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In Vivo Sodium Release and Saltiness Perception in Solid Lipoprotein Matrices. 1. Effect of Composition and Texture

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ABSTRACT: Reducing the sodium content in foods is complex because of their multidimensional sensory characteristics and the multifunctionality of sodium chloride. The aim of this study was to elucidate how food composition may influence in-mouth sodium release and saltiness perception. Lipoprotein matrices (LPM) were produced using milk constituents and characterized by means of rheological measurements, texture, and taste sensory profiles. Texture and taste perceptions were affected differently by variations in the salt level, dry matter, and fat contents. Composition and textural changes also modified temporal sodium release and saltiness perception recorded in five subjects, but the effects varied as a function of the salt content. The water content mainly appeared to influence the amount of sodium released, whereas saltiness perception was mainly related to fat content. Elasticity, coating, and granularity were found to be correlated with temporal sodium release and/or saltiness parameters.

KEYWORDS: sodium release, saltiness, time intensity, food composition, rheology, lipoprotein matrices, sensory profile

■ INTRODUCTION

Hypertension and other serious conditions are linked to an excessive sodium intake from foodstuffs.¹ To reduce the risk of such diseases, the health authorities recommend a reduction in the sodium chloride (salt) content of foods to a level closer to individual physiological needs.^{2–4} However, sodium chloride is a major ingredient in foods because of its numerous properties. For example, it is generally considered to be a taste enhancer, thus improving overall food acceptance and preferences. Consequently, any reduction in the sodium chloride content of foodstuffs may have only limited success because of the adverse sensory effects that might render such foods less attractive. These hurdles could be overcome if acceptable taste intensity was maintained while reducing sodium levels in foods. Numerous strategies have been adopted by food manufacturers to formulate low-salt foods: incremental salt reduction,^{5–7} mineral replacers,^{6,7} optimization of the physical form of salt,⁸ and saltiness enhancement.^{9,10} However, these solutions present several limitations that may restrict their use in all types of foods.

An alternative strategy consists of modifying the gross composition of foods to enable better salt release in the mouth during food oral processing leading to food breakdown. This implies a clear understanding of the relationships between food composition, texture, and flavor perception. Much less attention has been paid to the impact of product structure and composition on taste perception than on aroma perception, and these efforts have mainly focused on sweetness. These works on taste perception generally demonstrated that an increase in the viscosity or hardness of foodstuffs induced a

reduction in taste intensity.^{11–13} The use of model custard desserts with an identical composition but different viscosities achieved by means of a mechanical treatment demonstrated that an increase in viscosity induced a reduction in sweetness perception.¹⁴ The same phenomenon was observed in low-fat yogurts of the same chemical composition but different degrees of viscosity.¹³ This effect mainly resulted from reduced mass transfer of taste compounds from the food matrix into the saliva before the taste stimuli dissolved in saliva reached the taste receptors mainly located on the tongue. An increase in the viscosity of sucrose solutions induced a reduction in the diffusion coefficient and hence in sweetness perception.¹⁵ In the same way, the measurement of sucrose diffusion coupled to an evaluation of sweetness in carrageenan gels showed that as firmness increased, the diffusion coefficient of sucrose decreased.¹⁶ A stronger network structure of the food matrices thus induced a reduction in sucrose diffusivity and a decrease in sweetness.

The effects of a food matrix on taste perception change according to the type of taste compound. A reduction in bitterness and an increase in saltiness were observed in line with the degree of matrix breakdown in Camembert cheese.¹⁷ This increase in saltiness, attributed to a greater availability of minerals, corroborated findings that more salt was released from a soft cheese than from a hard cheese.^{18–20} A more recent

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Table 1. Formulation of Lipoprotein Matrices (LPM) (for 1 kg)

LPM name ^a	salt (%)	DM ^b (g/kg)	fat (%/DM)	pH at renneting	water (g)	fat (g)	PM ^c (g)	NaCl (g)	rennet ^d (mL)
S3D2F2P2	1.5	440	40	6.5	543	176	281	15	18
S2D2F2P2	1	440	40	6.5	543	176	281	10	15
S1D2F2P2	0.5	440	40	6.5	543	176	281	5	9
S2D2F2P1	1	440	40	6.2	543	176	281	10	10
S1D2F2P1	0.5	440	40	6.2	543	176	281	5	7.6
S3D2F1P2	1.5	440	20	6.5	537	88	375	15	18
S2D2F1P2	1	440	20	6.5	537	88	375	10	15
S3D2F1P1	1.5	440	20	6.2	537	88	375	15	12
S1D2F1P1	0.5	440	20	6.2	537	88	375	5	7.6
S3D1F2P2	1.5	370	40	6.5	616	148	236	15	18
S1D1F2P2	0.5	370	40	6.5	616	148	236	5	9
S3D1F2P1	1.5	370	40	6.2	616	148	236	15	12
S2D1F2P1	1	370	40	6.2	616	148	236	10	10
S2D1F1P2	1	370	20	6.5	611	74	315	10	15
S1D1F1P2	0.5	370	20	6.5	611	74	315	5	9
S3D1F1P1	1.5	370	20	6.2	611	74	315	15	12
S2D1F1P1	1	370	20	6.2	611	74	315	10	10
S1D1F1P1	0.5	370	20	6.2	611	74	315	5	7.6

^aLPM names are as follows: S1, S2, and S3 correspond to salt level (0.5, 1, and 1.5%, respectively); D1 and D2 correspond to DM level (370 and 440 g/kg, respectively); F1 and F2 correspond to fat to DM ratio of 20 and 40%, respectively; P1 and P2 indicate pH values of 6.2 and 6.5, respectively. ^bDM, dry matter. ^cPM, powdered milk. ^dThe rennet was previously diluted to 1/10 in pure water (Millipore system). The amount of rennet added to LPM varied as a function of the salt level and pH value.

study²¹ showed that sodium release in model cheese matrices mainly appeared to be affected by the water content, and saltiness by the fat content, with temporal intra-individual differences during the in-mouth process. However, this experiment was performed in a context of few composition variables; a more wide-ranging design would have been necessary to produce more consistent conclusions.

Even though the sodium concentration is globally related to saltiness perception, few direct relationships between temporal saltiness perception and in-mouth temporal sodium release parameters were found.^{21–23} One reason is that food composition differently affects the two phenomena. Very few studies have so far reported the effect of matrix on saltiness perception using an experimental design that integrated several defined composition factors.

The aim of this study was therefore to investigate the effect of changes in the food matrix composition on sensory and rheological properties, in-mouth sodium chloride release, and temporal saltiness perception. Because cheese is one of the foods most generally targeted as a sodium vector, the models chosen were dairy lipoprotein matrices, which were varied in terms of their fat, dry matter, and salt contents and pH at renneting.

MATERIALS AND METHODS

Experimental Food Products. Eighteen lipoprotein matrices (LPM) were developed to provide a simple dairy solid food model that could be studied under controlled conditions, without draining, and would enable acceptable reproducibility. The LPM were produced in accordance with an incomplete experimental design that included two levels of dry matter content (370 and 440 g/kg), two fat content levels (20 and 40%/dry matter), three salt content levels (0.5, 1, and 1.5%), and two pH at renneting levels (6.2 and 6.5). This experimental design is a D-optimal design, generated using the procedure Optex of the SAS software (SAS Institute Inc., Cary, NC, USA). The dry matter was mainly made of fat and crude powdered milk. The LPM recipes included anhydrous milk fat (Cormans, Goe-Limbourg, Belgium), skimmed milk powder (Eurial Poitouaine, Nantes, France), sodium chloride (Jerafrance, Jeufosse, France), rennet (Labo ABIA, Meursault,

France), pure water (Milli-Q system, Millipore, Bedford, MA, USA), δ -gluconolactone (GDL) (Sigma-Aldrich, Saint Quentin Fallavier, France) or 1 N NaOH (Chem-Lab NV, Zedelgem, France), and a solution of odorous compounds made up of 90 μ L of diacetyl (buttery aroma), 12 μ L of 2-heptanone (blue cheese aroma), and 9 μ L of ethyl hexanoate (fruity aroma) mixed in 1 mL of polyethylene glycol per kilogram of LPM (Sigma-Aldrich). The compositions of the 18 LPM are presented in Table 1. The aroma compounds were added to the LPM to achieve generic food matrices usable for further studies and allowing results to be compared.

To obtain each LPM, pure water, anhydrous milk fat, skimmed milk powder, and sodium chloride were stirred vigorously for 12 min at room temperature using a blender (Waring, Torrington, CT, USA). The mixture was poured in a beaker and placed in a thermostat-controlled bath at 32 °C. The pH was measured using a penetrometric electrode (Mettler-Toledo, France) and adjusted to a constant value of 6.2 or 6.5 by the addition of GDL or NaOH, respectively. After a rest period of 2 h (for a pH value of 6.2) or 30 min (for a pH value of 6.5), rennet (diluted 1/10 in pure water) was added to the solution of odorous compounds and mixed vigorously for 1 min. Prior to coagulation, the LPM was immediately poured into a plastic bag to form a roll, vacuum-sealed, and completely immersed in a thermostat-controlled bath at 32 °C for 3 h. The products were stored at 4 °C until use (3 days).

The equipment used to prepare the LPM was disinfected with ethanol (70% in water). The absence of total *Coliforms*, *Listeria monocytogenes*, *Salmonella*, and *Staphylococcus* was checked for each LPM by a certified food control laboratory (Laboratoire départementale de Côte d'Or, Dijon, France).

Rheological Measurements. The rheological properties of the 18 LPM (4 replicates) were determined by a uniaxial compression test at a constant displacement rate²⁴ to characterize the food structure. Moreover, these measurements enabled verification of the good reproducibility of LPM production from batch to batch.

Cylindrical pieces (3 cm high, 1.1–1.5 cm in diameter) were sampled from the LPM roll using a cork-borer. Before measurements, samples were stored for 15 min at 15 °C in hermetically sealed boxes, which allowed them to relax from cutting and prevented them from dehydration. The measurements were performed at 15 °C using a TA-XT2 texture analyzer (Stable, Micro Systems Ltd., Champlan, France). During the test, the sample was compressed in line with its main axis at

a constant crosshead speed and between two parallel plates. The force developed by the sample (i.e., the resistance of the sample during compression) was measured with a load cell and recorded according to the position of the upper plate. Samples were compressed to 80% maximum deformation at 0.8 mm/s between parallel plates lubricated with low-viscosity paraffin oil. Using the force and displacement data thus recorded, the engineering stress ($\sigma = F_t/A_0$, F_t = recorded force and A_0 = initial cross section) and Cauchy strain ($\epsilon = \Delta h/h_0$, Δh = displacement and h_0 = initial height) were calculated.²⁴ From these data, the modulus of deformability MD (kPa), the fracture stress σ_f (kPa) and strain ϵ_f (dimensionless), and the work to fracture Wf (kJ/m³) were determined.

Sensory Profiling. The sensory attributes of LPM were evaluated by a trained panel of 15 graduate students in food science (aged 18–20 years), using conventional sensory profiling.²⁵ Eight training sessions were carried out before measurement sessions. The panelists were trained to quote different concentration ranges of taste components dissolved in pure water, to recognize tastes in different commercial dairy products, to test texture references, and to quote taste, aroma, and texture perceptions of commercial products on a scale. Discussions with the judges were monitored to reach a consensus for the choice of each attribute to quote in the measurement sessions. The sensory panel evaluated the 18 LPM by sequential monadic profiling, wearing a nose-clip to prevent any influence of odor on texture and taste perception. The LPM samples were presented at 15 ± 1 °C. For each LPM sample, the panelists were asked to score texture (crumbly, firm, springy, coating, pasty, melting, grainy)²⁶ and taste intensities (salty, sour, sweet)²⁷ on linear scales from 0 to 10 (0 = none and 10 = extremely strong). Six products were evaluated per session in a well-balanced order, and each product was evaluated in duplicate. Between each sample, the subjects were asked to cleanse their mouth with apple, salt-free bread, and mineral water (Evian, France). Each subject participated in six 1 h sessions (two sessions per week). The panel, submitted to the CAP method,²⁸ showed good performances in terms of discrimination, repeatability and accordance between subjects.

The LPM were prepared 3 days (± 1 day) before each sensory session, and the sample pieces were prepared in the morning before each tasting afternoon and stored at 15 °C until evaluation. The samples (two pieces of 5 ± 0.2 g) were served following a designed order that differed for each subject. The tests were carried out in an air-conditioned room (21 °C), under red light and in individual booths. Data acquisition was performed manually on paper and entered using Excel Microsoft software.

In Vivo Temporal Saltiness and Sodium Release Measurements. Training of the Panel. Five subjects (three women and two men, 23–46 years old) were selected from a panel of 15 people as a function of their oral parameters: masticatory performance and salivary flow rate.^{21,29} These subjects differed from those involved in previous sensory profiling. In a food habits questionnaire (data not shown), subjects stated they consumed cheese products at least once a week. These subjects participated in two 1 h sessions per week and were paid for their participation. The subjects were introduced to the discontinuous time–intensity evaluation of saltiness during a number of training sessions prior to the measurement sessions.²¹ They were requested not to smoke or eat or drink flavored foods for at least 1 h before the sensory session.

Measurement Sessions. During a single session, sodium release, saltiness intensity, and chewing activity were recorded simultaneously during the eating of LPM. Before eating each LPM, a saliva sample was collected (blank). At different time points during the “normal” eating of a 5 g sample (20, 40, 60, and 80 s), the subjects were asked to spit out one saliva sample (around 0.5 mL) into a 5 mL plastic tube, 13 mm in diameter (Camlab Ltd., Cambridge, U.K.) and to evaluate saltiness intensity on a scale from 0 to 10 on the basis of a reference that represented one of the saltiest LPM (S3D2F2P2) situated at 80% on the scale. Saliva samples (18 model matrices \times 5 panelists \times 5 times \times 6 replicates = 2700 samples) were immediately put in an ice bath and centrifuged at 28600g for 5 min at 4 °C (2–16 KC, Sigma-Aldrich,

St Quentin Fallavier, France). The supernatants were stored at -20 °C until HPLC analysis.

Throughout eating of the sample, chewing activity was recorded by electromyography.³⁰ Experimental details and results are given elsewhere.²⁹

A total of nine 1 h sessions were completed by each subject (two sessions per week, each session taking place at the same time of day). During each session, six products were tested in duplicate (two blocks) in a well-balanced order. For each block, the LPM samples were presented at 13 ± 1 °C, in hermetically closed transparent coded cups, in random order, and under red light. Six replicates were performed for each of the 18 LPM. Between each sample, an interval of 90 s was allowed for the subjects to cleanse their mouths with apple, bread (salt-free), and mineral water.

Sensory data acquisition was ensured with FIZZ software (Biosystems, Coutermon, France).

Analysis of Sodium Levels in Saliva. The saliva sample supernatants were diluted to 1/20 (50 μ L saliva in 950 μ L filtered 18 m Ω Milli-Q-water (Millipore, Bedford, MA, USA)) and filtered through a membrane (pore size = 0.45 μ m, C.I.L., Sainte-Foy-La-Grande, France).

The amount of sodium in saliva was determined by HPLC ionic chromatography²¹ using a Dionex ICS2500 ion chromatographic system (Dionex, Voisins le Bretonneux, France). The system consisted of a GS50 quaternary pump, an ED50 electrochemical detector used in conductance mode, an ASS0 autosampler, a CRCS-ultra 2 mm suppressor, and an SRS controller. The loop injection was set at 25 μ L, the sample volume used throughout the experiments.

Sodium content was analyzed using a Dionex IonPac CS12-A column and an IonPac CG12-A guard column at 20 °C. Elution was achieved in isocratic mode with 22 mN sulfuric acid (Sigma-Aldrich, France) at a flow rate of 0.5 mL/min.

System control and data acquisition were achieved using UCI-100 Chromeleon software (version 6.8). Quantifications were performed using sodium standard solutions ranging from 0 to 30 mg Na/L prepared in 22 mN sulfuric acid.

Data Analyses. The rheological properties of LPM and sensory profiling data were analyzed using analysis of variance (ANOVA). Principal component analysis (PCA) was used to analyze the relationships between rheological and sensory parameters.

The effect of composition on sodium release and saltiness perception was analyzed using MANOVA (multivariate analysis of variance) and ANOVA. For each sodium release measurement sequence, the blank sample corresponding to time 0 was subtracted from all sodium measurements performed at the other time points. The following parameters were considered: slopeR1 and slopeI1, slope of the curve at the start of eating (between 0 and 20 s) for sodium release and saltiness, respectively; Cmax and Imax, maximum concentration of sodium release and maximum saltiness reached, respectively; TRmax and TImax, time to reach Cmax and Imax, respectively; slopeR2 and slopeI2, slope of the curve after reaching the maximum concentration of sodium release and maximum saltiness intensity, respectively. These decreasing slopes reflected the persistence of sodium or its taste in the mouth. The main effect of the composition and subject factors on sodium release and temporal saltiness were analyzed using ANOVA. When a significant effect ($p < 0.05$) was found by applying ANOVA, the Student–Newman–Keuls (SNK) test was used to compare the difference in least-squares (LS) means. To analyze correlations between variables, Pearson's correlation coefficients were calculated, and correlations were all graphically confirmed. MANOVA and ANOVA were performed using a General Linear Model (GLM) procedure. All data analyses were carried out using Statgraphics Centurion XVI.I software (version 15.2, Sigma-Plus, France). PCA was carried out with Uniwin Plus (version 6.1, Sigma-Plus, France).

RESULTS

Rheological and Sensory Properties of Lipoprotein Matrices (LPM). Effect of Composition on the Rheological

Properties of the LPM. Uniaxial compression tests were performed to evaluate differences in structure among the 18 LPM. One-way ANOVA (with products as fixed factors) was performed on the four rheological parameters: modulus of deformability MD, fracture stress σ_f , fracture strain ϵ_f , and the work to fracture Wf. This ANOVA revealed a significant product effect on the four rheological parameters. Consequently, four-way ANOVA (with DM, fat/DM, salt, and pH as fixed factors) with second-order interactions was performed on MD, σ_f , ϵ_f , and Wf. This ANOVA revealed a significant main effect of each composition factor on most rheological parameters (Table 2). The LPM with the highest DM content (D2), the lowest fat/DM ratio (F1), and the lowest salt content (S1) were characterized by the highest σ_f , Wf, ϵ_f , and MD values, indicating the hardest, most cohesive, and least elastic structure. The highest salt content (S3) had the opposite effect, whereas the intermediate salt content (S2) generated intermediate rheological values. The LPM with the lowest pH (P1) presented the highest σ_f , Wf, and ϵ_f values, indicating the hardest and most cohesive structure. Significant correlations ($p < 0.05$) were observed between all rheological parameters (data not shown).

Significant DM \times fat/DM interactions were found for σ_f ($F(1;56) = 29.6$, $p < 0.001$) and Wf ($F(1;56) = 42.8$, $p < 0.001$), but not for ϵ_f ($p = 0.7$) and MD ($p = 0.3$). For both σ_f and Wf, the DM effect was more marked with the lowest fat/DM ratio (F1), indicating that the order of firmness was D2-F1 > D2-F2 > D1-F1 > D1-F2.

Significant interactions involving the salt factor were also found, such as significant DM \times salt interactions for ϵ_f ($F(2;56) = 5$, $p < 0.001$), σ_f ($F(2;56) = 34.5$, $p < 0.001$), and Wf ($F(2;56) = 45.4$, $p < 0.001$), and a trend for MD ($F(2;56) = 2.5$, $p = 0.10$). The DM effect was less marked with the highest salt level (S3) than with intermediate and low salt levels (S2 and S1, respectively).

Significant fat/DM \times salt and pH \times salt interactions were observed only with respect to the parameter σ_f ($F = 23.5$, $p < 0.001$; and $F = 3.5$, $p < 0.05$, respectively). The fat/DM or pH effects were less marked when the salt level was the highest. Significant fat/DM \times pH interactions were also observed only with respect to mechanical resistance parameters. The effect of pH was less marked in products containing low fat/DM levels (data not shown).

Effect of Composition on the Sensory Characterization of LPM. The aim was first to evaluate relationships between rheological and textural properties of LPM and second to determine correlations with taste and composition.

Global Analysis of the Relationship between Taste, Texture, and Composition. An initial three-way ANOVA (with panelists as a random factor and products and replicates as fixed factors) using second-order interactions was performed on each attribute. The results revealed a significant effect of the product factor on all taste and texture attributes ($p < 0.001$ for each attribute), except for sourness ($p = 0.3$). This latter attribute was subsequently removed from the analyses.

Sensory data from the nine most discriminating attributes and instrumental data (on supplementary variables) were analyzed by PCA to establish possible correlations between the rheological and sensory characteristics of LPM (Figure 1). The first component accounting for 68% of variance could be ascribed to texture attributes. It contrasted grainy and firmness attributes ($r = 0.7$, $p < 0.01$) to coating and melting ($r = 0.9$, $p < 0.001$). The firmest and grainiest products contained the

Table 2. Least Squares Means (\pm Standard Error) and Fixed ANOVA Model of Rheological Parameters for Each Composition Factor (Dry Matter Content, Fat/DM Ratio, pH, and Salt Levels)^a

	DM			fat/DM			pH			salt			p
	D1	D2	p	F1	F2	p	P1	P2	p	S1	S2	S3	
Wf	10.9a \pm 0.4	24.8b \pm 0.4	***	22.8a \pm 0.4	12.9b \pm 0.4	***	18.8a \pm 0.4	16.9b \pm 0.4	*	21.5 \pm 0.5	18.9b \pm 0.4	13.1c \pm 0.4	***
σ_f	62.4a \pm 1.6	125.5b \pm 1.7	***	117.0a \pm 1.6	70.9b \pm 1.6	***	99.1a \pm 1.6	88.8b \pm 1.7	**	109.1a \pm 1.9	99.8b \pm 1.9	72.9c \pm 1.9	***
MD	82.8a \pm 3.7	162.2b \pm 3.8	***	142.9a \pm 3.7	102.2b \pm 3.7	***	124.0a \pm 3.7	121.0a \pm 3.7	ns	131.8a \pm 4.4	125.8a \pm 4.3	110.0b \pm 4.3	*
ϵ_f	0.42a \pm 0.0	0.44b \pm 0.0	**	0.45a \pm 0.0	0.42b \pm 0.0	***	0.44a \pm 0.0	0.43b \pm 0.0	*	0.45a \pm 0.0	0.44b \pm 0.0	0.41c \pm 0.0	***

^aD1 and D2, 370 and 440 g/kg; F1 and F2, 20% and 40%; P1 and P2, 6.2 and 6.5; S1, S2, and S3, 0.5, 1, and 1.5%, respectively. Wf, work to fracture; σ_f , stress at fracture (mechanical resistance); MD, modulus of deformability (elasticity); ϵ_f , strain at fracture (cohesiveness). Different letters following entries indicate significant differences between least-squares means (SNK test). p values: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

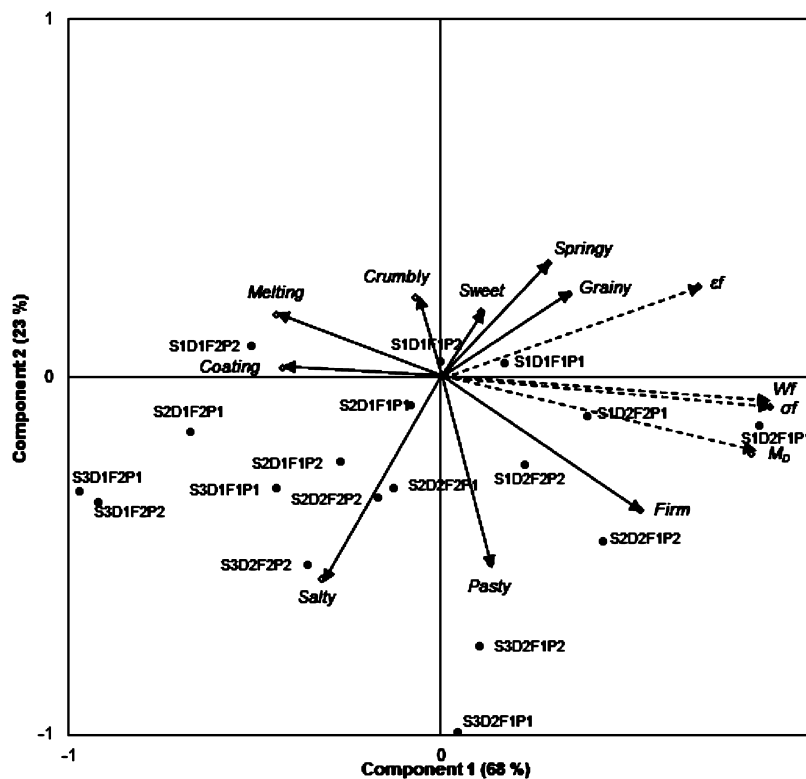


Figure 1. Principal component analysis of rheological parameters (dotted lines) and sensory attributes (continuous lines) with 18 LPM as individuals. The LPM were coded by their salt level (S1, 0.5%; S2, 1%; S3, 1.5%), dry matter content (D1, 370 g/kg; D2, 440 g/kg), fat/DM ratio (F1, 20%; F2, 40%), and pH level (P1, 6.2; P2, 6.5).

highest DM content (D2, at right), and products with the most coating and melting attributes contained the lowest DM content (D1, at left). The second component accounted for 23% of variance and could be ascribed to taste and two texture attributes. It contrasted saltiness and sweetness ($r = -0.9$, $p < 0.001$) and crumbly and pasty ($r = -0.6$, $p = 0.02$). The springy attribute contributed to both dimensions. The saltiest products contained the highest salt content (S3, left and bottom of the map). PCA revealed an interaction between texture and salt content. Products with a high salt level seemed to be perceived as being less firm and less grainy but more coating and more melting. Pearson's coefficients between salt content and rheological/texture data for each salt content confirmed these results (Table 3). For both textural and rheological parameters, the highest significant correlations were found with the highest salt content (1.5%), except for ϵf , which was only negatively correlated with saltiness intensity for S2. In addition, no correlation was observed for S1. Our results showed that the harder the texture (determined positively by σf , Wf , and firmness and negatively by coating and melting attributes), the less intense was the salty perception.

Relationships between Texture Perception and Rheological Behavior. PCA (Figure 1) and the data reported in Table 4 show the coherence between rheological and texture evaluations. As expected, σf and Wf , characterizing mechanical resistance, were strongly ($p < 0.001$) and positively correlated with firmness and negatively with melting and coating attributes. Interestingly, MD displayed the same correlations. These three rheological parameters were also all positively correlated with springy attributes, but MD to a lesser extent than σf and Wf . ϵf was highly correlated with springiness ($p < 0.001$), which shows that this attribute reflected the

Table 3. Pearson's Coefficients between Saltiness and Rheological/Texture Data for Each Salt Content^a

	S1 (0.5%) (n = 6)	S2 (1.0%) (n = 6)	S3 (1.5%) (n = 6)	all (n = 18)
σf^b	-0.25	-0.83*	-0.93**	-0.42
Wf^c	-0.29	-0.87*	-0.93**	-0.42
$\epsilon f^{d,e}$	-0.40	-0.85*	-0.44	-0.64**
MD ^e	-0.08	-0.72	-0.82*	-0.27
crumbly	0.12	0.09	0.35	-0.28
firm	-0.08	-0.84*	-0.90*	-0.36
springy	-0.38	-0.34	-0.57	-0.77***
coating	0.12	0.70	0.95**	0.59**
pasty	0.81	-0.71	-0.76	0.21
melting	0.03	0.85*	0.84*	0.51*
grainy	-0.19	-0.49	-0.81*	-0.70**

^a p values: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$. ^b σf , stress at fracture. ^c Wf , work to fracture. ^d ϵf , strain at fracture. ^eMD, modulus of deformability.

cohesiveness of the product. ϵf was also negatively correlated with melting and coating ($p < 0.01$) and weakly positively correlated with firmness ($p < 0.01$). MD was the only parameter significantly linked to pastiness ($p < 0.01$). Surprisingly, graininess was strongly positively correlated with the mechanical resistance and cohesiveness parameters.

Effect of Composition on Texture Attributes. All of these results revealed by PCA were confirmed by six-way ANOVA (with panelists as a random factor; DM, fat/DM, salt, pH, and replicates as fixed factors). However, the six-way ANOVA highlighted two results that were not evident from the PCA map: (i) Products with more fat/DM were more melting and

Table 4. Pearson's Coefficients between Rheological Parameters and Texture Attributes^a

	σ_f^b	Wf ^c	$\epsilon_f^{d,e}$	MD ^f
firm	0.95***	0.92***	0.58*	0.97***
grainy	0.79***	0.77***	0.76***	0.64**
springy	0.70**	0.70**	0.81***	0.51*
pasty	0.37	0.32	-0.18	0.60**
melting	-0.89***	-0.86***	-0.61**	-0.89***
coating	-0.89***	-0.85***	-0.64**	-0.85***
crumbly	-0.47*	-0.47*	-0.10	-0.57*

^a $n = 18$. p values: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$. ^b σ_f , stress at fracture. ^cWf, work to fracture. ^d ϵ_f , strain at fracture. ^fMD, modulus of deformability.

coating than the corresponding products with less fat/DM. An interesting DM \times fat/DM interaction was observed, that is, in

products with a low DM, more fat/DM reduced springy and grainy perceptions, whereas in products with a high DM, more fat/DM reduced firmness. (ii) The pH at renneting was the factor with the lowest influence on sensory perception.

Effect of Composition on Taste Attributes. The mean sensory profiles were plotted for each composition factor (Figure 2). Six-way ANOVA (with panelists as a random factor; DM, fat/DM, salt, pH, and replicates as fixed factors) and SNK tests were performed on saltiness and sweetness. No replicate effect was observed on these sensory attributes.

For saltiness, ANOVA revealed a significant effect of salt ($F(2;421) = 62$, $p < 0.001$) and of the fat/DM ratio ($F(1;421) = 5.3$, $p = 0.03$). A trend effect of DM ($F(1;421) = 3.9$, $p = 0.06$) was also found, whereas the pH had no effect on saltiness perception ($p = 0.3$). Post hoc analyses showed that saltiness significantly increased when the salt content or fat/DM ratio

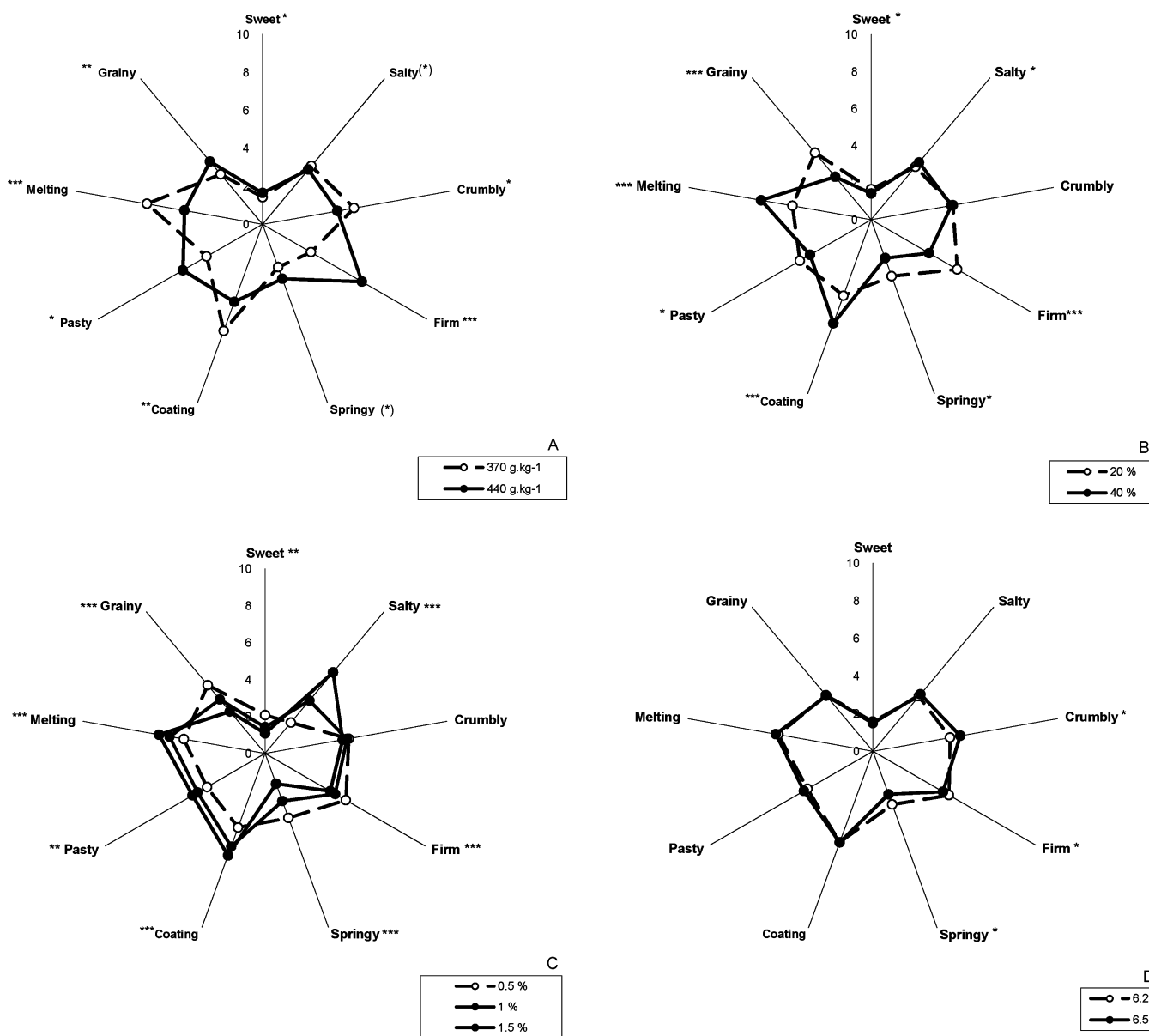


Figure 2. Sensory map of texture and two taste attributes obtained during the sensory profiling of 18 lipoprotein matrices (LPM) varying in terms of their (A) dry matter content (in g/kg), (B) fat/DM ratio (in %), (C) salt content (in %), and (D) pH at renneting. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; (*) $p < 0.1$.

increased (from 0.5 to 1.5% and from 20 to 40%, respectively) or when the DM level decreased (from 440 to 370 g/kg).

For sweetness, a significant effect of the salt content was observed ($F(2;421) = 8.3, p = 0.0013$), DM level ($F(1;421) = 4.5, p = 0.04$), and fat/DM ratio ($F(1;421) = 4.3, p = 0.05$). Sweetness significantly increased when the salt content or fat/DM ratio was lower (S1 and F1, respectively) or when the DM level was higher (D2). pH had no effect on sweetness perception ($p = 0.6$).

Relationships between Composition, Texture, Temporal Sodium Release, and Temporal Saltiness Perception. The curves for the amount of sodium released in the saliva and for saltiness perception displayed a rising phase at the beginning of the chewing process, a peak, and a more or less rapid decline at the end of this process (data not shown). An initial analysis was performed by two-way MANOVA (subjects, products) on all sodium release and saltiness perception data. The subject factor was found to be significant (Wilks' λ value = 0.03, $F = 89.4, p < 0.001$). MANOVA also showed significant effects of the product (Wilks' λ value = 0.11, $F = 9.5, p < 0.001$), indicating differences in temporal sodium release and saltiness perception values between the products.

Composition Factors Influencing Sodium Release. Four separate five-way ANOVAs (with subjects, DM, fat/DM, salt, and pH levels as fixed factors) and SNK comparison tests were performed on each sodium release parameter (slopeR1, Cmax, TRmax, and slopeR2) (Table 5). As expected, the Cmax

Table 5. Effect of Salt Level on Temporal Sodium Release in the Mouth and on Saltiness^a

	salt level ^b		
	0.5%	1%	1.5%
sodium release			
slopeR1 (g/100 g saliva/s)	0.1a	0.1a	0.2b
Cmax (g/100 g saliva)	0.1a	0.2b	0.3c
TRmax (s)	46a	44ab	42b
slopeR2 ($\times 10^4$)	3a	1a	-7a
saltiness			
slopeI1 (AU/s)	3a	6b	7c
Imax (AU)	3.5a	6.9b	8.4c
TImax (s)	37a	38a	39a
slopeI2 ($\times 10^{16}$)	1a	2a	4a

^aA five-way ANOVA model (with subjects, DM, fat/DM, salt, and pH levels as fixed factors) and SNK tests were performed on each sodium release and saltiness parameter. Different letters indicate the existence of a significant difference between samples (Student–Newman–Keuls, 5% confidence level). Cmax and Imax, higher sodium concentration and intensity, respectively; TRmax and TImax, time corresponding to Cmax and Imax, respectively; slopeR1 and slopeI1, increasing slope from the beginning to TRmax and TImax, respectively; slopeR2 and slopeI2, decreasing slope after TRmax and TImax, respectively. ^bLevel of salt incorporated in lipoprotein matrices.

(maximum concentration of sodium released) increased linearly with the salt content. The Cmax rose significantly from 0.1 to 0.2 and 0.3 g per 100 g of saliva for 0.5, 1, and 1.5% salt, respectively. A similar pattern was observed for slopeR1 (representing sodium release at the beginning of product breakdown), which was strongly correlated to Cmax ($r = 0.92, p < 0.001$). For TRmax (time required to reach Cmax), a trend effect of salt was also observed ($F(2;448) = 2.6, p = 0.07$). The TRmax was lower for the highest salt level. No significant salt

effect was observed on slopeR2, which reflected the sodium clearance rate from saliva after the Cmax had been reached.

Some interactions between salt content and other composition factors were found. Therefore, four-way ANOVA (with subjects, DM, fat/DM, and pH as fixed factors) was performed for each level of salt content on slopeR1, Cmax, TR_{max}, and slopeR2. The influence of formulation factors on mean values of sodium release parameter Cmax and saltiness parameter Imax is presented in Figure 3. At low salt contents (0.5 and 1%, respectively), no significant effect of composition was found on Cmax, TR_{max}, and slopeR2.

For slopeR1, only the DM level ($F(1;166) = 5.1, p = 0.03$) was a significant factor for the lowest salt content (0.5%). Post hoc analyses showed that slope 1 was higher for products with a lower dry matter content (370 g/kg). However, at the highest salt level (1.5%), the DM level ($F(1;169) = 4.4, p = 0.04$) and fat/DM ratio ($F(1;169) = 9.4, p = 0.003$) were significant. Post hoc analyses showed that slopeR1 was higher for products with a lower dry matter content (370 g/kg) and lower fat/DM ratio (20%). No effect of the pH factor was found ($p = 0.11$).

At low salt contents (0.5 and 1%, respectively), no effect of composition on Cmax was found, suggesting that the variation in texture did not induce a variation in sodium release ($p > 0.2$). At the highest salt content (1.5%), the DM level ($F(1;169) = 11, p = 0.001$), fat/DM ratio ($F(1;169) = 10.5, p = 0.002$) and pH ($F(1;169) = 6.6, p = 0.01$) all exerted a significant effect. Post hoc analyses showed that the C_{max} was higher for products with a lower dry matter content (370 g/kg), lower fat to dry matter ratio (20%), and higher pH level (6.5) (Figure 3).

At low salt contents (0.5 and 1%, respectively), no significant effect of composition on TR_{max} was found ($p > 0.2$). When the salt level was the highest (1.5%), a trend was observed for only the DM level ($F(1;169) = 3.2, p = 0.08$). Post hoc analyses revealed that the TR_{max} varied on average from 40.3 to 45.2 s for 370 and 440 g/kg, respectively.

At the highest salt content (1.5%), a trend was observed for slopeR2 regarding the pH level ($F(1;169) = 3.1, p = 0.08$), showing that sodium was released for a longer period when the pH was higher (6.5).

Composition Factors Influencing Saltiness Perception. Four separate five-way ANOVAs (with subjects, DM, fat/DM, salt content, and pH as fixed factors) were performed on each saltiness parameter (slopeI1, Imax, TImax, and slopeI2; Table 5). As expected, the Imax (maximum saltiness perceived on a scale from 0 to 10) increased in line with the salt content, from 3.5 to 6.9 and 8.4 with salt contents of 0.5, 1, and 1.5%, respectively. A similar pattern was observed for slopeI1 (representing the rate of increase in saltiness at the beginning of product breakdown), which was strongly correlated to Imax ($r = 0.9, p < 0.001$). No significant salt effect was observed for TImax (time required to obtain the Imax) and slopeI2 (representing the persistence of saltiness in the mouth).

Interactions were also found between the salt content of products and other composition factors. We therefore performed four-way ANOVA (with subjects, DM, fat/DM, and pH as fixed factors) for each level of salt content on slopeI1, Imax, TImax, and slopeI2. ANOVA results are shown in Figure 3.

At lowest salt content (0.5%), the ANOVA revealed a significant effect of the DM level ($F(1;161) = 5.1, p = 0.02$) and of the fat/DM ratio ($F(1;161) = 9.1, p = 0.003$) on slopeI1. No effect of pH was found ($p = 0.4$). Post hoc analyses showed that saltiness perception increased more rapidly when the DM level

Table 6. Pearson's Coefficients between Sodium Release and Saltiness for Each Time Intensity Parameter and Each Salt Level^a

	salt level		
	0.5%	1%	1.5%
Cmax/Imax	0.42*** (176)	0.22** (177)	0.35*** (179)
TRmax/TImax	0.24** (176)	0.26*** (177)	0.36*** (179)
slopeR1/slopeI1	0.20** (176)	0.20** (177)	0.44*** (179)
slopeR2/slopeI2	0.15* (176)	0.25*** (177)	-0.19** (179)

^a*p* values: ***, *p* < 0.001; **, *p* < 0.01; *, *p* < 0.05. Numbers in parentheses indicate sample size. Cmax and Imax, higher sodium concentration and intensity, respectively; TRmax and TImax, time corresponding to Cmax and Imax, respectively; slopeR1 and slopeI1, increasing slope from the beginning to TRmax and TImax, respectively; slopeR2 and slopeI2, decreasing slope after TRmax and TImax, respectively.

Table 7. Pearson's Coefficients between Temporal Sodium Release and Temporal Saltiness Parameters and Texture Parameters^a

temporal parameter	springy	coating	melting	grainy
saltiness				
slopeI1	-0.61**	0.41	0.32	-0.53*
Imax	-0.62**	0.44	0.35	-0.53*
TImax	0.068	-0.05	-0.09	0.03
slopeI2	0.20	-0.36	-0.29	0.25
sodium release				
slopeR1	-0.73***	0.49*	0.40	-0.60**
Cmax	-0.73***	0.48*	0.39	-0.59**
TRmax	0.41	-0.51*	-0.51*	0.40
slopeR2	0.62**	-0.32	-0.24	0.43

^a*p* values: ***, *p* < 0.001; **, *p* < 0.01; *, *p* < 0.05. Cmax and Imax, higher sodium concentration and intensity, respectively; Tmax and TImax, time corresponding to Cmax and Imax, respectively; slopeR1 and slopeI1, increasing slope from the beginning to TRmax and TImax, respectively; slopeR2 and slopeI2, decreasing slope after TRmax and TImax, respectively.

salty perception was slower with more springy and grainy LPM (slopeR1 and slopeI1), and the maximum amount of sodium released and maximum saltiness intensity was lower (Cmax and Imax). With the more elastic LPM, persistence of sodium in the mouth was lower, but this had no effect on temporal perception (slopeR2 and slopeI2). With LPM perceived as being more coating, sodium was released more rapidly and its peak concentration was higher and reached in a shorter time. With LPM perceived as being more melting, only sodium was released earlier during eating. However, coating or melting perceptions had no effect on saltiness parameters. Although correlations were observed between rheological and texture parameters, no correlation was obtained between sodium release and saltiness and rheological parameters.

Correlations between sodium release parameters and saltiness were observed, with slopeR1 and Cmax being positively correlated to saltiness perception ($r = 0.96$, $p < 0.001$) and slopeR2 negatively correlated to saltiness perception ($r = -0.85$, $p < 0.001$).

DISCUSSION

Rheological and Sensory Characterization of the Texture of Lipoprotein Matrices. Analysis of the rheological and texture perception data indicated that the rheological

behavior of a LPM could be a predictor of texture perception. Satisfactory formulation and reproducibility of a broad range of LPM were obtained in various compositions. These different LPM displayed different mechanical characteristics inducing differences in texture perception. The mechanical and textural characteristics of LPM were mainly dependent on the interaction between the DM and fat/DM contents of the products. Products with higher DM (D2) and lower fat/DM (F1) levels, which contained the largest quantity of milk powder, were physically characterized as the hardest and perceived as the firmest. An increase in the DM level caused by an increase in the protein content led to the formation of a more compact and more granular protein matrix with fewer open spaces that would be occupied by fat globules.^{31–33} Milk powder contained not only proteins but also minerals. Consequently, the protein network of these products was more reticulated by cross-linkings and, hence, more compact. By contrast, the melting and coating characteristics of LPM with low DM (D1) and high fat/DM (F2) contents were due to both their low milk powder content and high fat/milk powder ratio. The protein network was therefore less dense and fat globules fitted into it, acting as a lubricant. The microstructure study of these LPM using scanning electron micrographs, carried out by other authors,³⁴ confirmed our results. The microstructure of the protein networks was denser and more branched and displayed a higher degree of cross-linking in products containing the highest protein concentration. By contrast, the microstructure of matrices with a lower protein content was weaker, giving a frothier aspect to the LPM.

In parallel, an increase in salt content and pH induced a reduction in hardness and firmness. A negative relationship between hardness and salt content in cheeses is well documented.³⁵ One explanation may be the action of both salt and pH on chymosin (rennet) activity.^{36,37} Nevertheless, the amount of rennet was calculated according to variations in salt and pH, and the same mechanical resistance should therefore be measured after coagulation. This was probably due to the effect of both salt adjunct in milk and the reduction of pH on casein binding. In a rennet-induced gel, caseins are bound together by phosphocalcium links, involving the phosphorus from serine residues and the calcium from milk.³⁸ First, the Na⁺ ions added in milk may replace Ca²⁺ ions in the bonds, leading to their destruction; because it is a monovalent ion, Na⁺ cannot bind two phosphorus ions in the same way as Ca²⁺, a divalent ion, does. Second, a lower pH favors the soluble form of Ca²⁺ ions, compared to the form immobilized in bonds. This pH effect was only observed with the lowest and intermediate salt concentrations (0.5 and 1%) and not with the highest (1.5%), as revealed by a pH × salt interaction for hardness parameters. At high levels, the effect of salt on phosphocalcium links may prevail over the pH effect.

Impact of Composition and Texture on Sodium Release and Saltiness Perception. The principal aim of this study was to investigate the impact of the composition (fat, DM, salt, and pH) and texture of the food model on sodium release and saltiness perception. The sensory profile of LPM showed that saltiness intensity increased in line with the salt concentration, as did the temporal saltiness perception evaluated by TI assessment. This was expected and confirmed by a higher level of sodium release in saliva when the salt concentration increased. This finding suggests that saltiness perception is governed by the concentration of sodium present

in the aqueous phase of a product³⁹ and further extracted by saliva during mastication.

However, some relationships were observed between other composition factors and sodium release. Our results revealed that sodium release increased when the fat/DM decreased. Fat may act as a barrier against sodium in the matrix, slowing its mass transfer into the saliva phase,²¹ or it hypothetically may favor the formation of water in fat emulsion at the beginning of the in-mouth breakdown process that could be able to retain more concentrated salt solution within the water phase²¹ than after more important dilution with saliva later in the mouth process.

Our results showed that sodium release was highest at the lowest DM, probably because more water enhanced the solvating capacity of sodium, favoring its extraction by saliva.²¹ These hypotheses need to be confirmed during more specific studies.

Such relationships were mainly observed with the highest salt level. There may be two explanations for this. First, at lower and intermediate salt levels, highest sodium quantity was bound to protein and thus little was present in the aqueous phase. Second, the increase in salt content led to an increase in salivary flow rate^{40,41} and because water enables a good solvating capacity for sodium, this favored its extraction by the saliva, as mentioned above. These hypotheses need to be confirmed by more specific studies, for example, by measuring the salivary flow rate when specific LPM are eaten.

Similarly, when the salt content was constant, our results showed that saltiness perception was mainly influenced by the fat/DM ratio. With a low salt content (0.5%), an increase in fat/DM lowered the I_{max} . One theory is that a higher fat level may have created a barrier between the aqueous phase and taste receptors.⁴² However, when the salt content was the highest (1.5%), an opposite result was obtained. The I_{max} was higher when the fat/DM ratio was higher (40%). As such observation in the same conditions was not reported for sodium release, perceptual hypotheses can be put forward. The most plausible is perceptual interactions between saltiness and texture perception as the LPM sensory profile showed clearly that the intensity of sensory texture attributes significantly changed according to salt level.⁴³ However, such interactions are difficult to show because changes in texture or rheology properties are always linked to changes in composition. Another explanation could be a perceptual interaction between sweetness and saltiness, as suggested by the results obtained with respect to saltiness intensity. Indeed, at the highest fat level, the amount of milk protein (and consequently the amount of lactose supplied by the milk powder) was lowest. This could explain the lower sweetness intensity at high fat level and consequently an increase in saltiness perception. However, sweetness intensity, when perceptible, remained low compared to saltiness intensity.

Our results therefore suggest that saltiness perception could not solely be explained by the quantity of sodium available in the mouth; other factors were involved. The phenomenon is more complex. Composition factors exerted a different influence on the maximum amount of sodium released (C_{max}) and on maximum saltiness perception (I_{max}). As mentioned above, at the lowest salt level, we did not observe any significant effect of the perceived composition or texture on C_{max} , and composition affected only I_{max} . We suggest that the more firm and cohesive the product (having a high level of dry matter, a low fat to dry matter ratio, and a low pH), the greater the chewing force required, which led to a similar amount of

sodium being released in the mouth as with a softer product.^{44,45} Thus, a variation in composition influenced texture; texture influenced chewing activity, which in turn influenced sodium release. Results related to chewing activity and more generally to oral parameters of the subjects are presented in the accompanying paper.²⁹ When all salt levels were considered, we found correlations between certain texture attributes and temporal saltiness and sodium release parameters, but no such correlations were found with rheological parameters, even though most texture and rheological parameters were found to be highly correlated. In fact, rheological measurements were performed on the crude product, whereas texture perception was determined during LPM consumption and the overall score integrated texture perception throughout LPM breakdown in the mouth. Although a correlation could be found between rheological and texture parameters, we can hypothesize that the perception of some texture attributes such as springy, coating, melting, and granularity influenced the mastication process, which in turn affected sodium release and saltiness. However, we cannot exclude the possibility that perceptive interactions between saltiness and texture perceptions were not, at least partially, responsible for the high correlations between saltiness parameters and texture perception.

Another remarkable finding was that the amount of sodium released and saltiness perception increased immediately when the subject started to chew the LPM samples. However, curiously, the peak of sodium release was reached after about 40–45 s, whereas maximum saltiness was perceived after about 35–40 s, even toward the end of chewing. It is possible that human papillae become very rapidly saturated, which would explain why sodium was still present in the saliva. This hypothesis needs to be confirmed by more specific studies.

Thus, variations in food composition (dry matter, fat to dry matter ratio, salt, and pH) exerted different effects on structure, texture, sodium release, and taste perception as a function of the salt content in lipoprotein matrices. Reducing the level of dry matter or increasing the fat to dry matter ratio, pH level, or salt content led to a softer structure and texture. In addition to the salt level, sodium release was mainly influenced by the water and casein contents, whereas saltiness perception was mainly influenced by the fat content. Our results suggest that the effects of food composition may differ as a function of the amount of salt incorporated in lipoprotein matrices. A balance needs to be found between the salt concentration and food matrix composition to produce the low-salt cheeses targeted by public health organizations while maintaining a good salty perception. Moreover, marked individual differences were also observed regarding temporal sodium release and saltiness perception, probably due to differences in chewing behavior.^{21–23} Our next paper will be devoted to investigating the relationship between temporal sodium release, saltiness perception during consumption, LPM characteristics, and oral parameters.²⁹

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