ADDITIVES AND PROCESSING AIDS USED IN GRAPE PRODUCTS: A NEW APPROACH TO PROTECT ALLERGIC SUBJECTS

P. Restani[(1)], C. Ballabio[(1)], D. Fuggetta[(1)], A. Martelli [(2)], L. Terracciano[(2)], A. Fiocchi[(2)]
[(1)]Dept. Pharmacological Sciences
via Balzaretti 9, Milano, Italy
patrizia.restani@unimi.it
[(2)] Dept. Child and Maternal Medicine, The Melloni University Hospital
via Melloni 52, Milano, Italy
allerg@tin.it

SUMMARY

Since the prevalence of food allergy and intolerance is increasing in the world, several Food Safety Authorities asked to improve the labeling to protect consumers suffering from these diseases. New international Directives include alcoholic beverages making mandatory, also for this category, the labeling of all ingredients with allergenic effects.

At present, there is no recognized protocol to perform studies capable to ensure the safety of treated wines for allergic subjects. Between processing aids and additives used in enology, egg and milk proteins are the most dangerous since the percentage of adults suffering from these allergies cannot be ignored (more or less 0.3 % for both allergens). Moreover, other categories, such as patients suffering from celiac disease, must be protected by negative effects of toxic cereal derivatives. On these bases, the aim of this research was the identification of the most suitable approach to evaluate the safety of wines treated with allergenic proteins, considering different possible combinations with secondary fining agents.

RIASSUNTO

Con l'aumento della prevalenza dei casi di allergie ed intolleranze alimentari, diverse Autorità internazionali deputate alla valutazione della Sicurezza Alimentare hanno stilato nuove indicazioni relativamente agli obblighi di etichettatura per gli allergeni e ha reso obbligatoria le dichiarazioni anche per le bevande alcoliche. Punto critico per questo problema è l'assenza di un protocollo riconosciuto per la valutazione della sicurezza delle sostanze allergeniche usate nella produzione del vino. Tra gli additivi e i coadiuvanti tecnologici usati in enologia, particolare attenzione deve essere rivolta alle proteine di uovo e latte, in quanto il numero di soggetti adulti allergici a questi due alimenti non è trascurabile (circa 0.3% per entrambi). Inoltre, nel caso in cui si usino proteine da cereali si deve assicurare la protezione dei soggetti celiaci che devono evitare prodotti contenenti glutine. Scopo di questa ricerca è la messa a punto di un protocollo tossicologico che permetta la valutazione della sicurezza dei vini trattati con sostanze allergeniche, tenendo conto dei chiarificanti secondari che possono essere usati in associazione.

INTRODUCTION

Since the prevalence of food allergy and intolerance is increasing in the world, several Food Safety Authorities asked to improve the labeling to protect consumers suffering from these diseases. New international Directives include alcoholic beverages making mandatory, also for this category, the labeling of all ingredients with allergenic effects.

In wine production, the common practice of clarification consists in adding to the must or wine organic or inorganic substances that interact with elements responsible for turbidity and organoleptic imbalances. Among organic agents, animal proteins (fish gelatin, egg albumin, and milk proteins) are the most widely used because of their excellent fining properties, their ability to eliminate tannins, which are responsible for wine astringency, and their comparative cheapness. In same case, proteins can be added to wine for their antimicrobial activity; among allergenic proteins, lysozyme (from egg white) is used to prevent malolactic fermentation.

Milk and egg are responsible for adverse clinical reactions mediated by immunological mechanism, known as food allergies. The reported prevalence of milk allergy ranges widely according to the diagnostic test used and the geographical area considered. It has been reported to be 1.6–2.8% in randomly selected children younger than 2, and even though clinical tolerance is frequently acquired by 50–90% of the children under the age of 6, severe milk allergy can persist into adulthood (Host, Halken 1990). Similar pattern of sensitization and progressive clinical tolerance is observed in children suffering from egg allergy that is normally associated with egg white proteins.

The clinical symptoms, reported also in adult patients, can be classified as immediate or delayed reactions, according to the time of onset after the milk or egg ingestion. They range from mild to severe and can involve the skin (atopic dermatitis, eczema, lip edema, hives, angioedema), gastrointestinal tract (oral syndrome, vomiting, colic, diarrhea or constipation, oropharyngeal itching), respiratory tract (cough, wheezing, broncospasm, asthma, dyspnea) and other groups of symptoms (insomnia, recurrent otitis media, psychological disturbances). The most dangerous clinical event is anaphylaxis, an acute systemic and potentially fatal reaction.

REASONS FOR DEVELOPING A COMMUN EXPERIMENTAL PROTOCOL

Both red and white wines are treated with proteins for fining purposes, in other words additives and processing aids having allergenic potential are deliberately used in wine production. These proteins do not represent a danger for healthy people but they are potentially lethal for those patients who are highly sensitized to specific allergens. To minimize the risk for allergic subjects suffering from these severe allergic symptoms, producers must control the enological procedures carefully.

At present, there is no recognized international protocol to perform studies capable to ensure the safety of treated wines for allergic subjects; as a consequence, the aim of this project is the identification of the most suitable approach to evaluate the safety of wines treated with allergenic proteins, considering different possible combinations with secondary fining agents.

Previous experiences of our group showed that red wines are normally characterized by lower protein residues, while the residues in white wines are strictly dependent on the specific enological practices (Cattaneo et al. 2003a and 2003b).

From these bases, we suggest some points that should be considered in establishing an international research coordinated by OIV.

Production

Wine production, involving the use of allergenic proteins, is a potential risk for allergic patients so that the absence of significant residues must be ensured by specific experimental studies aimed to identify the most suitable enological procedures, allergen by allergen. The critical points are described in Table 1.

Table 1 - Critical points in enological practices for wines treated with allergenic proteins

Class of factors	Parameter	Consequences
Fining agent or additive	Allergenic source and product characterization	Presence of one or more proteins Stability of proteins could be different (loss of activity during processes) Specific clinical characteristics must be considered
Specific wine characteristics	White or red grape pH Tannin content Etc	All these factors can modified the extent of protein elimination, by changing their solubility and/or their binding to natural substances present in grape
Enological treatments	Secondary fining agents Solphites Other enological treatments (chemical addition and/or physical treatments)	All these factors can be responsible for, or interfere with, the protein elimination

As stated before, protection of allergic subjects requires suitable enological processes, experimentally defined for each specific allergen, and the defined practice must be compulsory applied by all wine producers to receive exemption from labeling.

Analytical Methods

No suitable enological treatment can be established without a specific, sensitive and reliable analytical method. Different analytical approaches can be developed but the most useful technique is ELISA test. This method is widely applied in food industry and presents all the

necessary characteristics described above. The cost is normally acceptable for routine controls and trained personnel easily perform it.
The immunochemical method must be specifically developed for wine and must be reliable enough to protect consumers at risk.

Clinical Approaches
All optimized enological practices must be verified by performing clinical studies including Skin Prick Test, detection of specific circulating IgEs (RAST, immunoblotting) and double blind placebo controlled food (wine) challenge.
The Skin Prick Test (Figure 1) and the detection of specific IgEs (Figure 2) are not very complicate objectives to reach, but different difficulties are associated with the performance of oral challenges as shown in Table 2.

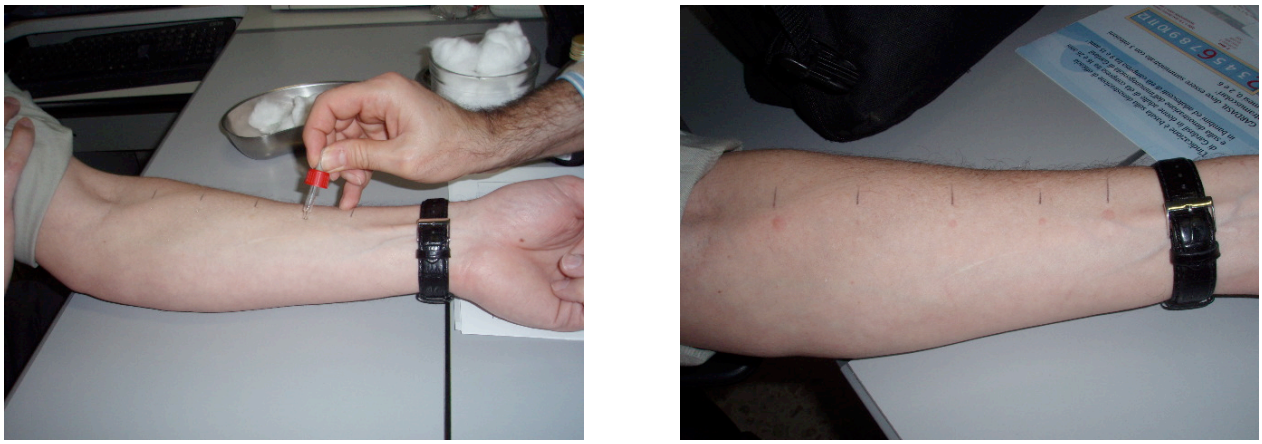

Figure 1 - Skin Prick Test performed with purified fining agent and treated wine

Critical point is the number of allergic subjects who must be enrolled for the oral challenge; in fact, (Muraro et al. 2004) according to the definition of hypoallergenic product as established by EAACI, 90% of subjects, allergic to any specific allergenic fining agent, should tolerate treateed wines.

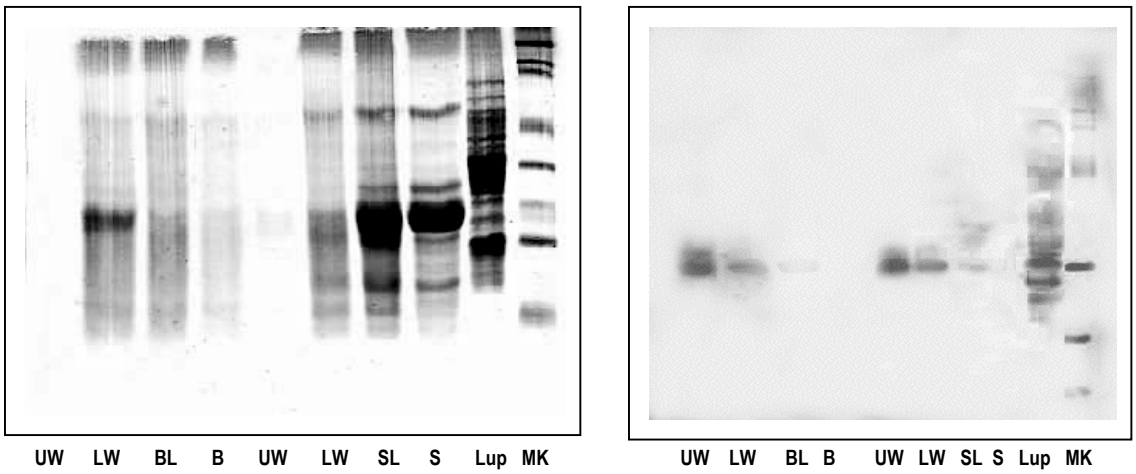

Figure 2 SDS-PAGE (left) and IgE-immunodetection with the serum from a patient allergic to lupin (right) of *Pinot Noir* wine samples, untreated (UW) or treated with lupin protein isolate alone (LW) or combined with bentonite (BL) or silica gel (SL).
To evaluate nonspecific reactivity, samples clarified with bentonite alone (B) or silica alone (S) were also loaded. MK = molecular weight marker solution; Lup = lupin protein isolate.
Data from Cattaneo et al, 2003b.

Table 2 - Critical points and limitations in performing oral challenges

Topic	**Comments**
Patient included	Allergy is more frequent in childhood but children cannot be enrolled in studies where wine administration is necessary
Number of patients	Adult patients are relatively few and it is difficult to enroll the number necessary to reach statistical significance (see text)
Cost	Patients must be hospitalized twice (day hospital) for several hours, to perform challenges with treated and control wines

CONCLUSIONS

Food allergy is a frequent pathology in children but its prevalence decreases in adult people, who consume alcoholic beverages. Even though, the figure of adult patients suffering from severe allergic reactions is relatively low, high level of control must be asked to wine producers if suitable dossiers must be presented to International Toxicological Committees in order to obtain the exemption from labeling of fining agents or additives. On these bases, the best strategy is the definition of international collaborative studies to define the best enological procedure to minimize dangerous residues in treated wines.

REFERENCES

Cattaneo A., Ballabio C., Bertelli A.A.E., Fiocchi A., Isoardi P., Terracciano L., Galli C.L., Restani P., 2003a. Evaluation of residual immunoreactivity in red and white wines clarified with gluten or gluten derivatives. *Int. J. Tissue React.* 25: 57-64.

Cattaneo A., Ballabio C., Bernardini R., Bertelli A.A.E., Novembre E., Vierucci A., Restani P., 2003b. Assessment of residual immunoreactivity in red or white wines clarified with pea or lupin extracts. *Int. J. Tissue React.* 25: 159-165.

Host A, Halken S. 1990. A prospective study of cow milk allergy in Danish infants during the first years of life. *Allergy* 45: 587-596.

Muraro A., Dreborg S., Halken S., Host A., Niggermann B., et al. 2004. Dietary prevention of allergic diseases in infants and small children. Part I. Immunologic backgroud and criteria for hypoallergenicity. *Pediatr Allergy Immunol.* 15: 103-111.