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Grana Padano cheese: thermoanalytical techniques applied to the study of ripening

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Abstract

In the present work, modifications of water–matrix interactions in Grana Padano (GP) cheese were studied using thermogravimetric techniques in order to provide an index of ripening. These techniques represent a simple and rapid method for determining water content and for monitoring its behaviour in ripening GP cheese. It is possible to evaluate the quantity of water present in the matrix, as well as the different types of water bound to it both qualitatively and quantitatively. Samples of traditional GP cheese produced in the winter season (same day), and collected at different ripening stages (5,10,14,19 months), were investigated. When statistical models of the STARMAX (Space Time Autoregressive Moving Average eXogenous variables) class, augmented with surface trends, were fitted to thermoanalytical data, valuable insights into the chemical nature of the ripening were acheived with good predictive performances. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

The ripening of a cheese involves complex modifications of the interactions between the water and other components. These modifications are due to different interrelated changes but, above all, proteolysis and lipolysis, salt diffusion and water migration.

In the present work the changes are studied using thermogravimetric techniques which represent a simple and rapid method for determining water content and for following its behaviour during the various stages of ripening of Grana Padano cheese. Statistical models of the STARMAX type are applied for the purpose of describing and quantifying the migratory flows of water.

Thermoanalytical techniques provide detailed information on the cooperative activity of all the various water–matrix interactions and allow all the different types of water present in a system to be accounted for. Different types of water include those with different energies of bonding to the matrix. Thermogravimetric measures can be used to make a detailed analysis of the water contained in the matrix, making both qualitative and quantitative distinctions among the various different types of water.

It has been demonstrated that water can be bonded to the matrix with different energies and so, when the matrix is heated, the water is lost in successive stages, depending on the amount of activation energy required to break the bonds (hydrogen bonds, Van der Waals forces, London forces, etc.) formed between the water and the matrix. The temperatures at which the various activation energies are reached can even be much higher than the boiling point of water. In particular, the presence of strong bonds in the water contained in a foodstuff can mean that several different kinds of water are bonded by means of such stable interactions that, in the conditions used for analysis using official methods, it is impossible to attain a sufficiently high activation energy to break the bonds in question (Curini, D'Ascenzo, Lucchetti, & Wendlandt, 1989).

This type of methodological approach has led to significant changes in the field of commodities both at the analytical and the production level, for example, qualitative and quantitative determination of total water in food systems, the study of the effect of ripening on foods and the determination of ripening period based

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on the effects induced by this event in the water-matrix interaction, as well as the optimization of food preservation processes, such as conventional and freeze-drying.

In Grana Padano type cheese, the ripening period is essential to the product's quality. It is therefore useful, to establish ideal conditions for ripening and to identify a simple and rapid method for determining the degree of ripening of the cheese.

The methods used to determine the age of a cheese are based on: quantitative determination of the various nitrogenous forms (Carini & Muchetti, 1985); electrophoretic analysis of the various casein species (Corradini & Battistotti, 1973; Resmini, Dieci, Volonterio, & Saracchi, 1973); statistical processing of the areas of the various peaks detected in the peptide profile (Pham & Nakai, 1984). These methods yield only an approximate estimate of the age.

Recently, other methods have been proposed, based on the determination of: pyroglutammic acid (Panari, 1985), free amino acids (Resmini, Pellegrino, Pazzaglia, & Hogenboom, 1985; Resmini, Hogenboom, Pazzaglia, & Pellegrino, 1993), and soluble and total nitrogen using dialysis techniques (Sciancalepore & Zannoni, 1987)

2. Materials and methods

2.1. Instrumentation

The thermogravimetric analyses were performed on a TGS-2 Perkin–Elmer thermobalance equipped with a Data Station 3600.

The cheese samples, in the form of small disks each weighing about 20 mg, were placed in the thermobalance sample pan and heated over the temperature range 30–750°C, at a scanning rate of 10°C min^{-1}, in a flow of air of 50–100 ml min^{-1}. The TG-FTIR analyses were performed on a TGA-7 Perkin–Elmer thermobalance coupled by transfer line to an FTIR spectrophotometer IR operating in Fourier Transform mode (Perkin–Elmer model 1760X). The IR spectra were performed over the temperature range 25–200°C in an air flow.

2.2. Sampling

For the present research, aimed at evaluating modifications in the water–matrix interactions for the purpose of providing an index of the cheese's ripening, Grana Padano round cheeses, produced during the winter season (same day), were used and samples taken at various stages of ripening (5,10,14 and 19 months).

A wedge-shaped sample was taken from each whole cheese, this wedge was obtained by cutting the cheese along the equatorial axis so as to obtain two symmetrical disks, and then cutting one of the two disks radially into eight equal parts. To avoid errors, the wedges were sampled at 30 different points 2 cm apart, lying on the plane of symmetry of the wedges. The thirty samples were obtained by subdividing the surface into a grid of horizontal lines parallel to the base of the cheese and vertical lines running at right angles to the base and parallel to the rind side. Then, using a special probe, 'cores' were removed at each point of intersection on the grid, excluding the outermost points, which were subject to shocks during cutting and preparation.

The samples thus obtained were placed in special containers and kept in liquid nitrogen until used in order to maintain their characteristics and avoid degradation. In order to verify interactions, preliminary tests were carried out using fresh cheese. This treatment was validated by the consistency of the thermoanalytical results, using fresh samples, with those obtained using refrigerated samples.

Cheese samples in the form of disks, each weighing about 20 mg, were placed in the thermobalance sample pan and heated over the temperature range 30–750°C at a scanning rate of 10°C min^{-1}, in an air flow.

3. Results and discussion

Examination of the thermogravimetric curves and their first derivatives shows that water loss always occurs through two main, partially overlapping, processes, each of which is the result of the convolution of a series of subprocesses corresponding to interactions between the water and different components of the matrix over the temperature range 30–200°C. Two different types of water can thus be identified: free water and bound water.

Free water, that is water bound with less energy to the matrix, is released over the temperature range between 30 and 90–110°C, corresponding to the first slight thermogravimetric step. *Bound water*, that is water more strongly linked to the matrix, is lost through a more extensive process over the temperature range 110–200°C. The anhydrous sample then decomposes thermally until ashes are obtained at a temperature exceeding 700°C. (Fig 1a and b).

Grana Padano samples were also tested using TG-FTIR analysis. The gases produced in the furnace were conveyed through a transfer line to the IR cell. The IR spectra were recorded (about 3 spectra per minute) over the temperature range 25–200°C. During the decomposition process, the gases given off were monitored and the IR spectra confirmed the presence of water over the range 25–200°C. At the same time, at around 150°C, bands fainter than those of water were observed, typical of CO_2 and of the amines probably deriving from protein degradation (Fig. 2).

The mean values of total, bound and free water were thus calculated as a function of the distance from the

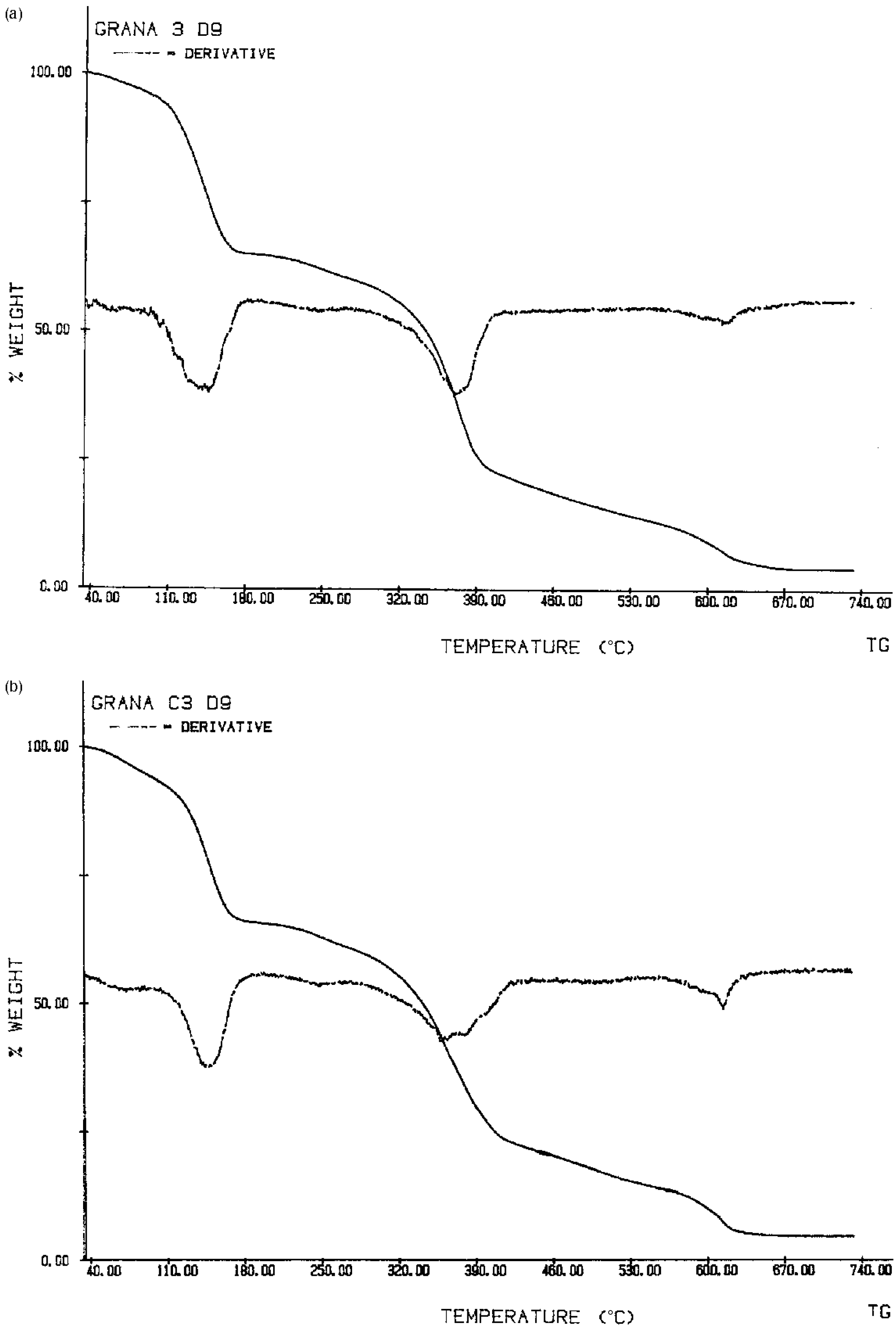

Fig. 1. TG and DTG curves of Grana Padano samples cheese at different ripening ages: (a) 5 months; (b) 19 months. Heating rate: 10°C/min. Atmosphere: air at flow rate of 50–100 ml min^{-1}.

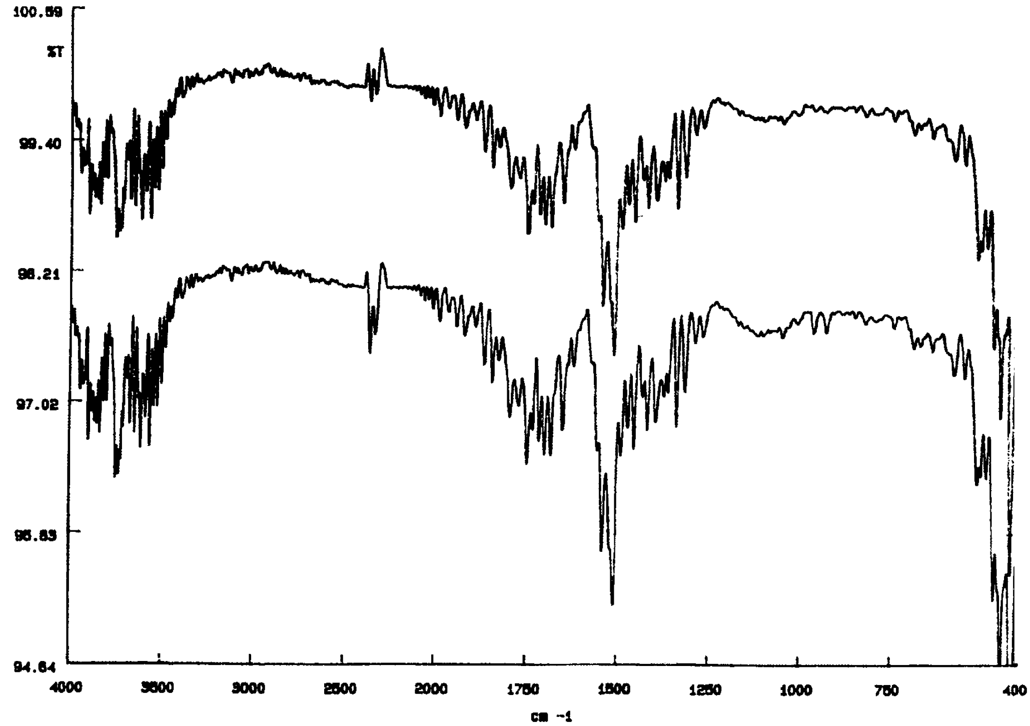

Fig. 2. FT-IR spectrum of Grana Padano sample cheese.

rind side. These values were then compared with those for samples at different ripening stages.

From the diagrams it can be seen that, during the ripening process, the total water content is reduced. This loss is certainly due to migration-evaporation processes, which are much stronger on the peripheral part of the whole cheese. The trend for the bound water (Table 1 and Fig. 3) is similar to that of total water; a reduction occurs in the course of ripening. Furthermore, observing the same sample at different points on the wedge, the water loss is found to increase from the centre towards the exterior.

The amount of free water present at the various sampling points is affected by a much larger number of parameters than those affecting the behaviour of bound water (i.e. evaporation, diffusion, proteolysis, etc.). Nevertheless, a fractal mathematical treatment of the problem clearly shows that the behaviour of the free water affects the behaviour of the bound water through a series of kinetic and thermodynamic equilibria.

Table 1
Mean values of the bound water at different ripening ages and at different points on the wedge

	0 cm (%)	2 cm (%)	4 cm (%)	6 cm (%)	8 cm (%)	10 cm (%)
5 Months	24.7	26.8	28.0	29.3	30.1	29.9
10 Months	24.6	25.9	26.3	28.0	28.54	27.3
14 Months	23.7	25.4	25.7	25.8	27.0	26.7
19 months	22.4	23.4	23.0	25.6	25.9	25.2

The total water displays a behaviour as a function of time and distance from the rind side that is comparable to that of the bound water.

This phenomenon may be interpreted at the level of the percentage differences in the bound and free water present, where the free water represents a small percentage of the bound water and the fluctuations in free water, as viewed on a macromolecular scale, are offset by the differential excess of bound water, with a resulting decrease in the differential between successive situations expressed as a function of time and of the distance from the rind side.

This behaviour may be accounted for by examining the following phenomena: (1) proteolysis and lipolysis; (2) salt diffusion.

1. During ripening, proteolysis increases until it reaches a plateau at around 14 months. Protein hydrolysis leads to the release of bound water; this accounts for the observed behaviour of bound water during ripening, which decreases with time (from 5 to 19 months). Moreover, the percentage of free amino acids (an indicator of the proteolysis process) increases from the outer part of the whole cheese towards its centre. This accounts for the observed behaviour of the bound water, i.e. the release of a smaller amount of water in the peripheral part of the cheese (where proteolysis is weaker), compared with its centre, where the proteolysis is stronger. A further contribution to the decrease in bound water comes from lipolysis, although this process is secondary to proteolysis.

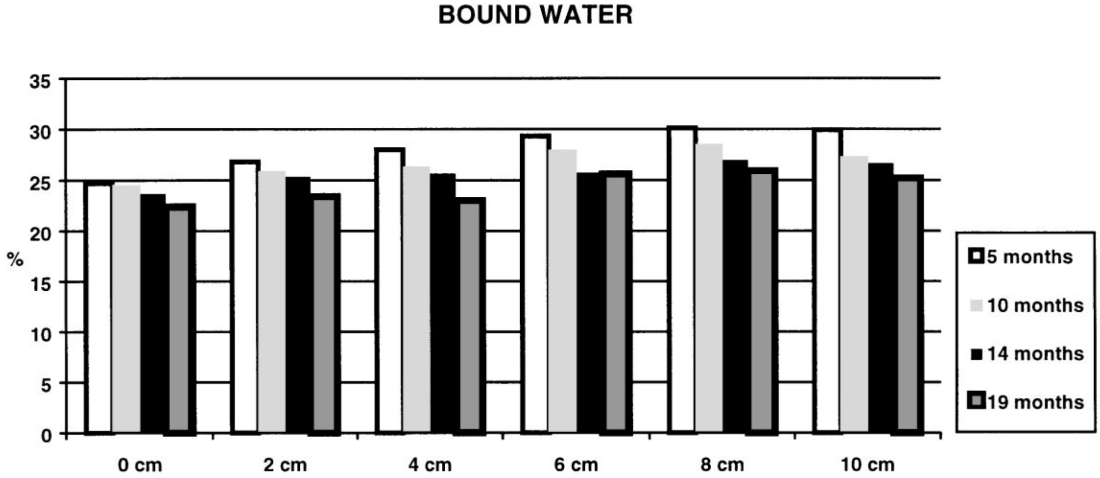

Fig. 3. Plots of bound water at different ripening ages and at different points on the wedge.

2. Salting can slow down the processes of protein degradation by inhibiting enzymatic activity; indeed, in the saltier areas, a lower level of proteolysis and of bound water release is found. When the salt has been uniformly distributed (after 10 months), the inhibiting effect on degradation becomes negligible. The lower salt concentration leads to reduction in the hydrophic ions capable of bonding coordination water, with a consequent increase of available free water.

The sum total of the processes governing ripening and affecting the water's behaviour was evaluated by processing the thermoanalytical data using the STARMAX (Space Time AutoRegressive Moving Average eXogenous variables) statistical model employed successfully in ecology and biology (Bennet, 1979; Ripley, 1981) to describe the behaviour of phenomena observed in space. The principle underlying this type of model is that the intensity of a particular phenomenon in a point or area, in a given time, can be explained, first of all, by the intensity of the phenomenon itself at the same point in the immediately preceding period (due to an obvious time drift effect) as well as in adjacent points (due to a spatial contagion effect: in our case, due to migration of water towards the exterior) and by exogenous variables.

At any stage of the ripening, the free water content in any given portion of the cheese depends on various factors, such as the transformation of the bound water into free water, migration processes and the initial conditions given by the salt distribution (which strongly affects the water). The creation of a model thus requires a careful analysis to be made of the spatial structure of the data (free and bound water). The latter may be represented as points (X, Y) on a plane. If the origin of the axes is placed in the centre of a cheese wedge, the X axis represents the equatorial axis of the wedge itself. For each point, two measurements are available for water content. The aim was to apply a statistical model to evaluate the distribution of water in both time and space.

More precisely, we postulated that the amount of free water, F_{xys}, present at a given point (x, y) where x, y are, respectively, the measure of the horizontal and vertical distance from the centre of the wedge, at a given maturity stage s, depends on the following factors, that represent the initial conditions:

1. the amount of free water present at the same point at a previous stage of ripening, F_{xys-1}, as part of the water that will migrate;
2. the amount of bound water present at the same point at a previous stage of ripening, B_{xys-1}, as part of the water that will be transformed into free water;
3. the amount of free water present in the vicinity of the point (x, y), which will affect the migration processes;
4. the actual position of the point in the plane.

Once the phenomenon is formalised in this way, a natural modelling candidate is the STARX (Space Time AutoRegressive exogenous variables) class augmented with a surface trend. Fitting models of this class allows evaluation of both the absolute and relative relevance of the various factors listed above, thus hopefully gaining a better understanding of the maturing process.

STARX models augmented with a cubic trend have been separately fitted to the data for cheese at maturity 10 and 14 months. After a selection process, based on the Schwartz Criterion (SC) and both joint and individual significance tests, with the randomness of residuals checked through Moran's I, we reached the final preferred specification reported in Tables 2 (10 months maturity data) and 3 (14 months data).

Some interesting conclusions can be drawn. First of all, both models fit the data very well, with no systematic

Table 2
Starx model application to Grana Padano cheese at maturity stage of 10 months

	B_{xys-1}[a]	x^2	y^2
	0.15	0.07	0.08
$R^2 = 0.97$[d]	[9.90][e]	[2.74][e]	[2.13][e]
	SC = 1.47 [2.01][b]	$I_s = 0.97$[c]	

[a] Note: dependent variable is the amount of free water at 10 maturity months; B_{xys-1} is the amount of bound water measured at the same point at 5 months.
[b] SC: Schwartz Criterion, with value for the unrestricted model in brackets.
[c] I_s: standardized Moran index of first order spatial autocorrelation of residuals, asymptotically $N(0,1)$.
[d] R^2: raw moment R^2, ratio of the sum of squares of the estimates and of the dependent variable.
[e] t-Ratios in brackets.

Table 3
Starx model application to Grana Padano cheese at maturity stage of 14 months

	F_{xys-1}[a]	B_{xys-1}[G]
	0.35	0.15
$R^2 = 0.98$	[2.51]	[4.87]
	SC = 0.85[1.65]	$I_s = 0.60$

[a] Note: dependent variable is the amount of free water at 14 maturity months; F_{xys-1} and B_{xys-1} are respectively the amount of free and bound water measured at the same point at 10 months.

pattern left in the residuals. Second, their structure is very different. Although the flow of bound water passing into the free state is stable relative to the amount of free water globally present, location, as measured by the surface trend, matters at 10 but not at 14 months. The opposite happens to the previous content of free water. The higher content of water in the most external parts of the wedge, implied by the quadratic trend, is presumably explained by the initially very high salt content of the surface (salt attracts water). This is in line with a priori expectations: the influence of initial conditions fades with time, while, as the system approaches more stable conditions, its persistence grows. Finally, migration processes do not appear to have significant effects at either maturity. Although the results are promising, the importance of initial conditions suggests that our results should be validated by analyses carried out with different samples. This, together with analyses at longer maturity periods, is the object of our current work.

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