

*Full Length Research Paper*

# Microwave treatment modify antigenicity properties of bovine milk proteins

KADDOURI Hanane, EI MECHERFI Kamel Eddine, KHEROUA Omar, SAIDI Djamel\*

Laboratoire de Physiologie de la Nutrition et de Sécurité Alimentaire, Faculté des Sciences, Département de Biologie, Université d'Oran, Oran, Algérie.

Accepted 2 May, 2006

**This work is aimed to assess the effect of a microwave heating on cow's milk protein antigenicity. The heating protocol is established on the power/time relationship. A first share of milk samples were treated at 300 and 400 watts for 10, 15 and 20 min. The second share of milk and whey samples were treated at 500, 600 and 700 watts for 10 min. The antigenicity of proteins is evaluated by a study of milk sample reactivity towards the IgG anti- $\beta$ -Lg (obtained from immunized rabbits) using ELISA method. Microwave treatment of whole milk cause significant decrease of whey proteins concentration than samples of fresh whey treated. Electrophoresis analysis reveals that whole milk treated by microwave induces more changes in whey proteins composition. Microwave heating of entire cow's milk seems to diminish its whey proteins reactivity towards the specific antibodies (IgG).**

**Key words:** Cow's milk allergy, microwaves, whey proteins, antigenicity.

## INTRODUCTION

Cow's milk allergy (CMA) is one of the major causes of food hypersensitivity. Approximately 2.5% of infants exhibit cow's milk hypersensitivity in the first year of life (Host, 1997). It is often manifested by gastrointestinal dysfunction including increased permeability and ions secretion (Saidi et al., 1995; Moneret-Vautrin, 1999). The treatment of cow's allergy is complete avoidance of cow's milk allergens. In infants, it is necessary to use substitute formulae.

Milk formula based on extensively hydrolysed protein is used for preventive and therapeutic treatment. Their manufacture follow two different approaches to abolish whey protein allergenicity: heat denaturation and enzymatic hydrolysis of protein. Although, normally well tolerated, these hypoallergenic products have been reported to cause serious immunological reactions in very sensitive subjects. This study is undertaken to examine the effects of microwave heating at different power levels on cow's milk protein antigenicity.

## MATERIAL AND METHODS

### Preparation of whey sample

Raw cow's milk was obtained from a local dairy farm. It was fractionated into two volume; the first one is used to obtain the fresh whey by precipitation of caseinate at pH 4.6 using 1 N HCl. The whey obtained was then treated with microwave. The second aliquot of raw milk was treated directly with microwave, and then precipitated to obtain whey.

### Microwave treatment protocol

The samples of fresh whey and cow milk were treated with an alternative - flow microwave heating using 2450 Mhz Whirlpool oven. The treatment protocol was performed as shown in Table 1. After microwave treatment the samples were kept frozen at -20°C until analysis.

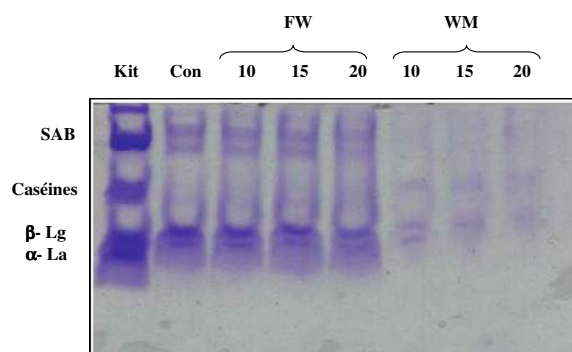
### SDS -PAGE

Sodium dodecyl sulfate gel electrophoresis (SDS-PAGE) of milk samples was performed under denaturing conditions as described by Laemmli (1970). Proteins were separated in polyacrylamide gel (12.5%) and stained with Coomassie Blue R250. All materials and instruments were purchased from Bio-Rad.

\*Corresponding authors E-mail. [djamsaidi@gmail.com](mailto:djamsaidi@gmail.com).

**Table 1.** Microwave irradiation protocol of fresh whey and whole milk.

Samples	Period (min)	300 Watt	400 Watt	500 Watt	600 Watt	700 Watt
Fresh whey (FW)	10	x	x	x	x	x
	15	x	x	-	-	-
	20	x	x	-	-	-
Whole milk (WM)	10	x	x	x	x	x
	15	x	x	-	-	-
	20	x	x	-	-	-



**Figure 1a.** SDS PAGE of milk (WM) and fresh whey treated (FW) by microwave irradiation at 300 Watts during 10, 15 and 20 minutes. **Kit:** Molecular weight Kit marker: bovin serum albumin (68 000 Da), caseine (24 000 Da),  $\beta$ - Lactoglobulin ( $\beta$ - Lg, 18 000 Da), and  $\alpha$ -Lactalbumin ( $\alpha$ - La, 14 000 Da). **Control:** fresh whey no treated.

### Polyclonal antibodies production

Polyclonal antibodies were produced from rabbits. A group of 12 New Zealand female rabbits were housed in the laboratory (University of Es-Senia Oran). Immunization was performed according to the method of Walker et al. (1973). A pre-immunization sample was collected from each rabbit at days 0. At day 1, rabbits received a primary immunization and booster immunizations at days 7, 20 and 30. For the primary injection, 2 mg of native antigen ( $\beta$ -Lg purchased from Sigma) dissolved in 0.5 ml of physiologic serum was emulsified with 0.5 ml of Freund's complete adjuvant (FCA). Each animal was injected subcutaneously with 1 ml of this emulsion into 6-8 sites on the animal's shaved back (0.1 – 0.2 ml/site).

For three booster immunizations, Freund's incomplete adjuvant (FIA) was used in place of FCA to avoid possible adverse reactions by the sensitized rabbits to the bacterial components of Freund's complete adjuvant (FCA). One week after the last injection, blood was collected by a heart puncture and each antiserum was separated by centrifuging at 3000 rpm for 10 mn at 4°C. All sera were stored at -20°C.

### IgG titers measured by ELISA

IgG anti whey proteins and anti  $\beta$ -Lg were assayed in serum samples by an enzyme-linked immunosorbent assay (ELISA). Multi well microtiter plate (Nunc, polylabo, France) was coated for 1 h at 37°C with 100  $\mu$ l of  $\beta$ -Lg, whey or milk (2 mg/l). The plates were

then washed with phosphate buffered saline containing 0.05% Tween 20, pH 7.4. After further washing of the plates, 100  $\mu$ l of serially diluted sera (1/30 - 1/65610) was added in triplicate to each well and incubated for 1 h 30 mn at 37°C. The plates were again washed and 100  $\mu$ l of goat anti-rat IgG peroxidase conjugate (1:3000, Sigma, France) was added to the wells and the plates were left for 1 h at 37°C. After further thorough washing of the plates, peroxidase activity was assayed by staining with 0.2 mg/ml of diamine-orthophenylene in 0.05 M citrate buffer, pH 4 containing 0.05% H<sub>2</sub>O<sub>2</sub>. The staining of the well contents was developed in the dark with the external stirring at room temperature. The reaction was stopped with 6 N H<sub>2</sub>SO<sub>4</sub> (50  $\mu$ l/well) and absorbance measured at 492 nm with Uniskan plate reader (Labsystem). Positive titers were given as the last dilution with optical density, which was above to the background.

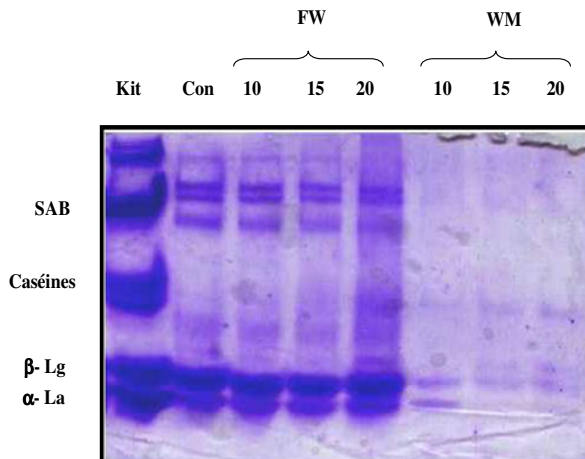
### Statistical analysis

Results were expressed as means  $\pm$  standard error (ES). All experiments were repeated five or six times. Obtained data were statistically analysed using student's test as programmed by MSII.

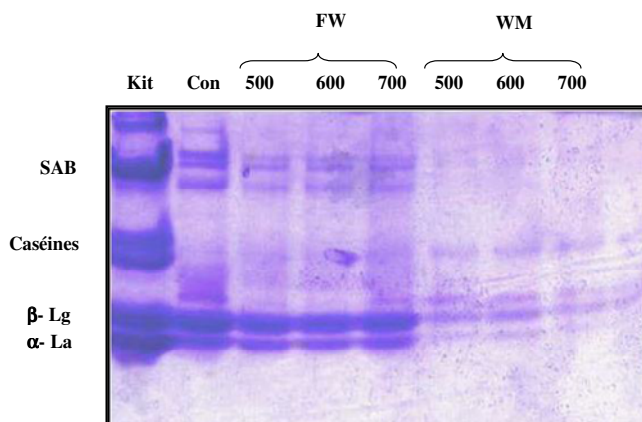
## RESULTS AND DISCUSSION

### Effect of microwave heating on composition of whey proteins

In order to analysis the effect of microwave treatment on whey proteins, an electrophoresis of different samples of fresh whey and whey descended from whole milk, were performed. The different electrophoretic distribution of sample's whey proteins in the 5% - 12.5% gel is shown in Figure 1. Figure 1-A shows profiles of fresh whey proteins, and those obtained from whole milk treated by microwave at 300 Watts for 10, 15 and 20 min compared to milk proteins. No differences were observed between the profiles of fresh whey samples treated at 300 Watts for 10, 15 and 20 min and those of milk proteins standard. In contrary, in samples obtained from whole milk treated at the same power and period, the intensity of band corresponding to whey proteins were diminished dramatically. In the case of treatment at 400 Watts for 10, 15 and 20 min (Figure 1b) the observation was identical; the profile did not reveal any different with the milk proteins. But the performance of microwave treatment of

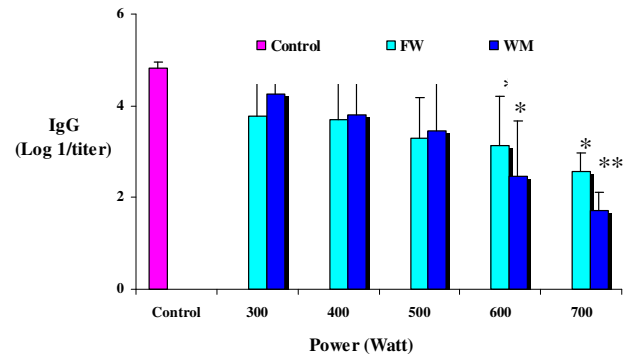


**Figure 1b.** SDS PAGE of milk (WM) and fresh whey treated (FW) by microwave irradiation at 400 Watts during 10, 15 and 20 min. **Kit:** Molecular weight Kit marker: bovin serum albumin (68,000 Da), caseine (24,000 Da),  $\beta$ -Lactoglobulin ( $\beta$ - Lg, 18 000 Da), and  $\alpha$ -Lactalbumin ( $\alpha$ - La 14,000 Da). **Control:** fresh whey no treated.

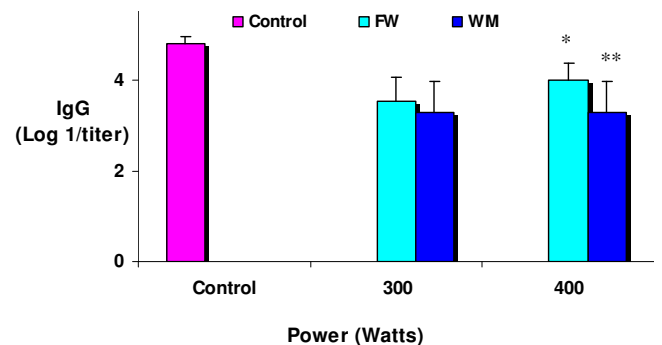


**Figure 1c.** SDS PAGE of milk (WM) and fresh whey treated (FW) by microwave irradiation at 500, 600 and 700 Watts during 10 min. **Kit:** Molecular weight Kit marker: bovin serum albumin (68 000 Da), caseine (24,000 Da),  $\beta$ -Lactoglobulin ( $\beta$ - Lg, 18,000 Da), and  $\alpha$ -Lactalbumin ( $\alpha$ - La, 14,000 Da). **Control:** fresh whey no treated.

whole milk at the same conditions (power/period) can alter the qualitative composition of its whey protein, as revealed by the electropherogram (1b). Figure 1c shows the electrophoresis of fresh whey samples treated at 500, 600 and 700 Watts for 10 min, and those obtained from whole milk treated at the same power and period. The obtained results were the same either for fresh whey samples or those descended from whole milk treated, with those obtained with microwave treatment at 300 and 400 Watts. The electrophoretic profiles were identical for the samples of fresh whey treated at 500, 600 and 700 Watts for 10 min. In the case of whey obtained from whole milk treatment, the intensity of the bands corresponding to whey proteins decrease considerably.



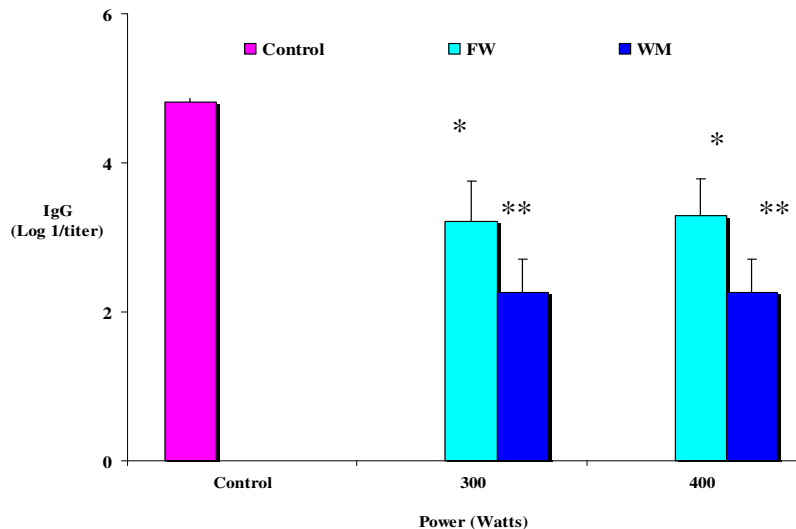
**Figure 2a.** Binding ability (antigenicity) of  $\beta$ -Lg rabbit IgG to fresh whey (FW) and whey descended from whole milk (WM) samples irradiated by microwave at 300,400,500,600 and 700 Watts during 10 min determined by ELISA. Values are given as mean  $\pm$  SE (standard error) from independent measurements (n=6). (\* : 0,01 < p < 0,05 ; \*\* : p < 0,01 ; \*\*\* : p < 0,001)



**Figure 2b.** Binding ability (antigenicity) of  $\beta$ -Lg rabbit IgG to fresh whey (FW) and whey descended from whole milk (WM) samples irradiated by microwave at 300,400 Watts during 15 minutes determined by ELISA. Values are given as mean  $\pm$  SE (standard error) from independent measurements (n=6). (\* : 0,01 < p < 0,05 ; \*\* : p < 0,01 ; \*\*\* : p < 0,001)

This results shows that whole milk treated by microwave induce more changes in whey proteins composition than fresh whey treatment; this modification can be result from denaturation and loss of solubility of whey proteins. Microwave treatment of whole milk cause significant decrease of whey proteins concentration than samples of fresh whey treated. That concentration decrease depends both on power and period of microwave treatment; so the higher the power, the lower is their concentration. Similarly, the whey protein concentration decreases following a long period treatment at lower power (data not shown).

This decrease due to microwave treatment can be explained by the loss of whey protein solubility. The stability of whey proteins is very weak when it is in the molten globule state, which is an intermediate structure between the native and the denatured form (Harb, 2002).



**Figure 2c.** Binding ability (antigenicity) of  $\alpha$ -La rabbit IgG to fresh whey (FW) and whey descended from whole milk (WM) samples irradiated by microwave at 300,400,500,600 and 700 Watts during 10 min determined by ELISA. Values are given as mean  $\pm$  SE (standard error) from independent measurements (n=6). (\* : 0,01 < p < 0,05 ; \*\* : p < 0,01 ; \*\*\* : p < 0,001).

Kuwajima et al. (1990) demonstrated that disulfide bridge is much reactive in these conditions, so several intermediate species can be produced allowing whey proteins, of which the structure is highly changed to polymerize, and consequently to become less soluble (Ewbank, 1984).

### Immunoreactivity of whey samples treated by microwave

ELISA was used to assess residual immunoreactivity of different whey samples treated by microwave at different power and period, with  $\beta$ -Lg and  $\alpha$ -La specific antibody produced by new-Zealand rabbit immunization. The results obtained with rabbit anti  $\beta$ -Lg and IgG on samples of fresh whey and those obtained from whole milk treated by microwave at different power period are shown in Figure 2. The figure (2a and c) show that immunoreactivity of whey samples descended from whole milk treated by microwave at 300 and 400 Watt decrease considerably as long as the period of the treatment is long (20 min). The same result is obtained at the higher power, 600 and 700 Watt, microwave treatment for 10 min as shown in Figure 2a. This confirms that structural changes of protein occurred during microwave exposure (Lawrance, 2000). Also, the whey proteins kinetic denaturation is accelerate by a microwave heat gradient (Bohr, 1999). These modifications are related to disulfide bridges breach (Manderson, 1999). Furthermore, the results obtained by Pomerai (2003) shows that microwave increase the denaturation rate of whey proteins such bovin serum albumin.

### ACKNOWLEDGEMENTS

This work was financially supported by the Ministère de l'Enseignement Supérieur et de la Recherche Scientifique.

### REFERENCES

- Bohr H, Bohr J (1999). Microwave enhanced folding and denaturation of globular proteins. *Am. Phys. Rev. Issue 4*: 4310-4314.
- Ewbank JJ, Creighton TE (1984). Structural characterization of the disulfide folding intermediates of bovine  $\alpha$ -Lactalbumin. *Biochemistry*. 32: 3677-3693.
- Harb Bertrand C, Baday A, Dalgalaronto J-M, Chobert, Haertlé T (2000). Thermal modification and co-denaturation of  $\alpha$  - Lactalbumin and  $\beta$ - Lactoglobulin induce changes of solubility and susceptibility to proteases. *Nahrung/Food*. 4: 283-289.
- Host A (1997). Cow's milk allergy. *J. R. Soc. Med.* 90: 39-40.
- Kuwajima K, Ikeguchi M, Sugawara T, Hiraoka Y, Sugai S (1990). *Biochem.* 29: 8240-8249.
- Laemelli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 227: 680-685.
- Manderson GA, Hardman MJ, Creamer LK (1999). Effects of heat treatment on bovine beta-lactoglobulin A, B and C explored using thiol availability and fluorescence. *J. Agric. Food. Chem.* 47: 3617-27.
- Moneret-Vautrin DA (1999). Cow's milk allergy. *Allergie et Immunologie*. 31(6):201-207.
- Pomerai D, Smith B, Dave A, North K, Smith T, Archer DB, Duce IR, Jones D, Candido EP (2003). Microwave radiation can alter protein conformation without bulk heating. *FEBS. Lett.* 543 (1-3): 93-7.
- Saidi D, Heyman M, Kheroua O, Boudraa G, Bylsma P, Kerroucha R, Chekroun A, Maragi J-A, Touhami M, Desjeux JF (1995). Jejunal response to  $\beta$ -lactoglobulin in infants with cow's milk allergy. *C.R. Acad. Sci. Paris Life Sci.* 318: 683-689.
- Walker WA, Isselbacher KJ, Bloch RJ (1973). Intestinal uptake of macromolecules. Effect of parenteral immunization. *J Immunol.* 111: 121-226.