergens are end-products, not ingredients.

For data interpretation we used analytical software SPSS for Windows.

The analysis method using ELISA kits represents a viable alternative to HPLC and fluorimetric methods for the determination of allergens, which are more expensive methods and require much more time working and more sophisticated equipment as well.

Certain specific conditions are imposed in order to determine the milk allergens in foodstuffs, namely:

→ milk allergens must be extracted separately from those for other food allergens, like peanuts or egg residues; reagents are specific for each target food allergen, too;

→ although partially digested milk proteins may be undetectable by ELISA analysis, the residues of allergenic proteins could be active;

→ cross contamination must be avoided.

Results and Discussion

The samples evaluated during experiments consist of different food matrices, as follows:

1. instant coffee assortments,
2. dressings – vinaigrette sauces, and
3. wafer with lemon and chocolate cream.

The analysis samples were taken from the foodstuffs available in stores.

The overall results indicated lower values for milk allergens present in the above-mentioned samples and were registered in instant coffee, while the highest values were registered in cream wafers.

As it is shown in figure 1 and table 1, the majority of values obtained in determining milk allergens from coffee varied between 0 and 1 ppm, with an average around 0.745 ppm. Exception made the eighth sample, namely instant coffee C-3, in which a higher concentration of 2.3 ppm milk allergen was registered, as well as the seventh sample, instant coffee C-2 that was free from milk allergen.

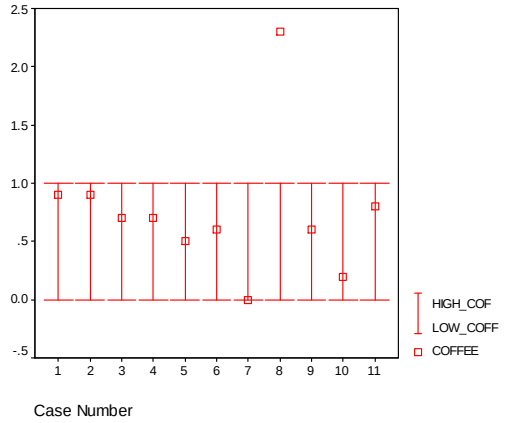

Figure 1. Low-high chart of set of coffee samples

Table 1.

Statistics of coffee set

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
COFFEE	11	.00	2.30	.7455	.5854
Valid N (listwise)	11				

The results obtained from the analysis of vinaigrette sauces with milk contaminants are listed in the table 2. Figures indicated a contamination with more than 1 ppm milk allergen for each sample.

Table 2.

Quantitative determination of milk allergens in vinaigrette sauces

No.	Matrix	Results (ppm)
1	vinaigrette sauce natural -1	1.0
2	vinaigrette sauce natural -2	1.4

The highest contamination with milk allergen was registered in cream wafers. For the set of samples the minimum value was 15.3 ppm, while the maximum one was 57.4 ppm, and the mean reached 38.875 ppm (see also figure 2). Generally speaking, the wafers with chocolate cream had a higher content of milk allergens in comparison with lemon ones.

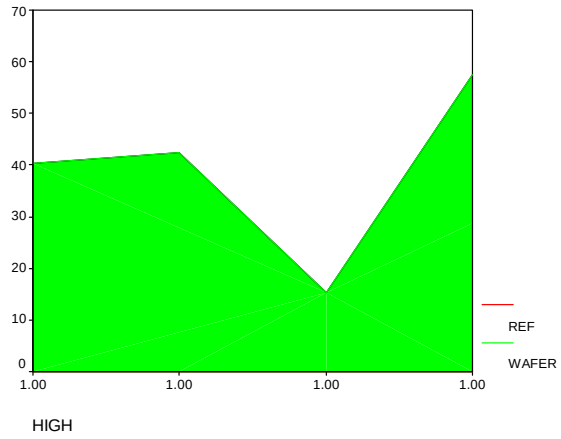

Figure 2. Graph for set of cream wafers samples

Conclusion

The testing of milk proteins occurrence in foodstuffs ensures food manufacturers that a potentially unsafe/allergenic ingredient, unspecified on the label is not really found in those foods.

In this purpose, some types of foodstuffs declared as not containing milk or its derivates were tested for milk allergens. Consequently, several samples of instant coffee, dressings and cream wafers were assessed by using VERATOX ELISA method for determining the total milk allergens.

After analysis performing, there was solely a coffee sample free from milk allergen. The lowest values for milk allergen natural occurred in samples were registered in coffee samples, in general less than 1 ppm. The analyzed vinaigrette sauces samples were characterized by a maximum contamination of 1.4 ppm milk allergens. Also the data showed an allergen milk contamination at maximum level in the case of wafers samples.

Although the prevention of cross-contamination with food allergens is a major component of HACCP system, our results indicate that there is plenty of foodstuffs in stores that could be contaminated by food allergens, which are not described on the label goods in accordance with the law.

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