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Research paper

Predicting water activity in long-ripened cheeses using moisture, salt, free amino acids and lactate

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ABSTRACT

Water activity (a_w) is a critical physico-chemical parameter that influences microbiological and biochemical properties during cheese ripening. This study assessed the use of Raoult's law in estimating a_w based on four chemical components (moisture, NaCl, total free amino acids [ΣFAAs] and lactate) across 14 commercial Swiss hard and semi-hard cheeses. A strong correlation (r = 0.979) was observed between the measured and calculated a_w values. Non-protein nitrogen was tested as an alternative to ΣFAAs but yielded a lower correlation. Additionally, o-phthaldialdehyde (OPA) analysis emerged as a simple, cost-effective alternative to ΣFAAs for estimating a_w values, achieving a comparable correlation coefficient (r = 0.980). The study proved that although moisture and NaCl were primary determinants of a_w, ΣFAAs (or OPA reactive substances) resulting from proteolysis and lactate were also crucial for accurate a_w values prediction in ripened hard/semi-hard cheeses. This study provides a robust model for a_w prediction in ripened cheeses, particularly when instrumental measurements are unavailable.

1. Introduction

Water activity (a_w) plays a decisive part in cheese manufacturing, influencing its physical (soft and creamy or crumbly and firm texture), chemical (moisture content), biochemical (enzymes and aroma compounds) and microbiological (growth of bacteria, pathogenic and spoilage-causing microorganisms) properties, and therefore linked to flavour development, quality, stability, shelf-life and safety standards of the final cheese (Marcos, 1993). Water in cheese is present in two forms: "bound" and "free" water. The a_w is an index of the "free" water that is available for bacteria ("freely available" water in a product that microorganisms can use for their growth) and is responsible for the water vapour pressure of the product (Hickey et al., 2013), which is represented as:

$$a_w = p/p_o, \tag{1}$$

where p and p_o are the vapour pressure of the water present in the cheese and pure water at the same temperature (usually at 25 °C), respectively. Due to the presence of various solutes in cheese (salts, lactates, free amino acids and others), the vapour pressure of water in a food system is always less than that of pure water, that is, $a_w < 1.0$. Ionic solutes such as NaCl interact with H_2O via hydrogen bonding or ion-dipole interactions and lowers a_w. Ionic compounds (NaCl) reduce a_w more strongly than hydrophilic or hydrophobic compounds. Lower aw values (e.g. in hard cheeses such as Sbrinz, aw ≈ 0.88): inhibit the growth of many pathogenic and spoilage-causing microorganisms (e.g. listeria, salmonella, mould) and significantly extend shelf life.

Ingredients in raw-milk cheese production are typically simple for most varieties (milk, starter cultures, rennet, NaCl), and the main players in cheese microbiology are primary and secondary starter and adjunct lactic acid bacteria, the milk microbiota and the cheese-manufacturing environment (non-starter lactic acid bacteria). As cheese is a dynamic system, the amount of water is continuously reduced due to dehydration and new solutes arise from microbiological and biochemical processes (e.g. by glycolysis, proteolysis, lipolysis). Consequently, understanding and predicting a_w based on key compositional changes is essential.

The calculation of a_w values from chemical compositional data has been investigated by several authors, driven by academic interest, research needs, practical application using literature data and situations where a_w analysis equipment is unavailable (Grummer & Schoenfuss, 2011; Hickey et al., 2013; Marcos et al., 1981). The a_w values of Cheddar

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cheese (270 d) have been correlated with moisture content (r = 0.65), NaCl (r = −0.09), lactate (r = 0.10) and other low-molecular-weight solutes, such as pH 4.6-soluble N (r = 0.31) or free amino acids (FAAs) (r = −0.42), as found by Hickey et al. (2013). Marcos et al. (1981) observed a correlation between the a_w values of cheese and their chemical characteristics of moisture (r = 0.556), NaCl (r = −0.808) and non-protein nitrogen (NPN, r = −0.671) and calculated a_w values of cheese with the following empirical linear function:

$$a_w = 1.0048 - 0.0386M, \tag{2}$$

where M was the molality (mol kg^{-1}) of NaCl in the aqueous phase of the cheese. The formula was correct for soft cheeses with a moisture content >40 %. The authors suggested that NPN compounds released by proteolysis could be responsible for lowering the a_w values of cheese with a moisture content <40 %. Several empirical equations from multiple regression analysis for estimation of a_w values of various types of cheese have been reviewed (Marcos, 1993), some depending on ash, NaCl, NPN and pH (Rüegg, 1985). For ripened Emmental-type cheese, an empirical model by linear multiple regression was found by Saurel et al. (2004), who incorporated free NH_2 groups as indicators for cheese proteolysis (Pajonk, 2001), as expressed in Formula 3.

$$a_w = 1.066 - 0.194X_{water} - 3.490X_{NaCl} - 0.331X_{NH2} + 6.509X_{water}{*}X_{NaCl} + 0.571X_{water}{*}X_{NH2}, \tag{3}$$

with the a_w values expressed as a function ($R^2 = 0.92$) of three contents: X_{water}, X_{NaCl} and X_{NH2}. The parameters X_{water}, X_{NaCl} and X_{NH2} represent the content of water, NaCl and free NH_2, expressed as mole equivalent glycine per kg cheese.

Although empirical models exist, a more fundamental approach using Raoult's law was explored by Grummer et al. (2011) to calculate a_w values for Cheddar cheese with 1.6 % salt and 36.8 % moisture (w/w) (Formula 4).

$$a_w = \gamma s \frac{n_{water}}{(n_{water} + n_{solutes})} = 1x\frac{20.4263}{20.4263 + 2x0.27379} = 0.974, \tag{4}$$

where γs = activity coefficient (=1), n_{water} = number of moles water and $n_{solutes}$ = number of moles solutes (Grummer et al., 2011).

However, using only moisture and salt, the calculated a_w value of 0.974 was much higher compared with the measured values for similar Cheddar cheeses with an a_w of 0.955 (Grummer et al., 2011), 0.96 (Marcos et al., 1981) or 0.956 (Hickey et al., 2013). This indicates that other osmotically active solutes significantly contribute to a_w reduction. Indeed, during ripening, lactose decreases while total lactate increases. Simultaneously, pH 4.6-soluble "N" increased from 3.4 to 24 % (% total nitrogen (TN)) and total FAAs increased from 91 to 1075 mg 100 g^{-1} cheese, while the mean a_w values decreased from 0.964 to 0.956. These observations highlight a gap in accurately predicting a_w using basic Raoult's law, particularly in ripened cheeses, in which proteolysis generates significant amounts of low-molecular-weight solutes.

This hypothesis guiding this study is that extending Raoult's law by incorporating not only moisture and NaCl but also lactate and FAAs (renormalisation of mole fractions) would lead to a more accurate prediction of a_w in long-ripened cheeses. The aim was, therefore, to develop and validate such an extended model using 14 different commercial hard and semi-hard cheese varieties from the Swiss market and to explore simpler analytical alternatives, such as OPA value and NPN, for quantifying proteolysis products.

2. Materials and methods

2.1. Cheese sample collection and gross composition

A total of 14 different cheese samples were randomly collected from the Swiss market, with ripening times from 4 to 30 months (Table 1). Moisture content was determined by the dry matter method ISO 5534:2004 (IDF 4:2004) by calculating the weight difference of the cheese sample after drying at 102 °C for 4 h. NaCl was calculated after analysing chlorine by an argentometric method according to ISO 5943:2006 (IDF 88:2006). The sum of the FAAs was analysed according to an in-house HPLC method (Kopf-Bolanz et al., 2012). The total lactate was analysed enzymatically according to ISO 8069:2005 (IDF 69:2005) with device-specific adaptation of the instructions of the kit manufacturer (R-Biopharm, Darmstadt, Germany).

Fat in cheese was analysed according to Gerber van Gulik (ISO 3433: 2008). To determine the levels of primary amines with o-phthaldialdehyde (OPA method), we followed Frister et al. (1986), Kopf-Bolanz et al. (2012) and ISO/CD 24167:2024(E) and IDF 261:2024 (E) (Annex E). Non-protein nitrogen (NPN) was analysed as 120 g kg^{-1} Trichloroacetic acid-soluble nitrogen (ISO 27871/IDF 224). TN was analysed according to the Kjeldal nitrogen method (ISO 17837).

2.2. Sample preparation

The samples were prepared according to Fig. 1. The blue spot was reserved for a_w sample preparation. The cheese material around the blue spot (inside the square) was used for chemical analysis to overcome the zonal variations. All analyses were performed in duplicate (n = 2).

2.3. Analysis of the a_w value

To determine the a_w values, we followed ISO Norm 18787:2017 using the a_w Sprint system and an electrolytic resistance sensor (Novasina, CH-8853 Lachen, Switzerland) together with a redox filter. The redox filter was installed during the calibration with six standards from 0.11 to 0.98 a_w [−] (Novasina, CH-8853 Lachen, Switzerland) and during analysis of the cheese samples. Novasina advised the use of a redox filter for cheese to reduce the interference of the sensor with volatile aroma compounds, due to high metabolic activity in the cheese. The a_w values of the cheese samples are reported as mean values by successively analysing two individual cheese samples from the same cheese.

2.4. Calculation of a_w values from cheese compositional content

The following formulas (5 - 8) were applied for a comparison of the analysed a_w values and the calculated a_w values compared by formula 4. In chemistry, this process is often referred to as renormalisation of mole fractions when an existing mixture of substances (e.g. water + NaCl) is expanded (e.g. with amino acids, lactate, etc.) so that the denominator is adjusted accordingly.

$$a_w = \frac{nMoisture}{(nMoisture + I{*}nNaCl + J{*}n\sum FAAs + K{*}nLactate)} \tag{5}$$

$$a_w = \frac{nMoisture}{(nMoisture + I{*}nNaCl + J{*}nOPA + K{*}nLactate)} \tag{6}$$

$$a_w = \frac{nMoisture}{(nMoisture + I{*}nNaCl + J{*}nGlutamate + K{*}nLactate)} \tag{7}$$

$$a_w \frac{nMoisture}{(nMoisture + I{*}nNaCl + J{*}nNPN + K{*}nLactate)} \tag{8}$$

where n is the number of moles of the components, and I, J and K are the van't Hoff factors, which describe the extent to which a substance (e.g. $NaCl \rightarrow Na^+ + Cl^-$) dissociates in a solution. Sodium chloride dissociates into two ions (Na^+ and Cl^-) in water, so its van 't Hoff factor is 2. For non-electrolytes, e.g. I would be approximately 1, as they don't dissociate or associate. The van 't Hoff factor is the ratio between the actual concentration of particles produced when the substance is dissolved and

Table 1
The 14 different cheese samples from the Swiss market and their main contents. Percentages refer to weight/weight specifications. (Average ± standard deviation from n = 2 determinations, for relevant compounds.)

Cheese type (n = 14)	Age (Months)	Moisture [%]	NaCl [%]	NaCl-in-moisture[f] [%]	FDM[a] [%]	pH [−]	NPN[b] [mol kg^{-1}]	TN[c] [%]	ΣFAA[d] [mol kg^{-1}]	Fat [%]	OPA[e] [mol kg^{-1}]	D-lactic acid [mmol kg^{-1}]	L-lactic acid [mmol kg^{-1}]	Lactate [mol kg^{-1}]
Gruyère PDO (doux)	6	35 ± 0.0	2.24 ± 0.01	33.5	51.5	5.51	0.556 ± 0.001	4.01	0.237 ± 0.031	33.5	0.3192 ± 0.007	75.2	51.1	0.1263 ± 0.001
Gruyère PDO (mi-salé)	8	36.2 ± 0.0	2.12 ± 0.0	32.5	50.9	5.57	0.751 ± 0.003	3.98	0.366 ± 0.011	32.5	0.4506 ± 0.005	75.3	34.5	0.1098 ± 0.001
Vacherin Fribourgeois	4	42 ± 0.0	1.98 ± 0.01	30.9	53.3	5.49	0.486 ± 0.004	3.62	0.131 ± 0.002	30.9	0.2050 ± 0.006	11.7	55.6	0.0673 ± 0.00
Tête de Moine PDO	4	35.1 ± 0.1	1.96 ± 0.0	34.8	53.6	5.78	0.48 ± 0.001	4.01	0.209 ± 0.004	34.8	0.2664 ± 0.001	46.7	32	0.0787 ± 0.00
Tête de Moine PDO	6	34.7 ± 0.1	1.92 ± 0.01	34.6	53.0	5.88	0.623 ± 0.002	4.14	0.26 ± 0.003	34.6	0.3283 ± 0.005	41.3	34	0.0753 ± 0.001
L'Etivaz	11	32.7 ± 0.1	2.18 ± 0.01	34.8	51.7	5.55	0.646 ± 0.019	4.12	0.277 ± 0.016	34.8	0.3740 ± 0.004	62.8	63.1	0.1258 ± 0.00
Sbrinz PDO	30	29.7 ± 0.0	2.31 ± 0.01	31.8	45.2	5.59	0.797 ± 0.00	4.89	0.363 ± 0.004	31.8	0.4778 ± 0.001	99.1	60.4	0.1595 ± 0.002
Parmesan	24	35.1 ± 0.0	1.29 ± 0.01	27.7	42.7	5.49	0.888 ± 0.008	4.85	0.431 ± 0.004	27.7	0.5476 ± 0.002	90.5	79.3	0.1698 ± 0.001
Berner Alpkäse PDO	10	28.1 ± 0.1	1.41 ± 0.01	39.8	55.4	5.5	0.61 ± 0.001	4.15	0.212 ± 0.004	39.8	0.3103 ± 0.005	136.6	46.3	0.183 ± 0.001
Emmentaler PDO	5	37.7 ± 0.1	0.36 ± 0.01	30.9	49.6	5.54	0.374 ± 0.00	4.33	0.116 ± 0.004	30.9	0.1647 ± 0.000	39.2	42.3	0.0815 ± 0.00
Emmentaler PDO	8	35.1 ± 0.1	0.33 ± 0.01	32.2	49.6	5.76	0.724 ± 0.004	4.57	0.245 ± 0.001	32.2	0.3519 ± 0.001	0	50.7	0.0507 ± 0.00
Appenzeller PDO Surchoix	5	37.1 ± 0.1	1.42 ± 0.01	32.4	51.5	5.57	0.463 ± 0.005	4.12	0.132 ± 0.008	32.4	0.2036 ± 0.007	54.2	54.2	0.1084 ± 0.00
Appenzeller PDO	9	37.3 ± 0.0	1.72 ± 0.05	31.6	50.4	5.85	0.863 ± 0.002	4.23	0.308 ± 0.038	31.6	0.4575 ± 0.006	19.8	19.8	0.0396 ± 0.00
Raclette	4	41.8 ± 0.0	2.32 ± 0.01	27.0	46.4	5.8	0.475 ± 0.008	4.18	0.122 ± 0.013	27.0	0.1993 ± 0.000	15	30.3	0.0453 ± 0.00

[a] FDM: fat in dry matter.
[b] NPN: non-protein nitrogen.
[c] TN: total nitrogen.
[d] ΣFAA: total of free amino acids.
[e] OPA: o-phthaldialdehyde.
[f] NaCl in moisture: refers to *the ratio of sodium chloride (NaCl) to moisture content in cheese.*

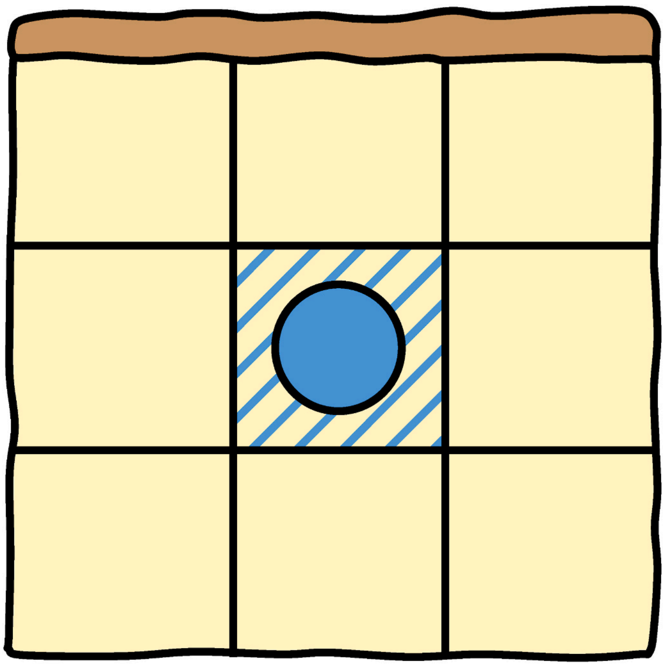

Fig. 1. Schematic top-down view of a cheese divided into nine equal sections. The blue circle indicates the location where water activity was measured (n = 2). The surrounding central section (blue-striped) was used for further chemical analyses in different laboratories, including moisture, NaCl, ΣFAAs, lactate and other parameters as listed in Table 1.

the formal concentration that would be expected from its chemical formula.

Instead of $\sum FAAs$, (formula 5), OPA, Glutamate or NPN was tested supplementary in formulas 6-8 as alternatives. OPA, glutamate or NPN are cheaper analyses compared to $\sum FAAs$.

2.5. Statistical analysis

Correlations were calculated using R (www.r-project.org, version: 4.4.2) and the library (correlation). The *p*-value adjustment method was used according to Holm, after multiple testing (Holm, 1979). Calculated and analysed data were also compared using Root Mean Square Deviation (RMSD). RMSD is calculated by taking the square root of the average of the squared differences between measured and calculated a_w results of the 14 cheese samples.

The data were also used to determine possible correlations between a_w values (response variable) and explanatory variables (e.g. NaCl, moisture, ∑FAA and lactate) by multiple linear regression with df as the degree of freedom.

3. Results and discussion

We begin by characterising the cheese samples before evaluating the proposed models for a_w prediction.

3.1. Cheese composition

Table 1 presents the ripening time, pH and main chemical components, such as moisture, NaCl, NaCl-in-moisture, ΣFAAs, D-, L-lactate, fat, fat in dry matter (FDM), primary amines with OPA, NPN and TN of the 14 different cheese samples. The samples were all prepared using the same procedure, according to Fig. 1, to ensure comparable analysis of the cheese samples. The ranges for moisture and NaCl were in the expected range, exhibiting Sbrinz and "Berner Alpkäse" (Jakob et al., 2007), which are known for very low moisture contents, and Emmentaler PDO (5 and 8 months) displaying very low NaCl (Fröhlich-Wyder et al., 2022; Fröhlich-Wyder et al., 2025) contents (0.33–0.36 %) (Table 1). Gruyère, L'Etivaz, Sbrinz and Raclette (Marcos, A., Alcalá, M., León, F., Fernández-Salguero, J. & Esteban, 1981) are known to have high NaCl contents, depending on their age and their specifications. The ΣFAA values depend on the ripening age and biochemical processes. The longer the cheese ripening process, the higher the ΣFAA value. Sbrinz and Parmesan are generally very long-ripened cheeses; therefore, they showed high ΣFAA values in this study. The lactate content of cheeses is highly dependent on biochemical processes. We found low levels for Emmentaler PDO, Appenzeller PDO and Raclette. Eye formation, mainly in Emmentaler, is a process, where the lactate is reduced by fermentation and CO_2 is produced. Fat, FDM and pH were in the expected ranges but were not relevant for a_w values based on initial correlations.

Analysis of OPA, NPN and TN (Table 1) showed values in the expected ranges. A conversion factor of 6.38 times TN was used to calculate the protein content. The protein, moisture and fat content were almost 100 %. The lowest OPA value was found for an Emmentaler AOP cheese with 5 months of ripening. Parmesan cheese (ripened for 24 months) showed the highest value for OPA. The compositional data (moisture, NaCl, ΣFAA and lactate) formed the basis for subsequent modelling.

3.2. a_w values of commercial cheese samples

The analysed a_w values are presented in Table 2. The lowest value was found for Sbrinz (a_w = 0.885, ripened for 30 months), whereas Emmentaler PDO with low NaCl content was high in a_w (a_w = 0.976 or 0.954), as expected. Most of the a_w values from the analysed cheese samples can be compared to a_w values published earlier in the literature (Marcos, 1993; Marcos et al., 1981; Bisig et al., 2025).

3.3. Correlation of analysed a_w values and main parameters of 14 cheese samples

The a_w values of the cheese samples were positively correlated with moisture content and negatively correlated with age and NaCl, NaCl-in-moisture, OPA, NPN, lactate and ΣFAA levels (Table 3). The highest correlation was found between a_w and NaCl-in-moisture, with a correlation coefficient of r = −0.84, as expected, due to the standardisation of the NaCl content by the moisture content. Marcos et al. (1981) found in his data set (34 European cheese varities) a similar correlation coefficient r = - 0.81. However, Hickey et al. (2013) reported decreasing or lower correlation coefficients (r = −0.74 => r = −0.28) for Cheddar cheese during the ripening from day 1 to day 270. During ripening the

Table 2
The 14 different cheese samples from the Swiss market and their analysed a_w – values (Average ± standard deviation from n = 2 determinations).

Cheese type (Name)	Age (Months)	a_w value [−] analysed	Cheese number
Gruyère PDO (doux)	6	0.921 ± 7.07E-04	1
Gruyère PDO (mi-salé)	8	0.916 ± 1.41E-03	2
Vacherin Fribourgeois	4	0.950 ± 1.41E-03	3
Tete-de-Moine PDO	4	0.931 ± 2.12E-03	4
Tete-de-Moine PDO	6	0.928 ± 2.12E-03	5
L'Etivaz	11	0.912 ± 7.07E-04	6
Sbrinz PDO	30	0.885 ± 7.07E-04	7
Parmesan	24	0.923 ± 7.07E-04	8
Berner Alpkäse PDO	10	0.922 ± 2.83E-03	9
Emmentaler PDO	5	0.976 ± 7.07E-04	10
Emmentaler PDO	8	0.954 ± 7.07E-04	11
Appenzeller PDO Surchoix	5	0.950 ± 1.41E-03	12
Appenzeller PDO	9	0.928 ± 7.07E-04	13
Raclette	4	0.943 ± 7.07E-04	14

Table 3
Parameters correlating with a_W values; the p-value adjustment method was used according to Holm for the 14 cheese samples.

Parameter:	Correlation coefficient (r)	p-value
Moisture	0.63	n.s.
Age	−0.67	n.s.
NaCl	−0.67	n.s.
NaCl-in-moisture	−0.84	0.003**
OPA[a]	−0.72	<0.05*
NPN[b]	−0.62	n.s.
Lactate	−0.58	n.s.
ΣFAAs[c]	−0.73	<0.05*

n.s.: not significant, *: $p < 0.05$; **: $p < 0.005$.
[a] OPA: o-phthaldialdehyde.
[b] NPN: non-protein nitrogen.
[c] ΣFAAs: total of free amino acids.

interplay of moisture and salt and newly produced proteolysis products must be taken into account.

In our study, no correlation was found for fat, TN and pH, supporting their exclusion from the primary a_w prediction models.

3.4. Multiple linear regression analysis with independent variables (moisture, NaCl, ΣFAAs and lactate)

To further understand the combined influence of the selected parameters, a multiple linear regression model was applied. Regression analysis aims to predict the value of a dependent variable (a_w values) based on one or more independent variables, whereas correlation analysis quantifies the strength and direction of the linear relationship between two variables.

The model, estimating the relationship between the quantitative dependent variable (a_w values) and four independent variables (moisture, NaCl, ΣFAAs and lactate), showed a sufficient explanatory contribution ($F(4, 9) = 99.79$, $p < 0.001$). The model quality was high ($R^2 = 0.978$, adjusted $R^2 = 0.968$). Moisture ($b = -2.62 \times 10^{-4}$, $p < 0.001$), NaCl ($b = 2.03 \times 10^{-3}$, $p < 0.001$) and ΣFAAs ($b = -8.98 \times 10^{-5}$, $p < 0.001$) were significant predictors of the a_w values. Lactate ($b = -6.55 \times 10^{-6}$) was not a statistically significant predictor in this specific regression model ($p > 0.05$), although its role in the Raoult's law-based model was still considered important due to its known osmotic activity. NaCl exerted the greatest influence on a_w, followed by moisture, ΣFAAs and lactate.

3.5. Comparison of analysed and calculated a_w values using formula 5 (moisture, NaCl, ΣFAAs, lactate)

We tested the primary hypothesis that incorporating moisture, NaCl, ΣFAAs and lactate into a model of an extended Raoult's law (Formula 5) would accurately predict a_w. The analysed a_w values were generally slightly lower than the calculated a_w values according to Formula 5 (Fig. 2a). Two exceptions were found for Parmesan cheese and young Emmentaler PDO (aged for 5 months), both of which had calculated a_w values that were slightly lower than the analysed values. For Parmesan, the low NaCl content was associated with a very high content of ΣFAAs, whereas the young Emmentaler PDO, which showed a very low NaCl content, had a high moisture content. These data are shown in Fig. 2a and b. A strong correlation coefficient of $r = 0.979$ and $RMSD = 0.0086$ was achieved between the analysed and calculated values, supporting the validity of this extended model. A comparison to Formula 4, without ΣFAAs and lactate resulted in $r = 0.83$ and a RMSD of 0.042.

For Formula 5, the Van't Hoff factors I, J and K were set to 2, assuming complete dissociation for NaCl and analogous behaviour for total FAAs and lactate under these modelling conditions. Lactic acid's dissociation in cheese is governed by its low pKa of approximately 3.86 and the higher cheeses pH (5.49–5.88). At pH 6, over 99 % of the lactate would be dissociated. Additionally, hard and semi-hard cheese contains different types of ΣFAAs, each with its own set of acidic and basic side chains and associated pKa values. The specific amino acids and their quantities vary depending on the type of cheese, but hard cheeses are particularly rich in free glutamate (Jakob et al., 2007). In the slightly acidic environment of hard or semi-hard cheese (5.49–5.88), free amino acids, like those found in cheese, exist primarily as zwitterions, where the carboxyl group has lost a proton (becoming $-COO^-$) and the amino group has gained a proton (becoming $-NH_3^+$).

This result suggests that these four components (moisture, NaCl, ΣFAAs and lactate) are indeed major contributors to a_w in long-ripened cheeses.

3.6. Comparison of analysed and calculated a_w values using formula 6 (OPA as an alternative to ΣFAAs)

Given the complexity of FAA analysis, a simpler alternative using OPA values was explored (Formula 6). The correlation coefficient with OPA was found to be $r = 0.980$, which was comparable to the correlation with ΣFAAs or slightly higher and $RMSD = 0.0042$, which is even lower (and therefore better) than RMSD of Formula 5 (Fig. 3a and b). The indexes I, J and K in Formula 6 were set with 2 (number of ions). The difference between ΣFAAs and OPA could be explained by the fact that

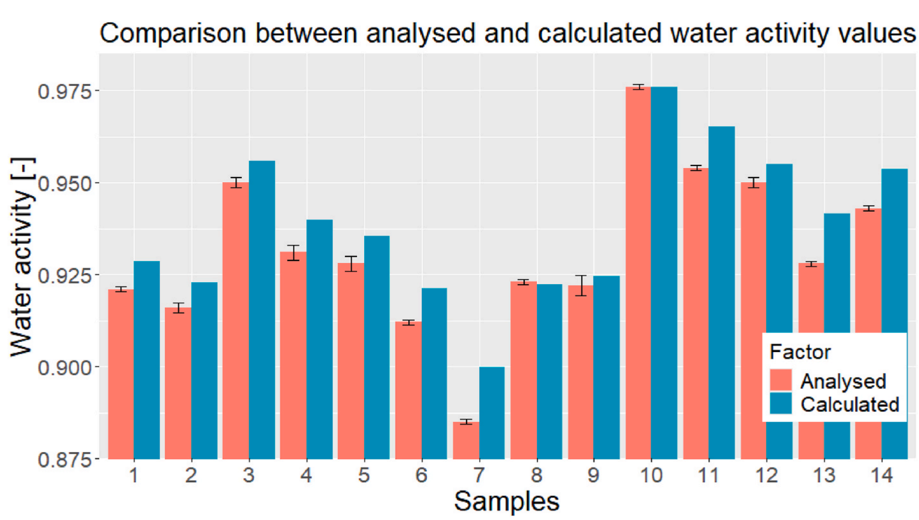

Fig. 2a. Comparison of analysed and calculated water activity values using Formula 5 with 4 parameters: moisture, NaCl, ΣFAAs and lactate.

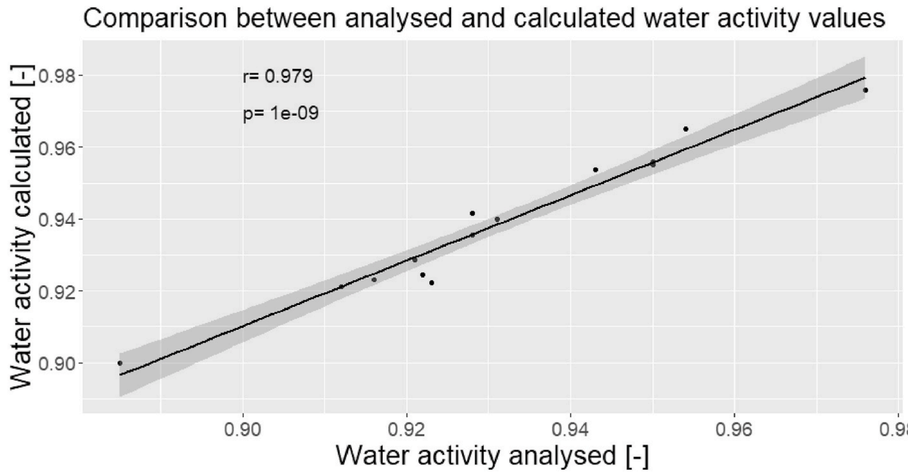

Fig. 2b. Correlation of analysed and calculated water activity values using Formula 5 with 4 parameters: moisture, NaCl, ΣFAAs and lactate.

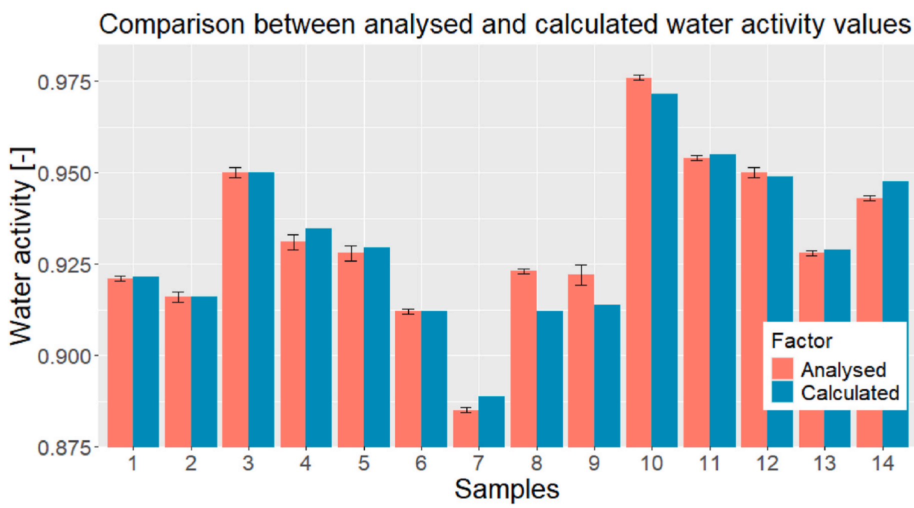

Fig. 3a. Comparison of analysed and calculated water activity values using Formula 6 with four parameters: moisture, NaCl, OPA and lactate.

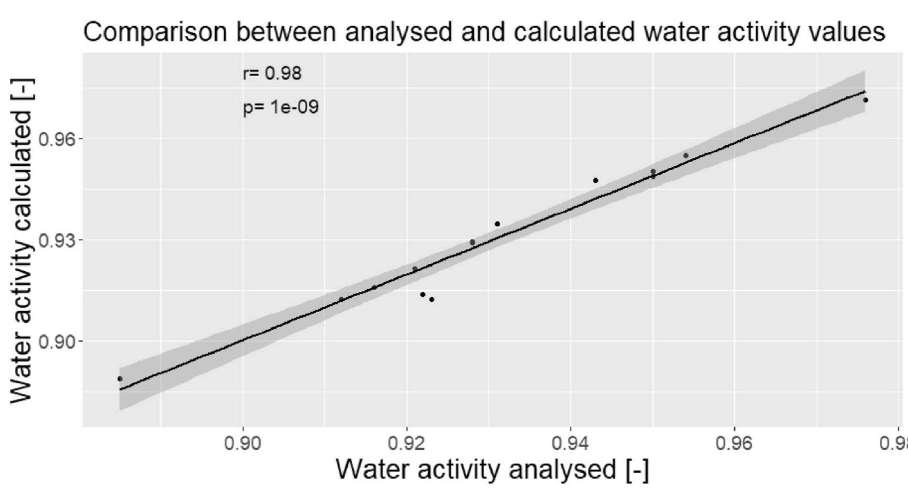

Fig. 3b. Correlation between analysed and calculated water activity values using Formula 6 with four parameters: moisture, NaCl, OPA and lactate.

the OPA values also included small peptides and possibly biogenic amines that would additionally decrease the a_w values. Thus, the OPA method is comparable to or even better than formula 5. Therefore, OPA analysis demonstrated its potential as a valid and cost-effective alternative to the more elaborate FAA method for a_w prediction, offering a practical advantage.

3.7. Comparison of analysed and calculated a_w values using formulas 7 & 8 (NPN as an alternative)

NPN, another measure of proteolysis, was also tested. The comparison of analysed a_w and calculated a_w values according to Formula 7 (using NPN values transformed into equivalents of glutamate, the major amino acid in cheese) and Fig. 4a and b yielded a lower correlation of r = 0.932 or RMSD = 0.026. The indexes *I*, *J* and *K* in Formula 7 were set at 2. The calculated contribution of NPN (as glutamate equivalents) obviously underestimated the quantity of ΣFAAs.

We tested an alternative approach with Formula 8 (Fig. 5a and b), using a van't Hoff factor of $J = 1.15$ instead of 2. The value of *J* corresponds to around 57 % of free R-NH_2, expressed as mole equivalent nitrogen per kg of cheese. The reason for selecting a smaller number of index *J* was justified by the fact that NPN not only considers free amino acids but also other compounds, such as ammonia, urea, sialic acid and others. Using NPN together with a $J = 2$ for a_w value estimation resulted in a clear overestimation.

To further investigate this outcome, we conducted a total amino acid analysis in NPN solutions, which showed that the amino acid content in NPN varied from 51 to 78 %, with a mean value of 67 % (data not shown). These results suggest that NPN is of limited use for accurately calculating a_w values in this specific Raoult's law context, primarily due to the variable proportion of osmotically active FAAs within the NPN fraction and the presence of other compounds.

3.8. Factors not included in the models

Generally, we found that TN, lactose, FDM, fat (compounds that are not dissociated) and the age or pH of the cheese were not relevant or not necessary for the calculation of a_w values in these extended Raoult's law models. Further, free Ca^{2+} and other minerals (Mg^{2+}, K^+ and Zn^{2+}) were not considered or found to be necessary for the estimation of a_w values. Free volatile carbonic acids were also not considered relevant, as these acids were partly present in an undissociated form (pKa acetic acid = 4.75, compared to pKa lactic acid = 3.86). Other compounds, such as biogenic amines, citrates, dipeptides, tripeptides (considered only by OPA) or other minor compounds in the cheeses were also not considered relevant due to the low contents or absence of studied in light of their overall osmotic impact. The focus on moisture, NaCl, ΣFAAs/OPA and lactate for robust a_w prediction appears justified in these cheese types. Additionally, for all calculations with formulas 5–8, an activity coefficient of 1 was chosen, supposing that the components moisture, NaCl, ΣFAAs/OPA and lactate behave as "ideal" components. A model with 4 components and $\gamma = 1$ was found to be a reasonable and simple approximation.

4. Conclusion

This study aimed to provide further knowledge on the usefulness of calculating a_w values from compositional data in hard and semi-hard cheese, specifically by extending Raoult's law. Cheese is a dynamic (non-steady state) and complex system, making precise interactions between solutes and the water–protein matrix difficult to predict. However, the primary hypothesis was supported: the suggested Formula 5, incorporating moisture, NaCl, ΣFAAs and lactate with van't Hoff factors set to 2, successfully predicted a_w values for the 14 long-ripened hard or semi-hard cheese samples (r = 0.979). This demonstrates that these four components are key osmolytes that determine a_w in such cheeses and that the postulated Van't Hoff factor of 2 was indeed realistic. Although direct instrumental analysis of a_w values is straightforward and inexpensive, this calculation method offers a valuable tool when direct measurements are unavailable (e.g. when using literature data or in settings with limited analytical capabilities), particularly for long-ripened cheeses.

Furthermore, the OPA method (Formula 6) emerged as a highly promising, cost-effective and equally accurate alternative to ΣFAAs analysis for a_w estimation (r = 0.980). Compared to the ΣFAAs analysis, OPA values include slightly more amines (small peptides and possible existing biogenic amines), which supports its utility as a cheaper and simpler analytical option for this modelling purpose. By contrast, NPN showed limited applicability for this specific modelling approach. These models enhance the understanding of the factors governing a_w activity and provide practical, validated tools for its estimation in long-ripened cheeses.

The models mentioned above only includes the 14 hard and semi-hard cheeses mentioned, all of which are very different and have strong links to Switzerland. Soft cheeses and cream cheeses were deliberately not included, as there are already published models for predicting their a_w values. Mould-ripened cheeses are also not included (such as Formaggio d'Alpe Ticinese or Tomme de Savoie). The models were not tested in these cases.

CRediT authorship contribution statement

Dominik Guggisberg: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Reto Portmann:** Writing – review & editing, Validation, Investigation, Formal analysis, Conceptualization. **Lotti Egger:** Writing – review & editing, Validation,

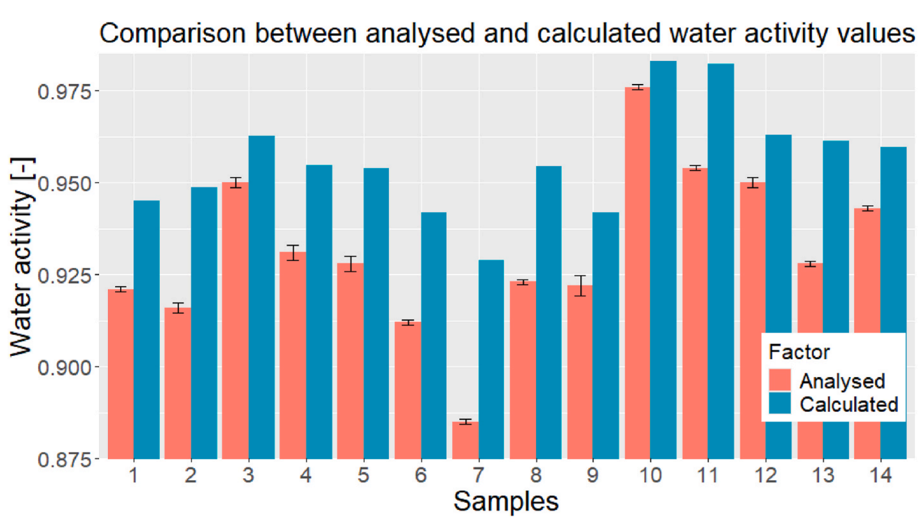

Fig. 4a. Comparison of analysed and calculated water activity values using Formula 7 with four parameters: moisture, NaCl, NPN (glutamate) and lactate.

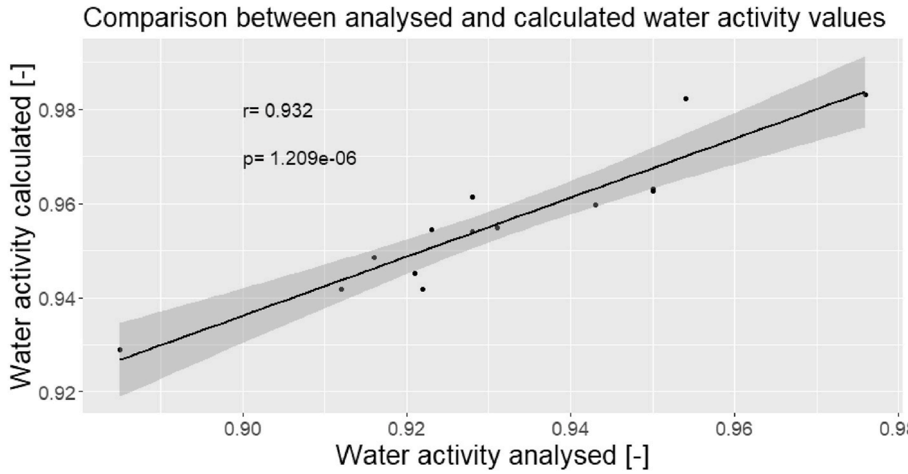

Fig. 4b. Correlation between analysed and calculated water activity values using Formula 7 with four parameters: moisture, NaCl, NPN (glutamate) and lactate.

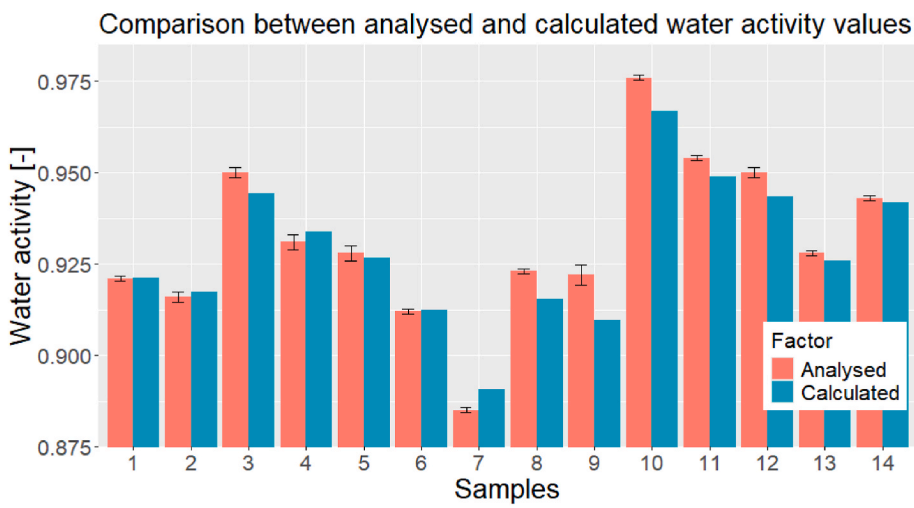

Fig. 5a. Comparison of analysed and calculated water activity values using Formula 8 with four parameters: moisture, NaCl, NPN (1.15 mol) and lactate.

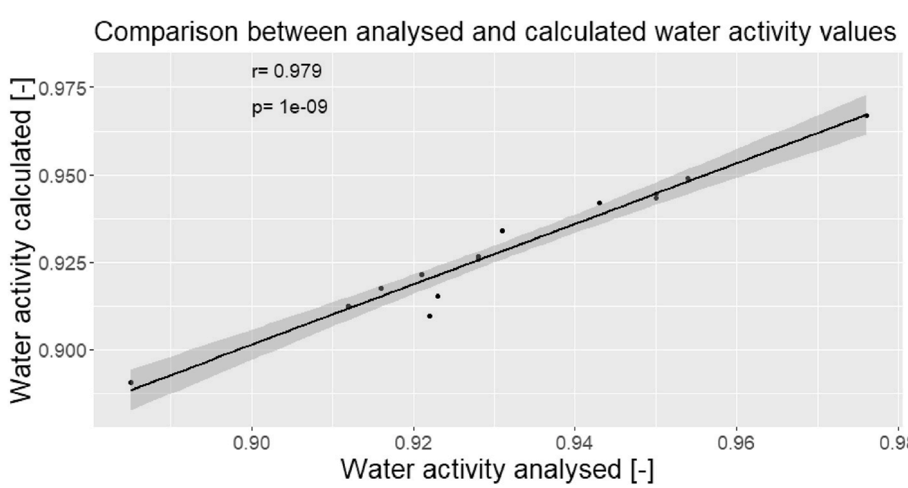

Fig. 5b. Correlation between analysed and calculated water activity values using Formula 8 with four parameters: moisture, NaCl, NPN (1.15 mol) and lactate.

Investigation, Data curation. **Remo S. Schmidt:** Writing – review & editing, Supervision, Resources, Project administration.

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Declaration of competing interest

The authors declare that there is no financial interest that could have influenced the work in this paper.

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Data availability

Data will be made available on request.

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