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# Interrelationships among physical and chemical traits of cheese: Explanatory latent factors and clustering of 37 categories of cheeses

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## **ABSTRACT**

Cheese presents extensive variability in physical, chemical, and sensory characteristics according to the variety of processing methods and conditions used to create it. Relationships between the many characteristics of cheeses are known for single cheese types or by comparing a few of them, but not for a large number of cheese types. This case study used the properties recorded on 1,050 different cheeses from 107 producers grouped into 37 categories to analyze and quantify the interrelationships among the chemical and physical properties of many cheese types. The 15 cheese traits considered were ripening length, weight, firmness, adhesiveness, 6 different chemical characteristics, and 5 different color traits. As the 105 correlations between the 15 cheese traits were highly variable, a multivariate analysis was carried out. Four latent explanatory factors were extracted, representing 86% of the covariance matrix: the first factor (38% of covariance) was named Solids because it is mainly linked positively to fat, protein, water-soluble nitrogen, ash, firmness, adhesiveness, and ripening length, and negatively to moisture and lightness; the second factor (24%) was named Hue because it is linked positively to redness/blueness, yellowness/greenness, and chroma, and negatively to hue; the third factor (17%) was named Size because it is linked positively to weight, ripening length, firmness, and protein; and the fourth factor (7%) was named Basicity because it is linked positively to pH. The 37 cheese categories were grouped into 8 clusters and described using the latent factors: the Grana Padano cluster (characterized mainly by high Size scores); hard mountain cheeses (mainly high Solids scores); very soft cheeses (low Solids scores); blue cheeses (high Basicity scores), yellowish cheeses (high Hue scores), and 3 other clusters (soft cheeses, pasta filata and treated rind, and firm mountain cheeses) according to specific combinations of intermediate latent factors and cheese traits. In this case study, the high variability and interdependence of 15 major cheese traits can be substantially explained by only 4 latent factors, allowing us to identify and characterize 8 cheese type clusters.

**Key words:** multivariate cheese analysis, cheese classification, cheese texture, cheese chemical composition, cheese ripening length

## INTRODUCTION

Cheese is manufactured from milk of different animal species after coagulation and, in many cases, ripening. The main factors responsible for the wide variation in cheeses produced around the world are the type of milk, milk pretreatments, microbial starters, acidification method and intensity, coagulating agent and coagulation conditions, curd cooking, curd cutting, the addition of spices and herbs, curd draining and pressing, salting technique and level, microbial and mold injection or smear ripening, rind treatments, and ripening length and conditions (McSweeney and Fox, 2009; Fox et al., 2017). More than 1,000 cheese types have been described (Fox, 1999). This variability is particularly evident with regard to cheese shape and size, the presence and appearance of the rind, textural properties, chemical composition, and cheese color (Donnelly, 2017). All these attributes give rise to the specificities of different cheese types in terms of their sensorial characteristics (Bergamaschi and Bittante, 2018), consumer recognition and appreciation (Drake, 2007; Cipolat-Gotet et al., 2018), and ultimately the economic sustainability of the dairy chain (Ojeda et al., 2015).

Cheeses are classified according to several criteria: the species of lactating females (cows, buffalos, goats, and sheep), type of coagulant (rennet, plant, microbial, acid, or acid-heat), ripening length (very fresh, short, medium, or long), textural properties (very soft, soft, firm or semi-hard, hard, extra hard), and the proportion of fat to DM (fat-free, low fat, medium fat, full fat, high fat cheeses; Fox et al., 2017; Trmčić et al., 2017; International Daily Federation, 2021). This means that

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it is possible for a single cheese to be classified differently according to the different criteria. Moreover, none of these classification criteria are fully independent of the others, and large overlaps therefore exist among the categories (Law and Tamine, 2010). Moreover, various criteria differentiate cheeses in terms of composition, color, texture, and sensory properties, meaning that the characterization of a cheese requires many different traits to be recorded and analyzed.

Attempts for classifying cheeses using various compositional and ripening parameters have been proposed in the past, but seldom were they based on published research analyzing many categories of cheeses. Very few studies compare the many different types of cheeses (Kędzierska-Matysek et al., 2018), and the interrelationships between these numerous traits are not always well known and have not been quantified. Furthermore, no studies have identified the possible independent latent factors driving a large part of the variability between cheeses and explaining their similarities and differences.

The general objective of this study is to analyze the enormous variability of many cheese types and the complex relationships among their properties. Our specific aims are: (1) to explore the correlations among 15 different traits (size, ripening length, 5 chemical composition traits, 2 texture traits, and 5 color traits) of many types of cheese; (2) to identify and quantify the independent explanatory latent factors explaining great part of the variance-covariance matrix of the cheese traits; (3) to use multivariate analysis of cheese traits for clustering the many cheese types as a potential tool for cheese classification; and (4) to explore the ability of the latent factors to characterize the different cheese types clusters. For this purpose, because of the need of a very large number of cheeses belonging to many cheese types, we have used as a case study the database of cheese traits represented by the Caseus Veneti ("Cheese from Veneto," in Latin) project. This is a large project being run in northeast Italy with the aim of characterizing more than 1,000 different cheeses of 37 different cheese types produced by more than 100 cheese producers.

## **MATERIALS AND METHODS**

Because no human or animal subjects were used, this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

#### Experimental Design and Cheese Categories

The collection, sampling, analysis, and descriptions of the cheeses are given in a previous article (Bittante et al., 2022b). Secondary methods based on electromagnetic radiation absorbance in visible and infrared light for a rapid and low-cost prediction of the chemical and physical properties of cheeses were derived from the same database (Stocco et al., 2019). Briefly, Caseus Veneti is an annual cheese exhibition and competition (http://www.caseusveneti.it/) sponsored by the Veneto Regional Government (northeastern Italy) and organized by the Veneto Dairy Producers Association (Treviso, Italy; https://www.aprolav.com/) with the aim of assessing and promoting cheeses and producers. The Veneto dairy sector has a long tradition and produces a wide variety of cheeses.

During the course of 3 annual exhibitions, 37 categories from 107 producers comprising a total of 1,050 different cheeses (no more than one per category per producer per year) were recorded, sampled, and analyzed.

The cheeses in the competition are classified into 37 categories belonging to 3 main groups (Table 1): 16 categories (321 cheeses) with the European Union Protected Designation of Origin (**PDO**) status (European Union, 2012), 4 categories (136 cheeses) of traditional regional cheeses designated by the Veneto regional government, and 17 categories of other cheeses (593 cheeses) without official designation. For this study, we divided the third group into subgroups according to their principal characteristics:

- Pasta filata cheeses, which involve curd stretching in very hot water (44 cheeses; 3 categories)
- Flavored cheeses, with the addition of spices, herbs, beer, or wine to the curd or on the rind during refinement (128 cheeses; 5 categories)
- Goat cheeses produced exclusively from goat milk (54 cheeses; 2 categories)
- Other cheeses (367 cheeses; 7 categories)

#### Available Cheese Traits

For this study, we explored 15 traits available for each cheese:

- Cheese weight (in kg); as the cheeses differed in shape according to cheese category and producer (e. g. wheel, block, ball, braid, cylinder), linear measures were not comparable, so the only information on cheese size we considered here was weight.
- Average ripening length (in mo), as declared by cheese producers.
- 2 texture traits: firmness (in N) and adhesiveness (in J<sup>-3</sup>), both obtained with a texture analyzer (XT2i, Stable Micro Systems Ltd., Godalming,

United Kingdom) fitted with a Warner-Bratzler shear device (50-N load cell; 2 mm/s crosshead speed). For details, see our previous study within the same project (Stocco et al., 2019).

• 5 chemical composition traits: moisture (%), fat (%), total protein (%), water-soluble nitrogen (WSN; % of total nitrogen), and ash (%), all obtained with a Foodscan spectrometer (FOSS A/S, Hillerød, Denmark) using pre-installed nearinfrared calibrations for cheese composition, with some exceptions for blue cheeses and WSN, as reported in the previous article (Bittante et al., 2022b).

Table 1. Groups and	categories of cheeses	analyzed $(n = 1,050)^1$
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- pH, measured in triplicate on fresh cheeses using a portable pH meter (Crison Basic 25, Crison Instruments SA, Barcelona, Spain).
- 5 color traits: lightness (L\*), redness/blueness (a\*), yellowness/greenness (b\*), chroma (C\*), and hue (h°); color readings were taken at 3 different positions on the freshly cut surface using a portable spectrocolorimeter (CM 508) standardized with a white calibration cap (CM-A70; both from Minolta Co. Ltd., Osaka, Japan). The color traits were expressed according to the Commission Internationale de l'Éclairage colorimetric system.

Cheese type	Ν	Milk $fat^2$	Cheese ripening time (mo)	Notes
PDO cheeses	321			
Asiago, fresco	45	Full fat	<1	Soft PDO cheese
Asiago, stagionato (mezzano)	28	Skim	4-10	Firm PDO cheese
Asiago, stagionato (vecchio)	19	Skim	10 - 15	Hard PDO cheese
Asiago, stagionato (stravecchio)	23	Skim	>15	Very hard PDO cheese
Casatella Trevigiana	27	Full fat	<1	Fresh PDO cheese, no rind
Grana Padano	17	Skim	12-20	Very hard PDO cheese
Grana Padano, riserva	18	Skim	>20	Very hard PDO cheese
Montasio, fresco	29	Full fat	2-4	Soft PDO cheese
Montasio, mezzano	23	Full fat	5 - 10	Semi-soft PDO cheese
Montasio, stagionato	20	Full fat	>10	Hard PDO cheese
Monte veronese, latte intero	21	Full fat	0.8-1.5	Soft PDO cheese
Monte veronese, mezzano	18	Skim	3-6	Firm PDO cheese
Monte veronese, vecchio	13	Skim	>12	Hard PDO cheese
Piave	6	3.5%	1-24	Soft or hard PDO cheese
Provolone, dolce	7	Full fat	2-3	Soft PDO, stretched curd
Provolone, piccante	7	Full fat	>3	Hard PDO, stretched curd
Traditional regional cheeses	136	1 411 140		Hard T 2 0, birotolioa cara
Formaggio inbriago	45	Full fat	2-6	In marc must or wine
Malga, fresco	42	Skim	2-6	Firm Alpine pasture cheese
Malga, vecchio	19	Skim	>12	Hard Alpine pasture cheese
Morlacco del Grappa	30	Full fat	1-3	Soft, from Monte Grappa
Pasta filata cheeses	44	1 411 140	10	Sole, Holli Holice Grappa
Mozzarella	20	Full fat	<1	Stretched curd, fresh
Pasta filata molle	13	Full fat	1-3	Stretched curd, soft
Pasta filata	11	Full fat	3-12	Stretched curd, hard
Flavored cheeses	128	1 411 140	0 12	Strovenoù oura, nara
Formaggi affumicati	22	Full fat	Var	Smoked cheeses
Formaggi alla birra o balsamico	4	Full fat	Var	In beer or balsamic vinegar
Formaggi erborinati	19	Full fat	Var	Blue cheeses
Formaggi con erbe, fieno e spezie	43	Full fat	Var	With herbs, hay, spices
Formaggi al pepe o peperoncino	40	Full fat	Var	With pepper or chili
Goat cheeses	54	1 411 140	1.01	the popper of child
Caprino a coagulazione acida	19	Full fat	<1	Acid-coag. goat cheese
Caprino a coagulazione presamica	35	Full fat	Var	Rennet-coag. goat cheese
Other cheeses	367		* Col	boug. Sour encose
Formaggi freschi, freschissimi	57	Full fat	<1	Very fresh, no rind
Caciotta	55	Full fat	1-2	Soft cheeses $<1.0$ kg
Latteria	79	Full fat	2-4	Soft cheeses $>1.0$ kg
Crosta fiorita	25	Full fat	1-4	Moldy rind
Crosta lavata	18	Full fat	2-6	Washed rind
Pasta semidura	91	Var	$\frac{2}{3-6}$	Firm cheeses
Pasta dura	42	Var	>10	Very hard cheeses

 $^{1}\text{PDO} = \text{European Union Protected Designation of Origin status; traditional regional cheeses = designation conferred by the Veneto regional government; coag. = coagulated.}$ 

 $^{2}$ Full fat = milk not skimmed; skim = partly skim milk; var = varies according to producer.

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$\mathrm{Trait}^2$	Mean	$^{\mathrm{SD}}$	Minimum value	Minimum category	Maximum value	Maximum category
Ripening length, mo	5.9	6.0	0.5	Casatella; goat ac; fresh; mozzarella	>20.0	Grana Padano riserva
Cheese weight, kg	6.6	7.5	0.2	Goat ac; mozzarella	34.7	Grana Padano
Firmness, N	10.8	6.4	0.7	Goat ac	26.0	Piave
Adhesiveness, $J^{-3}$	7.6	3.8	0.5	Goat ac	17.0	Piave
Moisture, %	39.5	9.4	25.9	Asiago stravecchio	66.3	Goat ac
Fat, %	29.6	4.7	15.5	Goat ac	37.2	Piave
Protein, %	25.5	5.6	12.9	Goat ac	36.7	Grana Padano riserva
WSN, %	6.5	1.9	3.0	Mozzarella	10.4	Grana Padano riserva
Ash, %	1.9	0.2	1.2	Goat ac	2.1	Beer cheeses
pH	5.7	0.3	4.7	Goat ac	6.6	Blue cheeses
L*	76.6	7.1	65.3	Malga vecchio	91.9	Goat ac
a*	-1.0	0.5	-1.9	Pasta filata molle	0.3	Malga fresco
b*	12.5	2.2	8.6	Piave	18.3	Malga fresco
C*	12.6	2.2	8.7	Piave	18.4	Malga fresco
h°	95.5	2.7	89.2	Malga fresco	100.0	Piave

 Table 2. Descriptive statistics of the 15 traits available for every cheese category<sup>1</sup>

 $^{1}$ Asiago stravecchio = very hard European Union Protected Designation of Origin (PDO) mountain cheese; beer cheeses = Formaggi alla birra o balsamico and cheeses refined in beer or balsamic vinegar; blue cheeses = Formaggi erborinati; Casatella = Casatella Trevigiana PDO (very fresh, rindless cheese); fresh = formaggi freschi-freschissimi/fresh-very fresh cheeses; goat ac = Caprino a coagulazione acida/acid-coagulated fresh goat cheese; Grana Padano = very hard 12–24 mo-ripened PDO cheese; Grana Padano riserva = very hard >24 mo-ripened PDO cheese; Malga fresco = traditional cheese from summer highland pasture, ripened <12 mo; Malga vecchio = traditional cheese from summer highland pasture, ripened <12 mo; Malga vecchio = traditional cheese from summer highland pasture, ripened >12 mo; mozzarella = very fresh stretched curd cheese; Piave = hard PDO mountain cheese; Pasta filata molle = soft stretched curd cheeses. "Minimum category" is the category where the minimum value was found. "Maximum category" is the category where the maximum value was found.

 $^{2}$ WSN = water-soluble nitrogen; L\* = lightness; a\* = redness/blueness; b\* = yellowness/greenness; C\* = chroma; h° = hue.

The descriptive statistics of the 37 mean values of each trait are summarized in Table 2. The texture, chemical composition, pH, and color traits were all obtained within 3 d of sampling (starting with the fresh cheeses) in the Department of Agronomy, Food, Natural Resources, Animals, and Environment (DAFNAE) laboratories of the University of Padova (Legnaro, Padua, Italy).

## Statistical Analysis

Pearson Correlations and Latent Explanatory Factors of Cheese Traits. The correlations among the 15 selected traits were explored. Due to the high number and complexity of the relationships among the traits, we used a multivariate factor analysis (FA) to summarize the interrelated measured traits in a small number of unmeasured latent independent explanatory variables (factors). First, we performed the Kaiser-Meyer-Olkin and Bartlett tests on the selected traits after standardization, which showed that they were suitable for FA. The FA was carried out with Varimax rotation in the R environment using the psych package (available at CRAN: The Comprehensive R Archive Network; https://cran.r-project.org/) in 3 steps: (1) extraction of factors such that the minimum number of uncorrelated latent factors explained the greatest proportion of common variance; (2) rotation until each factor was defined by a few variables with high loadings; and (3) biological interpretation of the factors based on the strength of the loadings of the variables. The eigenvalues of the factors and the communalities of the variables after rotation were also determined. Four latent explanatory factors were extracted from the 15 cheese traits selected.

**Clustering of Cheese Categories.** The physical and chemical traits of the 37 cheese categories were analyzed with a hierarchical clustering approach based on the 15 standardized traits. The clusters that group cheese categories according to their similarities were evaluated using Ward's hierarchical clustering method with a Manhattan distance analysis in the R environment using the hclust function. A dendrogram was constructed using the distances calculated between the categories and a total of 8 clusters were individuated.

ANOVA of Cheese Factor Scores and Traits According to Cheese Clusters. Scores for each of the 4 latent explanatory factors and the values of each of the 15 traits available for each cheese category were analyzed using the SAS 9.4 GLM procedure (SAS Institute Inc., Cary, NC) according to the following linear model:

$$y_{ij} = \mu + \text{Cluster}_i + e_{ij},$$

where  $y_{ij}$  is the factor or trait per cheese category;  $\mu$  is the overall intercept of the model; Cluster<sub>i</sub> is the effect of the *i*th cheese cluster (i = 8 levels); and  $e_{ij}$  is the random residual ~ N( $0, \sigma_e^2$ ).

The least squares means of each cheese cluster were contrasted with the average of all the clusters to identify the clusters that were significantly (P < 0.05, P < 0.01, and P < 0.001) lower or higher than the average of every latent factor and cheese trait.

#### RESULTS

#### **Correlations Among Cheese Traits**

The 105 correlation coefficients between the 15 cheese traits considered are quite variable in sign and value. The length of ripening and weight of whole cheeses and the firmness and adhesiveness of the cheese after removal of the rind were all positively correlated with each other (Figure 1). The 2 textural traits, in particular, had a correlation of +92%, whereas the cheese weight had positive but lower correlations with the other 3 traits (38%-69%).

The positive correlations between these 4 traits explain why they also tend to have similar correlations with the other cheese traits (44 correlations). As shown in Figure 1, these traits were positively associated with cheese pH, fat, protein, WSN, and ash contents, and negatively associated with moisture content and lightness. Their correlations with the other color traits are much more modest and variable. Among these 4 traits, cheese weight had the lowest correlations with the other traits, and particularly moisture and fat content, because the moisture loss during processing and ripening depends not only on the surface to volume ratio of the cheese, but also on other factors (e.g. temperature and relative humidity of the ripening cellar, vacuum packaging, cloth wrapping, wax coating, oiled rind, and brine aging).

The 15 correlations between the 6 chemical traits of cheeses are shown in Table 3. As expected, the correlations between cheese moisture and the other chemical components are strongly negative (-80%) for ash to -93% for fat), whereas the correlations between fat, protein, WSN, and ash are all positive (57%-88%). Cheese pH was also negatively correlated with moisture (-56%) and positively with other traits, but the correlation coefficients (24%-62%) tended to be lower than those for the nutrient contents (i.e., fat, protein, WSN, and ash) and depends mainly on the comparison between fresh cheeses and hard cheeses.

The 10 Pearson correlations between the 5 color traits are shown in Table 4. As can be seen, L\* is almost independent from the other 4 color traits (-13%) for a\* to +15% for h°). In contrast, all other color traits are strongly correlated with each other: positively between a\*, b\*, and C\* (69%–99%), and negatively between h° and these same traits (-74%) to -93%). Lastly, Table 5 summarizes the 30 correlations between chemical traits and color traits. As the table shows, lightness is strongly correlated with all the chemical traits: positively with cheese moisture and negatively with fat, protein, WSN, ash, and pH. None of the other correlation coefficients appear relevant, as they range from -33% (ash content with h°) to +19%(protein and WSN with a\*).

## Latent Explanatory Factors

The complexity of the 105 correlations examined made difficult to evaluate individually the 15 cheese traits considered and stimulated the use of a multivariate approach for the contemporary analysis of all traits and cheeses. The multivariate FA identified 4 latent factors that together explain 86% of all covariance among the 15 cheese traits. As can be seen from Table 6, the first factor explained 38% of the covariance and is substantially defined by the content of nutrients of the cheese, particularly fat (loading 0.99), protein, WSN, and ash, and negatively with moisture. Therefore, we named this factor Solids. It is worth noting that this factor also includes relevant positive loadings of the 2 textural traits, ripening length (but not cheese weight), and pH (not relevant), and, with a strong negative loading, lightness (Table 6). The other color traits do not belong to this latent factor.

The second factor represents 24% of all covariance among the cheese traits and has relevant loadings of all the color traits apart from lightness (a<sup>\*</sup>, b<sup>\*</sup>, and C<sup>\*</sup> positively, and h<sup>°</sup> negatively). Therefore, we named this factor Hue because the hue angle showed the highest loading, which is directly derived by a<sup>\*</sup> and b<sup>\*</sup>, which are combined in C<sup>\*</sup>.

The third factor accounts for 17% of the covariance and is defined by the weight of the cheese, the length of the ripening, and also, but with less relevant loadings, the 2 textural traits, the protein content of the cheese and WSN contents, and L\* (the only trait with a negative load). Considering the high positive relation to the cheese weight and to the ripening length (which also is correlated with cheese mass), this factor was named Size.

The last factor, representing only 7% of covariance, is defined by a relevant loading of cheese pH and nonrelevant loadings of protein, WSN and C\* (all positive). Considering the positive correlation with the pH, this factor was named Basicity.

It is interesting that all traits are characterized by a communality coefficient of 0.50 for pH and 0.72 to 1.00 for the other 14 cheese traits, which means that all the individual traits could be considered well represented by the 4 latent explanatory factors.

Bittante et al.: PHYSICAL AND CHEMICAL TRAITS OF CHEESE -1.00-0.75 -0.50 -0.25 0.00 0.25 0.50 0.75 1.00 Ripening Weight Firmness Adhesiveness pН Moisture Fat Protein WSN Ash L\* a\* b\* C\* h° ■ Ripening ■ Weight ■ Firmness Adhesiveness

Figure 1. Pearson correlations among length of ripening, weight, firmness, and adhesiveness of the cheeses, and between these and the other traits recorded for 37 categories of cheeses. WSN = water-soluble nitrogen;  $L^* = lightness$ ;  $a^* = redness/blueness$ ;  $b^* = yellowness/greenness$ ;  $C^* = chroma$ ;  $h^\circ = hue$ .

Trait

 $L^*$ 

 $a^*$ 

**Table 3.** Pearson correlations among the chemical composition traits of 37 categories of cheese

**Table 4.** Pearson correlations among the color traits of 37 categories of cheese<sup>1</sup>

 $C^*$ 

0.03

0.69

 $\mathrm{h}^{\circ}$ 

0.15

-0.93

 $b^*$ 

0.02

0.70

Trait	Fat	Protein	$\mathrm{WSN}^1$	Ash	pН
Moisture Fat	-0.93	$-0.92 \\ 0.72$	$-0.82 \\ 0.63$	$-0.80 \\ 0.76$	$-0.56 \\ 0.44$
Protein			0.88	0.71	0.59
WSN				0.57	0.62
Ash					0.24

 $^{1}WSN =$  water-soluble nitrogen.

 $L^* = lightness; a^* = redness/blueness; b^* = yellowness/greenness; = chroma; h^o = hue.$ 

 $a^*$ 

-0.13

Table 5. Pearson correlations between the chemical composition and color traits of 37 categories of cheese<sup>1</sup>

Trait	$L^*$	$a^*$	b*	$C^*$	$h^{\circ}$
Moisture	0.93	-0.16	-0.14	-0.13	0.21
Fat	-0.81	0.10	0.12	0.11	-0.17
Protein	-0.91	0.19	0.09	0.07	-0.23
WSN	-0.76	0.19	0.06	0.05	-0.12
Ash	-0.69	0.19	0.08	0.06	-0.33
pH	-0.49	0.05	0.14	0.14	0.03

<sup>1</sup>WSN = water-soluble nitrogen;  $L^* = lightness; a^* = redness/blue$ ness;  $b^* = \text{yellowness/greenness}$ ;  $C^* = \text{chroma}$ ;  $h^\circ = \text{hue}$ .

## Clustering of the 37 Categories of Cheese and Their Characterization by the Latent Explanatory Factors

The hierarchical clustering procedure adopted allowed us to identify 8 main groups of cheese categories. These 8 clusters are depicted by the dendrogram in Figure 2.

The 8 clusters explained a large proportion of the total variance of the 15 cheese traits (80%). In fact, the ANOVA showed a highly significant effect (P < 0.001)effect of the cheese cluster for all traits and for latent factors. The least squares means are reported in Table 7 together with the ranges of their standard errors. Each latent factor is followed by the relevant traits characterizing it. The least squares means of each cheese cluster were compared with the average of all cheese categories, and the significantly positive (green) and negative (red) deviations are shown in bold in Table 7.

As the table shows, none of the cheese clusters deviated significantly from the average in the sole case of adhesiveness, although the difference between the 2 extreme clusters (cluster 2 vs. cluster 3) was highly significant (P < 0.01).

## DISCUSSION

## Cheese Trait Correlations and Latent **Explanatory Factors**

Beyond the several technical articles and books proposing different criteria for classification of cheese, very few, if any, scientific articles have focused on the topics covered in this study, that is, comparing a very large number of cheese categories, analyzing the correlations among the main characteristics of the cheeses, identifying their latent explanatory factors, and conducting multivariate analyses to cluster and characterize the cheese categories.

However, studies on one or a few cheeses have shown that the ripening length is a powerful tool for modifying the appearance, composition, and sensory characteristics of cheese. It is also known that prolonging the ripening of the cheeses increases proteolysis and lipolysis in cheeses (Leitner et al., 2006; Ardö et al., 2017) and the content of volatile organic compounds, thus modifying the aroma and substances that change the taste of the cheese (Bergamaschi and Bittante, 2018). Prolonged ripening also leads to further moisture loss in the case of cheeses with a rind exposed to the atmosphere (e.g. no vacuum packaging, cloth wrapping, wax coating, oiled rind, and brine aging).

As a consequence of different degrees of drying, strong negative correlations between moisture content on one side and fat, protein, and mineral contents on the other

Table 6. Loadings of the latent explanatory factors explaining the variability in 15 traits of 37 categories of cheese (relevant loadings >0.50 or <-0.50 are shown in bold)

$\operatorname{Trait}^1$	Factor 1	Factor 2	Factor 3	Factor 4	Communality
Ripening length	0.53		0.68		0.81
Cheese weight			0.83		0.76
Firmness	0.72		0.54		0.88
Adhesiveness	0.77		0.33		0.76
Moisture	-0.93				1.00
Fat	0.99				0.99
Protein	0.74		0.56	0.32	0.98
WSN	0.65		0.49	0.37	0.80
Ash	0.79				0.72
pH	0.41			0.54	0.50
L*	-0.84		-0.41		0.91
a*	_	0.87			0.87
b*		0.93			1.00
$C^*$		0.92		0.31	1.00
h°		-0.94			1.00
Variance proportion	0.38	0.24	0.17	0.07	
Cumulative variance	0.38	0.62	0.79	0.86	
Name of factor	Solids	Hue	Size	Basicity	

<sup>1</sup>WSN = water-soluble nitrogen;  $L^* = lightness; a^* = redness/blueness; b^* = yellowness/greenness; C^* =$ chroma;  $h^{\circ} = hue$ .

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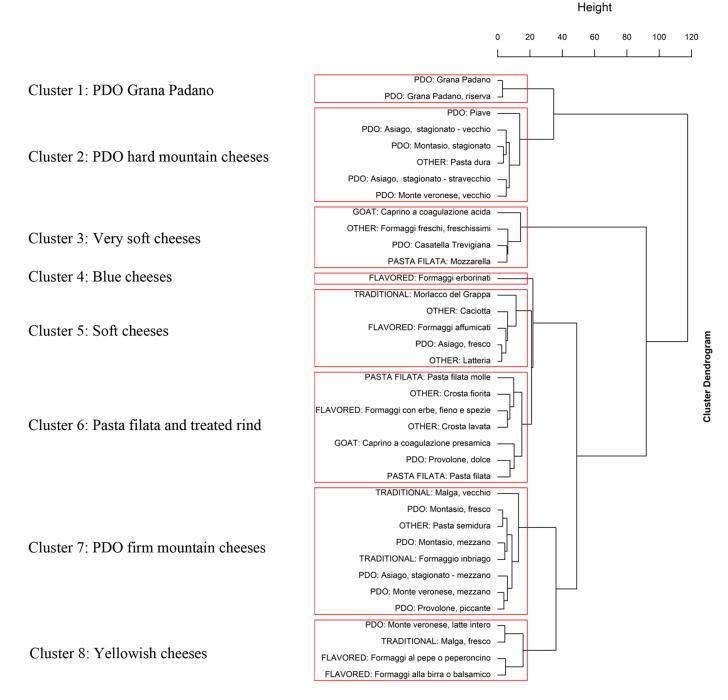


Figure 2. Clustering of 37 categories of cheeses. The red boxes group the categories assigned to each cluster. PDO = European Union Protected Designation of Origin.

are expected. Therefore, all these traits are driven by a common latent factor that we named Solids, which is the only factor that accounts for the variation in fat and ash, but not protein and WSN (Table 6). The latter 2 traits are also included in 2 other latent factors, providing clear evidence of the central role played by nitrogenous substances in cheesemaking and ripening. They are included in the latent factor Size due to the frequent use of partially skim milk (and then a higher protein:fat ratio) in producing large-wheel cheeses, and their inclusion in the latent factor Basicity could be explained by a greater proteolysis in cheeses with higher

				Cheese clusters (LSM):	rs (LSM):				
Factor and trait <sup>2</sup>	Cluster 1: Grana Padano	Cluster 2: Hard mountain	Cluster 3: Very soft cheeses	Cluster 4: Blue cheeses	Cluster 5: Soft cheeses	Cluster 6: Pasta filata	Cluster 7: Firm mountain	Cluster 8: Yellowish cheeses	SE
F1: Solids	-0.1	$1.3^{**}$	$-1.9^{***}$	-1.2	-0.3	-0.4	0.6	0.1	0.2 - 0.4
Moisture	32.8	28.7**	$60.2^{***}$	42.4	42.9	43.3	34.2	37.5	1.1 - 3.2
Fat	28.1	$35.1^{*}$	$20.2^{***}$	25.7	28.4	27.9	32.6	30.7	0.7 - 2.0
Protein	$36.1^{***}$	$30.2^{*}$	$14.5^{***}$	25.2	22.6	23.1	28.3	26.2	0.7 - 2.1
MSN	$10.1^{***}$	8.1	3.2***	$9.4^{*}$	5.2	5.6	6.9	7.1	0.3 - 0.8
$\operatorname{Ash}$	2.1	2.0	$1.4^{***}$	$1.4^{**}$	1.9	1.9	2.0	1.9	0.04 - 0.11
L*	$68.6^{*}$	$67.5^{**}$	88.5***	$85.2^{*}$	80.3	80.9	72.9	75.8	0.9 - 2.7
Adhesiveness	10.0	12.7	3.1	6.0	4.8	5.4	9.6	6.8	0.8 - 1.3
Firmness	$22.0^{**}$	$19.7^{*}$	4.1	6.7	5.1	7.2	12.6	9.2	1.1 - 3.1
F2: Hue	0.2	-1.0	-1.1	0.4	0.8	-0.3	0.1	$1.8^{**}$	0.2 - 0.5
a*	-0.6	-1.4	-1.4	-1.0	-0.7	-1.5	-1.0	$0.1^{***}$	0.1 - 0.2
$^{\mathrm{p}*}$	11.4	10.6	9.8	$16.1^{*}$	14.2	12.4	12.6	$16.1^{*}$	0.4 - 1.2
C*	11.4	10.7	9.9	$16.3^{*}$	14.3	12.5	12.7	$16.2^{*}$	0.4 - 1.2
H*	93.3	97.9	98.5	98.1	93.4	97.0	94.9	$90.8^{**}$	0.5 - 1.3
F3: Size	$3.4^{***}$	0.0	-0.2	-0.7	-0.4	-0.5	0.0	-0.1	0.2 - 0.6
Ripening	$21.0^{***}$	$13.7^{**}$	0.5	3.0	2.0	3.1	5.9	2.8	0.9 - 2.5
Weight	$34.7^{***}$	7.3	$0.6^{*}$	1.9	6.0	3.0	7.0	5.4	0.9 - 2.4
F4: Basicity	0.0	0.3	-0.9	3.8***	-0.4	0.4	-0.2	-0.3	0.3 - 0.7
pH	6.0	5.9	5.4	$6.6^{**}$	5.5	5.5	5.8	5.6	0.1 - 0.2
<sup>1</sup> LSM in bold and italics differ signific <sup>2</sup> WSN = water-soluble nitrogen; $L^* = *P < 0.05; **P < 0.01; ***P < 0.001$	talics differ signific ble nitrogen; $L^* =$ .01; *** $P < 0.001$ .	cantly from the aver : lightness; $a^* = rec$	<sup>1</sup> LSM in bold and italics differ significantly from the average of all the cheese categories, bold where greater, italics where lower $^{2}$ WSN = water-soluble nitrogen; L* = lightness; a* = redness/blueness; b* = yellowness/greenness; C* = chroma; h° = hue; SE * $P < 0.05$ ; ** $P < 0.01$ ; *** $P < 0.01$ .	categories, bold yellowness/gree	where greater, nness; $C^* = chn$	italics where low coma; $h^{\circ} = hue$ ;	ver. SE = range of star	<sup>1</sup> LSM in bold and italics differ significantly from the average of all the cheese categories, bold where greater, italics where lower. <sup>2</sup> WSN = water-soluble nitrogen; $L^* = lightness; a^* = redness/blueness; b^* = yellowness/greenness; C^* = chroma; h^\circ = hue; SE = range of standard errors of the LSM * P < 0.05; **P < 0.01; ***P < 0.001.$	M.



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pH, which is a consequence, in the case of mold-ripened cheeses, of the enzyme production of the molds, and the consequent increase of pH.

Studies that examine the effects of varying the length of ripening on a given type of cheese use serial analyses of cheeses from the same batch. Here, instead, we have compared different cheeses produced using different techniques according to the target ripening length, which more closely reflects commercial cheesemaking.

This can be clearly illustrated by the weight of the cheeses. The lengthening of the ripening of a given cheese type is expected to reduce the weight of the cheese (negative correlation between ripening length and cheese weight) due to the loss of both moisture as a result of drying (except in case of vacuum packaging) and nutrients due to microbial fermentation and enzymatic cheese metabolism. The positive correlation (+69%) found here reflects the tendency of the dairy industry to produce heavier cheeses when they are to be ripened over long periods to avoid excessive drying. An extreme case is the very heavy wheels of many hard cheeses, such as Grana Padano in this study, but also Parmigiano Reggiano, Emmentaler, and Gruyére. It is interesting that the weight of the cheese is not included in the latent factor Solids, whereas the ripening length is included in both the factors Solids and Size, but with a greater load in the latter (Table 6).

Regarding cheese color, we expected that the polar coordinates (C<sup>\*</sup> and h°) would be strongly related to the rectangular coordinates (a<sup>\*</sup> and b<sup>\*</sup>) from which they are directly derived and that these 4 traits would be driven by a common latent factor, called Hue. Less obvious is the substantial independence of the latter color traits from the lightness of the cheese, to the extent that they belong to different latent factors. The lightness of cheese is, in fact, strongly related to the latent factor Solids; that is, lighter cheeses are generally fresher, softer, moister, and acidic.

## Cheese Category Clusters and Their Characterization

The cheeses from northeast Italy (Caseus Veneti project) were used in our case study to see if a clustering of the many cheese categories based on multivariate analysis of their physical and chemical characteristics (Figure 2) would have been similar or different from a priori grouping of cheese categories based on PDO status, geographical area, and cheesemaking technology (Table 1). The data from the Caseus Veneti project were also used as a case study to see if the explanatory latent factors extracted with the multivariate analysis would have been able to characterize the clusters obtained. The first 2 clusters extracted from the 37 cheese categories studied contain hard cheeses that differ in size and origin.

PDO Grana Padano Cluster. The first cluster groups together 2 categories (Grana Padano and Grana Padano riserva) distinguished by cheese ripening length and by having the highest production level of all PDO cheeses of the European Union; they also represent approximately one-quarter of the Italian dairy industry's production (Bava et al., 2018). As Table 7 shows, this cluster has a much higher latent factor Size than all the other 7 cheese clusters. This is due to the Grana Padano wheels being the heaviest of all the cheese categories, with every copper vat containing about 1,000 L of partially skim milk producing 2 wheels of about 35 kg each. This is also the cheese cluster with the longest ripening period (particularly in the category Grana Padano riserva), and ripening length is the trait with the second highest loading on the latent factor Size. This latent factor is also characterized by high loadings of protein and WSN contents, and Grana Padano is the cluster with the highest values of these 2 components, reflecting the partial skimming of the milk and proteolysis resulting from the long ripening period. However, this cluster has an intermediate score for the latent factor Solids because the high protein and WSN values and the low L<sup>\*</sup> score are offset by the relatively low fat and moisture, although the latter trait is not as low as expected given the ripening period. Lastly, it is worth noting that the other 2 traits included in the latent factor Size are the 2 textural traits. As expected on the basis of the very long ripening time, the Grana Padano cluster has the greatest firmness of all the clusters, but not the greatest adhesiveness, despite the high correlation generally observed between these 2 traits. This is due to the typical granular structure (which gives rise to the name of the cheese) that gives resistance during mastication but little adhesion to the teeth and mouth.

The major difference between Grana cheese and the other very important Italian PDO hard cheese, Parmigiano Reggiano, is that silages (especially maize silage) are permitted in the dairy cows' feed for the first, but not the second cheese. The inclusion of silages results in the addition of lysozyme during cheesemaking to control the microbial spores that cause late blowing of the wheels (Mucchetti et al., 2017; Gobbetti et al., 2018; da Silva Duarte et al., 2022). The widespread use of maize silage is also the reason why Grana Padano is produced mainly by intensive dairy farms on the plains, although some niche production also takes place in the mountains, often without silages and lysozyme, such as Trentingrana (Bittante et al., 2011a,b). In any case, the physical and chemical traits observed for Parmigiano Reggiano are not much different from those observed here for Grana Padano, so we expect that they may cluster together.

**PDO Hard Mountain Cheeses Cluster.** The name of the second cluster was assigned because it comprises 6 hard cheese categories, 5 of which have mountain PDO status (Figure 2). The sixth category (Other cheeses: pasta dura) includes the hard cheeses without PDO status, produced mainly in the plains. It is worth noting that a large number of the PDO cheeses in this cluster are produced according to the typical cheesemaking procedures of the mountains, but using milk produced in an area that also includes nearby lowland regions (namely Asiago and Montasio cheeses). Consequently, the clustering discriminates more according to the origin of the cheesemaking technique than to the area of production.

As with the cheeses of the previous cluster, the PDO hard mountain cheeses also have a long ripening period and are very hard (although less than the Grana Padano cheeses). However, this cluster is characterized by the highest score on the latent factor Solids, rather than Size. In fact, the cheeses in this cluster have the lowest average moisture content and L\* values and the highest fat content (Table 7). The protein content is higher than average, but WSN is not, reflecting a different aging metabolism compared with Grana Padano cheeses. The different firmness to adhesiveness ratios of these 2 clusters should also be noted.

Very Soft Cheeses Cluster. The third cluster contrasts with the previous 2 clusters. It contains 4 categories with different origins and cheesemaking techniques, although all are rindless and are intended for consumption within a very short time after production. These cheeses have some similarities in appearance and composition, but they are obtained from different types of milk using different cheesemaking procedures (Figure 2). Casatella Trevigiana PDO cheese and cheeses in the Freschi-freschissimi category are obtained from cultured, rennet-coagulated bovine milk without curd cooking. Mozzarella is a pasta filata cheese, i.e., the manufacturing process involves stretching the curd in very hot water (Ah and Tagalpallewar, 2017). Lastly, capra coagulazione acida is made from acid-coagulated caprine milk, and the particularities of goat milk (Pazzola et al., 2018; Stocco et al., 2018; Bittante et al., 2022a) and cheese (Vacca et al., 2018; Pazzola et al., 2019) versus cow milk and cheese, and of acid versus rennet coagulation (Barbé et al., 2014), are well documented. This cluster had the lowest scores on the latent factor Solids because of very high moisture contents and L<sup>\*</sup> values, and very low fat, protein, WSN, and ash contents (Hashem et al., 2014). The cheeses in this cluster also had the lowest average weight (Table 7).

**Blue Cheeses Cluster.** The fourth cluster is very straightforward, as it is defined by a single cheese category: formaggi erborinati. This group of cheeses is distinguished by a very high score on the latent factor Basicity, mainly because of a much higher pH than any of the other clusters (Table 7). However, this latent factor is defined not only by pH, but also by protein, which is higher than expected based on the moisture content of these cheeses, and by WSN, which is very high given the relatively short ripening period. Their peculiarity is surely a consequence of this category of cheeses being inoculated with Penicillum. The last trait included in the latent factor Basicity is C\*, and blue cheeses are characterized by much higher than average  $C^*$  and  $b^*$  indices (Table 7). Blue cheeses have a further peculiarity in that some secondary analytical methods based on vibrational spectrography are unable to correctly predict their composition (Stocco et al., 2019; Bittante et al., 2022c).

The fifth, sixth, and seventh clusters are in some way intermediate for many aspects and do not differ significantly from the average of all the cheeses for any latent factor or individual trait. It is clear that the clustering procedure does not differentiate these groups of cheeses on the basis of an extreme value of a specific trait or factor, as with the other clusters, but rather on the basis of combinations of traits and factors representing the fingerprint of a cluster.

Soft Cheeses Cluster. The 5 categories included in the fifth cluster are characterized by a firmness that is numerically larger only than the very fresh cheeses but not significantly different from the general average of the model. The categories included were a PDO cheese (Asiago fresco), a traditional regional cheese (Morlacco del Grappa), 2 soft cheeses distinguished by wheel weight (Caciotta <1 kg, and Latteria >1 kg), and a category of similar soft cheeses that are smoked (Figure 2). They are all generally produced from full-fat milk with or without mild curd cooking and have large curd grains.

**Pasta Filata and Treated Rind Cluster.** The factor scores and least squares means of the individual traits of the sixth cluster are similar on average to the previous cluster, but the cheeses in this cluster are produced according to different processing methods. Of the 7 categories included in this cluster, 3 are made according to the *pasta filata* method, i.e., stretching the curd in very hot water (pasta filata, pasta filata molle, and Provolone dolce PDO), 2 are processed with rind treatments (crosta fiorita: moldy rind, and crosta lavata: washed rind), one has the rind infused with hay, herbs and spices, whereas the last one comprises rennet-coagulated goat milk cheeses, often with moldy rinds (Figure 2). Even though the 15 cheese traits do

PDO Firm Mountain Cheeses Cluster. The seventh cluster comprises 8 cheese categories, 4 of which are PDO firm mountain cheeses (Montasio fresco, Montasio mezzano, Asiago stagionato mezzano, and Monte Veronese d'allevo mezzano), one is a PDO firm cheese from the plains (Provolone piccante), one is a traditional regional ripened cheese produced only during summer on highland pastures (Malga vecchio), one is a traditional regional cheese matured in marc, must, or wine (Formaggio inbriago), and the last comprises firm cheeses without PDO status (Figure 2). Although as far as this cluster is concerned, no latent factor or individual trait differed statistically from the general average, it is worth noting that the average length of ripening is 5.9 mo, the average firmness is 12.6 N (Table 7), and the clustering procedure was able to capture the distinctive features of mountain cheese technologies, which explains why we named this cluster PDO firm mountain cheeses.

Yellowish Cheeses Cluster. The eighth cluster is composed of 4 cheese categories, 2 of which are produced in the mountains, where the animals are often pasture fed (traditional cheeses: Malga fresco and Monte Veronese latte intero PDO), whereas the other 2 are made with the addition of pepper or chili pepper (formaggi pepe, peperoncino) or matured in beer or balsamic vinegar (Figure 2). These 4 categories differ significantly from the others for the latent factor Hue, with high a<sup>\*</sup>, b<sup>\*</sup>, and C<sup>\*</sup> color indices and a low h<sup>°</sup> index. It is well known that cheese made from the milk of pasture-grazed cows is more yellow because of the high carotenoid and polyunsaturated fatty acid contents of the milk (Bovolenta et al., 2009; Bergamaschi et al., 2016; O'Callaghan et al., 2017). Therefore, we named this cluster Yellowish cheeses.

We expect that including specific information, such as the detailed fatty acid profile, microbiota composition, and volatile organic compounds, would greatly improve the discriminant ability of the multivariate analyses, and could be useful for authenticating specific types of cheeses (PDO, protected geographical indication, and traditional cheeses) and farming systems (e.g. organic farming, mountain products, pasture products, and hay products). Further research is needed on these issues.

#### **CONCLUSIONS**

In this case study, including over 1,000 different cheeses of 37 different cheese types produced by over

100 cheese producers, we have shown that the complex interrelationships of 15 different cheese traits can be largely represented by only 4 independent latent explanatory factors, here named Solids, Hue, Size, and Basicity. A multivariate approach also allowed 37 different categories of cheeses to be grouped into 8 clusters. The latent factors identified could clearly discriminate 5 of the 8 clusters: Grana Padano for high Size scores, PDO hard mountain cheeses for high Solids scores, very soft cheeses for low Solids scores, blue cheeses for high Basicity scores, and Yellowish cheeses for high Hue scores. The clustering procedure could also discriminate another 3 clusters, not on the basis of extreme values of a given latent factor or cheese trait, but for the combinations of factors that reflected differences in the cheesemaking techniques and areas of origin.

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