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A case of allergy and food sensitivity: the nasunin, natural color of eggplant

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Abstract: Allergies and food sensitivities can both be considered as "adverse reactions individualistic" to food. Are pathological and individual forms because they affect a few individuals in way rather serious; immediate or delayed reactions occur instead with simple effects histamine, or, in severe cases with respiratory and anaphylactic shock
The eggplant (Solanum melongena L.) is known to cause food allergies in some Asian countries, but detailed studies on allergies caused by eggplant are lacking, however, it was highlighted the presence of allergens in edible parts of eggplant with preponderance in the peel .
The purpose of this study was to propose an extraction method rapid, efficient and cost of natural dye from waste products from the food industry, such as the peels of eggplant, from which it was extracted, isolated and purified the nasunin,a colored molecule in red-fuchsia.
Nasusin was tested on 58 patients to evaluate the potential sensitizing effect on the skin. The results demonstrate that allergenic effects are negligible and therefore the nasunin can be used as a colorant in various industrial sectors with a certain safety margin

Keywords: Nasunin, natural dye,analytical methods, patch test, allergy

I. INTRODUCTION

The term *food allergy* is comprised of various pathological conditions that occur immediately or several hours after ingestion of certain foods [1]. *True food allergy* is a individual reaction, abnormal and immediate to a food or a component of it, usually a protein, triggered by the immune system with production of IgE antibodies (immunoglobulin E) [2].The most common allergic reactions such as those towards the milk, eggs, nuts, shellfish are represented by a variety of symptoms that can appear singly or in combination between them)[3]:

- Gastrointestinal: oral allergy syndrome, anaphylaxis, gastrointestinal;
- Cutaneous: urticaria, angioedema;
- Respiratory: acute rhinoconjunctivitis, bronchospasm;
- Generalized: anaphylactic shock.

Oral allergy syndrome is characterized by itching and burning of the oral mucosa and edema of the lips shortly after intake of foods, especially vegetables..
Anaphylaxis gastrointestinal typically presents with rapid onset of nausea, abdominal pain of colic, vomiting and diarrhea; usually it occurs in association with other allergic manifestations in target organs such as the skin and the respiratory tract
Urticaria / angioedema is characterized by acute heartburn, wheals that vary considerably in scope and duration. Angioedema (bloating of the skin) does not cause itching but tingling, warmth and feeling of tension on the skin edematous. These are among the most common symptoms of food-induced allergic reactions.
Rhinoconjunctivitis is very rare as food-induced allergic reaction although often it occurs in association with other symptoms of food allergy.
Asthma is an uncommon manifestation of food allergy, although the acute bronchospasm is observed with other symptoms induced by food [4]. The hyperreactivity of the respiratory tract and the worsening of asthma can be induced even in the absence of bronchospasm after ingestion of small quantities of allergens in sensitized individuals .Vapori and fumes containing proteins emitted by the food during cooking (eg.fish) may induce reactions of asthma and even anaphylaxis: about 1% of asthma in adults may involve reactions to inhalation for the exposure to food, especially in the workplace [5,6].
Anaphylaxis is the most severe allergic reaction: in addition to various forms of cutaneous, respiratory and gastrointestinal the patients may have cardiovascular symptoms including hypotension, vascular collapse and arrhythmias. It is essential hospitalization.
The exercise-induced anaphylaxis associated with foods is a form of anaphylaxis that occurs only when the patient performs physical activity within 2-4 hours after ingestion of foods. In the absence of exercise, the patient can ingest the food without showing reactions. It is much more common in young patients (15-35 years

old) female. The omega-5 gliadin present in the flour was found to be the most frequent cause of exercise-induced anaphylaxis dependent on food.
The symptoms observed in the allergic form are the results of the release of active substances ,such as histamine, pharmacologically by specific cells of our body, the mast cell, as a result of the interaction between immunoglobulin E (IgE) and food substances, that cause allergic reactions (allergens).
The *alimentary sensitivity or intolerance* can cause symptoms like the alimentary allergy including nausea, diarrhea and stomach cramps, but the reaction does not involve the immune system. It occurs when the body can not properly digest a food or food component, so that those who have an intolerance can often tolerate small amounts of food without developing symptoms, unlike allergy sufferers who have to avoid wholly the foods allergy cause.
Food sensitivities are indicated by various symptoms and depend on several factors:

- Enzyme deficiency is the lack of certain enzymes needed to digest food, such as lactose intolerance or gluten;it appears bloating, diarrhea, weight loss;
- Foods additive is the intolerance that some individuals have regard to particular substances such as sulfites, dyes, artificial sweeteners;
- Food idiosyncrasies are characterized by adverse reaction to a food for its particular component such as Chinese restaurant syndrome for the use of monosodium glutamate;
- Stress recurrent or psychological factors can be cause a reaction similar to those allergic only to the idea of taking a particular food: the causes of this phenomenon have not yet been clarified .

Our body ingests a large number of potentially pathogenic substances of which we do not are aware of their danger or of their presence in the foods. In our markets we find fruits, vegetables, meat and fish from all over the world; there is no longer the "change of seasons", because through greenhouse farming and import we can find the same food during the whole year. Consequently, we find an increasing number of preservatives, antioxidants, additives, etc. to which our body is completely helpless and this is a big problem, because it is not easy to distinguish if the manifestations of allergy and intolerance are due to foods or additives .Among the latter especially the dyes have been studied, for their massive presence not only in food, but also pharmaceuticals and cosmetics.
In recent years, the increasing use of synthetic dyes showed an increase of allergic dermatitis caused by contact with the tissues, the foods, the pharmaceuticals and cosmetics.
The dyes of synthesis, especially in the textile field, they have changed the perception of the color with their fixity and their sameness, eliminating the traces of time, light, water, wear. The products treated with synthetic dyes have a significant impact on the environment, both in the production phase and in that disposal, for the release of toxic substances in air and water, constituting a risk for the operators during the phase same production [7].
We have seen that many finished products have a carcinogenic effect, especially the type of synthetic azo- dyes, as the scarlet Sudan III that can evolve to liver tumors because of their metabolites, so as aromatic amines which are known carcinogens. Recently it is rediscovering the use of natural dyes, especially from vegetables, as they present significant advantages and attractive features regarding environmental protection and the preservation of health [8].
The purpose of this work was to propose a method rapid, efficient method and economic of natural dyes from waste products from the food industry, such as the peels of eggplant [9], from which has been extracted, isolated and purified the nasunin,a colored molecule in red-fuchsia

II. EXPERIMENTAL

Extraction method

G.100 of fresh eggplant peels have been cut into small pieces, put in a flask, covered with 200 mL of a solution of acetic acid at 10% in maceration for 24 hours with constant stirring. Once removed the solvent, the same peels were covered with glacial acetic acid and left to macerate for an additional 24 h. After filtration the peels were almost colorless, while the liquid of extraction was strongly colored violet. The extracts were combined and concentrated under vacuum at 40 °C. The crude extract was treated with methanol and, after removal of the solvent, the residue was treated with distilled water until completely dissolved. The solution was purified by the use of a C_{18} column Seepack activated with 5 mL of methanol and 5 mL of distilled water.
A first elution was performed with methanol (Solution B colored in brown), while subsequently an distilled water was used (Solution A colored in red, purple). Both eluates were concentrated in vacuo to respectively 0.6523grams as residue A and 0.2110 grams as residue B; both compounds were analyzed by high performance liquid chromatography (HPLC). Each sample was solubilized in water with trifluoroacetic acid (TFA) 1% (v/v); 10μL of the solution thus obtained were injected into an apparatus Sunicom Oy (Helsinki, Finland) connected to a detector UV / VIS variable wavelength Model 500 equipped with a degasser ERC3TT.

E 'it was used a column Luna 5 µ ODS size 250x4.60 mm and a system eluant gradient represented by a mixture of water with 0.5% TFA and from methanol with 0.5% TFA; the gradient and was now set: 75:25 v/v to a 30: 70 v/v for 25 minutes with a flow velocity of 1 mL / min; and the reading was performed at to □ □520 nm [10,11].

In the chromatogram (Fig.1) relative to solution A are detected two peaks most important: the peak 1 is compatible with that of the delfininidin 3- [4- (cis-p- cumaroil) -L-ramnosil (1.6) glucopyranoside] - 5-glucopyranoside (cis-nasunin), while the peak 2 is compatible with that of delphinidin 3- (4- (trans-p-cumaroil) -L-ramnosil (1.6) glucopyranoside) -5-glucopyranoside (trans-nasunin) [12].In the chromatogram relating to sample B (Fig. 1) were detected peaks consistent with the presence of other phenolic substances, not identified.

The presence of nasunin was also confirmed by GC-MS. The spectrum MS showed the peak of molecular mass m / z 919 and other peaks fragmentation of m / z 757 (loss of hexose group), m / z 465 (loss of the group p-cumaroil-hexose), m / z 303 (loss of the group of delphidin).

III. TEST DERMATOLOGICAL

In this study it was evaluated the potential irritant effect on the skin of an extract containing purified nasunin. Of this extract we have been set up some formulations 1 and 5% using water as a vehicle and petroleum jelly. Patch tests with these apteri were run on a heterogeneous sample of 58 volunteers, 41 female and 17 male, age ranging from 17 to 71 years. On all patients were tested with haptens standard series of Italian Society of Dermatology Allergology, Professional and Environmental (SIDAPA) and with the solution A purified through Seepack which had been identified nasunin. A method for assessing the irritancy of a substan is the patch test in vivo on volunteers, a reliable test that can identify substances responsible for a potential irritant effect on the skin. They were prepared four sets of samples using as vehicles the water and vaseline with concentrations 1 and 5% of the test substance. The solutions in water were deposited on supports consisting of cellulose disks of a diameter of 1 cm, in quantities of between 10 and 40µL, while for solutions in vaseline diskettes were of 1-4 mM. The diskettes have been applied, by means of adhesive strips, free from lesions on the backs of the volunteers and left in contact with the skin for 48 hours, after which time they were removed and was performed the evaluation . A type reaction irritating and strictly limited to the area of application of the substance and subsides in about 2 days. A reaction is regarded as a potentially allergic, if you have redness, swelling and blisters eczemiforme. The positivity is expressed by a sequence of + signs (Table 1)

IV. RESULTS AND DISCUSSION

Of the test subjects, 49 were negative patch test (Fig.2) made with the test sample (Table 2); seven showed a positive reaction irritative (Fig. 3) and two showed a positive reaction probably allergic. It is important to note that, among the 49 volunteers who did not show any kind of reaction to the extract, 17 were positive for the hapten series standard SIDAPA. Of the two volunteers who have reacted positively to extracts of eggplant,allergic contact dematitis,(DAC) one (+++) was negative to the hapten SIDAPA, while the other (++) was positive to haptens Balsam of Peru, p-phenylenediamine and Cobalt chloride (Table 2). Of the seven individuals who experienced a reaction of irritative contact dermatitis (DIC) with the extracts of eggplant, five were negative to haptens SIDAPA and two tested positive, one at a formaldehyde and other at p-phenylenediamine (Table 2). It should be emphasized that all positive subjects were treated with the concentration to 5% (a concentration considered remarkable for a dye), while no subject was positive at a concentration of 1%. The nasunin is the most important of anthocyanin present in the peels of eggplant at a concentration of 700 mg / 100 g and is responsible of the purple coloring of this vegetable. It 'was isolated for the first time in 1933 by Kuroda and Wada [13,14] and its structure was determined by Sakamura et al in 1963 [15]. It is an anthocyanin acylated rather stable, and is presented as a mixture of cis-trans isomers of delphinidin. Its structure was indeed identified as: delphinidin 3- (4- (p-cumaroil) -L-ramnosil- (1.6) glucopyranoside) -5-glucopyranoside. It had an high antioxidant activity [16,17], and a powerful scavenger of hydroxyl radicals and superoxide radicals [18]. Spectrophotometric studies have shown that the nasunin forms a complex with the Fe^{3+} attributing this its antioxidant action at the ability to inhibit the formation of free hydroxyl radicals for chelation with the iron. This is very important because superoxide radicals generated in vivo, they are generally converted into hydrogen peroxide and like other free radicals can damage lipids, proteins and DNA [19].

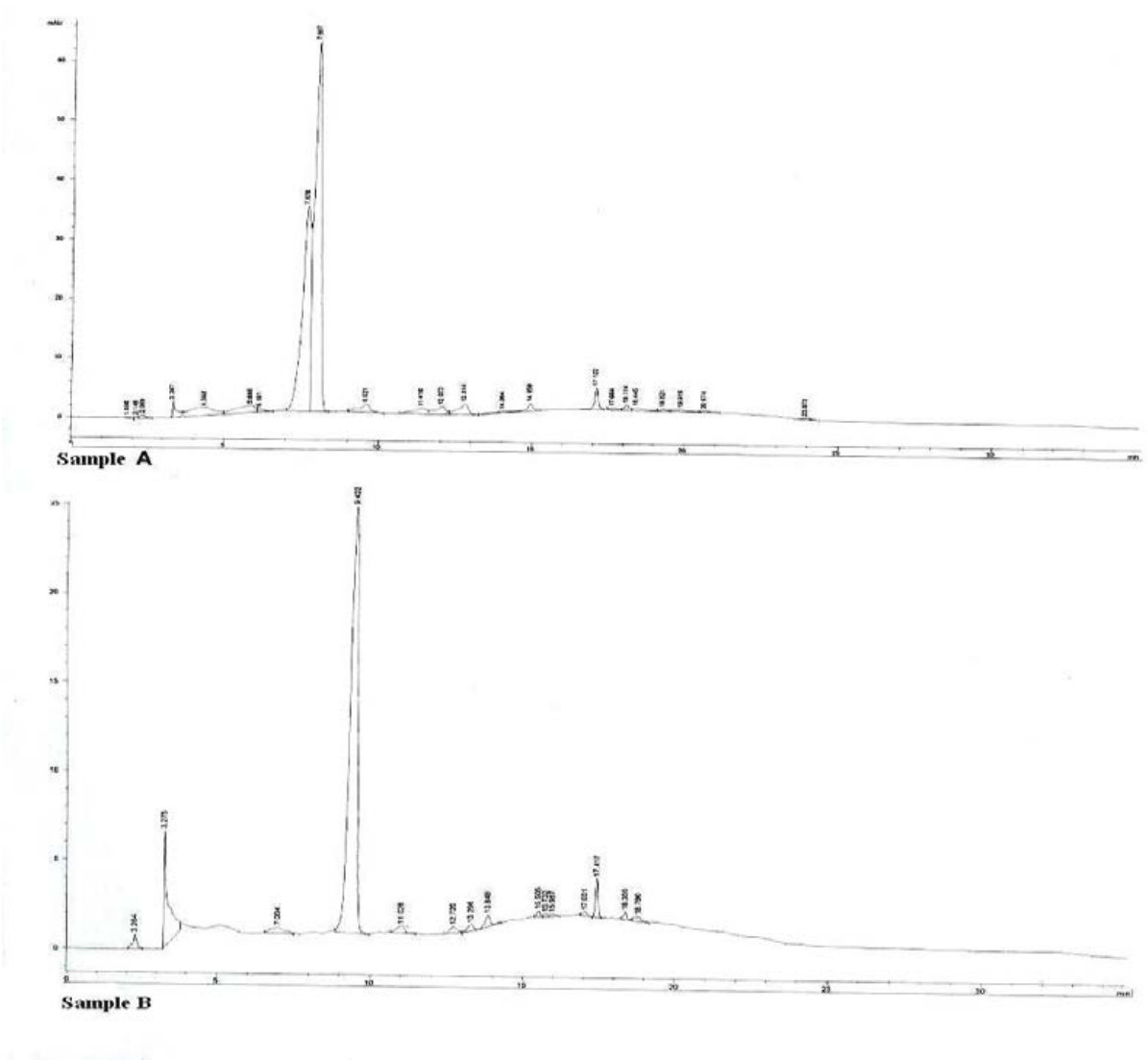
The nasunin has anti-angiogenic activity: in fact at a concentration greater than 10 mM is able to suppress the growth of microvessels [20] and is also involved in the prevention of other diseases such as atherosclerosis and diabetic retinopathy [21]. Several studies have also shown that the nasunin could prevent the toxic effects of paraquat, a pesticide that can cause damage (oxidative stress) in various organs such as liver, lungs, kidneys and heart [22]. For its ability to suppress both the increase of lipid peroxidation in liver, both the reduction of the activity catalase, induced by Paraquat precisely, the nasunin, preventing an increase in atherogenic factors, might be useful in preventing the development of the atherogenesis due to this pesticide

V. CONCLUSIONS

The patch tests on extracts containing nasunin have showed a moderate irritancy only at high concentrations (5%) and therefore can be used in concentrations sufficient to impart color, and namely below 1%, which concentration did not induce process of irritation to the skin in vivo. The development of a mining method which allows to obtain this anthocyanin from the peels of eggplant in a rapid and efficient, providing a great advantage because it makes available a dye for both the textile field, which for other sectors, such as cosmetic, pharmaceutical, food, thus reducing the problems related to the use of synthetic colorings

VI. FIGURES AND TABLES

Figure1 HPLC chromatograms of eggplant peels extracts of A solution (Panel A) solution B (Panel B).



+	Weak erythema
+	Uniform erythema with edema, possibly papules or vesicles
++	Erythema, edema, papules and vesicles evident that can spill over the area of application
+++	Erythema, edema, papules and vesicles very obvious, sometimes Confluent in bubbles
IR	Type reaction irritating to different morphology
NT	Not tested

Tab. 1 Quality and quantity assessment of allergic reactions according to SIDAPA

N. Patients	Nasunin	Haptens*
49	Negatives	
2	DAC	1 negative 1 positive: balsam of Peru, paraphenylenediamine, cobalt chloride
7	IR	5 negatives 1 positive (formaldehyde) 1 positive (paraphenylenediamine)

Tab. 2. Results of patch tests with haptens of ISDAPE standard series and with Nasunin performed on 58 volunteers.*Haptens of Italian Society of Dermatology, Allergology, Professional and Environmental (SIDAPA) standard series

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