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Original article

Grated Grana Padano cheese: new hints on how to control quality and recognize imitations

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Abstract – The sensorial and physico-chemical characteristics described in the product specification for most PDO cheeses are inadequate to verify the compliance of cheeses on the market with the registered designation, particularly for grated products. During the past few years, much research has indicated the analytical parameters suitable for distinguishing Grana Padano (GP) from other similar hard cheeses. The characterization of grated GP is currently based on 3 analytical parameters, related to different aspects of cheese processing, which are: (i) the measurement of alkaline phosphatase (ALP) activity, a marker for possible heat treatment applied to milk, in the outermost layer of the cheese, just below the rind; (ii) the identification of specific peptides, that are identified only in the rind, due to the very slow progress of proteolysis in the rind during GP cheese ripening; and (iii) the free amino acid (FAA) composition. In the present study, we developed an extraction method, based on density gradient centrifugation of solubilized cheese, to separate the outermost layer of the cheeses from the rest in grated cheese, and we proposed a simplified criterion to evaluate the "typicalness" of the FAA pattern. The quality control scheme based on ALP activity, detection of specific peptides and FAA pattern was applied to more than 300 samples of marketed grated GP collected over three years, 10% of which were collected outside Italy, and ~ 100 samples of grated generic ("Grana-type") hard cheeses. The results demonstrate that the simultaneous application of the three parameters allows one to distinguish grated GP from similar, non-PDO grated hard cheeses, and to recognize irregular GP cheeses.

Grana Padano cheese / quality control / alkaline phosphatase / cheese peptides / free amino acids

摘要 – 提高 Grana Padano 搓碎干酪粉质量和掺假识别的新方法。通过感官和理化特性来描述产品规格, 由于大多数 PDO 干酪难以验证其是否为已登记过的原产地保护产品, 特别是搓碎干酪粉。在过去几年中, 有研究发现根据分析特征参数能区分 Grana Padano 干酪粉 (GP) 和其他硬质干酪粉。目前, 用于表征搓碎 Grana Padano 干酪粉 (GP) 的 3 个特征参数都与干酪加工相关, 分别是 : (i) 测量干酪表皮下部分的碱性磷酸酶 (ALP) 活性, 以此检验是否进行过热处理; (ii) 鉴定表皮下的蛋白肽, 因为 GP 干酪在成熟过程中, 表皮下的蛋白质水解缓慢; (iii) 测游离氨基酸 (FAA) 成分。本研究基于干酪可溶性部分密度梯度离心的方法, 开发了一种新的提取方法, 用于从搓碎干酪中分离出干酪的最外层部分, 同时建立了一个简单的标准来评价 FAA 模式的"典型性"。基于 ALP 活性,特异性蛋白的检测和游离氨基酸模式建立了质量控制体系, 在 3 年内已经用于 300 多个市售的搓碎 GP 干酪粉的鉴定, 其中 10% 的样品是非意大利产的干酪粉, 100 多个样品为普通的 Grana 型硬质干粉。结果表明, 同时应用这 3 个参数可以有效地区分搓碎 GP 干酪粉和非 PDO 硬质干酪粉, 而且可以识别不合格的 GP 干酪。

Grana Padano 干酪粉 / 质量控制 / 碱性磷酸酶 / 干酪肽 / 游离氨基酸

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Résumé – Fromage Grana Padano râpé : nouvelles indications pour le contrôle de qualité et pour reconnaître les imitations. Les caractéristiques sensorielles et physico-chimiques indiquées dans le cahier des charges de la plupart des fromages AOP sont peu utiles pour vérifier la conformité des produits mis sur le marché avec l'appellation, surtout pour les fromages râpés. Au cours des dernières années, plusieurs recherches ont mis en évidence les paramètres analytiques capables de distinguer le fromage Grana Padano (GP) d'autres fromages à pâte dure similaires. Actuellement, la caractérisation du GP râpé se base sur trois paramètres analytiques, liés à différents aspects de la production : (i) l'activité de la phosphatase alcaline (ALP), indicateur d'un possible traitement thermique du lait, mesurée dans la pâte du fromage, juste en-dessous de la croûte ; (ii) l'identification de peptides spécifiques de la croûte, du fait de la protéolyse très réduite dans cette partie durant l'affinage ; et (iii) la composition en acides aminés libres (AAL). Dans la présente étude, nous avons développé une méthode d'extraction basée sur la centrifugation en gradient de densité du fromage solubilisé afin d'extraire la partie de la pâte située juste en-dessous de la croûte dans le fromage râpé. Nous proposons aussi un critère simplifié pour évaluer la « typicité » du profil des AAL. Le plan de contrôle qualité basé sur l'activité de l'ALP, la détection de peptides spécifiques et le profil des AAL a été appliqué à plus de 300 échantillons de GP râpé du commerce collectés en trois ans, dont 10 % en dehors de l'Italie, ainsi qu'à environ 100 échantillons de fromages non-AOP à pâte dure râpés. Les résultats montrent que l'application simultanée des trois paramètres permet soit de discriminer le GP des autres fromages à pâte dure râpés non-AOP, soit de reconnaître les échantillons de GP défectueux.

fromage Grana Padano / contrôle de qualité / phosphatase alcaline / peptides / acides aminés libres

1. INTRODUCTION

Grana Padano (GP) is an extra-hard, long-ripened cheese produced in northern Italy from semi-skimmed raw milk. As GP is registered at European Community level as a Protected Designation of Origin (PDO) cheese, its characteristics and production process must conform to the specification deposited with the Commission (Council Regulation (EC) No. 510/2006). Compliance near the product specification is guaranteed by the "Consorzio di Tutela del Formaggio Grana Padano" (Consortium for the Defence of Grana Padano Cheese), which constantly monitors farms, cheese factories, ripening conditions and packaging, thus assuring the origin, safety and quality of the commercialized products bearing the certification marks of GP.

While the reputation and the related high commercial value of a PDO cheese like GP are justified by its high nutritional value and quality, these may encourage the sale, as GP, of cheeses with similar appearance, but of different origin or obtained with methods other than those reported in the product specification for GP. In this regard, it should be noticed that the sensorial and appearance characteristics presently defined by the specification of GP cheese are generic and thus absolutely inadequate for assessing the correspondence of marketed cheeses to this designation, particularly when grated. Hence the need for objective analytical parameters suitable for characterizing GP and distinguishing it from similar, "Grana-type" cheeses [15].

Proteolysis represents one of the most important biochemical processes occurring during cheese ripening [2]. Through the action of various enzymes, naturally present in the milk or deriving from rennet, milk micro-organisms and natural starter micro-organisms [4], milk protein is hydrolyzed into peptides and free amino acids (FAA) [3]. Milk characteristics and cheesemaking technology determine specific proteolytic enzyme activities, leading to FAA patterns specific for different cheese types [2]. During the last 20 years several studies have been carried out on proteolysis behavior in some Italian PDO cheeses with

the aim of characterizing these products on the basis of their FAA patterns, and chemometric models have been defined, suitable for distinguishing traditional Parmigiano-Reggiano [17, 18], Fontina [16] and GP [15] from similar non-PDO cheeses. More recently, the possibility of detecting addition of extra rind to grated GP cheese, by evaluating the peptide pattern, has been studied [12].

On the basis of the results of the aforementioned research, the Consortium has decided to set up a quality control scheme for grated GP based on several parameters, and an extensive investigation into commercial products has been undertaken. During this research a simplified criterion for evaluating the FAA pattern, and for detecting the presence of cheese manufactured with heat-treated milk in grated cheese was identified, and is presented here for the first time. The results of the three-year survey on the analytical characteristics of genuine grated GP and of "Grana-type" cheeses, marketed in Italy and abroad, are briefly discussed as an example of a quality control scheme based on multiple parameters.

2. MATERIALS AND METHODS

The samples used in this study were:

- 29 experimental "Grana-type" cheeses produced with non-traditional technologies or produced outside of the PDO area;
- 71 samples of grated Grana Padano cheese of known age and origin, including 26 defective cheeses;
- 20 samples of grated GP manufactured from raw milk under controlled conditions;
- 5 grated "Grana-type" cheeses produced from heat-treated milk (55, 60, 62, 65 or 72 °C for 15 s). Milk was heat-treated using an industrial plate heat-exchanger (Alfa-Laval, Monza, Italy);
- 336 samples of grated GP collected, by the Consortium, on the market; principally in Italy, but also in other EU member states;
- 97 commercial grated "Grana-type" cheeses.

2.1. Extraction of the rind from the grated cheese

Five grams of grated cheese were dissolved in 25 mL of 0.45 mol·L^{-1} sodium citrate solution (pH 7.20) containing 200 g·L^{-1} sucrose and stirred for 30 min. The mixture was centrifuged at 8000× g for 30 min at 0–4 °C. The collected upper layer underwent a further solubilization with 25 mL of 0.90 mol·L^{-1} sodium citrate solution containing 200 g·L^{-1} sucrose. After centrifugation (in the conditions reported above), the upper layer was collected and washed with 50 mL distilled water. ALP activity was measured in the pellet, collected after further centrifugation (the same conditions as above) and freeze-dried.

2.2. Alkaline phosphatase activity determination

On 500 mg of freeze-dried product, by the fluorimetric method according to IDF Standard 155:1992.

2.3. Electron microscopy

Ultra-structure evaluation was carried out by transmission electron microscopy (Philips EM 201). Samples were prepared using a Moor ultramicrotom (Balzer BAF 301), adopting the freeze-fracturing technique [13].

2.4. Capillary electrophoresis (CE) of cheese protein fractions

Sample preparation on 900 mg of grated cheese according to the method described by Pellegrino et al. [12]; CE separation using a Beckman P/ACE™ system MDQ (Beckman Instruments Inc., Fullertone, CA, USA), fitted with a hydrophilically coated fused-silica capillary column, CElect P150 (Supelco, Bellafonte, PA, USA), 600 mm total length (effective length 500 mm) and 50 μm i.d., with slit opening of 100 × 800 μm and detection at 214 nm. CE buffers were prepared according to Recio and Olieman [14]. All samples were analyzed in duplicate. The area of peaks corresponding to α_{s2}'-CN and α_{s1}-PL1 (i.e. α_{s1}-CN f80–199) (see Fig. 2 in Results and Discussion) were measured and the following ratio calculated:

$$R_{\alpha s} = (\alpha'_{s2}\text{-CN}/\alpha_{s1}\text{-PL1}) \times 100$$

where:

α_{s2}'-CN = peak area of α_{s2}'-CN;

α_{s1}-PL1 = peak area of α_{s1}-CN f80–199.

2.5. Determination of protein content

On 350 mg of grated cheese, according to IDF Standard 20 B:1993.

2.6. Free amino acid determination

Extraction on 1.5 g of grated cheese with sodium citrate buffer pH 2.2, and homogenization and deproteination with 7.5% (w/v) sulfosalicylic acid according to the method described by Resmini et al. [18]. Separation by ion-exchange chromatography (IEC) using a Biochrom 30 Amino Acid Analyzer (Biochrom Ltd., Cambridge, CB4 0FJ, UK), following the conditions proposed by the manufacturer. Registration and integration of chromatograms with EZChrom Elite™ software (Agilent Technologies Inc., Palo Alto, CA 94306, USA).

2.7. Statistical analysis

χ^2 analysis of comparison between GP and "Grana-type" cheeses was performed in order to ascertain whether the observed frequencies of irregular samples differed significantly.

3. RESULTS AND DISCUSSION

3.1. Use of heat-treated milk

Generic cheeses are usually manufactured from pasteurized milk and therefore present a negative response to the ALP test. In contrast, according to PDO specification, GP cheese must exclusively be manufactured by using raw milk (Reg. EC 510/2006). In spite of this, it has already been pointed out [8, 10] that ALP is fully inactivated in the core of the GP cheese wheel, but not in the outer part. Therefore, use of raw milk in cheesemaking can be verified only by measuring residual ALP activity in the latter.

Taking this evidence into consideration, the Italian Ministry of Agriculture in 1998 established that ALP activity must not be lower than 300 $mU \cdot g^{-1}$ of cheese when measured in the outermost layer (1 cm depth) of the GP cheese wheel.

Of course, determination of ALP activity is not effective in grated cheese unless a preliminary extraction of the rind from the whole grated cheese is adopted.

Low water activity [9], high fat and salt contents [7], and a low level of proteolysis [12] are specific properties of the rind of long-ripened hard cheeses. By examining the ultra-structure of GP cheese rind (Fig. 1a), both intact and coalesced fat globules can be observed, entrapped in the protein network.

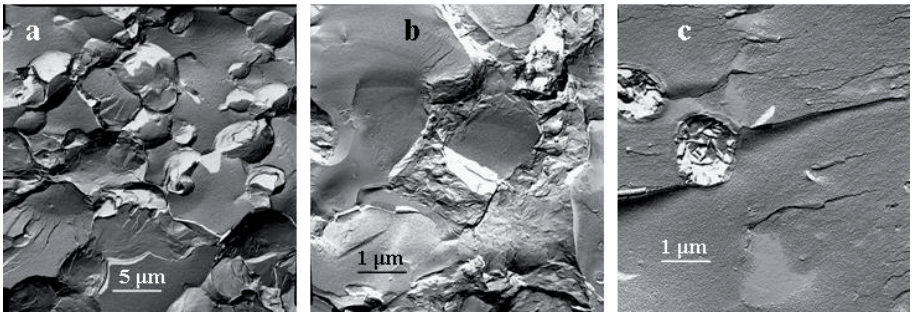

Figure 1. Ultra-structure of Grana Padano cheese rind (a) and of the upper layer (b) and pellet (c), both obtained by the sample centrifugation described in Materials and Methods.

The aforementioned characteristics make the GP cheese rind hardly soluble. Hence, the mild conditions (low sodium citrate concentration at neutral pH) adopted in the first step of the reported extraction procedure allow the solubilization of the cheese interior, but not the rind. Upon centrifugation in the presence of sucrose, the rind migrates into an upper layer while the solubilized cheese sticks onto the pellet. The ultra-structure of the upper layer (Fig. 1b) is close to that of the cheese rind (Fig. 1a), thus demonstrating that no disruption of its structure occurs during the adopted extraction procedure, while the pellet is mainly made up of protein (Fig. 1c).

The efficacy of the proposed extraction procedure is shown in Table I. The cheese rind concentrates into the upper layer, resulting in a higher ALP activity in this extracted fraction if compared with that revealed in the pellet. It is noteworthy that ALP values were always higher than 300 $mU \cdot g^{-1}$ (the established threshold value for GP cheese) when determined in the upper layer obtained by applying the described extraction procedure. On the contrary, ALP values determined in whole grated cheeses would suggest the use of heat-treated milk.

When heat treatment of cheese milk had occurred, ALP activity values below 300 $mU \cdot g^{-1}$ were observed in the extracted upper layer, even when mild conditions (55 °C/15 s) had been applied (Tab. I). Therefore, the legal threshold value stated for GP cheese could also be applied to controlling grated cheese on the condition that the proposed preliminary extraction procedure is performed.

3.2. Detection of extra rind addition to grated cheese

With the aim of characterizing the rind of GP, Pellegrino et al. [12] stratigraphically analyzed 9-month-old cheeses by CE. These authors pointed out that, while the main casein fractions are increasingly degraded in a centripetal direction, α_{s2}'-CN is almost completely lacking just below the rind (4 mm depth). This casein fraction belongs to the α_{s2}-CN cluster, whose polymorphism is related to the different number of phosphate groups. The CZE profiles in Figure 2 depict a 9-month-old GP cheese (pattern a), its rind (0–4 mm layer) (pattern b), and a GP after 48 h of moulding (pattern c). As already mentioned, the low a_w value in the GP cheese rind (a_w = 0.8) [8] largely inhibits enzyme activity even throughout a prolonged ripening. This explains why patterns b and c overlap and both show all main casein fractions, except κ-CN, to be almost intact, lacking the

Table I. Alkaline phosphatase (ALP) activity in different portions of grated Grana Padano and "Grana-type" cheeses.

Type of grated cheese	Heat treatment of cheese milk	ALP activity ($mU \cdot g^{-1}$)		
		Whole cheese	Extracted upper layer	Pellet
Grana Padano $n = 20$	raw	129–296	337–631	86–199
"Grana-type"	55 °C/15 s	110	251	n.d.
	60 °C/15 s	96	214	n.d.
	62 °C/15 s	48	72	n.d.
	65 °C/15 s	10	12	n.d.
	72 °C/15 s	4	5	n.d.

n.d.: not determined.

derived peptides. Among others, the peptide with tm 20.5 min (pattern a), here named α_{s1}-PL1 and corresponding to fragment α_{s1}-CN f80–199 [12], proved to be particularly useful in view of the rind characterization. In fact, it is clearly detectable from a few days after cheesemaking and increases in time (data not reported) within the cheese. Inversely, α_{s2}'-CN is progressively hydrolyzed during cheese ripening and it is almost completely absent in 9-month-old cheese.

While these changes occur within the cheese body, leading to pattern a of Figure 2 in ripened GP cheese, in the cheese rind (pattern b) α_{s1}-PL1 does not form and α_{s2}'-CN does not decrease. On this basis, the $R_{\alpha s}$ described in Materials and Methods was proposed as a characterizing index of rind in grated GP cheese.

In GP samples aged 9 months or more, the rind (0–4 mm) showed values of $R_{\alpha s}$ from 100 up to one million times higher than that of the corresponding whole cheese [12], thus proving the reliability of this parameter for detecting extra rind in grated cheese.

An experimental calibration curve was calculated by measuring $R_{\alpha s}$ values of grated cheeses containing amounts of rind from 0 to 30% (w/w) [12]. At a 99.7% confidence level, a maximum $R_{\alpha s}$ value of 7 was calculated for grated cheeses containing 18% of rind, corresponding to the pertinent rind amount of the whole wheel. This value was proposed as the higher threshold level for genuine grated GP cheese. Cheese manufacturers should take care of the homogeneity of the grated cheese before packaging. In fact, as expected, the rind amount can be higher in the first and last portions during the grating process, if the mixing step prior to conditioning is not sufficiently accurate to guarantee the homogeneity of each batch [12].

3.3. Free amino acid composition

As previously mentioned, during former research a chemometric model has been developed for the characterization of GP [15] on the basis of its FAA composition. Using a multivariate approach, 2 variables (Standardized Scores I and II), which can be represented in a bi-dimensional model, are extracted from the original data. Threshold values for both variables define an "area of typicalness" for traditional GP cheese, allowing the discrimination of GP from similar "Grana-type" cheeses.

During the present research the capability of this chemometric model to distinguish GP from similar cheeses was confirmed on 29 experimental "Grana-type"

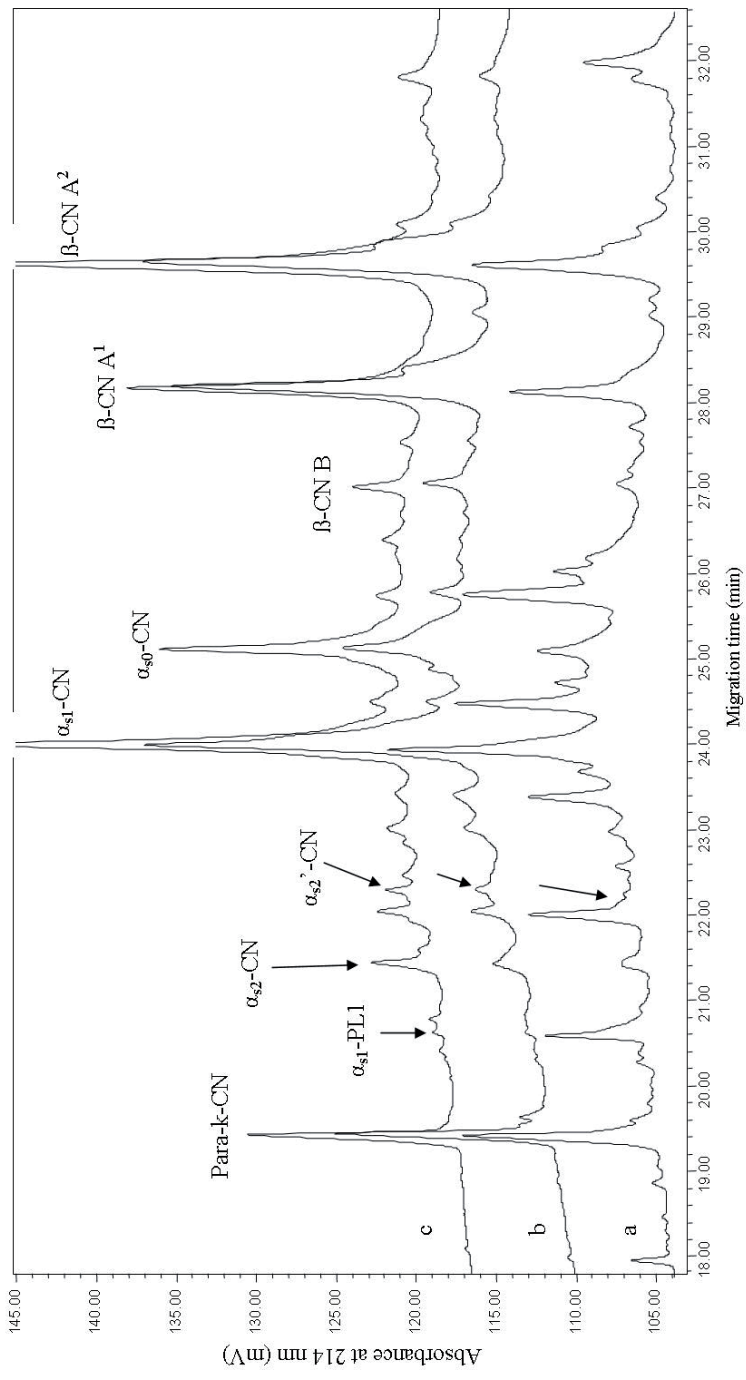

Figure 2. Capillary electrophoresis profiles of a 9-month-old Grana Padano cheese (a), its rind (b) and a GP after 48 h of moulding (c). α_{s2}'-CN = fraction belonging to α_{s2}-CN; α_{s1}-PL1 = α_{s1}-CN f80–199.

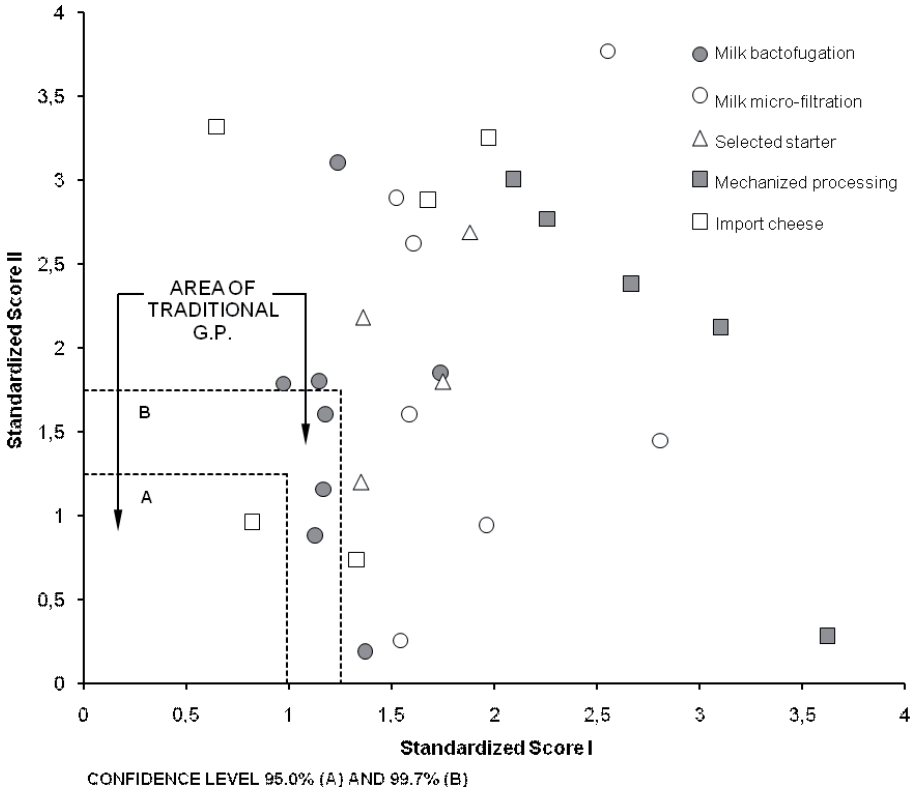

Figure 3. Distribution of experimental "Grana-type" cheese samples, produced with different non-traditional technologies, in the chemometric model for Grana Padano cheese.

cheeses produced outside of the PDO area or with technologies different from that of traditional GP (bactofugation or micro-filtration of milk; use of selected starters; mechanized processing). As shown in Figure 3, 25 out of 29 samples were excluded from the model at a confidence level of 99.7%, indicating that each of the investigated deviations from the traditional cheese processing induces significant changes in the FAA pattern.

Recently, the necessity of a "simplified" model, suitable for a routine, fast and easy evaluation of grated GP arose. For that purpose FAA composition was determined in 71 grated GP cheeses of known age and origin, including 26 defective samples. Mean values and standard deviations of all FAA data obtained for genuine, good quality GP were compared with those of defective GP cheeses and those of the aforementioned experimental "Grana-type" cheeses. On the basis of these data, a set of threshold values was found (mean value ± 2 standard deviations) for several FAA (Tab. II), allowing a rapid assessment of ripening period (total content of FAA), cheese typicalness (glutamic acid, lysine, serine, glutamine) and the possible presence of defects or undesired fermentations (γ-aminobutyric acid, ornithine) for grated GP.

As reported by Resmini et al. [15], the total content of FAA in GP rapidly

Table II. Free amino acids (FAA) suitable for quality assessment of grated Grana Padano cheese: mean values and standard deviations determined in 45 GP cheeses of known age and origin, and calculated threshold limits. All data expressed as % of total FAA, except total content of FAA (% of total cheese protein).

Amino acid	Mean value	Standard deviation	Threshold value
Total content of FAA	18.32	1.61	≥ 15.0%
Glutamic acid	18.52	1.28	≥ 16.0%
Lysine	12.13	0.57	≥ 10.8%
Serine	4.28	0.46	≥ 3.4%
Glutamine	1.37	0.45	≤ 2.3%
Ornithine	0.99	0.48	≤ 2.0%
γ-Aminobutyric acid	0.27	0.16	≤ 0.6%

increases during the first year of ripening; thus, the threshold value for total FAA (15% of protein) should assure reaching the minimum ripening period (9 months) provided for GP. Grated cheeses with a low amount of total FAA, especially if associated with a high glutamine content, are likely to contain short-ripened cheese [1]. Nevertheless, a slightly low content of FAA may be the result of slower proteolysis, due to particular milk characteristics which might partially inhibit the growth of lactic acid bacteria or favor non-dairy bacteria [5]. Another reason for low total FAA content is the addition of extra rind; in fact, as reported above, due to low a_w, proteolysis is very limited in the rind, without modifications of the relative content of individual amino acids.

Whereas relative contents of glutamic acid and lysine in GP remain surprisingly stable throughout ripening, the relative content of serine increases during proteolysis, while that of glutamine decreases [15]. Therefore, these amino acids are suitable for assessing the correct evolution of proteolytic processes throughout ripening of GP. No γ-aminobutyric acid is normally detected in GP; high contents of this amino acid, deriving from the decarboxylation of glutamic acid, indicate the presence of cheese with some defect, possibly due to undesired microbiological characteristics of the milk or starter [6]. Addition of hen's egg white lysozyme to cheese milk [11] largely inhibits growth of non-dairy bacteria.

3.4. Evaluation of grated cheeses by simultaneous application of all of the proposed parameters

As of 2005, a quality control scheme based on the described analytical parameters has been adopted by the Consortium as a measure to guarantee compliance of marketed cheeses to the product specification of GP. Evaluating the data obtained for the 336 grated GP samples analyzed in the 3-year testing period according to the aforementioned parameters, it was observed that all of the threshold limits allow one to individuate samples showing irregular values. In particular, 14 samples (4%) showed low values ($< 300\ mU \cdot g^{-1}$) of ALP activity, seemingly indicating the presence of cheese obtained from heat-treated milk, while 44 (13%) exhibited high values (> 7.0) for the $R_{\alpha s}$ ratio, denoting the addition of extra rind. As far as FAA composition is concerned, among the 47 (14%) irregular samples the most frequently observed irregularities

were high contents of γ-aminobutyric acid, related to the presence of undesired fermentations, or slightly low amounts of total FAA, related to ripening duration, whereas the frequency of samples with irregular values for the FAA more related to cheese typicalness, such as glutamic acid, serine or lysine, was much lower.

The situation emerging from the simultaneous application of all of the proposed parameters over the three-year survey can be summarized as follows: 74% of the 80 irregular GP samples were irregular for just 1 parameter and only 5% of the samples presented irregular values for all of them. Therefore, it can be reasonably supposed that irregular GP samples mainly derived from genuine GP with minor defects or a slightly short ripening period.

Among the 97 analyzed samples of "Grana-type" cheese, 40 samples (41%) showed low ALP activity, whereas 53 (55%) had high values for $R_{\alpha s}$ and 55 (57%) were irregular for parameters related to FAA composition. With regards to FAA, the most frequently observed irregularities were low amounts of total FAA and irregular values for glutamine and lysine, related to cheese typicalness. Applying simultaneously all of the proposed parameters to the scrutinized samples of "Grana-type" cheeses, a total of 82 samples (85%) were irregular, 67% of which showed abnormal values for at least 2 parameters, proving that these cheeses were obtained with processing technologies different from that of traditional GP.

As evidenced by the χ^2 test, the distribution of irregular samples was significantly different between GP and "Grana-type" cheeses. It should be noticed that although statistically it was possible to point out differences between defective GP and "Grana-type" cheeses, at present it is impossible to securely assign an irregular cheese sample to one of these two classes. Nevertheless, the presence of even one single anomalous value for any one of the considered parameters makes a sample irregular, no matter if it is a defective GP cheese or a "Grana-type" cheese.

4. CONCLUSIONS

By addressing multiple aspects of the traditional cheesemaking process, the analytical approaches proposed in the present work, combined together, appear to be a suitable tool for the quality control of GP cheese, allowing one to distinguish genuine GP from similar non-PDO cheeses, even when grated. The described analytical parameters also provide useful information to producers in order to monitor the compliance of their own production. The quality control scheme based on the results of this research work, adopted by the Consortium and included in the product specification, places GP among the most inspected and guaranteed PDO cheeses.

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REFERENCES

[1] Ardö Y., Thage B.V., Madsen J.S., Dynamics of free amino acid composition in cheese ripening, Austr. J. Dairy Technol. 57 (2002) 109–115.

[2] Choisy C., Desmazeaud M.J., Gripon J.C., Lamberet G., Lenoir J., The biochemistry of ripening, in: Eck A., Gillis J.C. (Eds.), Cheesemaking – From Science to Quality Assurance, Lavoisier, Paris, France, 2000, pp. 82–151.

[3] Desmazeaud M.J., Gripon J.-C., General mechanism of protein breakdown during cheese ripening, Milchwissenschaft 32 (1977) 731–734.

[4] Grapin R., Rank T.C., Olson N.F., Primary proteolysis of cheese proteins during ripening. A review, J. Dairy Sci. 68 (1985) 531–540.

[5] Limsowtin G., Inhibition of starter cultures, Austr. J. Dairy Technol. 47 (1992) 100–102.

[6] Nomura M., Kimono H., Someya Y., Furukawa S., Suzuki I., Production of gamma aminobutyric acid by cheese starters during cheese ripening, J. Dairy Sci. 81 (1998) 1486–1491.

[7] Panari G., Mariani P., Summer A., Guidetti R., Pecorari M., Change of the composition and evolution of proteolysis of Parmigiano-Reggiano cheese during ripening as regards the shape (outer and inner part) of the wheel, Sci. Tecn. Latt.-Cas. 54 (2003) 199–212.

[8] Pellegrino L., Battelli G., Resmini P., Ferranti P., Barone F., Addeo F., Effects of heat load gradient occurring in moulding on characterization and ripening of Grana Padano, Lait 77 (1997) 217–228.

[9] Pellegrino L., Resmini P., Cheesemaking conditions and compositive characteristics supporting the safety of the raw milk cheese Italian Grana, Sci. Tecn. Latt.-Cas. 52 (2001) 105–114.

[10] Pellegrino L., Resmini P., Fantuzzi U., Evaluation and interpretation of alkaline phoshatase activity in the control of Grana Padano and Parmigiano Reggiano cheese, Ind. Latte 1 (1995) 17–30.

[11] Pellegrino L., Tirelli A., A sensitive HPLC method to detect hen's egg white lysozyme in milk and dairy products, Int. Dairy J. 10 (2000) 435–442.

[12] Pellegrino L., Tirelli A., De Noni I., Resmini P., Valutazione del Grana Padano grattugiato attraverso la determinazione per elettroforesi capillare di frazioni caseiniche e di loro peptidi di degradazione, Sci. Tecn. Latt.-Cas 54 (2003) 321–333.

[13] Pellegrino L., van Boekel M.A.J.S., Gruppen H., Resmini P., Pagani M.A., Heat-induced aggregation and covalent linkage in β-casein model systems, Int. Dairy J. 9 (1999) 255–260.

[14] Recio I., Olieman C., Determination of denatured serum proteins in the casein fraction of heat-treated milk by capillary zone electrophoresis, Electrophoresis 17 (1996) 1228–1233.

[15] Resmini P., Hogenboom J.A., Pazzaglia P., Pellegrino L., Gli amminoacidi liberi nella caratterizzazione analitica del formaggio Grana Padano, Sci. Tecn. Latt.-Cas. 44 (1993) 7–19.

[16] Resmini P., Pellegrino L., Hogenboom J.A., The free amino-acid pattern in cheese characterization, SENS – Relating SENSory, Instrumental and Consumer Choice Studies, FLAIR – Concerted action No 2, Cost 902, Vol. 3, No 4, 1994, pp. 59–80.

[17] Resmini P., Pellegrino L., Hogenboom J.A., Bertuccioli M., Gli amminoacidi liberi nel formaggio Parmigiano-Reggiano stagionato, in: Ricerca triennale sulla composizione e su alcune peculiari caratteristiche del formaggio Parmigiano-Reggiano, Consorzio del Formaggio Parmigiano-Reggiano, Reggio Emilia, Italy, 1988, p. 41.

[18] Resmini P., Pellegrino L., Pazzaglia C., Hogenboom J.A., Gli amminoacidi liberi nella tipizzazione del formaggio Parmigiano-Reggiano ed in particolare del prodotto grattugiato, Sci. Tecn. Latt.-Cas. 36 (1985) 557–592.