



Food and Agricultural Immunology

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/cfai20>

Allergenic changes in $\beta\beta$ -lactoglobulin induced by microwave irradiation under different pH conditions

Djazia Zellal^a, Hanane Kaddouri^a, Hadria Grar^a, Hanane Belarbi^a, Omar Kheroua^a & Djamel Saidi^a

^a Laboratory of Physiology of the Nutrition and Food Safety, Department of Biology, Faculty of Science, University of Oran, Es-Séénia, Oran-31000, Algeria

Available online: 24 May 2011

To cite this article: Djazia Zellal, Hanane Kaddouri, Hadria Grar, Hanane Belarbi, Omar Kheroua & Djamel Saidi (2011): Allergenic changes in $\beta\beta$ -lactoglobulin induced by microwave irradiation under different pH conditions, Food and Agricultural Immunology, DOI:10.1080/09540105.2011.582094

To link to this article: <http://dx.doi.org/10.1080/09540105.2011.582094>



PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.tandfonline.com/page/terms-and-conditions>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan, sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Allergenic changes in β -lactoglobulin induced by microwave irradiation under different pH conditions

Djazia Zellal, Hanane Kaddouri, Hadria Grar, Hanane Belarbi, Omar Kheroua and Djamel Saidi*

Laboratory of Physiology of the Nutrition and Food Safety, Department of Biology, Faculty of Science, University of Oran, Es-Sénia, Oran-31000, Algeria

(Received 26 March 2010; final version received 12 April 2011)

The allergenicity of β -lactoglobulin (BLG) was assayed by Ussing chamber after microwave irradiation of whey proteins at different pH values, in a murine model of BLG allergy. BALB/c mice were sensitised intraperitoneally with BLG. Serum levels of BLG-specific IgG, IgG1, IgG2a and IgE were analysed by Enzyme-Linked Immunosorbent Assay (ELISA). Local anaphylactic responses and residual allergenicity of various treated whey proteins were performed *in vitro* in Ussing chamber. BLG immunisation was associated with strong IgG, IgG1, IgG2a and IgE production, a significant increase in short current circuit (Isc) and high conductance (G) response. The allergenic potential of BLG was markedly reduced after microwave irradiation at 300 or 700 W of whey proteins at pH values 6.8 or 4.6 (Isc and G remained unchanged after intestine challenge with treated whey proteins). The application of microwave irradiation of whey proteins at the natural milk pH (pH 6.8) or pH 4.6 induces a significant decrease in the BLG allergenicity.

Keywords: BLG; allergenicity; microwave irradiation; pH; Ussing chamber; BALB/c; ELISA

Introduction

β -Lactoglobulin (BLG) is a major food allergen, contributing significantly to the overall allergenicity of bovine milk (Besler, Eigenmann, & Schwartz, 2002). Sequential and conformational epitopes may play an important role in the allergenicity of this protein (Cocco, Jarvinen, Han, Beyer, & Sampson, 2007). Immunological investigations of BLG have reported six different IgE-binding regions (epitopes) of the protein that were linked with allergic reaction (Niemi et al., 2007).

Different attempts have been made to reduce the allergenicity of cow's milk protein (El-Agamy, 2007). Heat treatment was previously believed to be a good way to reduce the allergenicity of milk proteins, but later it became clear that for decreasing allergenicity heating is not adequate (Monaci, Tregoeat, Arjon, Hengel, & Anklam, 2006). Microwave processing has achieved a remarkable acceptance by the food industry (Sadeghi & Shawrang, 2007). It was suggested that microwave irradiation may replace conventional heating (Chi-Yue, Shui-Tein, Shyh-Horg, & Kung-Tsung, 1992). Published data showed that microwaves caused a significantly

*Corresponding author. Email: djamsaidi@gmail.com

higher degree of unfolding than conventional thermal stress for protein solutions heated to the same maximum temperature (George, Bilek, & McKenzie, 2008). Microwave heating is accomplished both by the absorption of the microwave energy by rotation of the dipolar water molecules and by the translation of the ionic components of food. Microwaves are shown to affect the kinetics of conformational changes of whey proteins and to accelerate their denaturation, but these effects are non-thermal (Bohr & Bohr, 2000a, b; Lerina et al., 1997). Using rabbit polyclonal antibodies, it was found that microwave-irradiated whey proteins (BLG and α -La) lost a part of their IgG-binding ability (Kaddouri, El-mecherfi, Kheroua, & Saidi, 2006) but this treatment was not satisfying since it could not lead to an acceptable hypoallergenic formula due to a non-sufficient reduction of the antigenicity of proteins. Microwave irradiation could be easily combined with chemical reactions and, in some case, accelerate the chemical reaction rate (Caddick, 1995). Interestingly, recent research from our laboratory has shown that application of microwave irradiation at 300 or 700 W of whey proteins at different pH values considerably modified the antigenicity of the major milk protein (BLG) (Grar et al., 2009). Authors of this study suggested that this treatment can remove conformational epitopes of BLG as a result of the loss of tertiary protein structure. Whether the BLG allergenicity may also be decreased upon the microwave irradiation of whey proteins at various pH values remains to be determined. The aim of this study was to assess the allergenic potency of BLG after microwave irradiation of whey proteins at different pH values in a murine model of BLG allergy.

Materials and methods

Immunisation of mice

Six- to eight-week-old female BALB/c mice were purchased from Pasteur Institute d'Alger. Mice were housed under standard animal husbandry condition with food and water *ad libitum*.

BALB/c ($n = 20$) were immunised at days 0, 14, 21 and 28 by intraperitoneal injection of 10 μ g BLG (Sigma, France) adsorbed to 2 mg of Alum [$\text{Al}(\text{OH})_3$] in 0.1 ml phosphate buffered saline (PBS). Non-sensitised mice ($n = 6$) were used as control. Mice were bled from the retro-orbital venous plexus one week after the last immunisation (days 35). After centrifugation, sera were stored at -20°C until assay.

Determination of BLG-specific serum antibody titres

BLG-specific IgG, IgG1, IgG2a and IgE titres were determined by Enzyme-Linked Immunosorbent Assay (ELISA). Microtitre plates (Maxisorp; Nunc, Denmark) were coated with BLG ($100 \mu\text{g ml}^{-1}$) in PBS pH 7.4 for 48 h. After overnight incubation at 4°C , plates were washed and blocked with 3% BSA PBS. After incubation for 1 h at 37°C and washing the plates, 50 μ l of serum samples were added to the plates and incubated for 2 h at 37°C . Plates were then washed and incubated for 1 h at 37°C with 50 μ l of 2 $\mu\text{g ml}^{-1}$ biotinylated polyclonal-specific antibody for IgG (Sigma, B9904, France), of biotinylated monoclonal specific antibodies (Pharmingen, France) for IgE (553387/R1915), IgG1 (553440/A85-1) and IgG2a (553387/R19-

15). Fifty microlitres of streptavidin:peroxydase (1:5000 dilution, E2886, Sigma; France) were then added to plate and incubated for 30 min at 37°C. The reaction was revealed by addition of 50 µl of H₂O₂ (30%, 0.25 ml l⁻¹; Sigma, France) associated with diamino-orthophenylene (OPD) (0.5 mg ml⁻¹; Sigma, France) in sodium citrate buffer 0.05 M pH 5.1. The reaction was stopped by adding H₂SO₄ (2N). The plates were read at 492 nm on an automated ELISA reader (EL × 800, Bio-Tek Instruments GmbH, Germany).

Whey proteins samples

To investigate the allergenic potential of BLG, treated whey proteins were used. The general procedures for whey proteins treatment were essentially performed as previously described (Grar et al., 2009). Briefly, whey proteins adjusted at various pH values of 2, 4.6, 9 and at the natural milk pH (6.8) were subjected to microwave irradiation at 300 W for 20 min or 700 W for 10 min.

Tissue preparation

Seven days after the last immunisation, mice were sacrificed by intraperitoneal administration of sodium pentothal (50 mg kg⁻¹). A segment of jejunum (10–20 cm) was removed and placed in ice-cold oxygenated Ringer solution containing ionic composition in millimolar (Na⁺ 140, K⁺ 5.2, Ca⁺⁺ 1.2, Mg⁺⁺ 1.2, Cl⁻ 120, HCO₃⁻ 25, HPO₄ 2.4, H₂PO₄ 0.4).

Ussing chamber experiments

Jejunal segments were opened longitudinally along the anti-mesenteric border and placed in ice cold oxygenated solution. The jejunum was mounted at flat sheets between two Lucite halves Ussing chamber having a flux area of 0.1 cm². Both sides of each mucosal sheet were bathed in the Ringer solution which was maintained at pH 7.4 and 37°C oxygenated continuously with 5% CO₂ and 95% O₂. The solutions bathing the serosal and mucosal sides of the tissue were connected to calomel electrodes via agar bridges (3M KCl) for measurement of transepithelial potential difference (PD) and to Ag–AgCl electrodes for the passage of current through the system. The PD was recorded continuously, and at regular intervals, the PD was turned off by passing a 10 µA current. Current pulses allowed measurement of the tissue I_{sc} (index of electrogenic ion transport; Gauthier & Reddix, 2000) and conductance at regular intervals. After electrical parameters stabilisation (20–30 min), the respective effects of BLG or treated whey proteins were measured by adding these proteins at the serosal side of the tissue.

Statistical analysis

All data were presented as means ± error. Student's *t*-test was used to determine the statistical significance between control and experiment group. A probability value of 0.05 or less was considered statistically significant.

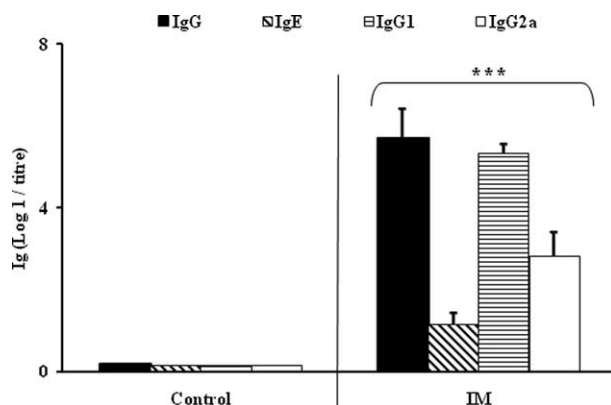


Figure 1. BLG-specific serum immunoglobulin IgG, IgE, IgG1 and IgG2a antibody titres in serum of control and immunised mice (IM). *** $p < 0.001$.

Results

BLG-specific serum antibody titres

Anti-BLG immunoglobulin titres were measured in sera from six mice immunised with BLG adsorbed on alum. Significantly higher titres of IgG, IgG1, IgG2a and IgE were detected in the serum (Figure 1), with positive IgG1/IgG2a ratio reflecting the Th2 orientation of the response.

BLG-induced ion transport in jejunum from sensitised mice

In Ussing chamber, jejunal segment from BLG-sensitised mice responded to serosal BLG challenge with an increase in I_{sc} (58.66 ± 7.85 to $91.37 \pm 10.34 \mu A/cm^2$) ($p < 0.001$) (Figure 2) and conductance (34.67 ± 8.76 to $46.83 \pm 4.43 \text{ mmho/cm}^2$) ($p < 0.01$) (Table 1).

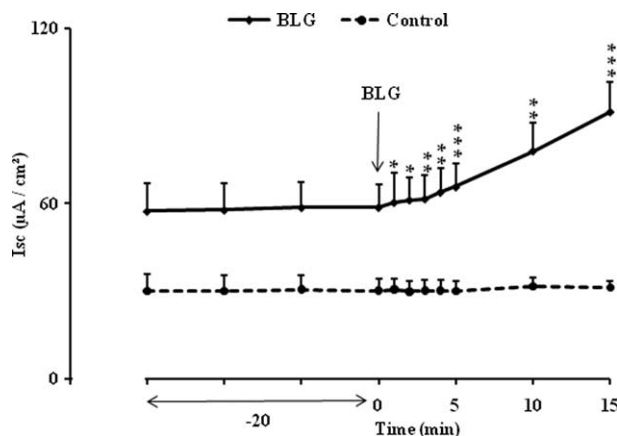


Figure 2. Changes in I_{sc} in response to BLG challenge in control and sensitised mice. Values are given as mean $\pm$ SE (standard error) for six mice ($n = 6$). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. p -Value represents the comparison of BLG response on I_{sc} before (T_0) and after challenge.

Table 1. Changes in conductance before (T0) and after (T15) BLG challenge in control and immunised mice.

Time	Conductance (mmho/cm ²)	
	T0	T15
Control (<i>n</i> = 6)	23.23 ± 4.58	23.13 ± 2.45
Immunised mice (<i>n</i> = 6)	34.67 ± 8.76	46.83 ± 4.43**

***p* < 0.01. *p*-Value represents the comparison of BLG response on conductance before and after challenge.

Note: Data are expressed as mean change ± SE (standard error) for six mice (*n* = 6).

Residual allergenicity

To investigate the residual allergenicity of BLG, treated whey proteins were challenged in serosal compartment of immunised BLG tissues. Our results indicate that serosal addition of microwave irradiated whey proteins (at 300 or 700 W) either at the natural milk pH (Figure 3a) or at pH value 4.6 (Figure 3b) produced no

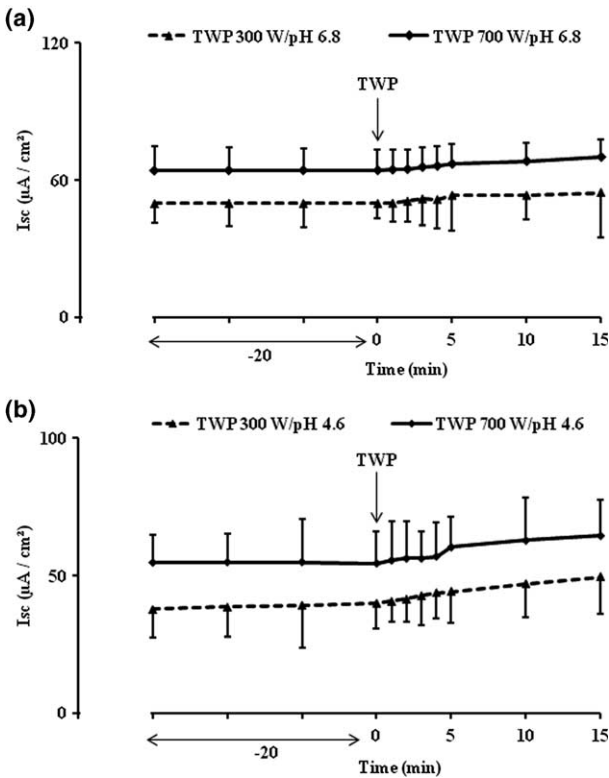


Figure 3. (a) Changes in *I*_{sc} in response to challenge with microwave irradiated whey proteins at the natural milk pH (pH 6.8) as determined by Ussing chamber. Values are given as mean ± SE (standard error) for six mice (*n* = 6). (b) Changes in *I*_{sc} in response to challenge with microwave irradiated whey proteins at pH 4.6 as determined by Ussing chamber. Values are given as mean ± SE (standard error) for six mice (*n* = 6).

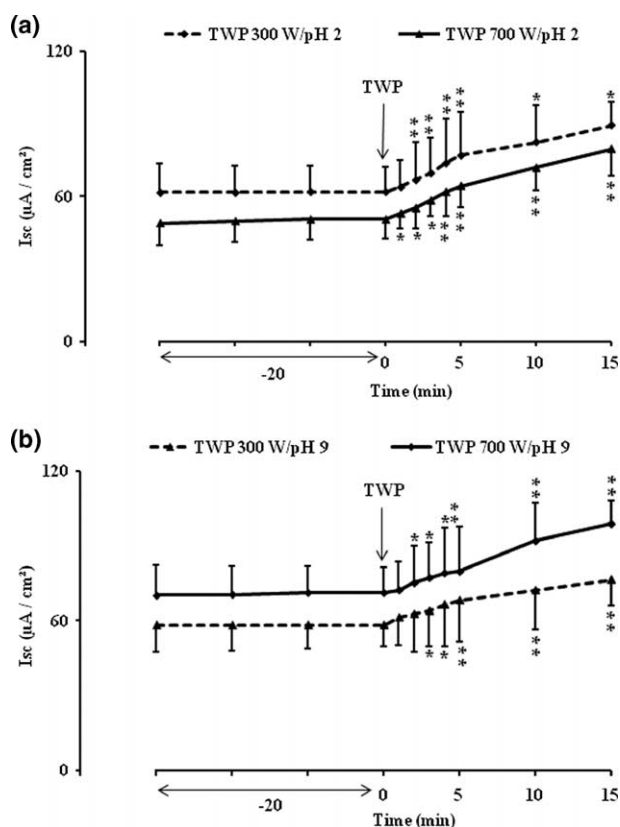


Figure 4. (a) Changes in I_{sc} in response to challenge with microwave irradiated whey proteins at pH value 2 as determined by Ussing chamber. Values are given as mean $\pm$ SE (standard error) for six mice ($n=6$). * $p < 0.05$, ** $p < 0.01$. p -Value represents the comparison of BLG response on I_{sc} before (T0) and after challenge. (b) Changes in I_{sc} in response to challenge with microwave irradiated whey proteins at pH value 9 as determined by Ussing chamber. Values are given as mean $\pm$ SE (standard error) for six mice ($n=6$). * $p < 0.05$, ** $p < 0.01$. p -Value represents the comparison of BLG response on I_{sc} before (T0) and after challenge.

change in I_{sc} and conductance, reflecting a decrease in BLG allergenicity. In contrast, challenge of irradiated whey proteins (at 300 or 700 W) at pH 2 to tissue from sensitised mice induced a marked increase in I_{sc} ($p < 0.05$ and $p < 0.01$, respectively) (Figure 4a) and conductance ($p < 0.01$ and $p < 0.05$, respectively) (Table 2). The same results were obtained in case of samples irradiated at pH 9, with an important increase in I_{sc} (Figure 4b) ($p < 0.01$) and conductance (Table 2) ($p < 0.05$). These findings suggest that microwave irradiation has no significant effect on the allergenic properties of BLG at these pH values (pH 2 and 9).

Discussion

In the present study, we sought to evaluate the effect of microwave irradiation of whey proteins at different pH values on BLG allergenicity. For this purpose, BALB/c mice were used as an experimental model of intestinal anaphylaxis.

Table 2. Changes in conductance before (T0) and after (T15) treated whey proteins (TWP) challenge in immunised mice.

Time	Conductance (mmho/cm ²)	
	T0	T15
TWP 300 watts (pH 6.8)	22.45 ± 3.29	22.17 ± 6.60
TWP 700 watts (pH 6.8)	21.45 ± 3.79	25.34 ± 5.25
TWP 300 watts (pH 4.6)	36.34 ± 7.44	35.17 ± 4.2
TWP 700 watts (pH 4.6)	29.62 ± 6.24	29.33 ± 3.86
TWP 300 watts (pH 2)	31.62 ± 4.82	45.50 ± 4.42**
TWP 700 watts (pH 2)	36.62 ± 6.46	50.33 ± 6.4*
TWP 300 watts (pH 9)	37.51 ± 3.55	48.67 ± 4.78*
TWP 700 watts (pH 9)	36.01 ± 5.03	46.50 ± 4.42*

* $p < 0.05$; ** $p < 0.01$. p -Value represents the comparison of treated whey proteins response on conductance before and after challenge.

Note: Data are expressed as mean change ± SE (standard error) for six mice ($n = 6$).

BLG-specific immunoglobulins were detected by using an Enzyme-Linked Immunosorbent Assay (ELISA). Our results showed that sensitised mice exhibited the characteristics of immediate hypersensitivity reaction in small intestine, with high levels of IgG, IgG1, IgG2a, IgE, and positive IgG1/IgG2a ratio. We also observed a significant increase in Isc reflecting, an antigen-induced change in transepithelial Cl⁻ transport (Caldwell, Harari, Hargis, & Castro, 2001) and a significant increase in tissue conductance (measure of integrity of the tight junctions) (Berin et al., 1998; Mehedi, Kaddouri, Saidi, & Kheroua, 2007) suggesting an alteration of intestinal epithelium with increase in para-cellular permeability. These results are consistent with previous reports (Adel-Patient, Creminon, Wal, & Chatel, 2000; Frossard, Tropia, Hauser, & Eigenmann, 2004; Negaoui, Kaddouri, Kheroua, & Saidi, 2009) and make BALB/c mice attractive for evaluating the allergenic potential of processed milk proteins.

As several groups have already demonstrated BLG immunoreactivity (Grar et al., 2009; Kaddouri et al., 2006), we found a significant decrease in BLG allergenicity when whey proteins were irradiated at the natural milk pH (pH 6.8). This result could be explained by the conformational changes of globular proteins (BLG) that occur during microwave irradiation (Homenko et al., 2009) which can lead to the loss of conformational epitopes. Homenko et al. (2009) reported that the stability of antigen-antibody complexes decreased after microwave irradiation at 100 GHz.

A marked reduction in BLG allergenicity was observed after microwave irradiation at 300 or 700 W of whey proteins at pH value 4.6. This is in agreement with the results of Grar et al. (2009) who found a dramatic decrease in BLG immunoreactivity applying the same treatment. Indeed, between pH 2 and 8, BLG exhibits a number of pH-induced structural transitions as well as changes in the association state and stability (Sakurai & Goto, 2007). When this protein heated at pH values in the maximum range 3–10, heat-induced protein aggregates were formed at all pH values (Hoffmann & Van-Mil, 1997; Hoffmann, Sala, Olieman, & De kruif, 1997). Our result confirmed that the thermal stability of whey protein and conformational change of BLG are significantly affected by pH (De Wit, 1990; Selo et al., 1999).

Conclusions

In the present work, we have shown that BLG allergenicity is markedly dependent on changes in the pH of the milk at microwave irradiation. Microwave processing of whey proteins at the natural milk pH or at acidic pH (pH 4.6) has been shown to decrease significantly the allergenic properties of BLG. Hence, microwave heat treatment alone or at pH 4.6 may be an effective approach for eliminating the allergenicity of BLG and development of hypoallergenic milk-based foods.

Acknowledgements

This research was supported by the Ministry of Higher Education and Scientific Research (MESRS).

References

- Adel-patient, K., Creminon, C., Wal, J.M., & Chatel, J.M. (2000). Prévention d'une réponse IgE spécifique de la beta-lactoglobuline bovine chez la souris par immunisation génique. *Revue Française d'Allergologie et d'Immunologie Clinique*, 40, 171–184.
- Berin, M.C., Kiliaan, A.J., Yang, P.C., Groot, J.A., Kitamura, Y., & Perdue, M.H. (1998). The influence of mast cells on pathways of transepithelial antigen transport in rat intestine. *The Journal of Immunology*, 161, 2561–2566.
- Besler, M., Eigenmann, P., & Schwartz, R.H. (2002). Cow's milk (*Bos domesticus*). *Internet Symposium Food Allergens*, 4, 19–106.
- Bohr, H., & Bohr, J. (2000a). Microwave enhanced folding and denaturation of globular proteins. *Physical Review E*, 61, 4310–4314.
- Bohr, H., & Bohr, J. (2000b). Microwave enhanced kinetics observed in ORD studies of a protein. *Bioelectromagnetics*, 21, 68–72.
- Caddick, S. (1995). Microwave assisted organic reactions. *Tetrahedron*, 51, 10403–10432.
- Caldwell, D.J., Harari, Y., Hargis, B.M., & Castro, G.A. (2001). Intestinal anaphylaxis in chickens: Epithelial ion secretion as a determinant and potential component of functional immunity. *Developmental and Comparative Immunology*, 25, 169–176.
- Chi-Yue, W., Shui-Tein, C., Shyh-Horg, C., & Kung-Tsung, W. (1992). Specific peptide-bond cleavage by microwave irradiation in weak acid solution. *Journal of Protein Chemistry*, 11, 45–50.
- Cocco, R.R., Jarvinen, K.M., Han, N., Beyer, K., & Sampson, H.A. (2007). Mutational analysis of immunoglobulin E-binding epitopes of β -casein and β -lactoglobulin showed a heterogeneous pattern of critical amino acids between individual patients and pooled sera. *Clinical and Experimental Allergy*, 37, 831–838.
- De wit, J.N. (1990). Thermal stability and functionality of whey proteins. *Journal of Dairy Science*, 73, 3602–3612.
- El-agamy, E.I. (2007). The challenge of cow milk protein allergy. *Small Ruminant Research*, 68, 64–72.
- Frossard, P., Tropia, L., Hauser, C., & Eigenmann, P. (2004). Lymphocytes in Peyer patches regulate clinical tolerance in a murine model of food allergy. *Journal of Allergy and Clinical Immunology*, 113, 958–964.
- Gauthier, C., & Reddix, R. (2000). Dual effect of [D-Pen2, D-Pen5] enkephalin on ion transport in guinea pig colon. *European Journal of Pharmacology*, 390, 335–338.
- George, D.F., Bilek, M.M., & Mckenzie, D.R. (2008). Non-thermal effects in the microwave induced unfolding of proteins observed by chaperone binding. *Bioelectromagnetics*, 29, 324–330.
- Grar, H., Kaddouri, H., Gourine, H., Negaoui, H., Kheroua, O., & Saidi, D. (2009). Microwave irradiation under different pH conditions induced a decrease in β -lactoglobulin antigenicity. *European Food Research and Technology*, 229, 779–783.

- Hoffmann, M.A.M., Sala, G., Olieman, C., & De kruif, K.G. (1997). Molecular mass distributions of heat-induced beta-lactoglobulin aggregates. *Journal of Agricultural and Food Chemistry*, 45, 5949–5957.
- Hoffmann, M.A.M., & Van-Mil, P.J.J.M. (1997). Heat induced aggregation of beta-lactoglobulin: Role of the free thiol group and disulfide bonds. *Journal of Agricultural and Food Chemistry*, 45, 2942–2948.
- Homenko, A., Kapilevich, B., Komstein, R., & Firer, M.A. (2009). Effects of 100 GHz radiation on alkaline phosphatase activity and antigen–antibody interaction. *Bioelectromagnetics*, 30, 167–175.
- Kaddouri, H., El-mecherfi, K.E., Kheroua, O., & Saidi, D. (2006). Microwave treatment modify antigenicity properties of bovine milk proteins. *African Journal of Biotechnology*, 5, 1267–1270.
- Lerina, I.V., Kovalenko, V.A., Belous, A.M., Rozanova, E.D., Leonov, B.N., & Morozova, T.F. (1997). Structural changes in serum albumin induced by SHF heating. *Biofizika*, 42, 1029–1034.
- Mehedi, N., Kaddouri, H., Saidi, D., & Kheroua, O. (2007). Effect of oral and parenteral sensitization to bovine BLG on intestinal structure in rats. *Dirasat: Pure Science*, 34, 58–66.
- Monaci, L., Tregoat, V., Arjon, J., Hengel, V., & Anklam, E. (2006). Milk allergens, their characteristics and their detection in food: A review. *European Food Research and Technology*, 223, 149–179.
- Negaoui, H., Kaddouri, H., Kheroua, O., & Saidi, D. (2009). A model of intestinal anaphylaxis in whey sensitized Balb/c mice. *American Journal of Immunology*, 5, 56–60.
- Niemi, M., Jylhä, S., Laukkanen, M.L., Söderlund, H., Mäkinen-kiljunen, S., Kallio, J.M., et al. (2007). Molecular interactions between a recombinant IgE antibody and the β -lactoglobulin allergen. *Structure*, 15, 1413–1421.
- Sadeghi, A.A., & Shawrang, P. (2007). Effects of microwave irradiation on ruminal protein degradation and intestinal digestibility of cottonseed meal. *Livestock Science*, 106, 176–181.
- Sakurai, K., & Goto, Y. (2007). Principal component analyses of the pH-dependent conformational transitions of bovine β -lactoglobulin monitored by heteronuclear NMR. *Proceedings of the National Academy of Science*, 104, 15346–15351.
- Selo, I., Clement, G., Bernard, H., Chatel, J.M., Creminon, C., Peltre, G., & Wal, J.M. (1999). Allergy to bovine β -lactoglobulin: Specificity of human IgE to tryptic peptides. *Clinical and Experimental Allergy*, 29, 1055–1063.