Chapter 18

Gliadins in Foods and the Electronic Tongue

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18.1 INTRODUCTION

Food allergy is an adverse, abnormal immune-mediated reaction to a certain food or food ingredient that appears in susceptible individuals, often requiring a strict avoidance of their ingestion (Amaya-González et al., 2013). Sometimes, people exhibit food sensitivity, including intolerance that is a nonimmune-mediated reaction. The incidence of these disorders is difficult to assess and the percentage of people self-perceived as food intolerant (up to 25%) is very different from that of confirmed cases (less than 3%). Most allergens are proteins that must be detected along the food chain, posing a real challenge for the development of analytical methods. Gliadin is a heat-stable allergen, known as the alcohol-soluble fraction of gluten, being the antigenic protein of wheat responsible for celiac disease. The ratio of gliadin to total gluten varies with the food matrix. The gliadin content usually corresponds to half of the gluten content (Peres et al., 2011), although this value is not consensual (EFSA, 2004; Tranquet et al., 2012). A daily intake of 100 mg of gliadin can induce clinical symptoms in celiac patients, being the prevalence of celiac disease (classical, oligosymptomatic, and silent forms) in children and adults around 1:200 in Europe (EFSA, 2004). Therefore, a threefold definition of gluten-free foods was proposed (EFSA, 2004): (1) foods in which ingredients do not contain any prolamin from wheat or *Triticum* species with a gluten level not exceeding 20 mg/kg (or ppm); (2) those consisting of ingredients which have been rendered "gluten-free" with a gluten level not exceeding 200 mg/kg; and (3) those resulting from a mixture of ingredients with a gluten level not exceeding 200 mg/kg. It should be noticed that the values previously mentioned are only indicative since there is not enough information to make a final decision on them. More recently, foods labeled as "very low gluten" or "gluten-free" must have gluten content lower than 100 and 20 mg/kg, respectively (Nassef et al., 2008; OJEU, 2009; Zeltner et al., 2009). However, commercial foods labeled as gluten-free may be contaminated by gluten in the range of 20–200 ppm (Collin et al., 2004; Scognamiglio et al., 2014).

Several commercial analytical tools have been developed, namely to detect gliadin/gluten, most of them relying in immunoassays, both competitive for hydrolyzed food and sandwich formats for complete proteins (Amaya-González et al., 2013). Moreover, emerging electrochemical techniques such as aptasensors and electronic tongues (e-tongues) have also been reported (Amaya-González et al., 2014; Meirinho et al., 2015; Peres et al., 2011).

18.2 GLIADINS AND THE CELIAC DISEASE

The celiac disease is classified as an autoimmune disease of the small intestine induced in genetically susceptible individuals that is caused by the ingestion of gluten proteins, which are important components of commonly used food sources like wheat, rye, and barley (Bai et al., 2013; Shan et al., 2002). The introduction of gluten-rich foods in the human diet led to the development of disease related to gluten exposure (Sapone et al., 2012; Troncone and Jabri, 2011). These reactions are not restricted to celiac disease, but also include nonceliac gluten sensitivity and wheat allergy, which combined affect about 10% of the general population (Battais et al., 2003, 2005; Lammers et al., 2014; Williams et al., 2010; Woodward, 2010). Moreover, they represent distinct pathophysiological reactions to gluten ingestion, with differing clinical presentations, serological markers, and long-term treatments (Bai et al., 2013; Briani et al., 2008; Ciclitira et al., 2005b). Although current

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research attempts to elucidate the frontiers between these reactions, their differences can be difficult to discriminate.

In the case of celiac patients, the exposure to gluten induces an inflammatory response that ultimately will lead to the destruction of the villous structure of the intestine (Shan et al., 2002; Williams et al., 2010; Woodward, 2010). It usually appears in early childhood with pronounced symptoms such as chronic diarrhea, abdominal distension, and failure to thrive. In some patients, symptoms are only revealed later in life and these may include fatigue, diarrhea, and weight loss due to malabsorption, anemia, and neurological symptoms (Ciclitira and Moodie, 2003). Celiac disease is a life-long disease and if untreated, it is associated with increased morbidity and mortality. Despite its high prevalence and severe symptoms, the only effective therapy is a strict dietary abstinence from the previously mentioned food grains (Briani et al., 2008; Fric et al., 2011; Tye-Din et al., 2010; Sapone et al., 2012).

Gluten is the main structural protein complex present in wheat with equivalent toxic proteins found in rye and barley (Sapone et al., 2012). The amino acid composition of gluten peptides with a high percentage of glutamine (up to 35%) and proline (15–20%) is unique (Fric et al., 2011). Immune-reactive protein fractions of gluten comprise gliadins and glutenins, with gliadins containing monomeric proteins and glutenins containing aggregated proteins (Bittner et al., 2008). Gliadins are complex glycoproteins rich in proline and glutamine (Lammers et al., 2014). Due to their structure, the intestinal enzymes cannot completely degrade the proteins. Actually, it is well known that undigested or partly digested gliadins can affect a broad range of human cells (eg, inhibit cell growth, induce apoptosis, and alter redox equilibrium).

The celiac disease is a model autoimmune disease, in which, contrarily to many other autoimmune diseases, the trigger (gluten), the tight genetic junction (HLA antigens: DQ2 and DQ8), as well as the primary autoimmune reaction [autoantibodies to tissue transglutaminase (tTG)] are known (Fric et al., 2011; Sollid et al., 2012; Tye-Din et al., 2010). This knowledge represents an advantage in the development of new diagnosis and treatment methods, as well as for the development of food analytical techniques that can easily and accurately detect the presence of gluten-related toxic protein fractions, such as gliadins. Indeed, the main clinical issues in the management of celiac disease are that the diagnostics are suboptimal and invasive, and that patients must rely on a complex, costly, and life-long therapy (Tye-Din et al., 2010). While intestinal biopsy is still considered the gold standard for diagnosing celiac disease, the presence of highly specific autoantibodies in patient serum has been clinically used as a marker for screening candidates for duodenal biopsy (Bizzaro et al., 2012; Ciclitira and Moodie, 2003; Williams et al., 2010; Woodward, 2010). Additionally, the relevance of antibody assessment in predicting celiac disease has increased along with the number of patients with minor or atypical symptoms.

As previously mentioned, from the human diseases related to gluten exposure, the best known are mediated by the adaptive immune system and include celiac disease and wheat allergy (Battais et al., 2003). In both conditions, the reaction to gluten is mediated by T-cell activation in the gastrointestinal mucosa (Han et al., 2013; Sapone et al., 2012). However, in wheat allergy, it is the cross-linking of immunoglobulin IgE by repeat sequences in gluten peptides that triggers the release of chemical mediators. Contrarily, the celiac disease is an autoimmune disorder as demonstrated by specific serologic autoantibodies [tTG and antiendomysium antibodies (EMA)] (Han et al., 2013). Besides these two conditions, there are cases of gluten reactions in which neither allergic nor autoimmune mechanisms are involved. These are generally defined as gluten sensitivity. Individuals exhibiting gluten sensitivity are unable to tolerate gluten and develop an adverse reaction different from the one observed in patients with celiac disease, that is, without damage in the small intestine (Bai et al., 2013; Troncone and Jabri, 2011). Although the symptoms may be similar to those associated with celiac disease, no tTG autoantibodies or other specific celiac-related antibodies are found.

Although a gluten-free diet is prescribed to patients suffering from diseases related to gluten exposure, this does not mean that they cannot tolerate gluten at all, as their clinical sensitivity varies significantly (Ciclitira et al., 2005a; Hischenhuber et al., 2005). Some individuals cannot tolerate trace amounts of gluten, whereas others appear to tolerate large amounts. In the standard Western European gluten-free diet, some gluten is accepted as a contaminant in wheat starch (Kupper, 2005). This starch improves the baking quality and palatability of the gluten-free diet and it is tolerated by most celiac patients (Collin et al., 2004; Fido et al., 1997; Goesaert et al., 2005; Peraaho et al., 2003). In other countries, such as United States of America, for example, wheat starch is not recommended. The US National Food Authority has decided that the label "gluten-free" can only be used for foods that contain no gluten at all (Kupper, 2005). Therefore, foods that contain wheat starch should be labeled as "low-gluten" (Fasano and Catassi, 2001). The proposed standard as formulated by the WHO/FAO organization *Codex Alimentarius*, has one limit at 0.02% for "rendered gluten-free" food, and another at 0.002% for "naturally gluten-free food" (Bai et al., 2013; Hischenhuber et al., 2005; Niewinski, 2008). These different practices reflect the fact that we do not know the exact limit of gluten intake that is tolerated long term without harmful effects by patients with celiac disease as a group (Ciclitira et al., 2005a). The acceptable gluten dose per day is still under debate, although the scientific and medical communities have suggested that around 50 mg/day is safe (Hischenhuber et al., 2005). As a reference, the normal

gluten intake by healthy individuals is about 13 g/day (Van Overbeek et al., 1997). Research indicates no significant differences in susceptible individuals undergoing a strict wheat starch-containing, gluten-free diet versus a naturally gluten-free diet (Kupper, 2005).

In summary, the awareness of the gluten-related diseases, its dietary restrictions, and the impact of adhering to a gluten-free diet warrant further research. Also, reliable detection and quantification methods for food allergens, such as gluten proteins, are required to ensure compliance with food labeling and to improve consumer protection.

18.3 SENSOR DEVICES FOR GLIADIN AND/OR GLUTEN DETECTION IN FOODS

The availability of fast, sensitive, and reliable analytical methods to detect specific food risks, ensuring food safety for people susceptible or intolerant to some food substances that may be allergens like gliadins is of huge importance and a real need. Indeed, in a recently market survey carried out in the United States (Sharma et al., 2015), it was reported that 3.6% of the gluten-free labeled foods evaluated contained 5.8–554 ppm of gluten, and 1.1% of those foods had gluten contents greater than the regulatory threshold (20 ppm). The limitations (eg, nonportability; strict operating conditions; and required highly qualified trained technicians) associated to the high-cost and time-consuming traditional methods (eg, gel or capillary electrophoresis, high-performance liquid chromatography, polymerase chain reaction) have encouraged the development of emerging sensor-based technologies. Nevertheless, it should be stated that those traditional techniques are complementary and sensitive tools that are commonly used to confirm the results of the immunological officially accepted methods (Rosell et al., 2014).

18.3.1 Factors Affecting Gliadins/Gluten Analysis

There are two issues that can make gluten analysis difficult, namely, the extraction yield of gliadin from the food samples and the use of a correct gliadin standard. These two factors may limit the development and/or implementation of novel analytical approaches for gluten-free food analysis (Rosell et al., 2014). Gluten extraction from processed foods is not an easy task, since, in some cases, during the food processing, high temperatures are used that contribute to the formation of isopeptide bonds between amino and carboxamide groups of the protein residues or to the formation of protein aggregates making gluten analysis quite difficult. Therefore, to ensure a complete extraction of both prolamins and glutenins, several cocktail recovery solutions have been proposed (Garcia et al., 2005; Mena et al., 2012), although some of them, namely those using β-mercaptoethanol, may be incompatible with some immunological-based techniques. On the other hand, in gluten analysis, the use of the most adequate standard plays an important role. The standard should be as representative as possible of the gluten proteins to be analyzed. Some standards are available, such as *The Working Group on Prolamin Analysis and Toxicity* (PWG) gliadin standard (Van Eckert et al., 2006). Nevertheless, its use is not consensual. Some authors have suggested that it would be more correct to use a hydrolyzed standard combined with a competitive assay to quantify peptides of partially hydrolyzed gluten in fermented wheat, rye, and barley products (Comino et al., 2012, 2013; Gessendorfer et al., 2009; Mena et al., 2012; Rosell et al., 2014).

Regardless of these pertinent questions, several works have reported the development of sensor-based analytical methodologies toward the detection of gliadins in foodstuffs using commercial gliadin or gluten standards and extraction approaches based on the use of aqueous–ethanolic solutions.

18.3.2 Immuno- and Aptasensors for Gliadin/Gluten Detection in Foods

In recent years, several optical and electrochemical biosensors, including immunosensors and aptasensors, have been developed to detect gliadin in food matrices, namely to evaluate gluten-free foods, since the amount of gluten must be lower than 20 mg/kg (or ppm), according to the legal requirements (Nassef et al., 2008; OJEU, 2009; Zeltner et al., 2009). De Stefano et al. (2006) used an optical sensor with a recombinant glutamine-binding protein to detect traces of gluten in food. Nassef et al. (2008) proposed an electrochemical immunosensor, based on the use of an antibody raised against the putative immunodominant celiac disease epitope, to measure the gliadin content in foods. Detection limits between 5.5 and 11.6 ng/mL (or ppb) could be achieved. Labelless impedimetric and antigliadin Fab-based amperometric immunosensors were also developed by Nassef et al. (2009), showing gliadin detection limits of 3.23 ng/mL. Mairal et al. (2009) developed a microfluorimeter with a disposable polymer chip with a gliadin detection limit of 4.1 ng/mL, by detecting the emission of a fluorophore-labeled monoclonal antigliadin antibody upon excitation with light. Laube et al. (2011) developed an electrochemical magneto immunosensor, coupled or not to ELISA, allowing the quantification of gliadin or small gliadin fragments in natural or pretreated food samples with detection limits ranging from 1.2 to 24.2 ng/mL (depending on the food matrix). Chu and Wen (2013) developed a sensitive liposomal fluorescence immunoassay with immunomagnetic beads for the detection and quantification of gliadin in gluten-free foods with a detection limit of 0.6 μg/mL (or ppm), although slight cross-reactions with barley and rye were found. Although the immunosensor technology seems

promising, limitations like long-term stability, surface effects, and interferences resulting from complex sample matrices are major concerns (Neves et al., 2010). Also, finding a single antibody able to react with different gliadin and glutenin subunits with similar affinity, as well as with prolamins from different cereals and from modified gluten is very challenging (Tranquet et al., 2012). Thus, due to the complexity of gluten proteins, the quantification of the total gluten content in foods is extrapolated from the gliadin concentration, assuming a constant gliadin–glutenin ratio equal to 1 within all samples (Tranquet et al., 2012). Gluten composition depends on multiple parameters such as the species, cultivars, agronomical conditions, as well as on the products processing (Wieser and Koehler, 2009). Therefore, some authors (Van Eckert et al., 2010; Wieser and Koehler, 2009) have suggested that the next step could be the use of a mixture of antibodies that could recognize gliadin and glutenin subunits at similar degrees. Still, the development of such assays, with two or more antibodies, is complex and may be expensive (Tranquet et al., 2012).

Hence, recently aptamers against hydrophobic immunotoxic peptides from gliadin from wheat that also recognize celiac disease related proteins from barley, rye, and oat have been investigated (Amaya-González et al., 2013; Pinto et al., 2014). Fernández et al. (2012) developed an electrochemical genosensor for the detection of a specific DNA sequence that encodes an immunogenic fragment of gliadin, being achieved a detection limit of 0.001 μM. More recently, Amaya-González et al. (2014) reported a competitive electrochemical magneto-assay without cross-reactivity with nontriggering celiac disease proteins from soya, rice, or maize. This device enabled the detection of 0.5 ppb in diluted gliadin standard solutions, which corresponds to a detection limit of 0.5 ppm of gluten, considering the dilution factor and assuming that gliadin constitutes 50% of gluten.

18.3.3 Electronic Tongue

Although optical and electrochemical immuno- and aptasensors proved to be a potentially fast and practical tool to accurately detect possible gliadin/gluten contamination of gluten-free labeled foods, their development has been very demanding, requiring a considerable amount of consumables, equipment, and skilled technicians. A possible and simpler alternative has been proposed by Peres et al. (2011) and is based on the use of an all solid-state potentiometric e-tongue with 36 polymeric membranes, not coupled to any antibody against gliadin, or aptamer against any immunotoxic peptides from gliadin. The device comprised two-sensor arrays, being the membranes prepared with organic compounds containing long carbon chain with different functional groups (lipid additive compounds). Each membrane contained polyvinyl chloride (PVC) as polymeric matrix, a plasticizer, and a sensor additive. The multisensor device enabled the semiquantitative discrimination of aqueous–ethanolic (30:70, v/v) mixtures, containing preestablished levels of dissolved gliadin standard, chosen in order to mimic food aqueous–ethanolic extracts of gluten-free, low-gluten content, or gluten-containing foodstuffs (<20; 20–200; and >200 mg/kg of gluten equivalent, assuming a gliadin/gluten ratio equal to 0.5) with a sensitivity of around 80%, corresponding to a gliadin detection limit around 1–2 mg/kg. Also, e-tongue was successfully applied to real samples, being able to correctly classify more than 80% of the gluten-free or gluten-containing foodstuffs evaluated.

The successful performance reported by the research team (Peres et al., 2011) may be tentatively explained based on the chemical composition of the polymeric membranes applied on the e-tongue. Indeed, the lipid polymeric membranes used contain hydrophobic and hydrophilic groups allowing the interaction with several chemical compounds (electrolytes and nonelectrolytes) via electrostatic or hydrophobic interactions (Kobayashi et al., 2010; Toyota et al., 2011a,b; Yasuura et al., 2014a,b). Hydrogen bonds or electrostatic interactions may also arise in the presence of mediating electrolyte substances, between carboxyl or phosphate groups in the lipid/polymer membrane and vicinal hydroxyl groups of the target molecules (Toyota et al., 2011b). Furthermore, it is accepted that lipids interact with proteins during gluten formation; thus, lipids could enhance the formation of large complex aggregates involving both gliadin and glutenin proteins (Carcea and Schofield, 1996). Besides, it is known that nonpolar lipids can be associated with glutenins through either hydrophobic interactions or hydrogen bonds, whereas polar lipids containing phosphate groups preferentially interact with gliadin (McCann et al., 2009). Moreover, when lipid/polymer membranes are applied for protein detection, namely gliadin, that does not bind directly, it is expected that their behavior could mimic that of protein–lipid interactions occurring in biological membranes, where unspecific hydrophobic association or electrostatic interactions between protein and lipid head groups occur (Thomas and Glomset, 1999; Zhao and Lappalainen, 2012).

To further evaluate and verify the possible interaction of the lipidic membranes toward gliadin or gluten from wheat, a new e-tongue was built. The electrochemical device consisted of a print-screen potentiometric array (Fig. 18.1), with 20 chemical sensors, with cross-sensitivity lipidic membranes and relative plasticizer-additive compositions (Table 18.1) identical to those previously used by the research team for gliadin qualitative and semiquantitative detection (Peres et al., 2011). Plasticizer bis(2-ethylhexyl) phthalate was replaced by dioctyl phenylphosphonate and the additives bis(2-ethylhexyl)phosphate and tridodecylmethylammonium chloride were not included in the new

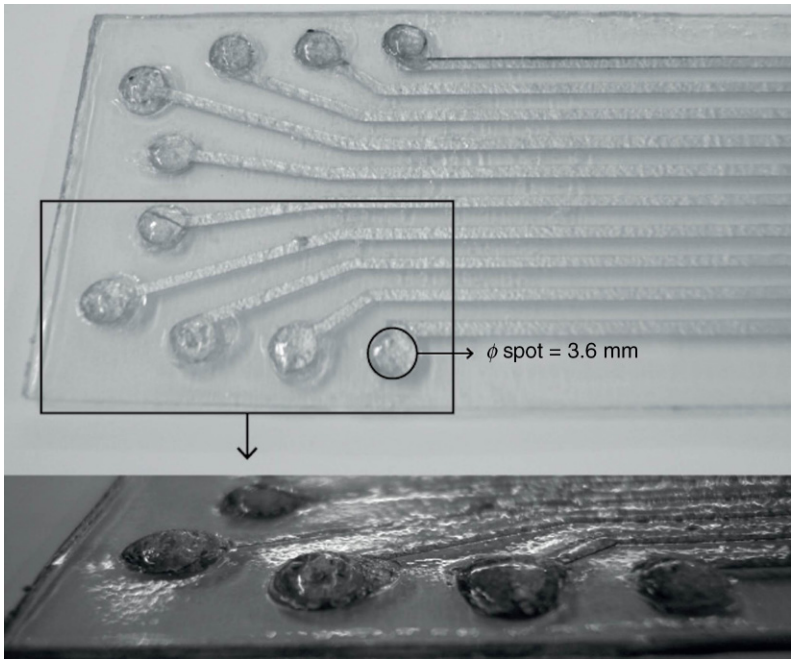

FIGURE 18.1 **Screen-printed scheme with conductive resin silver of the e-tongue multisensor device, containing 20 lipid/polymeric membranes, used for potentiometric analysis of aqueous–ethanolic gliadin or gluten standard solutions (surface isolated with acrylic resin).**

electrochemical device tested, since in a preliminary study sensors containing those compounds showed low response toward gliadin and other proteins (data not shown). Finally, each lipidic membrane contained PVC (≈32%), as the polymeric matrix, and a combination of each of the five plasticizers used (≈65%) and the four additive compounds (≈3%), as shown in Table 18.1. An identification code was used for each lipidic membrane, containing the letter S as the sensor followed by two numbers (the first identifying the plasticizer, from 1 to 5, and the latter the additive, from 1 to 4) separated by a punctuation mark (comma).

The e-tongue signal profiles were recorded in alkaline aqueous–ethanolic solutions (pH≈12; 30:70 v/v) containing standard gliadin (from Sigma-Aldrich) or gluten (from Sigma-Aldrich, protein content >80%), varying from +86.1 to +151.0 mV and +82.7 to +142.4 mV, respectively. In general, for all sensors, the corrected signal potential $[\Delta E(\text{mV}) = E^0_{\text{dissolved protein}} - E^0_{\text{solvent}}]$ increased with the

TABLE 18.1 Sensors Used in the E-Tongue: Identification of the Plasticizer and Additive Compounds Used in Each Lipidic-Polymeric Membrane

ID No.[a]	Name	Chemical Formula
Plasticizer compound[b]		
1	Bis(1-butylpentyl) adipate	$[\text{-}(CH_2)_2COOCH[(CH_2)_3CH_3]_2]_2$
2	Dibutyl sebacate	$[\text{-}(CH_2)_4CO_2(CH_2)_3CH_3]_2$
3	2-Nitrophenyl-octyl ether	$O_2NC_6H_4O(CH_2)_7CH_3$
4	Tris(2-ethylhexyl) phosphate	$[CH_3(CH_2)_3CH(C_2H_5)CH_2O]_3P(O)$
5	Dioctyl phenylphosphonate	$C_6H_5P(O)[O(CH_2)_7CH_3]_2$
Additive compound[c]		
1	Octadecylamine	$CH_3(CH_2)_{17}NH_2$
2	Oleyl alcohol	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CH_2OH$
3	Methyltrioctylammonium chloride	$[CH_3(CH_2)_6CH_2]_3N(Cl)CH_3$
4	Oleic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$

[a] *Sensor identification number.*
[b] *All plasticizers were Selectophore™ grade from Fluka, with purity ≥97%.*
[c] *All additives were from Fluka, with purity ≥97%.*

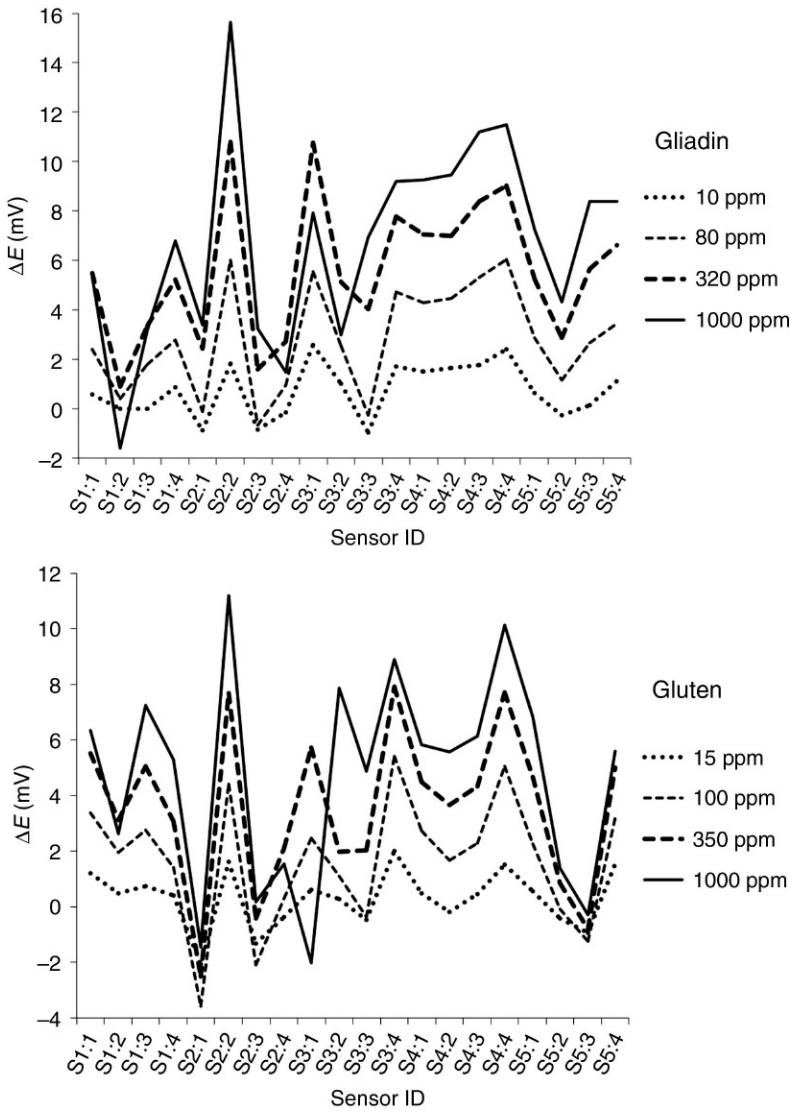

FIGURE 18.2 The e-tongue corrected signal profiles (ΔE, mV) variation with increasing concentration levels of gliadin (10–1000 ppm) or gluten (15–1000 ppm) in aqueous–ethanolic standard solutions (pH≈12).

gliadin or gluten content, because their responses evaluated in dynamic concentration ranges as can be seen in Fig. 18.2 (gliadin: 10–1000 ppm; gluten: 15–1000 ppm).

Linear correlations were obtained by plotting the sensors' signals against decimal logarithm of the concentrations [ΔE(mV) = $a + b \times \log_{10}$ (C, ppm)], although for different concentration intervals (Table 18.2 and Fig. 18.3). All sensors showed a quantitative response toward gliadin concentration ($0.967 \leq R \leq 0.997$) enabling the quantification of gliadin in standard solutions. For more complex matrices, such as foodstuffs, the possible quantification of gliadin content must be experimentally evaluated, but it is expected that the use of multivariate regression models (linear or nonlinear) based on a subset of the most informative sensors (chosen using a heuristic or a metaheuristic variable selection algorithm) will overcome possible modeling difficulties, namely due to signal interferences (Dias et al., 2014).

The results clearly show the capability of the e-tongue to quantify gliadin and, although only standard solutions were analyzed, a potential application to real samples can be foreseen. It should be noticed that, if gluten concentrations were converted into *apparent* gliadin contents (assuming a gliadin–glutenin ratio equal to 1) similar regression equations would be obtained for the dependence of ΔE with $\log_{10}(C)$, independently if the assays were made with gliadin or gluten solutions, as also exemplified in Fig. 18.3 for two e-tongue sensors (S1:4 and S4:4). This result suggests that, in principle, the lipidic membranes are responding preferentially to gliadin over glutenin proteins. This apparent preference may be tentatively explained taking into account that: (1) glutenin has a greater average molecular weight (70–90 kDa) compared to gliadin (30–50 kDa) (Wieser, 2008), which may favor the gliadin possible adsorption over glutenin and (2) lipidic membranes used preferentially interact with gliadin

TABLE 18.2 Parameters of the Linear-Logarithm Regressions and Dynamic Concentration Ranges for Each Sensor (S1:1 to S5:4) of the Potentiometric E-Tongue [ΔE(mV) = $a + b \times \log_{10}$ (C, ppm)]

	Gliadin		Gluten	
Sensor ID No.[a]	Concentration Range (ppm)	R[b]	Concentration Range (ppm)	R[b]
S1:1	[10, 320]	0.967	[15, 570]	0.999
S1:2	[10, 320]	0.992	[15, 570]	0.995
S1:3	[10, 560]	0.997	[15, 1000]	0.993
S1:4	[10, 1000]	0.993	[15, 1000]	0.996
S2:1	[80, 1000]	0.991	[100, 820]	0.989
S2:2	[36, 1000]	0.994	[15, 1000]	0.996
S2:3	[80, 1000]	0.993	[15, 820]	0.96
S2:4	[36, 810]	0.996	[15, 820]	0.997
S3:1	[3, 810]	0.982	[15, 820]	0.990
S3:2	[3, 560]	0.997	[15, 820]	0.984
S3:3	[80, 1000]	0.992	[100, 820]	0.975
S3:4	[10, 560]	0.996	[15, 570]	0.825
S4:1	[10, 1000]	0.997	[190, 570]	0.991
S4:2	[10, 1000]	0.996	[190, 570]	0.974
S4:3	[3, 1000]	0.996	[190, 820]	0.994
S4:4	[10, 1000]	0.999	[190, 1000]	0.991
S5:1	[10, 1000]	0.995	[190, 1000]	0.960
S5:2	[36, 1000]	0.995	[190, 1000]	0.990
S5:3	[36, 1000]	0.994	[190, 1000]	0.984
S5:4	[36, 810]	0.993	[190, 1000]	0.979

[a]*Sensor identification code number based on the information given in Table 18.1.*
[b]*Correlation coefficient.*

over glutenin proteins due to their polarity and presence of a phosphate group in some of them (McCann et al., 2009). A more detailed analysis of the results, shown in Table 18.2, indicate that apparently the type of additive has less influence in the potentiometric signal responses than the type of plasticizer. Indeed, two plasticizers [tris(2-ethylhexyl) phosphate and dioctyl phenylphosphonate] gave the best correlations, which was expected due to the presence of the phosphate group that enhances the gliadin–lipid interaction (McCann et al., 2009).

Globally, from the results reported by Peres et al. (2011) and those obtained in this work, both based on the use of potentiometric e-tongues with lipid/polymeric membranes, it can be inferred that this electrochemical approach exhibits a sensitivity of 1–3 ppm (≈2–6 ppm of gluten), which is quite satisfactory since an analytical method with a sensitivity of 10 ppm is suitable for gluten detection (Zeltner et al., 2009). Moreover, the e-tongue fulfills the requirements of gluten-free, low-gluten content, or gluten-containing food label verification, enabling gliadin content quantification in a wide dynamic range, varying from 3 to 1000 ppm. However, this quantitative potential must be further investigated by applying the device to real food samples. Nonetheless, this work together with the previous one (Peres et al., 2011) may be viewed as a proof-of-principle that a potentiometric e-tongue with lipidic membranes may be used as a practical, fast, simple, and sensitive tool toward the detection of gliadin.

18.4 CONCLUSIONS AND FINAL REMARKS

Several analytical techniques have been reported for gliadin detection in food samples. Recently, the use of sensors gained an increased attention, namely immunosensors and aptasensors, which exhibit gliadin detection limits (3 ppb–0.6 ppm, depending on the technique) much lower than the regulatory gluten threshold allowed in gluten-free foodstuffs (<10 ppm of equivalent gliadin). Nevertheless, these high-sensitive techniques are usually far beyond the economic and technical possibilities of the majority of the food industries, namely micro- and small familiar enterprises, reducing its routine application. Hence, in recent years the research team has developed electrochemical devices for gliadin detection in food samples. The potentiometric e-tongues developed have exhibited a suitable sensitivity toward gliadin (1–3 ppm) enabling

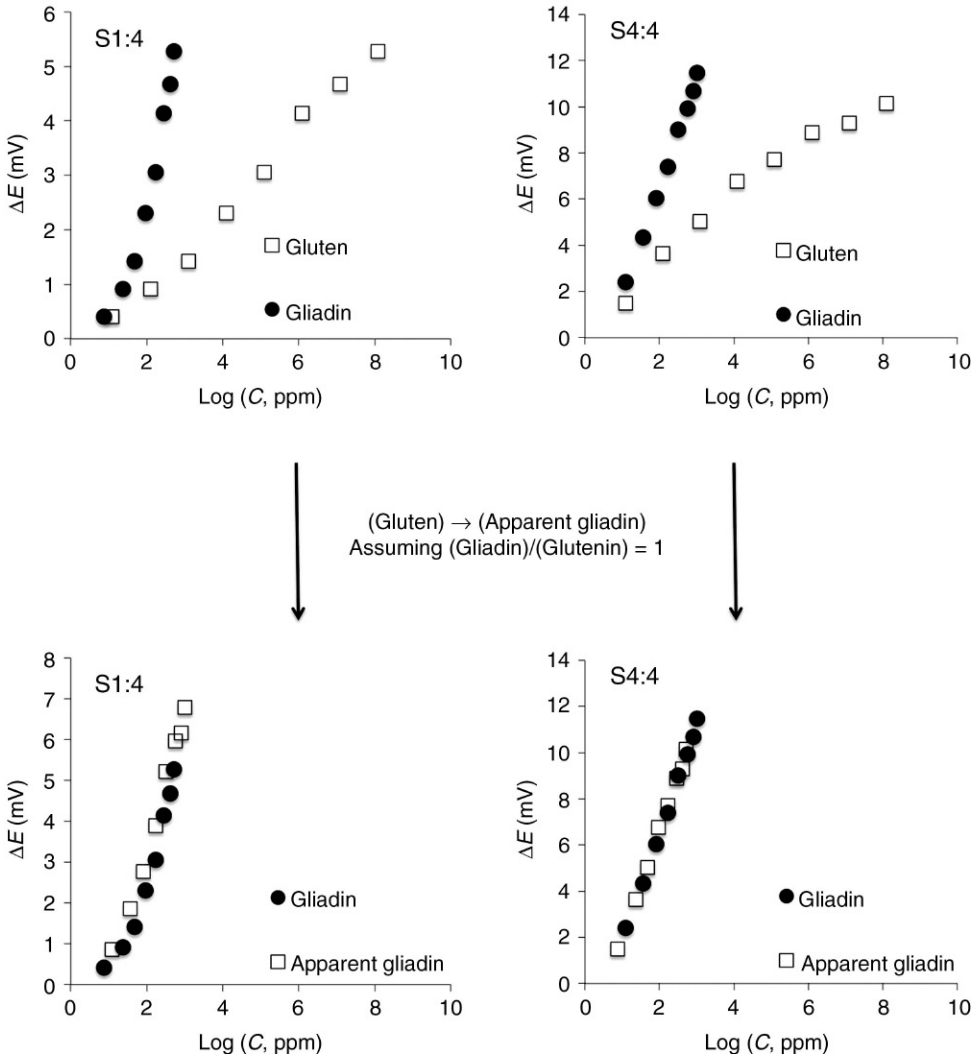

FIGURE 18.3 The e-tongue calibration: corrected potential signals (ΔE, mV) versus decimal logarithm of gliadin or gluten concentration, for two sensors [S1:4 and S4:4, corresponding to a plasticizer/additive combination of bis(1-butylpentyl) adipate/oleic acid and tris(2-ethylhexyl) phosphate/oleic acid, respectively].

its quantification, as well as the qualitative or semiquantitative discrimination of foods based on their gluten content and according to the legal thresholds. The satisfactory e-tongue performance suggests this device as a promising routine tool for gliadin detection in foodstuffs. Finally, the gliadin quantification capability could be attributed to the polar character of the lipidic/polymeric membranes applied in the e-tongue, and also to the presence of the phosphate group in some of the membranes. Nevertheless, a wider study is required, including the validation of the methodology using different liquid and food samples. Also, a future work should include the use of nonpolar lipidic membranes in the e-tongue since they preferentially interact with glutenins, enabling the direct quantification of the gluten content, thus avoiding the controversial use of the gliadin–glutenin ratio equal to 1 to extrapolate the gluten concentration.

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