

The use of carbonyl analysis to follow the main reactions involved in the process of deterioration of dehydrated dairy products: prediction of most favourable degree of dehydration

Théo EFSTATHIOU^{a*}, Claude FEUARDENT^a, Serge MÉJEAN^b,
Pierre SCHUCK^b

^a Analyses et Recherche, NUTRINOV, Z.A.C. des Trois Marches, 3 impasse de La Jonchée, 35132 Vezin-Le-Coquet, France

^b Laboratoire de Recherches de Technologie Laitière, INRA, 65 rue de Saint-Brieuc, 35042 Rennes Cedex, France

Abstract – Carbonyl analysis was used in order to follow the main reactions involved in the process of deterioration of milk powders. This method was applied after the milk had been processed using microfiltration and ultrafiltration, freeze-drying or spray-drying. The milk powders were then stored at a temperature of 45 °C and aw ranging from 0.7, 0.11, 0.23 and 0.33 to 0.5 for a period of twenty days. Water adsorption was influenced by powder composition on one hand, with a maximum water adsorption of whey proteins at 0.20 aw, and by the physical state of molecules on the other hand. At certain conditions such as 45 °C and 0.33 aw, amorphous lactose released the water molecules and turned into crystallised lactose. All powders studied exhibited a minimum deterioration, except those rich in lactose, at 0.20 ± 0.5 aw. For the products rich in lactose this minimal deterioration was observed at two aw values: 0.10 and 0.30. The determination of the monolayer moisture was carried out either theoretically with the BET model or experimentally so as to predict the most favourable degree of dehydration of dehydrated products.

Carbonyl / powder stability / sorption isotherm / water activity / monolayer value

Résumé – L'utilisation du dosage des carbonyles pour suivre les principales réactions intervenant dans la détérioration des produits laitiers déshydratés : prédiction du degré de déshydratation le plus favorable. Un dosage des carbonyles a été utilisé afin de suivre les principales réactions intervenant dans la détérioration des poudres de lait. Cette méthode a été appliquée à des

Communication at the 1st International Symposium on Spray Drying of Milk Products, Rennes, France, October 16–18, 2001.

* Correspondence and reprints

Tel.: (33) 02 99 14 70 50; fax: (33) 02 99 14 70 51; e-mail: tefstathiou@nutrinov.com

produits laitiers issus des procédés de microfiltration et ultrafiltration, lyophilisés ou séchés par atomisation. Les poudres étaient stockées pendant 20 jours à 45 °C à des aw de 0,07, 0,11, 0,23, 0,33 et 0,5. La fixation de l'eau est influencée d'une part par la composition des poudres, avec un maximum de fixation d'eau par les protéines sériques à 0,20 d'aw et d'autre part par l'état physique des molécules. À 0,33 d'aw et à 45 °C, le lactose amorphe libère les molécules d'eau et se transforme en lactose cristallisé tandis que les protéines se polymérisent. Toutes les poudres que nous avons étudiées, excepté les poudres riches en lactose, présentaient un minimum de détérioration à $0,20 \pm 0,5$ d'aw. Quant aux produits riches en lactose, ils présentaient deux minima (à 0,10 et à 0,30 d'aw). La détermination de la couche monomoléculaire d'eau a été effectuée soit avec le modèle théorique (BET) soit expérimentalement, afin de prévoir le degré de déshydratation le plus favorable des produits laitiers déshydratés.

Carbonyle / stabilité des poudres / isotherme de sorption / activité de l'eau / couche monomoléculaire d'eau

1. INTRODUCTION

During food storage or modification, lipid autoxidation is the main reaction in the deterioration of its organoleptic qualities (colour, flavour and texture change) as well as of its nutritional ones (essential fatty acids loss, nutritional value loss of proteins and co-oxidation of vitamin C) [10, 12, 23].

Autoxidation of milk lipids and milk by-products can be influenced by various factors such as oxygen, light, metal ions, ascorbic acid, tocopherols, carotenoids, thiols, heat treatment, water activity and storage temperature. Bearing in mind that regulation does not permit the addition of anti-oxidants to dairy products for human consumption, the essential prevention of oxidative changes will depend on the action performed to avoid catalysis and propagation of oxidation during storage. The realisation of sorption isotherms is of great importance in predicting food behaviour during treatment or storage [8].

From the sorption graphs and with the help of the Brunauer-Emmet-Teller (BET) or Guggenhen-Andersson-De Boer (GAB) theoretical models [27], it is possible to obtain the optimal water content for a higher stability of all powdered dairy products. In fact, it is considered that maximum stability is reached on the monomolecular water layer [17, 20].

The theoretical models, however, are often applied to the conventional milk products (whole or skimmed powdered milk) but they have not been confirmed either on products obtained after filtration or on formulated products.

In order to obtain the most favourable degree of dehydration for increased milk powder stability, an experimental study is necessary, based on the sorption isotherm in connection with the degradation compounds (issued either from the lipid, or protein autoxidation or the Maillard reaction) at different relative moisture levels [20]. To carry out the study, a number of analytical methods have already been tested.

The method measuring the Thiobarbituric Acid Reactive Substances (TBARS) is ideally suited to evaluating the lipid oxidation.

Lactulose is often proposed to indicate the occurrence of the Maillard reaction in dairy products.

Nevertheless, instead of using a number of analytical markers, sometimes difficult to adapt and which require sophisticated and expensive equipment, we proposed one single analytical method which, in combination with sensorial analysis, enables the main reactions of degradation in different milk powders to be followed.

Carbonyls belong to the chemical compound class also including aldehydes and

ketones. These compounds are derived from lipid oxidation on one hand [11, 13, 16] and protein oxidation on the other hand. The latter happens in the presence of free radicals issued from lipid autoxidation [9]. Finally, the reactions of photo-oxidation and irradiation can also induce lipid oxidation [7].

Furthermore, heat treatment, before and during drying, is another source of production of carbonyls.

These compounds are created from cetosamines during the second stage of the Maillard reaction [21, 24].

The present study consists of two parts.

The first part has as a task to establish a method of determining carbonyl quantity and to test it on powdered dairy products which have undergone an accelerated ageing, and to compare it with the other processing techniques usually applied to fix the speed graph of food deterioration according to water activity [19].

Products, based on a membrane separation technique (microfiltration and ultrafiltration) and dehydration (spray-drying and freeze-drying) on one hand and on milk fat formulation on the other hand, were made so as to examine their behaviour while manipulating aw.

The second part of the study compares the experimental results of the monolayer value of different products with the results obtained by the BET theoretical method.

2. MATERIALS AND METHODS

2.1. Powdered dairy products

The stage of preparation and the final products are represented in Figure 1.

2.1.1. Purified milk

A great variety of skimmed milk was used, supplied by Triballat Dairy (Noyal sur Vilaine, France).

A part of the milk was purified (Bactocatch procedure) by microfiltration using a mineral membrane with a pore diameter of 1.4 μm , as described by Trouvé et al. [32].

Another part of the milk was dehydrated by freeze-drying (CIRP, CS10, Argenteuil, France), which gave the powder P1.

2.1.2. Microfiltration of purified milk

The purified milk was then microfiltrated at a volume reduction factor (VRF) equal to 3, followed by a diafiltration at 4 volumes, and a new concentration at VRF equal to 2.50 so as to obtain a retentate richer in casein micelles (retentate 6.0) and a microfiltrate, as described by Pierre et al. [26].

A part of the sample of the microfiltrate and of the retentate 6.0 were dehydrated by freeze-drying (CIRP), which gave the powders P2 and P3, respectively. Another sample of the retentate 6.0 was spray-dried according to Schuck et al. [29], which gave the powder P4.

The rest of the retentate was added to milk cream (Triballat Dairy) so as to obtain a final product adjusted to contain 42 g·100 g⁻¹ milk fat with respect to the dry solid. The fat-enriched retentate was dehydrated either by freeze-drying (CIRP), which gave the powder P7, or by spray-drying according to Schuck et al. [29], which gave the powder P8.

2.1.3. Ultrafiltration of microfiltrate

The non-freeze-dried microfiltrate sample was filtered on a pilot (TIA, Bollène, France) with a spiral wound organic membrane of 9.7 m² and a molecular weight cut-off (MWCO) of 5000 (Koch International, Roissy, France) at a volumic reduction

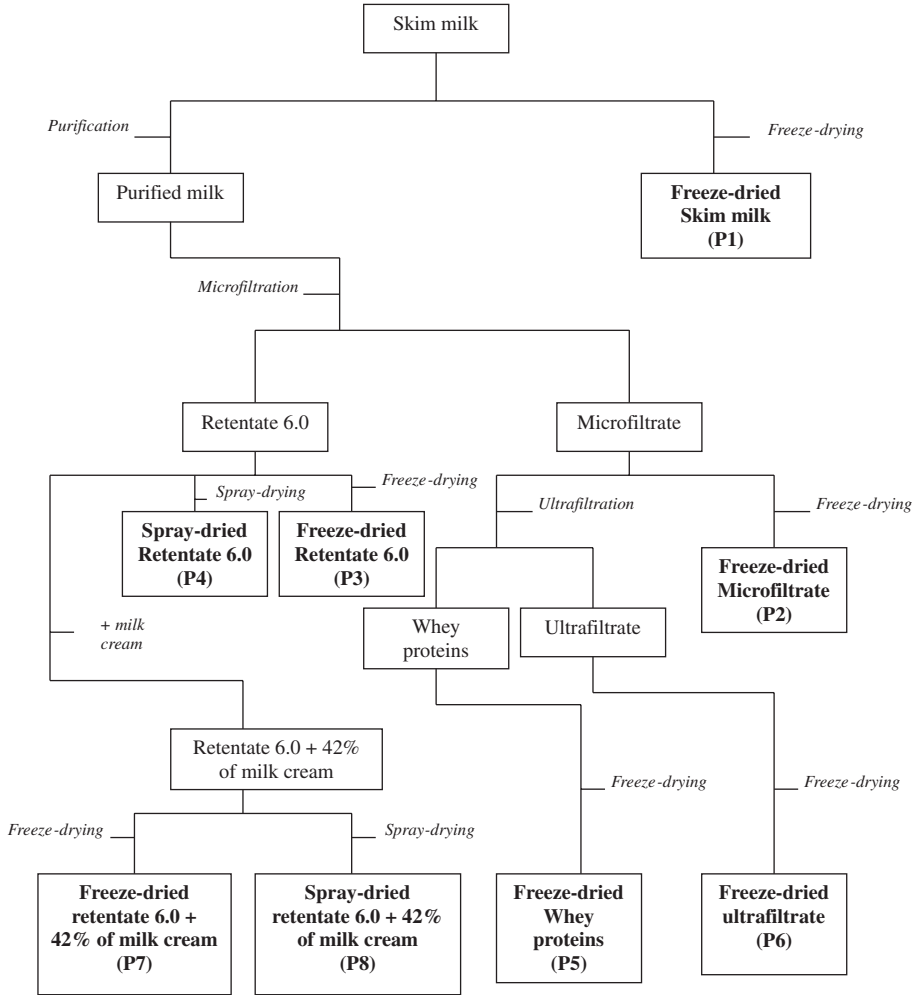


Figure 1. Technological diagram of powdered dairy products.

factor of 20.0 to obtain a concentrated whey protein and a concentrated ultrafiltrate.

These two products were then dehydrated by freeze-drying (CIRP) which gave the whey protein powder P5 and the ultrafiltrate powder P6.

2.2. Accelerated storage

Five grammes of products were weighed precisely in a Petri box and then sealed hermetically in a shell together with 100 g of

saturated salts. The saturated salts silica gel (Si), lithium chloride (CLi), potassium acetate ($C_2H_3KO_2$) and magnesium nitrate ($Mg(NO_3)_2$), are used to give moisture contents of 7, 11, 23, 33 and 53%, respectively [31]. The shells are then stored in a tank at 45 ± 1 °C for 20 d.

Total solids were determined by the AFNOR method [1], and aw was analysed [14] (with a Novasina RTD 200/0 aw meter, Pfäfficon, Switzerland, at 20 °C) before the accelerated storage of the products.

Two samples were taken at intervals of ten days during storage. The powders were weighed to measure the adsorbed and desorbed water content.

2.3. Brunauer-Emmet-Teller model (BET)

The BET theoretical model is represented by the following equation:

$$aw / E(1 - aw) = 1 / MC + aw(C - 1) / MC$$

where:

E = water content (g·100 g⁻¹ of solid);

M = monolayer value;

C = constant.

The BET model was chosen as it is better suited to water activities less than 0.5 [25].

2.4. Analytical methods

2.4.1. Carbonyls

The Levine et al. method [22], determining the quantity of carbonyls in biological surroundings, has been modified so as to determine the quantity of carbonyls in dairy products.

One gramme of milk powder was dissolved in 100 mL distilled water (40 °C). The mixture was stirred by a small magnetised bar for 10 min. One mL of the solution was placed in a screw-top tube of 20 mL and mixed with 1 mL solution of 2,4 dinitrophenylhydrazine (2,4 DNPH), supplied by Sigma (Saint Quentin Fallavier, France). The solutions were mixed with the help of a vortex for 30 s and then left to fall downward for 5 min. This stage of the process was repeated twice. 1.1 mL of 10% trichloroacetic acid was added to the mixture, thus precipitating the yellow-coloured complex (carbonyls – 2,4 DNPH). After centrifugation at 1000 rpm, for 10 min at room temperature, the surface layer was totally eliminated. The

residue was taken again by 10 mL of urea 8 mol·L⁻¹.

The measurement was carried out with the help of a spectrophotometer (Uvikon 922, Kontron Instruments, Saint-Quentin-en-Yvelines, France) in 370 nm quartz tanks against zero urea 8 mol·L⁻¹.

The results were represented in OD·mg⁻¹ of protein.

2.4.2. Total malondialdehyde (MDA)

The total malondialdehyde, the final product of lipid oxidation, was determined according to the Knight et al. method [18] which consists of separating on a HPLC column the thiobarbituric acid - malondialdehyde complex (TBA-MDA) before quantity determination. This method allows the TBA-MDA complex individual detection, which is less than TBARS [6] in value in most of the samples.

2.4.3. Lactulose

The quantity of lactulose, being a specific marker of the Maillard reaction, was determined by an enzymatic method [15]. The released lactulose is 340 nm, measured by a spectrophotometer after enzymatic hydrolysis.

2.4.4. Lactose

The lactose quantity determination was carried out by the AFNOR V 04-213 method [4].

2.4.5. Total nitrogen

In order to measure the total nitrogen content we used the Kjeldahl method [3]. The total nitrogen contents were converted to proteins using a conversion factor of 6.38.

2.4.6. Fats

Fat content was analysed using the ethero-chlorhydric extraction method [2].

2.4.7. Sensory analysis

The milk powders were dissolved in water at 40 °C for 30 min, then chilled down to 20 °C and put in plastic odourless glasses before being tested by a tasting panel of five specialists. The organoleptic quality was assessed on a scale ranging from 8 to 15 points.

3. RESULTS AND DISCUSSION

3.1. Precision of carbonyl content determination

A study of repeatability and reproducibility was carried out to assess the precision of the method. The results, as well as the average

(M), the standard deviation (SD) and the relative standard deviation (RSD %) are shown in Table I. The demonstrated values in RSD are 1.28% for repeatability and 1.89% for reproducibility.

These results are well within the 5% level accepted as a limit for analysis to be considered as reliable. Thus, it is deduced that this analysis is repeatable and reproducible and can be applied for the assessment of the carbonyl content in dairy products.

3.2. Chemical composition of powders

The biochemical composition of dehydrated dairy products at T0 is represented in Table II.

Table I. Precision of carbonyl content determination method in skimmed milk powder.

	Assay 1 ¹	Assay 2 ¹	Assay 3 ¹	Assay 4 ¹	Assay 5 ¹	M	SD	RSD %
Repeatability	1.329	1.331	1.297	1.296	1.312	1.313	16.78	1.28
Reproducibility	1.468	1.451	1.517	1.480	1.472	1.479	27.99	1.89

¹ Carbonyl content (units of OD / mg of product).

Table II. Biochemical composition (in g·100 g⁻¹ of product) of powdered dairy products at T0.

Compound	P1 ¹	P2 ¹	P3 ¹	P4 ¹	P5 ¹	P6 ¹	P7 ¹	P8 ¹
Total water	3.37	6.70	4.54	8.20	2.54	5.26	2.66	5.10
Free water	1.54	2.58	1.88	8.15	0.73	4.20	1.54	2.58
aw	0.08	0.33	0.08	0.30	0.08	0.17	0.14	0.15
Lactose	57.00	92.00	0.50	0.80	0.74	99.50	< 0.50	< 0.50
Crystallized α lactose	-	85.10	-	-	-	20.20	-	-
Proteins	36.09	9.81	96.90	85.70	81.40	2.95	46.20	47.33
Milk fat	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50	< 0.50	41.35	40.90
Carbonyl ²	2.12	3.75	1.37	1.54	1.72	-	-	-
Total MDA ³	-	-	-	-	-	-	53.12	83.52
Lactulose	-	-	-	-	-	1.29	-	-

¹ P1 = freeze-dried skim milk. P2 = freeze-dried microfiltrate. P3 = freeze-dried retentate 6.0. P4 = spray-dried retentate 6.0. P5 = freeze-dried whey proteins. P6 = freeze-dried ultrafiltrate. P7 = freeze-dried retentate 6.0 + 42% of milk cream. P8 = spray-dried retentate 6.0 + 42% of milk cream.

² Carbonyl content (units of OD / mg of protein).

³ Total MDA ($\mu\text{g}\cdot 100\text{ g}^{-1}$ of product).

(-) = not determined.

These powders show free water content ranging from 0.73 to 8.15 g·100 g⁻¹ of solid, total water content from 2.54 to 8.20 g·100 g⁻¹ of product and *a_w* from 0.08 to 0.33. The differences are caused by the biochemical composition of the powders, and by the technologies applied [8].

The lactose content in free and bound water displays a monohydrated α -lactose content of 85.1% for P2 and of 20.2% for P6 affecting water activity.

In fact, for the same quantity of lactose, the more crystallized it is, the higher the *a_w* [27]. Moreover, the protein and fat contents are consistent with the results obtained by Schuck et al. [29] and Pierre et al. [26].

3.3. Importance of the composition, physical state and milk powder dehydration mode for water adsorption

3.3.1. Importance of the composition and physical state of powders

The results of sorption isotherms on freeze-dried powders at T10 and T20, and at 45 °C are shown in Table III.

The sorption isotherm evolution of freeze-dried powders after storage for 10 and 20 d at 45 °C is illustrated in Figure 2. After storage for 10 d (0.07 *a_w*, 45 °C) the freeze-dried products lose a certain quantity of water due to the phenomenon of desorption, which is not true for P5 and P7.

Between 0.10 and 0.20 *a_w* at 45 °C, all freeze-dried products continue to adsorb water. Yet the protein-rich P3 and P5 (Tab. III) adsorb a greater quantity of water: at 0.20 *a_w*, for example, 10.96 and 11.34 g of water/100 g of solid is adsorbed compared to 6.31, 4.05, 7.11 and 6.39 for P1, P2, P6 and P8, respectively.

Between 0.2 and 0.3 *a_w* at 45 °C, P3, P5 and P6 lose water. It is probable that a change of structure could occur during the water freezing. It is also probable that a

fraction of proteins from the whey protein concentrate (P5) are polymerised [30]. The product P6 practically only consists of lactose, where 20% is crystallised (Tab. II). Here the initiation of lactose crystallisation coincides with the freezing of water [28].

The product P2, however, contains 85% lactose in crystallised form and a loss of water does not recur. We can observe the same phenomenon with P7 (P3 + 42% fat) which does not lose free water during the first ten days of storage at *a_w* ranging from 0.10 to 0.40. Since the chemical composition of P3 has been modified, the behaviour of the water adsorption on the product has changed as well.

We can note that between 0.10 and 0.30 *a_w* the form of the sorption isotherms changes with time.

The observed slight discontinuity on the graphs after 20 d of storage (0.10 *a_w* for the products P3 and P5 and 0.20 *a_w* for the products P7 and P6) might correspond to the initiation of a change in structure and a freezing of water, as already observed by Berlin and Anderson [5].

P6 shows the most clear discontinuity, which is probably the result of the transformation of amorphous lactose into α -crystallised lactose [28].

3.3.2. Importance of the method of dehydration

Table IV shows the results of ten sorption isotherms (45 °C) after 10 and 20 d of storage of the spray-dried P4 and P8.

After 10 d of storage (between 0.07 and 0.20 *a_w*), the protein-rich product, identical to the structural level of P3 (Tab. III) and P4 (Tab. IV) but dehydrated by different methods, did not have the same behaviour with regard to water adsorption.

In fact, the lyophilised product P3 retains more water than P4 which is in spray form (10.96 and 8.91 g of water/100 g of solid, 0.20 *a_w*). However, if we compare the

Table III. Sorption isotherm values at 45 °C after 10 and 20 d of storage of freeze-dried products.

Products	Time of storage (in days)		Water activity (aw) and water content (E)				
P1 ^a	10 d	aw	0.07	0.12	0.21	0.26	0.44
		E ^b	3.03	4.95	6.32	7.34	7.97
	20 d	aw	0.08	0.18	0.21	0.33	0.45
		E ^b	2.93	5.25	6.19	8.21	8.43
P2 ^a	10 d	aw	0.07	0.13	0.20	0.31	0.45
		E ^b	1.59	2.80	4.05	6.35	6.57
	20 d	aw	0.08	0.13	0.21	0.31	0.47
		E ^b	1.59	2.77	4.61	6.35	6.42
P3 ^a	10 d	aw	0.07	0.10	0.19	0.26	0.38
		E ^b	3.20	7.52	10.96	9.70	11.89
	20 d	aw	0.07	0.12	0.18	0.29	0.38
		E ^b	2.99	8.43	8.16	9.74	13.04
P5 ^a	10 d	aw	0.07	0.10	0.18	0.29	0.38
		E ^b	4.04	6.59	11.34	10.27	12.88
	20 d	aw	0.07	0.11	0.21	0.27	0.39
		E ^b	3.53	6.50	8.58	11.77	12.72
P6 ^a	10 d	aw	0.11	0.18	0.21	0.26	0.43
		E ^b	2.19	3.10	7.11	6.36	6.73
	20 d	aw	0.12	0.20	0.21	0.26	0.37
		E ^b	2.09	7.39	5.07	2.31	6.40
P7 ^a	10 d	aw	0.08	0.19	0.23	0.24	0.37
		E ^b	3.47	5.15	6.39	6.88	7.98
	20 d	aw	0.09	0.17	0.21	0.25	0.31
		E ^b	3.19	4.59	8.42	6.29	8.07

^a Abbreviations see Table II.

^b Water content (g of water / 100 g of solid).

products P7 (Tab. III) and P8 (Tab. IV) we can see that it is P8, in spray form, that adsorbs more water (6.39 and 8.94 g of water/100 g of solid for P7 and P8, respectively at 0.20 aw).

After 20 d of storage the freeze-dried P3 still remains the most hygroscopic product (8.16 and 6.60 g of water/100 g of solid for P3 and P4, respectively, at 0.20 aw).

At the same time, P7 and P8 show practically the same degree of hygroscopicity (8.40 and 7.96 g of water/100 g solid at 0.20 aw).

We can observe that for P4 the two-sorption isotherms, after 10 and 20 days of storage, are continuous between 0.07 and 0.10 and then become irregular between 0.10 and 0.40 aw.

Table IV. Sorption isotherm values at 45 °C after 10 and 20 d of storage of spray-dried products.

Products	Time of storage (in days)	Water activity (aw) and water content (E)					
P4 ^a	10 d	aw	0.07	0.10	0.20	0.26	0.38
		E ^b	0.87	3.91	8.91	8.06	11.92
	20 d	aw	0.07	0.10	0.17	0.26	0.39
		E ^b	0.74	4.89	6.60	7.24	10.24
P8 ^a	10 d	aw	0.09	0.12	0.22	0.26	0.39
		E ^b	3.27	4.93	8.94	6.10	7.89
	20 d	aw	0.08	0.13	0.22	0.26	0.36
		E ^b	3.18	4.38	7.96	5.99	7.07

^a Abbreviations see Table II.

^b Water content (g of water / 100 g of solid).

The same discontinuity, however, is not found for P8.

Moreover, P4 (Fig. 3) and P3 (Fig. 2) show an irregular starting point at about 0.13 aw for both products. We can deduce that the way of drying does not affect the initiation of structure change.

3.4. Effect of the aw on the reactions of deterioration of milk powders

3.4.1. In terms of composition and physical state of milk powders

Table V displays the results on the study of the evolution of freeze-dried dairy

Table V. Carbonyl content in terms of aw at 45 °C after 20 d of storage of freeze-dried products.

Products	Water activity (aw) and carbonyl content					
P1 ¹	aw	0.08	0.18	0.21	0.33	0.45
	Carbonyl ²	2.96	2.87	2.61	2.98	2.99
P2 ¹	aw	0.08	0.13	0.21	0.31	0.47
	Carbonyl ²	15.84	17.04	19.55	14.65	13.61
P3 ¹	aw	0.07	0.12	0.18	0.29	0.38
	Carbonyl ²	2.28	2.32	1.81	2.14	2.37
P5 ¹	aw	0.07	0.11	0.21	0.27	0.39
	Carbonyl ²	1.86	1.74	1.64	1.65	1.54
P6 ¹	aw	0.11	0.18	0.21	0.26	0.43
	Carbonyl ²	11.20	11.83	11.25	11.13	11.51
P7 ¹	aw	0.09	0.17	0.21	0.25	0.31
	Carbonyl ²	3.63	3.46	2.94	2.98	3.38

¹ Abbreviations see Table II.

² Carbonyl content (units of OD / mg of protein).

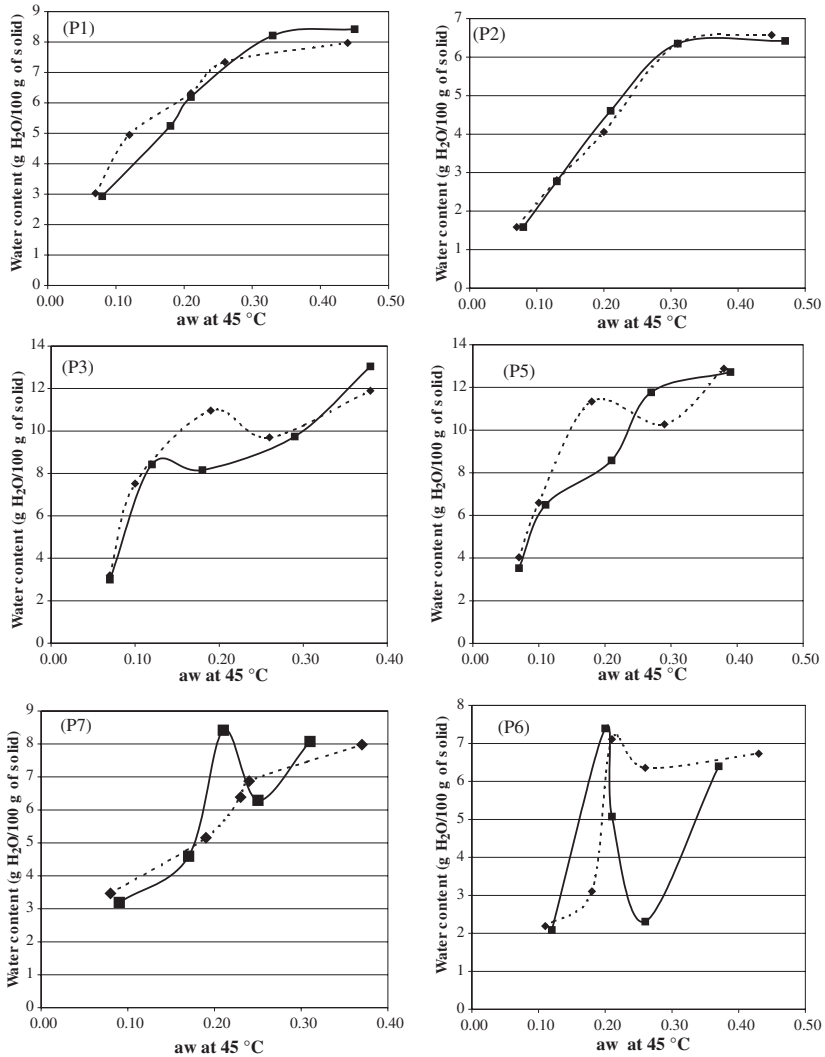


Figure 2. Sorption isotherm at 45 °C after 10 d (---◆---) and 20 d (—■—) of storage of freeze-dried dairy products.

products' deterioration in terms of aw at 45 °C after 20 d of storage.

It can be seen that the graph of deterioration is not proportional to the aw. The curves run through a minimum (corresponding to the lower rate of carbonyls), situated at about 0.20 ± 0.03 for P1, P3, P5 and P7. Yet the graph area, where we have maxi-

imum stability, is not very large and the initial aw (T0) is not within this area for all the products.

As regards P2 and P6, their minimum is situated either before 0.10 aw or after 0.30 aw. In order to determine which of the two zones is characterised with an optimum stability, we turned to sensory analysis.

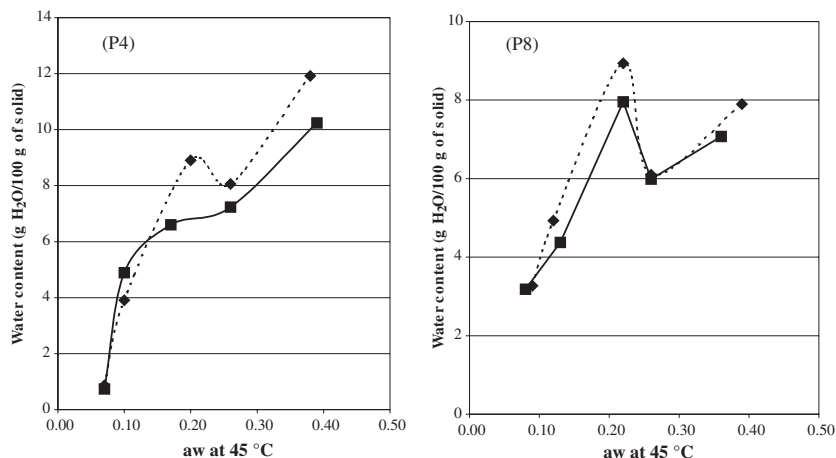


Figure 3. Sorption isotherm at 45 °C after 10 d (---◆---) and 20 d (—■—) of storage of spray-dried dairy products.

Three out of five trained sensory panelists preferred the products preserved at 0.07 and 0.10 aw, while the two others liked the products preserved at 0.30 aw better, their choice being influenced by the caramel flavour due to the Maillard reaction. All panellists rejected the products preserved at 0.20 aw (P2 and P6).

It is also interesting to note that the rate of carbonyl development of P2 and P6 became very high after 20 d of storage at 45 °C, as follows: for P2: OD units ranging from 3 200 to 19 000-mg⁻¹ of proteins for T0 and T20 at 0.2 aw; for P6: OD units from 9 400 to 11 700-mg⁻¹ of proteins for T0 and T20 at 0.20 aw.

On the other hand, if we compare P3 and P7 (Fig. 4) we can notice that the point of

minimum deterioration of P7 has been moved to the right of the profile (0.18 and 0.23 aw for P3 and P7 respectively).

3.4.2. In terms of method of dehydrating dairy products

Table VI and Figure 5 allow us to follow the evolution of the deterioration of spray dairy products in terms of aw after twenty days of storage at 45 °C. The comparison between the two identical products which were dehydrated by different methods, exhibits an optimum aw (where the products are more stable), which is very close for both products: P3 – 0.18 aw (Fig. 4) and P4 – 0.16 aw (Fig. 5). Both products are rich in protein (Tab. I).

Table VI. Carbonyl content in terms of aw at 45 °C after 20 d of storage of spray-dried products.

Products		Water activity (aw) and carbonyl content				
P4 ¹	aw	0.07	0.12	0.21	0.26	0.44
	Carbonyl ²	1.73	1.76	1.54	1.63	1.69
P8 ¹	aw	0.07	0.13	0.20	0.31	0.45
	Carbonyl ²	2.10	2.02	1.68	2.09	1.93

¹ Abbreviations see Table II.

² Carbonyl content (units of OD / mg of protein).

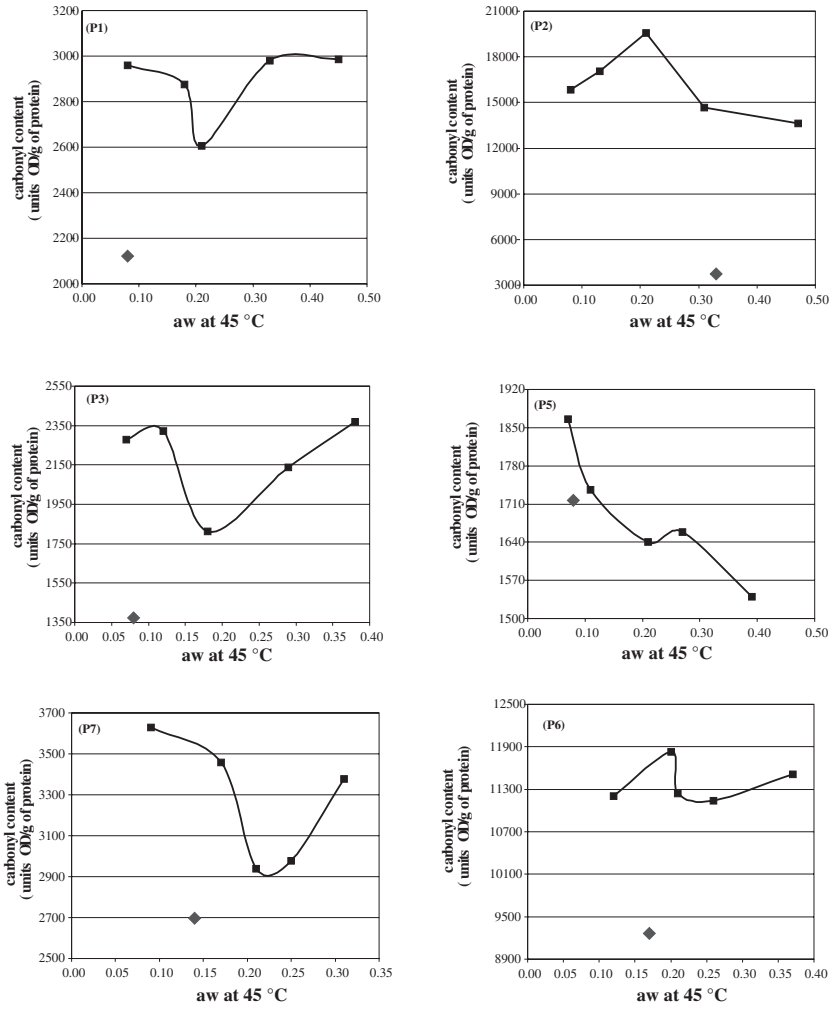


Figure 4. Evolution of carbonyl content in terms of aw at 45 °C after 20 d (—■—) of storage of freeze-dried dairy products. ◆ Initial aw.

The same conclusion is valuable for P7 (freeze-dried) and for P8 (spray-dried), the only ones to have fat among the eight products studied (Tab. I). The optimum aw is 0.22 (Figs. 4, 5) for both products.

The above described observations - allow us to deduce that the way of dehydrating the product does not affect the aw, thus not affecting the reactions of deterioration of dehydrated dairy products.

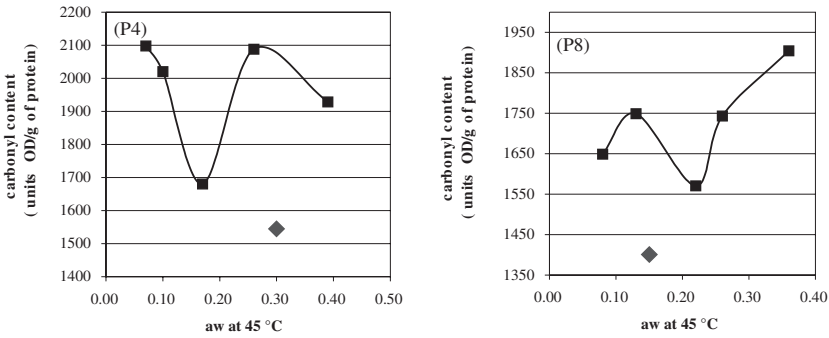


Figure 5. Evolution of carbonyl content in terms of aw at 45 °C after 20 d (—■—) of storage of spray dairy products. ◆ Initial aw.

3.5. Determination of the monolayer value

The monolayer value was determined experimentally (Figs. 6 and 7) and by the

BET theoretical model at 45 °C after 20 d of storage on all the products we studied.

The experimental, as well as the theoretical values of the monolayer are shown in Table VII.

Table VII. Experimental (Mex) and theoretical (Mth) monolayer value (in g of water · 100 g⁻¹ of solid) of dehydrated dairy products.

	P1 ¹	P2 ¹	P3 ¹	P4 ¹	P5 ¹	P6 ¹	P7 ¹	P8 ¹
Mex	4.30 ±0.30	2.95	8.10 ±0.30	6.30 ±0.30	8.20 ±0.30	2.40	7.80 ±0.30	8.0 ±0.30
Mth	6.60	1.30	6.20	6.30	8.00	-	8.00	8.00

¹ Abbreviations see Table II.

Table VIII. Lactulose and total MDA content in terms of aw after 20 d of storage at 45 °C for P6 and P7, respectively.

P6 ¹		P7 ¹	
aw	Lactulose ^a	aw	Total MDA ^b
0.12	5.15	0.09	66.30
0.20	1.28	0.17	63.00
0.21	1.32	0.21	62.74
0.26	2.61	0.25	68.44
0.37	7.63	0.31	54.21

¹ Abbreviations see Table II.

^a Lactulose content (g·100 g⁻¹ of product).

^b Total MDA content (µg·100 g⁻¹ of product).

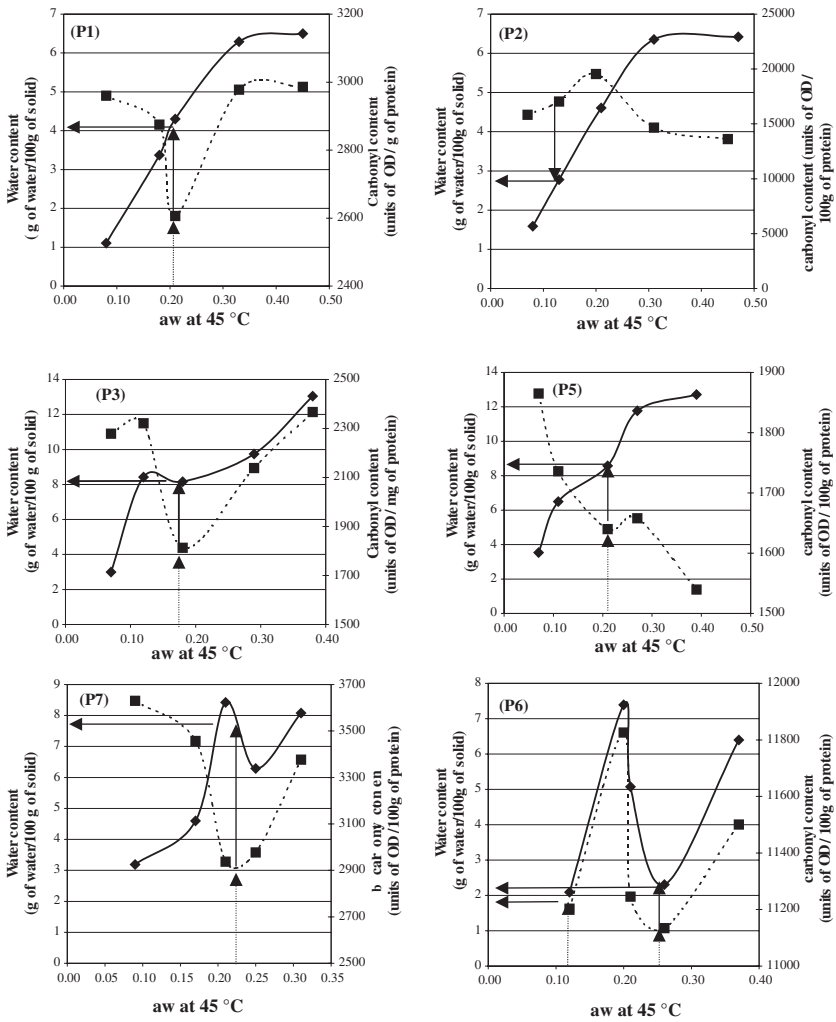


Figure 6. Experimental determination of the monolayer value of freeze-dried dairy products. (---■---) Reaction rate; (—◆—) sorption isotherm; (---▶) optimal aw; (—▶) optimal water content.

The comparison between the experimental and theoretical quantity of monomolecular layer exhibits a significant variation for P1, P2 and P3 while for P4, P5, P7 and P8 the experimental and theoretical quantities are very close.

It is important to mention that the BET theoretical model cannot be applied to P6

since its deterioration graph is represented in a sinusoidal form.

It is concluded that the theoretical model is not sufficient in itself to determine the quantity of optimal water. It could be well advised to accompany the determination with an experimental study.

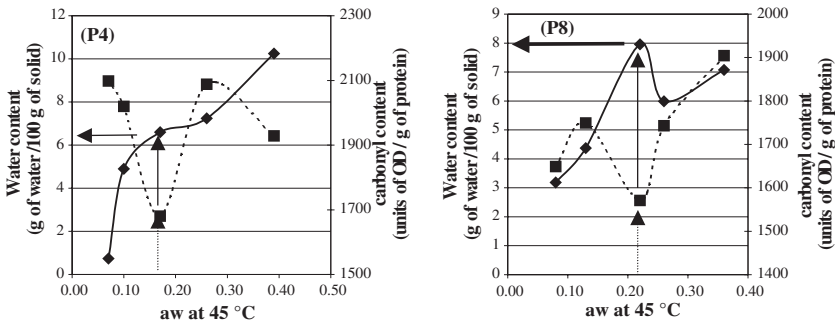


Figure 7. Experimental determination of the monolayer value of spray-dried dairy products. (---■---) Reaction rate (—◆—) sorption isotherm; (---▶) optimal aw; (—▶) optimal water content.

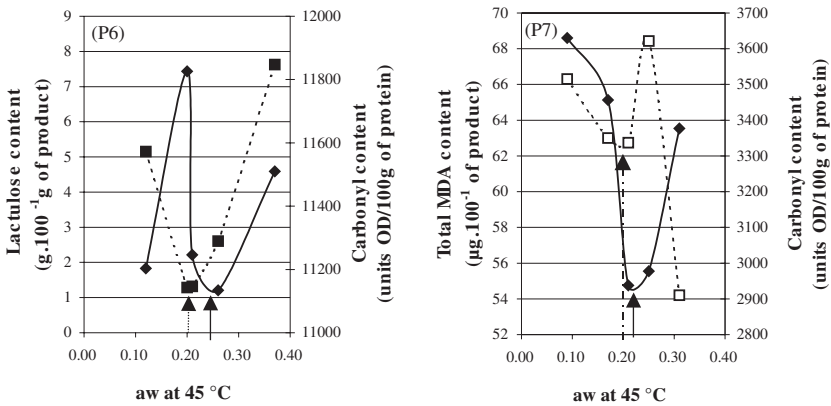


Figure 8. Comparison of the monolayer values obtained at 45 °C after 20 d of storage, of the P6 - product with the help of carbonyls and lactulose, and carbonyls and total MDA for the P7 product. (---■---) Evolution of lactulose; (—◆—) evolution of carbonyl; (---□---) evolution of total MDA; (---▶) optimal aw obtained with lactulose marker; (---▶) optimal aw obtained with total MDA marker; (—▶) optimal aw obtained with carbonyl marker.

3.6. Comparison of the carbonyl content determining method with the one usually used in order to follow the deterioration reactions of milk powders

3.6.1. In fat-rich products

The total MDA quantity in terms of aw after twenty days of storage at 45 °C of P7 is shown in Table VIII.

Figure 8 allows us to compare the evolution of carbonyls and the evolution of total MDA of P7 in terms of aw after twenty days of storage at 45 °C.

The arrows, dotted for the curve of the total MDA evolution and continuous for the evolution of carbonyls, permit us to situate the monolayer on the deterioration curve of the product.

The graph of the evolution of total MDA and carbonyls of P7 (Fig. 8) represents the

same outline as the quantities in optimal aw (0.20 and 0.22 optimal aw for the graphs of evolution of carbonyls and total MDA, respectively).

3.6.2. *In lactose-rich products*

Table VIII and Figure 8 allow us to compare the evolution of carbonyls and the evolution of lactose of P6. We can see that the optimal aw is close in the graphs of evolution both of lactulose and carbonyls (0.21 and 0.23, respectively) of P6.

4. CONCLUSION

The present study proved that protein-rich products and, more precisely, the whey protein were the most hygroscopic and that at 0.33 aw and 45 °C the products lost water, coinciding with a probable polymerisation of the proteins. The lactose-rich products, especially those containing amorphous lactose, released free water and underwent structural change at 0.20 aw and 45 °C. The amorphous lactose was transformed into crystallised lactose after 20 d of storage (0.25 aw, 45 °C). As regards the optimal aw of the studied powders, the minimum is situated at about 0.2 ± 0.05 (optimal aw) for all the products, which agrees with Labuza et al.'s work [20] except for the lactose-rich products.

The optimal aw is not affected by the method of drying for both protein-rich and formulated products with fat.

Our study showed that the theoretical model could not by itself determine the optimal water content, thus allowing the assessment of the most favourable conditions of storage of powdered dairy products, but it was necessary to verify the method by an experimental determination.

It also proved that the established method, using carbonyl determination as markers facilitates the determination of the most important biochemical reactions

(lipid and protein oxidation, and the Maillard reaction) intervening in the deterioration of milk powders on one hand, and on the other hand the determination of the experimental monolayer value, and so the degree of dehydration most favourable for the stability of milk powders.

Moreover, carbonyl content determination is a global marker and can replace the total MDA for the fat-rich products and the lactulose for lactose when desiring to study the graphical evolution of deterioration.

ACKNOWLEDGEMENTS

The authors acknowledge the Region Bretagne for financial funding which partially supports this research.

REFERENCES

- [1] AFNOR, Lait. Détermination de la matière sèche. NF VO4 207, in: AFNOR (Ed.), Recueil de normes françaises. Laits et produits laitiers. Méthodes d'analyse, Paris La Défense, 1980, pp. 33–34.
- [2] AFNOR, Lait. Détermination de la teneur en matières grasses. NF V 04-215, in: AFNOR (Ed.), Recueil de normes françaises. Laits et produits laitiers. Méthodes d'analyse, Paris La Défense, 1980, pp. 244–245.
- [3] AFNOR, Lait. Détermination de la teneur en azote total. NF V 04-211, in: AFNOR (Ed.), Recueil de normes françaises. Laits et produits laitiers. Méthodes d'analyse, Paris La Défense, 1980, pp. 212–215.
- [4] AFNOR, Lait. Détermination de la teneur en lactose. NF V 04-213, in: AFNOR (Ed.), Recueil de normes françaises. Laits et produits laitiers. Méthodes d'analyse, Paris La Défense, 1980, pp. 222–225.
- [5] Berlin E., Anderson B.A., Reversibility of water vapor sorption by cottage cheese whey solids, *J. Dairy Sci.* 58 (1975) 25–29.
- [6] Bird R.P., Draper H.H., Comparative studies on different methods of malondialdehyde determination, in: Packer L. (Ed.), *Methods in enzymology, Oxygen radicals in biological systems*, Vol. 105, Academic Press, Orlando, USA, 1984, pp. 299–319.
- [7] Bosset J., Gauch R., Effet protecteur de l'emballage contre la photo-oxydation, *Trav. Chim. Aliment. Hyg.* 79 (1988) 165–174.

- [8] Cheftel J.C., Introduction à la biochimie et technologie des aliments, Lavoisier, Tec & Doc, Paris, France, 1977, pp. 3–31.
- [9] Davies K.J., Lin A.S., Pacifici W., Protein damage and degradation by oxygen radicals, *J. Biol. Chem.* 262 (1987) 9914–9920.
- [10] Gardner H.W., Lipid hydroperoxide reactivity with proteins and amino acid: a review, *J. Agric. Food Chem.* 27 (1979) 220–229.
- [11] Gardner H.W., Reactions of hydroperoxides – Products of high molecular weight, in: Chan H.W.S. (Ed.), *Autoxidation of unsaturated lipids*, Academic Press, London, UK, 1987, pp. 51–93.
- [12] Grosch W., Lipid degradation products and flavour, in: Morton I.D., MacLeod A.J. (Eds.), *Food flavours*, Elsevier, Amsterdam, The Netherlands, 1982, pp. 325–398.
- [13] Halliwell B., Gutteridge J.M.C., Role of free radicals and catalytic metal ions in human disease: An overview, in: Packer L., Glazer A.N. (Eds.), *Oxygen radicals in biological systems*, *Methods in Enzymology*, Vol. 186, Academic Press, San Diego, USA, 1990, pp. 1–85.
- [14] Haugaard Sorensen I., Krag J., Pisecky J., Westergaard V., *Méthodes d'Analyses des Produits Laitiers Déshydratés*, Niro A/S, Copenhagen, Denmark, 1978.
- [15] IDF, Détermination de la teneur en lactulose, Standard 175, Int. Dairy Fed., Brussels, Belgium, 1995.
- [16] Kanner J., German J.B., Kinsella J.E., Initiation of lipid peroxidation in biological systems, *CRC Crit. Rev. Food Sci. Nutr.* 25 (1987) 317–365.
- [17] Kinsella J.E., Fox P.F., Water sorption by proteins, *CRC Crit. Rev. Food Sci. Nutr.* 24 (1986) 91–139.
- [18] Knight J.A., Pieper R.K., McClellan L., Specificity of the thiobarbituric acid reaction: its use in studies of lipid peroxidation, *Clin. Chem.* 34 (1988) 2433–2438.
- [19] Labusa T.P., Oxydative changes in foods at low and intermediate moisture levels, in: Duckworth R.B. (Ed.), *Water relations of foods*, Academic Press, London, UK, 1975, pp. 455–459.
- [20] Labusa T.P., Tannenbaum S.R., Karel M., Water content and stability of low-moisture and intermediate-moisture foods, *Food Technol.* 24 (1970) 543–550.
- [21] Lee H.S., Nagy S., Quality and nonenzymic browning intermediates in grapefruit juice during storage, *J. Food Sci.* 53 (1988) 168–176.
- [22] Levine L.R., Williams J.A., Stadtman E.R., Shacter E., Carbonyl assays for determination of oxidatively modified proteins: An overview, in: Packer L. (Ed.), *Oxygen radicals in biological systems*, *Methods in Enzymology*, Vol. 233, Academic Press, San Diego, USA, 1992, pp. 346–357.
- [23] Love J.D., Pearson A.M., Lipid oxidation in meat and meat products. A review, *J. Amer. Oil Chem. Soc.* 48 (1971) 547–549.
- [24] Mauron J., The Maillard reaction in food: a critical review from the nutritional stand point, *Prog. Food Nutr. Sci.* 5 (1981) 5–35.
- [25] O'Connor T.P., O'Brien N.M., Lipid oxidation, in: Fox P.F. (Ed.), *Advanced Dairy Chemistry-2: Lipids*, Chapman & Hall, London, UK, 1994, pp. 309–347.
- [26] Pierre A., Fauquant J., Le Graët Y., Piot M., Maubois J.L., Préparation de phosphocéinate natif par microfiltration sur membrane, *Lait* 72 (1992) 461–474.
- [27] Roos Y.H., Water in milk products, in: Fox P.F. (Ed.), *Advanced Dairy Chemistry –3: Lactose, water, salts and vitamins*, Chapman & Hall, London, UK, 1997, pp. 304–345.
- [28] Saltmarch M., Labusa T.P., Influence of relative humidity on the physicochemical state of lactose in spray-dried sweet whey powders, *J. Food Sci.* 45 (1980) 1231–1236, 1242.
- [29] Schuck P., Piot M., Méjean S., Le Graët Y., Fauquant J., Brulé G., Maubois J.L., Déshydratation par atomisation de phosphocéinate natif obtenu par microfiltration sur membrane, *Lait* 74 (1994) 375–388.
- [30] Stapelfeldt H., Nielsen B.R., Skibsted L.H., Effet of heat treatment, water activity and storage temperature on the oxidative stability of whole milk powder, *Int. Dairy J.* 7 (1997) 331–339.
- [31] Stokes R.H., Robinson R.A., Standard solutions for humidity control at 25 °C, *Ind. Eng. Chem.* 41 (1949) 2013–2016.
- [32] Trouvé E., Maubois J.L., Piot M., Madec M.N., Fauquant J., Rouault A., Tabard J., Brinkman G., Rétention de différentes espèces microbiennes lors de l'épuration du lait par microfiltration en flux tangentiel, *Lait* 71 (1991) 1–13.