1.1 INTRODUCTION TO FOOD ALLERGY

Food allergies are abnormal responses of the immune system towards proteins in certain foods that most individuals can eat safely. It is estimated that food allergies which affect up to 6% of young children and 3–4% of adults (Wang and Sampson, 2011), is distinct from food intolerance which is a non-immunological reaction that can be caused by enzyme deficiencies, pharmacological agents and naturally occurring toxins. Food allergy occurs when oral tolerance fails to develop normally or ‘breaks down’ in genetically susceptible individuals. These reactions may be immediate (occurring within minutes or 2 hours of ingesting an allergen) or delayed (up to 2 days after ingesting), and accounts for a considerable number of emergency room visits each year. Allergic reactions are often unexpected and involve cutaneous reactions (urticaria, erythematous rashes, angiooedema, and eczema flare), gastrointestinal reactions (vomiting, abdominal pain, diarrhoea, oral burning/itching), respiratory reactions (wheeze, rhinitis, cough, stridor, voice change, dyspnoea) and cardiovascular reactions (hypotension, profound floppiness). Potentially life-threatening allergic reactions, called “anaphylaxis,” may also include a drop in blood pressure and loss of consciousness. An effective and safe treatment for such allergic reactions is yet to be developed (Burks et al., 2013).

Allergy or hypersensitivity was classified into 4 types by Gell and Coombs half a decade ago. Type I hypersensitivity reactions are predominantly mediated by immunoglobulin E (IgE) class of antibodies bound to mast cells and basophils. It is also suggested that these types of reactions are basically designed to prevent multicellular metazoan parasites from taking up residence in respiratory and GI systems (Rajan, 2003). Type II reactions are characterized by antigen–antibody interactions resulting in local tissue injury, wherein IgG antibodies are mainly involved. Type III and type IV reactions are mediated by antigen–antibody complexes and immune cells (T cells) respectively.
While food allergies are mainly IgE–mediated type I hypersensitivity reactions, wherein ingested food proteins elicit allergic symptoms in predisposed individuals, non–IgE mediated, or a combination of both also exits. These allergenic proteins have antigenic sites or epitopes which recognize and bind to specific IgE antibodies in the host immune system. The IgE antibodies are mainly found in lungs, skin and mucous membranes, and are often in high levels in people suffering from allergy. These IgE antibodies are mostly fixed on to the surface of mast cells and basophils via the high–affinity IgE receptors (FcεR1–fragment crystallizable region R1). These mast cells and basophils are found at perivascular sites in tissues; basophils are also circulating in peripheral blood which directly get exposed to the environment and function as first line of defence.

As described by Kumar et al. (2012), during primary exposure, the food allergens are captured by antigen–presenting dendritic cells (DC) of the intestinal mucosa and internalized. The allergens are then detected by ubiquitin, a 76-residue protein within the DC cytosol and degraded to peptide fragments. These fragments are presented by major histocompatibility complex class–II (MHC II) and recognized by naïve CD4+ T cells or T helper cells, which differentiate into Th2 cells in the presence of adequate amounts of IL–4. The differentiated Th2 cells secrete a spectrum of cytokines which induces class switching to IgE (Fig. 1.1). The IgE attaches to FcεR1 of mast cells or basophil cells (Fig. 1.2).

After the allergen–IgE–FcεR1 complex formation during secondary exposure, immunoreceptor tyrosine activation motifs (ITAMs) of FcεR1 induce mast cell signaling and degradation. Mediators which include prostaglandins, cytokines, leukotrienes, histamine, slow reacting substance of anaphylaxis (SRS-A), heparin, platelet–activating factor of anaphylaxis and proteolytic enzymes are secreted during degranulation. These mediators cause smooth muscle dilation, capillary disruption, local swelling and other clinical symptoms. Although the basic mechanism leading to an allergic response has been elucidated, additional molecular components of the immune system have also been observed to play a
Fig. 1.1 Presentation of an allergen to Th cells by dendritic cells during primary exposure. Dendritic cells (DCs) are antigen-presenting cells, (also known as accessory cells) of the mammalian immune system. Their main function is to process antigen material and present it on the cell surface to the T cells of the immune system. Ubiquitin is a small protein that exists in all eukaryotic cells. It performs its myriad functions through conjugation to a large range of target proteins. A variety of different modifications can occur. The ubiquitin protein itself consists of 76 amino acids and has a molecular mass of about 8.5 kDa. Mature T helper 2 cells (Th2) express the surface protein CD4 and are referred to as CD4+ T cells. CD4+ T cells are generally treated as having a pre-defined role as helper T cells within the immune system. For example, when an antigen presenting cell expresses an antigenic fragment on MHC class II, a CD4+ cell will aid those cells through a combination of cell to cell interactions and through cytokines.
Immunoglobulin E (IgE) is a class of antibody (or immunoglobulin (Ig) "isotype") that has been found only in mammals. All immunoglobulins have a 4 chain structure as their basic unit. They are composed of 2 identical light chains (23kDa) and 2 identical heavy chains (50-70kDa). The heavy and light chains and the 2 heavy chains are held together by inter–chain disulfide bonds and by non–covalent interactions. The number of inter–chain disulfide bonds varies among different immunoglobulin molecules. Within each of the polypeptide chains there are also intra–chain disulfide bonds. Both heavy and light chains have variable regions ($V_h$, $V_l$) and constant regions ($C_h$, $C_l$). Digestion with papain breaks the Ig molecule into $F_{ab}$ and $F_c$ fragments. The high–affinity IgE receptor, also known as FceR1, or Fc epsilon R1, is the high–affinity receptor for the Fc region of IgE. Cross–linking of the FceR1 via IgE–antigen complexes leads to degranulation of mast cells or basophils and release of inflammatory mediators. Under in vitro conditions, degranulation of isolated basophils can also be induced with antibodies to the FceR1α subunit, which crosslink the receptor.
deciding factor in the culmination of a reaction (Barbour et al., 2013). Fig. 1.3 summarizes the outline of the mechanism of food allergy.

![Summary of the mechanism of IgE mediated food allergic reactions](image)

**Fig. 1.3** Summary of the mechanism of IgE mediated food allergic reactions (Kumar et al., 2012).

Although an individual can be allergic to any food (plant or animal source), the following 8 foods have been found to account for 90% of all food–allergic reactions (FARRP Database–www.farrp.org): milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat and soybean (Fig. 1.4). Apart from these 8 foods, other foods including cereals and grains, legumes, fruits and vegetables also contribute toward food allergy cases. Fruits and vegetables causing allergic reactions have often been found to result in clinical symptoms which are mild and observed in the oral cavity with itching of the oral mucosa, swelling of lips, tongue and throat. Occasionally skin, respiratory and more severe symptoms are reported. Such localized oral cavity and pharyngeal reactions are referred to as oral allergy syndrome (OAS).
Vegetable allergies have been described for celery, asparagus, avocado, bell pepper, cabbage, carrot, lettuce, potato, pumpkin, turnip, and zucchini. Fruits and vegetables form a major part of the food pyramidal system (Fig. 1.5). An inquiry into the fruits causing allergy leads one to the listing of around 15–20 fruits to be commonly associated. Most of these are available worldwide in fruit markets; however, a few rare fruits, especially tropical fruits and berries, also can be observed to cause allergy in susceptible individuals.

1.2 ORAL ALLERGY SYNDROME (OAS) AND POLLEN–FOOD CROSS-REACTIVITY

OAS is found to be the most common form of allergy in adults with symptoms towards fresh fruits and vegetables. OAS results in localized symptoms such as pruritus and inflammation of lips and mouth, voice hoarseness, etc. OAS is generally termed as due to cross-reacting, homologous proteins found in the plant food proteins and pollens. Pollen–fruit–vegetable associations have similar characteristics, namely, localized oropharyngeal symptoms following ingestion of plant foods. Since conserved proteins are found
Recommended dietary compositions represented as a food pyramid. A food guide pyramid is a pyramid shaped guide of healthy foods divided into sections to show the recommended intake for each food group. The first food pyramid was published in Sweden in 1974. The most widely known food pyramid was introduced by the United States Department of Agriculture (USDA) in the year 1992, was updated in 2005, and then replaced in 2011. “MyPlate” is the current nutrition guide published by the USDA, depicting a place setting with a plate and glass divided into five food groups. MyPlate is divided into sections of approximately 30 percent grains, 20 percent fruits, 20 percent vegetables, 20 percent protein, accompanied by a smaller circle representing dairy, such as a glass of low-fat/nonfat milk or a yogurt cup.
throughout the plant kingdom, expression of homologous proteins in plant foods is not surprising (Wang, 2008).

Regional variations have been observed in OAS. In a study of 274 adults in England who were allergic to at least one pollen (birch, grass, and/or mugwort), 34% were sensitive to apple, 25% to potato, 23% to carrot, 23% to celery, 22% to peach, and 16% to melon (Bircher et al., 1994). In contrast, OAS was most commonly due to hazelnut, kiwi, apple and celery root (*Apium graveolens*) in Italy (Osterballe et al., 2005). Pollen–allergic adults in Sweden most often reported symptoms with hazelnut, apple, tomato, carrot, and peanut (Ghunaim et al., 2005). In Spain, peach is the most common fruit which causes allergy (Cuesta–Herranz et al., 1999).

Pollen–food syndromes have been observed to be associated with specific plants. One of them is birch–fruit–vegetable syndrome. Foods belonging to the order Rosaceae, which include apple, pear, peach, and almond, most commonly cause symptoms in birch-allergic patients. The prevalence of birch–fruit–syndrome is variable depending on geographic location (Breiteneder et al., 1989). Another is celery–birch–mugwort–spice syndrome. Celery has been found to have cross–reactivity with both birch and mugwort pollens. In areas where birch trees are prevalent, celery allergy is due to Bet v 1 homologs. However, celery allergy does exist in birch–free areas; in these cases, mugwort pollen allergens may be the primary sensitizier (Wang, 2008). Bet v 1 and profilins have also been identified in various spices (Scholl and Jensen-Jarolim, 2004), including anise (Pim a 1 and 2), coriander (Cor s 1 and 2), cumin (Cum c 1 and 2), fennel (Foe v 1 and 2), and parsley (Pet c 1 and 2). Cross–reactivity between mugwort and mustard has also been demonstrated, and accordingly celery–birch–mugwort–spice syndrome has been used to describe these cross–reactivities (Figueroa et al., 2005).

Apart from these, the ragweed–melon–banana association has also been observed. Up to 50% of ragweed–allergic patients have specific IgE to at least one member of the gourd family Cucurbitaceae, e.g. watermelon, cantaloupe,
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honeydew, zucchini, and cucumber (Enberg et al., 1987). These cross-reaction syndromes have also been observed for mugwort–peach association, plantain–melon association, pellitory–pistachio association, goosefoot–fruit association, and Russian thistle–saffron association (Egger et al., 2006).

Latex–fruit syndrome was first reported by M’Raihi et al. (1991), wherein an allergic reaction to banana was observed in a latex allergic patient. Soon thereafter, cross-reactivity between latex and various fruits was demonstrated, and this was termed latex–fruit syndrome (Blanco et al., 1994). Some studies have reported that up to 88% of latex–allergic adults have evidence of specific IgE to plant–derived foods (Beezhold et al., 1996; Ebo et al., 2003). The primary sensitization in these cases is believed to be due to latex, generally via inhalation (Wang, 2008), and several homologous proteins are found to be present in both allergenic plant foods and in latex allergens which include Hev b 2 (β-1,3-glucanase), Hev b 11 (class I chitinase) and profilin as Hev b 8 (Blanco et al., 1999; Reindl et al., 2002; Wagner and Breiteneder, 2002; Wagner et al., 2004).

1.3 ALLERGY TO COMMON FRUITS AND THEIR ALLERGENS

Fruit allergy is an adverse reaction to the consumption of fruit or fruit-based products (Hoffmann-Sommergruber, 2000). Plant allergens can be classified into two groups depending on how the primary sensitization takes place, via the GI or via the airways. The first group is generally more robust and resists degradation in the GI. It consists of storage proteins (prolamins, cupins) or cysteine proteases, and is more commonly a problem for young children. The other large group of plant allergens is the pollen–related allergens, for which primary sensitization takes place via inhalation.

Fruit allergy has been explained by cross-reactive IgE to pollen and, thus, allergy to fruits has also been linked to particular pollens, for instance, apple to birch, banana/melon to ragweed, hazelnut to hazel, celery to birch/mugwort, tomato/melon/watermelon to grass, fennel/celery to birch, kiwi to grass or birch, melon to plantago/grass etc. Some of these associations have been
demonstrated to be due to cross-reactivity between homologous protein structures shared between pollens and fruits.

Allergic reactions to fruits and fruit–based processed products are caused by distinct epitopes, which bind IgE antibodies (usually on mast cells) and initiate a series of clinical symptoms (Fernandez-Rivas et al., 2006). Different fruit manufacturing conditions may induce alterations of immune–reactive epitopes on allergenic proteins. Processing was shown to destroy existing epitopes on a protein and generate new ones (formation of neoallergens) as a result of conformational changes (Sathe et al., 2005). As one surveys through these kinds of studies and other fruit allergen characterization studies, the following fruits and their allergens can be observed to be at the center of the current allergy research on fruits.

**Apple** (*Malus domestica*)

Allergy to apple, a fruit of the Rosaceae botanical family, is usually demonstrated with mild oropharyngeal symptoms. Apple accounts for nearly 60% of the world fruit production. Apple is the most widely grown and consumed fruit in the Northern hemisphere. Together with other plant–derived foods, apple has been shown to have a positive effect on human health by reducing the risk of chronic diseases such as heart disease, strokes and some forms of cancer. However, the consumption of apples may also impose a serious allergenic risk for sensitized individuals.

An allergen homologous to the major birch pollen allergen Bet v 1 (17.5 kDa, pathogenesis–related protein family 10, PR-10) was the first apple allergen to be characterized (Vanek-krebitz et al., 1995; Vieths et al., 1995). Later other allergens were identified which include the thaumatin–like protein (TLP, 23 kDa, PR-5), the non–specific lipid transfer protein (nsLTP, 9 kDa, PR-14) and the profilin (14 kDa). These allergens have been officially named by the WHO/IUIS allergen nomenclature subcommittee (www.allergen.org) as Mal d 1, Mal d 2, Mal d 3 and Mal d 4, respectively (Andersen et al., 2011).
In Northern and Central Europe, the occurrence of allergy to apple is frequently related to birch pollinosis and sensitization is due to cross-reactivity between Bet v 1 and Mal d 1, whereas in Southern Europe this fruit allergy is observed together with allergy to peach caused by the allergens Pru p 3 and Mal d 3. Symptoms related to Mal d 1 are generally mild and local, representative for a chemical labile protein. Mal d 3, on the other hand, is a highly stable protein due to the presence of four disulfide bonds. Mal d 3 and its homologs in other fruits and vegetables have been repeatedly mentioned as main elicitors for true food allergy (Fernández-Rivas et al., 1997; Ma et al., 2006). Systemic manifestations mainly occur in the Mediterranean area and are observed to be based on cross-reactivity between apple LTP and peach LTP, with the latter considered as primary sensitizer. LTPs resist denaturation and degradation by thermal food processing and digestive proteases, long enough to induce sensitization in the GI tract and to provoke systemic symptoms (Sicherer and Sampson; 2010).

Little is known about the way of sensitization to Mal d 2. However, due to the presence of 8 conserved disulfide bridges, TLPs are expected to be resistant to pH or heat. Mal d 4 is a minor allergen and seems to be pollinosis-related. Bet v 2, the birch pollen profilin, sensitizes approximately 20% of the pollen-allergic patients. Profilins have recently been described as highly cross-reactive with other fruits and vegetables of the Rosaceae, Vitaceae and Solanaceae families as well as with pollen. Although profilins were shown to be strong sensitizers and highly cross-reactive, frequently they do not provoke symptoms and are believed to be of limited clinical relevance (Oberhuber et al., 2008).

**Sweet Orange (Citrus sinensis)**

Anaphylaxis to orange, a Rutaceae fruit has been observed to depend mainly on sensitization to a LTP. In one case, positive prick-to-prick tests with both raw and boiled orange and 2 apple varieties suggest heat-stability of the allergens involved. Two classes of plant allergens possess such stability, LTPs
and TLPs. Manifestations of orange allergy range from OAS to mainly IgE-mediated anaphylaxis. A case was reported wherein a dose-dependent anaphylactic reaction to orange juice occurred with no detectable specific IgE to orange allergens. They showed that the culprit allergen was at least partly heat-labile, because the patient reacted only after consuming a large amount (500 mL) of boiled orange juice. This allergen does not belong to any of the 3 identified major orange allergens – profilin (14 kDa, Cit s 2), germin–like protein (Cit s 1), and LTP (9 kDa, Cit s 3), because these were all found to be heat-resistant (Tsiougkos and Vovolis, 2013; Vovolis and Koutsostathis, 2009).

The presence of carbohydrate epitopes, recognized by IgE, on the orange protein Cit s 1 was described by Ahrazem et al. (2006). It indicated that the carbohydrate of Cit s 1 is a major epitope for the IgE of orange-allergic patients, because deglycosylation of this protein abolished the binding of IgE from pooled and individual sera. Cit s 3 had a ~35% prevalence and behaved as a minor allergen (Ahrazem et al., 2005; Pöltl et al., 2007).

**Peach (Prunus persica)**

Rosaceae fruit allergy is the most common food allergy in Europe in patients over 5 years of age. The two fruits most frequently involved are peach and apple. Different clinical phenotypes of peach allergy are observed across Europe in relation to different allergen sensitization patterns to the peach allergens. In a series of studies on Rosaceae fruit allergy in the Mediterranean area, peach has been shown as the first triggering food to subsequently associate with other Rosaceae fruits, such as apple, due to cross-reactivity of their LTPs. To date, allergy to this fruit has been reported in three distinct settings (Asero and Cecchi, 2011):

- In subjects primarily sensitized to birch pollen as the result of cross-reactivity between the major birch pollen allergen, Bet v 1, and a homologous protein in the fruit, named Pru p 1.
• In subjects characterized by IgE reactivity to multiple pollen extracts both *in vivo* and *in vitro* that co-recognizes the plant pan-allergen profilin (called Pru p 4 in the peach)
• Particularly in Mediterranean countries, in patients allergic to nsLTPs, a family of heat and pepsin-resistant plant food pan-allergens.

In areas rich in birch trees of Central and Northern Europe, peach allergy is linked to birch pollinosis and apple allergy. These patients present mild oropharyngeal symptoms (OAS) upon peach ingestion. In patients mono-sensitized to Pru p 3, the phenotype is severe and the great majority experience systemic reactions including anaphylaxis. In subjects mono-sensitized to Pru p 4, peach allergy is linked to a pollen allergy, generally to grass pollen, and the phenotype is mild with OAS in all the patients. When sensitization to Pru p 3 and Pru p 4 are combined, systemic reactions are less frequently observed than in patients mono-sensitized to Pru p 3, and OAS is more frequently reported (Fernandez-Rivas, 2011).

Sensitization to Pru p 2, the TLP in peach, may be found in combination with Pru p 3 and 4, but its clinical relevance has not yet been established. TLPs have been reported as relevant allergens in cherry, apple, kiwi, banana, bell pepper, grapes and peach. In a Spanish cohort of peach-allergic patients about 50% recognized TLP *in vitro*. However, the clinical relevance of TLPs is still ill-defined, because TLP reactivity is in most cases low and associated with hypersensitivity to other distinct allergens (Chen et al., 2008). Peach allergy across Europe is an interesting model food allergy that illustrates how different allergen sensitization profiles determine the clinical phenotypes of fruit allergy. The reason for the (almost) lack of sensitization to Pru p 3 in Central and Northern Europe still remains unclear.
**Musk melon (Cucumis melo)**

The fruit, musk melon (*Cucumis melo*) belongs to Cucurbitaceae family; this family includes several warm season vegetables (squash, cucumber and pumpkin) and fruits (watermelon). Musk melon has been reported as a frequent cause of fruit allergy, both in some areas from USA and the European Union. Profilin (Cuc m 2) has been identified as a major allergen from this fruit. In a study (López-Torrejó et al., 2005) it was observed that native (nCuc m 2) had a blocked N-terminus, whereas recombinant (rCuc m 2) rendered the expected N-terminal amino acid sequence, its full protein sequence being highly similar (98–71% identity) to those of profilins from plant foods and pollens. The natural allergen displayed a slightly higher IgE-binding capacity than its recombinant counterpart. Other allergens described from this fruit are Cuc m 1 (cucumisin), a 67 kDa subtilisin–like protease, and Cuc m 3; a 16 kDa PR protein belonging to the PR-1 family. No plant allergen homologous to Cuc m 3 has been detected until now.

The Cuc m 1 serine protease is present in the melon extract in several molecular forms originated during the process of maturation and subsequently as degradation fragments and, similar to Hev b 6, they have been named Cuc m 1.01 (67 kDa), Cuc m 1.02 (54 kDa) and Cuc m 1.03 (36 kDa). Cucumisin and several N–terminal cucumisin fragments are major allergens of melon. The ubiquitous distribution of this protein family (cucumisin–like proteases) in many plant species, its high structural similarity and the inhibition data suggest its potential role as a new panallergen in plant foods. The 36 kDa band was the most frequent IgE–binding band (100% of the sera), showing the highest reactivity with the sera of the melon–allergic patients and indicating that this N–terminal fragment of cucumisin is highly allergenic. Other bands corresponding to native (67 kDa) and mature (54 kDa) cucumisin were also found to be major allergens, although they were recognized with lower frequency (Cuesta-Herranz et al., 2003).
Gold Kiwi, Green Kiwi (*Actinidia chinensis, A. deliciosa*)

The kiwi fruit is a popular fruit very rich in vitamin C. There are two varieties, one with green flesh and another with yellow flesh. Allergy to green kiwi fruit (*Actinidia deliciosa*), which was first documented in the early 1980s, has been reported increasingly in recent years. Moreover, a closely related species, gold kiwi fruit (*Actinidia chinensis*), became available in the international market in 1999 and shares IgE cross-reactivity and the presence of common allergens with green kiwifruit. Although allergy to kiwi fruit is commonly associated with mild and local symptoms (mainly OAS) and with hypersensitivity to pollens, severe anaphylactic reactions are rather common. Additionally, kiwi allergy also has to be considered in relationship with the latex–fruit syndrome, together with sensitization to avocado, chestnut and banana, which are the main plant foods linked to latex allergy (Bublin et al., 2013). In spite of this extensive background on kiwi allergy, its objective immunological evaluation is limited by the low predictive value of the commercial tests used currently, both *in vitro* (UniCAP System, Phadia, Uppsala, Sweden) and *in vivo* (SPT).

Several putative kiwi fruit allergens have been detected in recent years. However, only two of them have been studied sufficiently in different groups of kiwi–allergic patients until now. Act d 1 (originally Act c 1) corresponds to the 30 kDa thiol–protease actinidin, which is well established as a major kiwi allergen (Palacin et al., 2008). Although recently reported data point to its lack of *in vitro* reactivity in the case of the kiwi–sensitized population from the UK, Act d 2 is a 24 kDa TLP, whose sensitization prevalence is still controversial. Besides, N-terminal amino acid sequences of putative relevant allergens, namely an 11 kDa cystatin (Act d 4), a 28 kDa kiwellin (Act d 5), 43 and 45 kDa proteins (Act d 3) have been reported, their actual importance in kiwi sensitization is not fully clarified. On the other hand, none of these allergens appears to be primarily responsible for the latex–kiwi cross–sensitization described in the context of the latex–fruit syndrome (Cremer and Mennicken et al., 2011). Class I chitinases with an N-terminal hevein–like domain and latex hevein have been identified as the major cross–reactive components involved in this syndrome (D’Avino et al.,
Identification of major allergens in kiwi fruit has so far resulted in conflicting and confusing results both in terms of number and relevance of allergens. In fact, different studies reported different dominant allergens, probably due to differences in both experimental procedures and study population used (Lucas et al., 2003).

**Sweet Cherry (Prunus avium)**

Cherry (Prunus avium) allergy is often reported in the context of allergy to other fruits of the Rosaceae family and pollinosis to trees because of cross-reactive allergens. Allergic reactions to cherry are reported by 19–29% of birch pollen–allergic patients (Fuchs et al., 2006). Pru av 2, identified as a TLP from sweet cherry, was recognized by the majority of cherry–allergic patients. Pollinosis is one of the prevailing allergic diseases in adults throughout Europe. Allergy to pollen of trees of the order Fagales is often combined with food allergy to fruits of the Rosaceae family including cherries. Based on a patient questionnaire, 29% of Swedish patients with birch pollen allergy were defined as being allergic to cherries. Even in Japan, it was reported that 61% of 54 birch pollen–allergic patients were allergic to fruits and vegetables, and 58% from the food–sensitive group stated adverse reactions after ingestion of cherries. OAS was frequently reported as the most frequent clinical manifestation (Fuchs et al., 2006).

Pollen–related cherry allergy is caused by the presence of cross–reactive IgE epitopes on homologous proteins. Four allergens from sweet cherry have been identified so far. Pru av 1 and Pru av 4 are homologous to the birch pollen allergens Bet v 1 and Bet v 2, respectively, and are in part responsible for the cross-reactivity between birch pollen and cherry. Pru av 3 is a nsLTP sharing high amino acid sequence identities with nsLTPs from other fruits of the Rosaceae family. Pru av 2 was first discovered in 1996 as the most abundant soluble protein in ripe cherries accumulating during the ripening process; it was purified and described as a non–sweet 29 kDa polypeptide. TLP from cherry was described as a potential major allergen of 30 kDa and named as Pru a 2, later
modified to Pru av 2 (Inschlag et al., 1998). A high degree of sequence similarity to thaumatin, an intensely sweet–tasting protein of the fruits of a West African shrub (miracle fruit), was observed in TLPs.

**Olive (Olea europaea)**

Proteins in the pulp of olive (*Olea europaea*) constitute a minor fraction. They have been sparsely studied despite their suggested role in oil stability and olive allergenicity. Olive is one of the main crops in Mediterranean countries. Olive fruit may be used for direct consumption as table olives, as well as for the production of oil. Olive oil is one of the most representative foods of the Mediterranean diet, and its consumption has been related with a decrease in the incidence of cardiovascular diseases, cancer, and Alzheimer’s disease. Numerous studies have been carried out on the determination of the main components in both olives and olive oil, producing a vast body of information. Nevertheless, minor pulp components, such as proteins, have been poorly studied, despite their suggested role in oil stability. Furthermore, there are studies showing that proteins present in olive fruit and oil may elicit allergic reactions in sensitive individuals.

Although allergy to olive fruit or oil is uncommon, some cases of allergy associated with olive or olive oil ingestion have been reported. Moreover, some patients presented cross-reactivity of olive pollen allergen and olive fruit allergen. Reliable detection methods for olive allergens are necessary to improve consumer protection (Esteve et al., 2011). In contrast to olive seed proteins, pulp proteins have been insufficiently studied. For a long time, an oleosin–like polypeptide of 4.6 kDa was described as the main protein in olive pulp and oil. Extraction of proteins from the olive pulp is problematic because of the high content of interfering compounds (lipids and phenolic compounds) and due to the low amount of proteins (~2%). This probably explains why research related to the identification and characterization of pulp proteins is scarce. The olive fruit is frequently consumed as food and used as raw material to obtain olive oil. Olive oil is pressed in mills, where workers are exposed daily to inhalation of
particles derived from this processing. Immunoblotting with the occupational allergic patient’s serum revealed a reactive 23 kDa band in olive–fruit protein extract that was purified by size–exclusion chromatography and RP–HPLC. The purified protein was IgE–reactive, and Edman degradation gave the amino acid sequence ATFIVNQXTYTVXAAASP, which showed homology to allergenic TLPs from plant foods and pollens. It was reported that occupational asthma can be caused by a TLP from olive fruit (Palomares et al., 2008).

**Grape (Vitis vinifera)**

*Vitis vinifera* is one of the oldest cultivated plants all over the world. It grows in a temperate climate, especially around the Mediterranean, and its fruit, the grape, is consumed either directly or as wine. Western Europe is the world’s biggest producer of grapes; France, Italy, and Spain are the major producers of wine that is consumed throughout the world. The effects of grapes and wine on health are always viewed with much interest. For example, it has recently been observed that moderate consumption of red wine is beneficial to the cardiovascular system on account of its high content of polyphenols, which behave as radical scavengers and antioxidants.

Allergic reactions to wine are commonly believed to be mainly caused by sulfites (Pastorello et al., 2003). Allergic reactions to grape are described as case reports: one case of urticaria–angioedema caused by white grapes, one case of exercise–induced grape anaphylaxis, and several anaphylactic reactions in one patient selectively sensitized to the Americana grape (*Vitis labrusca*). A possible relationship between allergic reactions to grape and other botanically unrelated fruits appears from other studies. Giannoccaro et al. (1998) reported a patient allergic to grape and cherry. A single case of rhinoconjunctivitis and asthma caused by vine pollen has also been reported (Feo Brito et al., 1999). No immediate reactions to wine have been described nor have grape major allergens been properly investigated. Only in one report (Pastorello et al., 2003), it was found that an unidentified protein of ~30 kDa bound IgE antibodies from 3 patients with grape anaphylactic reactions.
Pastorello et al. (2003) characterized the grape major allergens and attempted to identify the allergen in wine. Documented histories of allergic reactions to grape and wine were collected and purified grape allergens were identified by SDS-PAGE and immunoblotting. Cross-reactivity with peach and cherry was evaluated by means of cross-wise inhibition experiments. Eleven patients with reactions to grape and 3 with anaphylactic reactions to wine were recruited. The major allergens were an endochitinase 4A (~30 kDa) and a LTP that was homologous to and cross-reactive with peach LTP and a 24 kDa TLP was reported as minor allergen. Endochitinase 4A is very likely the allergen in \textit{vino novello} and in \textit{vino Fragolino}.

Researchers have observed several patients with severe allergic reactions after eating grapes and, in some of them, also after drinking 2 particular kinds of red wine, namely \textit{vino Fragolino} and the young wine \textit{vino novello}. Some technical differences in the process of making non-aged wine might explain why the patients were allergic only to \textit{vino novello} or \textit{vino Fragolino}. The composition of red wines is affected by both the wine-making process and aging before it is sold. \textit{Vini novelli} (\textit{vins nouveaux} or young red wines) have become popular in recent years. These wines are intended to be consumed within a short time, so that polymerization of the polyphenols present in red wines cannot occur, and any proteins present remain in solution. Polymerization of polyphenols causes the tiny residual proteinaceous material in red wines to coalesce, so that it can be filtered off once the wine has aged, thus theoretically explaining why the patients tolerated older wine. \textit{Fragolino} wine, obtained from a blue \textit{Vitis labrusca} grape, is also consumed young (Pastorello et al., 2003).

Grape chitinases account for 50% of the soluble proteins in grapes, persisting through the vinification process. Thus far, 4 chitinases have been purified (eg, from Muscat of Alexandria fruit), and 3 of them had pyroglutamate residues as the N-terminal group, indicating that they are N-terminally blocked (Waters et al., 1998). The other proteins persisting in wine throughout vinification are TLPs; it was found that this 24 kDa protein was another
important allergen in grapes. The amino acid sequence of this allergen proved to be highly homologous to cherry (Pru av 2) and apple TLP (Mal d 2).

The identification of a 9 kDa LTP as a major grape allergen seems very interesting because it could explain why grape allergy is often associated with food allergic reactions to fruits, such as peach and cherry. The high rate of sequence homology between grape and peach LTPs gives the molecular basis of the observed high cross-reactivity. The history of symptoms to latex gloves only in patients with a strong IgE positivity to the latex 14 kDa allergen suggest, however, that the above-mentioned cross-reactivity is not clinically expressed (Pastorello et al., 2003).

**Strawberry** (*Fragaria ananassa*)

Strawberries are not only eaten fresh but are also used as a common ingredient in many food products such as jam, yoghurt, ice-cream, and breakfast cereals. Strawberry is an important ingredient in the food industry. The strawberry Fra a 1 allergen is a homologue of the major birch pollen allergen Bet v 1. Mass spectrometry pointed out the presence of strawberry homologues to the Bet v 1 allergen in both the 20 and the 18 kDa protein bands. It is synthesized by red ripe strawberry fruits while white strawberry fruits of a mutant genotype, which is known to be tolerated by individuals affected by strawberry allergy, are devoid of it (Karlsson et al., 2004; Radauer and Breiteneder, 2007; Musidlowska Persson et al., 2007). The presence of a strawberry homologue to the 35 kDa Bet v 6 allergen, an isoflavone reductase, was also suggested to be a strawberry allergen. A 9 kDa LTP with 74% homology to apple LTP (Mal d 3) has been detected, which could be a possible strawberry allergen (Munoz et al., 2010).

**Banana** (*Musa acuminata*)

Banana is a frequent cause of food allergy, particularly in latex-sensitized patients. The relevance of banana as a source of food allergy was confirmed in
two patients by double-blind food challenge. In 1 of 2 patients, in whom banana allergy was not a consequence of latex sensitization, a 70-kDa protein was identified as a banana allergen, and in the other patient profilin was detected as a putative cross-reactive allergen. The commonly occurring hypersensitivity to banana in patients allergic to natural rubber latex (NRL) could be explained by cross-reacting IgE antibodies binding to epitopes in hevein and in a hevein–like domain of a previously undescribed endochitinase in banana. It has been observed that 20% to 50% of patients allergic to NRL have experienced symptoms after eating banana. Evidence for cross-reacting allergens in NRL and banana has also been reported. It has been shown that hevein (Hev b 6.02), the 43 amino acid chitin–binding domain of prohevein (Hev b 6.01), is a major IgE-binding allergen for patients allergic to NRL in relation to endochitinase (Mikkola et al., 1998).

**Custard apple (Annona squamosa, A. cherimola)**

Allergic cases reported for custard apple have, for most of the cases been in cross-reaction to latex. Cross-reactivity with latex allergy was found for protein bands of 40–45 kDa; the 45 kDa band was identified as chitinase. It has also been reported that the N-terminal hevein–like domain of the chitinase is responsible for cross-reactivity with latex (Santos-magadan et al., 2012). The first case of allergy to custard apple was reported in 1997 wherein a 20–25 kDa band was detected. A 14 kDa acyl carrier protein was also reported as an allergen but not confirmed. Several reports of allergy to custard apple have appeared in the literature (Gonzalo et al., 1997; Sánchez-Guerrero et al., 2000; Sánchez-Morillas et al., 2003). The 20–25 kDa protein identified as the allergen by IgE–immunoblotting is likely to be a TLP.

**Mango (Mangifera indica)**

Mango belongs to the Anacardiaceae family (Sumac species), which also includes cashews and pistachios. Rubin and Shapiro (1965) were the first to report an anaphylactic reaction following the ingestion of mango; since then, the
fruit has been reported to cause both type 1 allergy (anaphylaxis or oral allergy syndrome) and type 4 allergy or contact dermatitis (Miell et al., 1988). Mango allergens have also been shown to cross-react with mugwort/celery, latex (via class I chitinases), papaya, tomato, and banana (Brehler et al., 1997). Renner et al., (2008) identified 2 major allergens with a molecular mass of 27 kDa in two patients, in addition to a 15 kDa allergen in 1 patient and a 32 kDa allergen in another. Mango anaphylaxis and mugwort (Artemisia vulgaris) pollen cross-reactivity has been described by Silva et al. (2009). Mango profilin has been shown to cross-react with birch pollen profilin Bet v 2 (Song et al., 2008).

**Pomegranate (Punica granatum)**

Pomegranate fruits are commonly consumed in raw and processed forms such as juice, wines, flavors, and extracts, but they have rarely been reported to cause immediate hypersensitivity after ingestion (Zoccatelli et al., 2007). Allergy to pomegranate was first reported by Igea et al. (1991) wherein an IgE mediated allergy could not be demonstrated, and Valsecchi et al. (1998) reported contact hypersensitivity to pomegranate. A case of anaphylaxis to mannitol present in pomegranate was described by Hegde et al. (2002). A 29 kDa protein allergen was detected as an allergen in 3 cases of multisystemic reactions to pomegranate (Gaig et al., 1999). LTP from this fruit was shown to react with a pomegranate allergic patient (Zoccatelli et al., 2007). The limited number of cases obscures any conclusion on the possible presence and identification of common allergens in this fruit, and there is not a general consensus on which is the major pomegranate allergen(s). However, cross-reactivity has been demonstrated for LTPs present in different fruits including pomegranate (Enrique et al., 2006).

### 1.4 IMPORTANT ALLERGENS CAUSING FRUIT ALLERGY

From the foregoing description of fruit allergy, it is seen that fruit allergy is more frequently associated with pollen cross-reactivity, and birch pollen-associated allergy in relation to fruits is a well known clinical phenomenon especially in northern Europe. Following a primary sensitization to birch pollen
allergen, a subsequent IgE cross–reaction with homologous proteins in the consumed fruit occurs. Bet v 1, the major birch pollen allergen, shares common epitopes with major food allergens in a large number of different fruits and berries, e.g., cherry (Pru av 1), apple (Mal d 1), pear (Pyr c 1), celery (Api g 1). Patients suffering from type I allergy caused by birch pollen frequently demonstrate allergy to other fruits. A summarized list of all the fruits reported to cause allergy and their identified allergens is shown in Fig. 1.6.

Plant nsLTPs are a widely distributed superfamily of related proteins. They are divided into two subfamilies according to their molecular masses: the 9 kDa nsLTP1 and the 7 kDa nsLTP2. They have been classified as PR-14 defense proteins and several nsLTPs with allergenic activity have been identified in fruits and pollens so far. The most frequently implicated foods belong to the Rosaceae fruits, but nsLTPs with allergenic activity have also been detected in tree nuts, peanut, beer, maize, mustard, asparagus, grapes, cabbage, dates, orange, fig, kiwi, lupine, fennel, celery, tomato, eggplant, lettuce, chestnut, and pineapple. Because of their stable structure and resistance to proteolytic digestion and heat treatment, nsLTPs are regarded as primary sensitizers responsible for severe allergic reactions leading to the definition of nsLTPs as true food allergens. An Italian study performed on 1100 food allergic patients showed that nsLTPs are the most important allergens causing food–induced anaphylaxis, where peach was the most frequently offending food.

The family of TLPs, also referred to as PR-5 protein, plays an important role in the plant’s defense against pathogens. Several members of the TLP family have been identified as major allergens in cupressaceae pollens such as Jun a 3, Cup a 3, and Cry j 3 as well as in plant foods such as cherry, apple, kiwi, banana, orange, grape and bell pepper. Another factor contributing to the allergenic potential of TLP is resistance to denaturation by heat treatments. Processed foods like juices, sauces, and jams that are prepared from fruits are frequently subjected to thermal treatments. Unfolding increases the likelihood of additional allergenic epitopes becoming accessible during food processing. Recombinant TLPs have been characterized as allergens of several fruits such as kiwi, apple,
23.0 kDa - TLP (Mal d 2)
17.5 kDa - Bet v 1 homolog (Mal d 1)
14.0 kDa - Profilin (Mal d 4)
09.0 kDa - LTP (Mal d 3)

Apple (Malus domestica)

23.7 kDa - germin–like protein (Cit s 1)
14.0 kDa - Profilin (Cit s 2)
09.0 kDa - LTP (Cit s 3)

Sweet Orange (Citrus sinensis)

17.5 kDa - Bet v 1 homolog (Pru p 1)
23.0 kDa - TLP (Pru p 2)
14.0 kDa - Profilin (Pru p 4)
09.0 kDa - LTP (Pru p 3)

Peach (Prunus persica)

67.0 kDa - serine protease (Cuc m 1.01)
54.0 kDa - serine protease (Cuc m 1.02)
36.0 kDa - serine protease (Cuc m 1.03)
14.0 kDa - Profilin(Cuc m 2)
16.0 kDa - PR-1 protein (Cuc m 3)

Musk melon (Cucumis melo)
35.0 kDa - Bet v 6 homolog (isoflavone reductase)
17.5 kDa - Bet v 1 homolog (Fra a 1)
09.0 kDa - LTP (Fra a 3)

Strawberry (Fragaria ananassa)

43 - 45 kDa - Chitinase (Act d 2)
30.0 kDa - Actinidin (Act d 1)
28.0 kDa - Kiwellin (Act d 5)
23.0 kDa - TLP (Act d 2)
11 kDa - Cystatin (Act d 4)

Kiwi (Actinidia deliciosa)

17.5 kDa - Bet v 1 homolog (Pru av 1)
23.0 kDa - TLP (Pru av 2)
14.0 kDa - Profilin (Pru av 4)
09.0 kDa - LTP (Pru av 3)

Sweet cherry (Prunus avium)

23.0 kDa - TLP

Olive (Olea europaea)

30.0 kDa - Endochitinase
23.0 kDa - TLP
09.0 kDa – LTP

Grape (Vitis vinifera)

Fig. 1.6 (Continued)  List of common fruits and their known allergens.
cherry, bell pepper, grape, and orange as well as of cypress, mountain, and Japanese cedar pollens.

The latex–fruit syndrome is the result of cross–reactivity between NRL proteins and fruit proteins. Class 1 chitinases (Hev b 6, hevein–like proteins), profilins (Hev b 8), β–1,3–glucanases (Hev b 2), and other cross–reactive polypeptides have been implicated. The *Hevea brasiliensis* latex profilin is cross–reactive with allergens of plant foods and pollens. The commonly reported cross–reactive foods include banana, avocado, kiwi, and chestnut. The group of defense–related plant proteins, class 1 chitinases, cross–react with the panallergen hevein. Cross–reactivity with these proteins is noted with banana, avocado, kiwi, chestnut, papaya, tomato, cherimoya, passion fruit, mango, and wheat. Prohevein (Hev b 6) behaves as a major allergen, since it reacts to IgE in most of the sera of patients with latex allergy (Wang, 2008).

Plant allergens, being one of the most widespread allergenic substances, are hard to avoid. Therefore, their identification and characterization is one of the keys for the diagnosis and treatment of allergic diseases. It appears that the relatively high amount of these proteins in plant–derived foods play an important role in sensitizing genetically predisposed patients (atopic). In most cases, protocols for the diagnosis of food allergy make use of total protein extracts. However, depending on the experimental procedure used and on the food characteristics (e.g., the ripening stage of a fruit), protein extracts may be variable in both the number and amount of the allergenic components. This heterogeneity may be at least one of the causes of some conflicting and confusing results reported in this field. Moreover, results obtained by using total protein extracts do not provide information about individual sensitivity towards single allergenic components of the investigated food, which should be particularly useful in planning and monitoring desensitizing immunotherapy. Availability of purified and characterized allergens would help solving these problems, and also allow controlled and reproducible production of hypoallergenic derivatives (Bohle and Vieths, 2004).
1.5 ALLERGY TO SAPODILLA FRUIT

Based on the extensive survey of fruit allergy in literature, it is seen that many of the widely available fruits have been studied with reference to their allergenicity. Although allergic reaction to uncommon fruits like Chinese bayberry (*Myrica rubra*), sharon fruit (*Diospyros* spp.), papaya (*Carica papaya*), pineapple (*Ananas comosus*), blueberry (*Vaccinium* spp.) and ackee fruit (*Blighia sapida*) do occur, their reports are scarce (Bolhaar et al., 2005; Gebhardt et al., 2009; Lebo et al., 1996; Reindl et al., 2002; Sharda et al., 2010; Wang et al., 2012). These exotic fruits are consumed commonly in a particular region and have not been well studied in terms of individual allergens and cross-reactivity among fruits.

Sapodilla (*Manilkara zapota* syn. *Achras zapota*), also known as sapota, chikoo and by various other names, yields delicious fruits that are round, ovoid, ellipsoid or conical, flattened at the stem end, 5 to 10 cm wide. The fruit is highly perishable and sensitive to cold storage; hence, the bulk of the produce is used for table purpose. Sapodilla is cultivated in the tropical parts of the world with India being the largest grower. Commonly available varieties in India are the round cricket ball variety and the oval Kalipatti variety. The fruit is becoming popular as a specialty menu item in restaurants across Europe and North America. The fruits are rich in sugars (10.2%) and fiber (11.5%); however, the protein content is very low (~0.4–0.7%).

Allergic reactions to the ingestion of sapodilla fruit are rare; only 3 cases of sapodilla allergy displaying oral allergy syndrome have been described in a report, wherein a ~21 kDa protein was identified as the causative allergen responsible for OAS in sapodilla-allergic subjects. Since sapodilla fruit is widely consumed in India, it appeared important to study and characterize this major allergen in detail. The present investigation focuses on biochemical and immunological characterization of the 21 kDa major allergen, and the study was carried out under the following objectives:
CHAPTER 1

1. To characterize the sapodilla major allergen for its biological activity, thermal stability and stability to simulated gastric fluid.

2. To examine the allergenic potential of sapodilla major allergen upon intra–gastric administration in mice as an animal model.

3. To clone the gene coding for sapodilla major allergen and its characterization.

These objectives were investigated by using various *in vitro*, *in vivo* and *in silico* methods commonly used in allergology. The research work carried out towards fulfilling these objectives are presented in 6 chapters and compiled in the form of a thesis.