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Article

Development of Reference Standards for the Sensory Analysis of Cheese: An Innovative Approach

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Abstract: A sensory profile provides important information on the qualitative and quantitative properties of cheeses. Thus, tasters must be trained with reference standards that allow the calibration of the panel around shared sensory definitions. The ideal standard is simple to prepare, easily reproducible, discriminant and specific for the product evaluated. Therefore, commercial products and pure chemicals, which are currently implemented as reference standards, are not suitable. Furthermore, the lack of common practices in the dairy sectors has been widely highlighted. This study aimed to establish new and reproducible reference standards for the gustatory and olfactory descriptors of cheeses. Using casein, maize starch, powder skimmed milk and *k*-carrageenan, a tasteless and odourless product like a fresh cheese was produced. At this matrix, 20 odour and taste compounds have been added to obtain standards that have been evaluated by 60 tasters so as to define their recognisability and perceived intensity. These cheese standards are easy to reproduce, are stable and exhibited good recognisability. Overall, the reference standards proposed are suitable for the panel training and are a starting point for standardising practices and lexicon, thus enabling the comparability of sensory studies on cheese.

Keywords: cheese; sensory analysis; reference standards; descriptive analysis; panel training

1. Introduction

Sensory analyses are fundamental for multiple applications in cheese technology such as classification [1], quality assurance, new product development, research and investigation of drivers behind consumers' choice and liking [2,3]. In addition, sensory evaluation is extremely important for cheeses with the Protected Denomination of Origin or Protected Geographical Indication whose product specification must include a sensory description useful for controlling production and ripening [4].

The key outcome of a sensory analysis is the sensory profile, which is defined as the 'description of the sensory properties of a sample, consisting of the sensory attributes in the order of perception, and with assignment of an intensity value for each attribute' [5]. It is determined by descriptive tests, which focus on the differentiation and description of products based on their qualitative and quantitative sensory aspects [6]. Descriptive methods have been considered the most powerful sensory tool for cheese flavour research [7], and their application to determine the sensory profile of cheeses is largely documented [3,8].

The fundamental element of descriptive techniques is the sensory panel, a group of tasters that have been trained to identify, describe and rate the perceived sensory aspects of a sample [6]. The use of reference standards during tasters' training and calibration procedures is crucial [9,10] and required by the international standards [11]. The ideal reference standard should provide both qualitative and quantitative information, be stable and reproducible over time [11], be simple in its preparation, identify only one term and not be of commercial origin to avoid production variability or supply difficulties [12]. Consequently, commercial products and food ingredients are not appropriate as they limit the reproducibility of studies worldwide and over time, although their use as standards for gustatory and olfactory descriptors has been reported in several studies on cheese [13–25]. Such a limitation is overcome by pure chemicals, which have been used as reference

standards for the gustatory and olfactory descriptors of cheese in several studies [13–15,18,19,21,25–30]. However, because the assessor must be able to recognise the descriptor among the complex sensations of the stimulus [11], the use of pure chemicals is not recommended for the panel training. Indeed, the assessors should learn sensory descriptors directly from a medium like the food matrix that is going to be evaluated instead of using external reference standards, as in the case of pure chemical compounds [31].

A possible solution is represented by food models, which are reconstructed foods that mimic the food structure to be evaluated, but with sensory properties as bland as possible, to include within them compounds representative of the typical sensory descriptors of the product. Such an approach has already been implemented for orange juice [32], beer [33], tomatoes [34], wine [35–41], fruit juice [42] and balsamic vinegar [43], in which the proposed food models exhibited improved panel performance and the superiority of product-specific training was demonstrated [31].

In the case of the dairy sector, cheese models (CMs) have been scarcely used as reference standards. Among the reconstructed CMs available in the literature, the one developed by Salles et al. [44] was described as a potential starting point to create effective and objective standards [31]. This CM was obtained using a mixture of water, milk fat, milk powder, casein, sodium chloride and glucono- δ -lactone. It was exposed to the action of rennet, and overall, the preparation lasted more than 3 h. However, this study did not focus specifically on the development of reference standards for the descriptive analysis of cheese, and the intensity scales for panel training have not been elaborated [44].

Some researchers have attempted to harmonise both the lexicon and the reference standards involved in the training process by comparing the terms used by different research groups for the descriptive sensory analysis [2,3,8,15]; however, the reported reference standards did not include any CM. The lack of homogeneous procedures for the training of tasters, the absence of common validated reference standards for the gustatory and olfactory descriptors of cheese and the need to develop a universal sensory lexicon for cheese have also been highlighted [3,8,31].

Therefore, this study aimed to develop reference standards that are easily replicable and feasible for panel training procedures. To this end, a tasteless and odourless CM was produced and then added with compounds representative of the typical taste and odour/aroma of cheese. The CM was tested by cheese tasters to collect information on their perceived intensity and recognisability. The implementation of such reference standards in panel management practices is expected to have further potential applications. In particular, it would allow for a uniform sensory lexicon, which is an essential prerequisite for the comparison of the different sensory profiles of cheeses and between different tasting panels, even cross-countries, a procedure that has encountered difficult applications to date [8]. Furthermore, reference standards are a critical element for cheese products under the EU quality schemes. Their implementation would facilitate the drafting procedures of product specifications and improve conformity verification by producers and authorities [45,46].

2. Materials and Methods

2.1. Materials

Milk casein concentrate was purchased from EkoPura (Hoofddorp, Netherlands). Maizena maize starch was provided by Unilever (London, United Kingdom). Kappa-carrageenan was purchased from SaporePuro (Torino, Italy), skimmed milk powder (carbohydrate, 56.0%; protein, 34.0%; fat, 1.2%; salt, 1%) from ProntoFoods (Montichiari, Italy) and natural mineral water from the market (Sant'Anna, Vinadio, Italy). Furthermore, food-grade flavours (almond, ananas, cooked egg yolk, milk, hazelnut, honey, mushroom, olive oil, cooked ham, raw ham, smoked) were furnished by FlavourArt (Oleggio, Italy). Tartaric acid, caffeine anhydrous and glutamic acid monosodium salt were purchased from Fluka Chemie GmbH (Buchs, Switzerland), sodium chloride from 'Compagnia Italiana Sali' (Porto Viro, Italy) and lactose from Carlo Erba Reagents Srl (Cornaredo, Italy). In addition, UHT skimmed milk (carbohydrate, 5.0%; protein, 3.2%; fat, 0.1%; salt, 0.1%) was purchased from Granarolo S.p.a. (Bologna, Italy). Butter ghee was provided by Khanum (Hertford, United

Kingdom). Pasteurised fresh cow cream (carbohydrate, 3.0%; protein, 2.0%; fat, 38.0%; salt, 0.07%) was furnished by ‘Centrale del latte d’Italia S.p.a.’ (Torino, Italy). Low-fat yogurt (carbohydrate, 5.6%; protein, 4.3%; fat, 0.1%; salt, 0.1%) was purchased from ‘Cooperativa Latteria Vipiteno’ (Vipiteno, Italy).

2.2. Sensory evaluation

The sensory evaluation (identification of attributes and perceived intensity) was conducted by 60 cheese tasters (men = 53.33%, age range: 24–70 years) from the Italian National Organisation of Cheese Tasters. Each assessor underwent a training in cheese sensory analysis for at least 20 h that included a final exam. Every taster received four cheese standards with different attributes (one for taste and three for odour/aroma) in a randomised, anonymous, and blinded scheme. Therefore, each descriptor was evaluated 12 times by different tasters.

The samples were provided in transparent plastic plates (R.F. Distribuzione Srl, Mercato San Severino, Italy) with no information. For the sensory analysis, a special evaluation sheet was used where the perceived sensory odour/aroma or taste and the intensity were reported (Figure 1). For the intensity evaluation, a continuous, unipolar 15-cm linear scale with anchors at the two ends (‘none’ and ‘very high’) was used [6].

The panellists rinsed their mouths with natural mineral water (Sant’Anna, Fonti di Vinadio, Italy) after tasting each sample. The panellists took a 1-min break between sample evaluations. The evaluation was performed in a room with white light at a temperature of 22°C ± 2°C. The samples were prepared in the preceding 2 h and served at 15°C ± 1°C.

SAMPLE CODE: _____

IDENTIFIED DESCRIPTOR: _____

PERCEIVED INTENSITY: (mark with a line /)

None
(min.)

Very high
(max.)

Figure 1. Evaluation sheet provided to the tasters for the sensory evaluation.

When the odour/aroma or taste was correctly identified by the taster, the perceived intensity was manually measured according to the method described by Meilgaard et al. [6]. The minimum and maximum values of the perceived intensity were determined for each reference standard.

3. Results and Discussion

3.1. Cheese-like base production

To produce a cheese-like base, 8 g of maize starch, 5 g of casein, 4.3 g of powder skimmed milk and 1 g of *k*-carrageenan were mixed and then added to 81.7 g of water. The obtained mixture was heated to 80 °C using a bain-marie with water pre-heated to 90 °C and stirred for 2 min. Subsequently, the mixture was poured into hemisphere silicone moulds (E-CrossStu GmbH, Frankfurt am Main, Germany) and allowed to cool at room temperature (20 °C ± 2 °C). A Medidor PH BASIC 20 pH-metre (Hach Lange Srl, Lainate, Italy) was used to evaluate the pH of the samples.

Approximately 110 different formulations were tested, each aimed at achieving a structure resembling fresh cheese, devoid of any distinctive taste or odour. Through a rigorous evaluation, the formulation that exhibited the most accurate mimicry of fresh cheese while lacking discernible taste and odour was selected. Notably, in the trial without carrageenan, a poor structure and a grainy mouthfeel were noted. Furthermore, the sample without starch and with a higher amount of casein felt strong on the palate with an odour of casein, and while it was not completely tasteless, it deviated

from the desired characteristics. Although the specific data obtained from these trials were not presented in this manuscript, the final selected formulation was presented in detail above.

The CM obtained through the proposed formulation was comparable to a generic fresh cheese but was odourless and tasteless (Figure 2). Compared with the study by Salles et al. [44], the proposed formulation was able to obtain a cheese-like base without sensory properties in a shorter time and with the simplified use of ingredients and equipment.

Coagulation with acids or rennet was avoided to limit their impact on the sensory properties of the standard, to allow greater reproducibility of the production and reduce the preparation time. Alternatively, *k*-carrageenan was used owing to its gelling and thickening properties. Indeed, its historical use in combination with milk has been reported [47] as well as specific interactions with *k*-casein and their charged amino acids [48] and maize starch [49]. Similarly, maize starch has shown positive interactions with caseins that resulted in a stronger density of the gel network structure [50,51]. The temperature range of 70–80 °C, which was selected for the heating process, enabled a complete gelatinisation [52] and the transfer into the silicone moulds to cool, it firmed up completely to the desired texture.

Unlike the study by Salles et al. [44], Ultra-Turrax T25 (IKA-Labortechnik, Staufen, Germany) was not used for this preparation as they negatively affected the texture in the preliminary trials.



Figure 2. Cheese-like base used for the development of reference standards of cheese.

3.2. Reference standard production

According to the same ‘food-models’ approach already implemented for several food products [32–44], chemicals and food ingredients were added to the cheese-like base to obtain a standard product that has cheese odour/aroma and taste.

According to the descriptors used for the sensory analysis of cheese [2,3,8,14–31] 20 reference standards were selected: 5 for taste and 15 for odour/aroma (Table 1). Although the use of food ingredients may be subjected to the limits of global supply and production changes over time [12], the ones selected (low-fat yogurt, pasteurised cow cream, butter and UHT skimmed milk) are easily available in different parts of the world [53], and therefore, the reproducibility of standards is guaranteed.

Solid substances were mixed with the other solid ingredients of the CM, whereas liquid compounds were added to the mix before the heating process.

For the ‘cooked milk’ odour/aroma, the UHT skimmed milk was boiled for 5 min and then added to the mixture before the heating process, whereas for the ‘yogurt’ odour/aroma, the low-fat yogurt was centrifuged at $8000 \times g$ for 15 min at 10°C and then 6 mL of whey was added to the mixture also before the heating process.

Standard concentrations were defined in the preliminary tests to obtain the mean intensity according to the ISO 11035:1994 [11] to properly train the sensory panel.

The inclusion of these ingredients did not affect the CM structure. Only the standard for acid was less humid and soluble than CM. The reason can be related to the reduction in pH values from 6.64 ± 0.01 of the CM to 5.20 ± 0.03 after the addition of L-tartaric acid. However, the obtained product maintained a solid and stable structure without significant differences from the CM. The alternative use of *k*-carrageenan with maize starch, casein and skimmed milk in an aqueous medium to obtain a

solid matrix similar to a fresh cheese was effective and the obtained products is adaptable to a different shape. This provides a solid standard to train tasters, allowing mastication with an improved retro-olfactory aroma perception [54,55], compared with liquid pure chemicals currently used as cheese reference standards. The versatility of the base matrix in including substances representative of the 20 gustatory and olfactory descriptors developed would also allow future use of this formulation for additional attributes not involved in this study.

Table 1. Reference standard composition.

Attributes	Addition ¹
Acid	0.40 g of L-tartaric acid
Bitter	0.05 g of caffeine
Salty	0.20 g of sodium chloride
Sweet	0.40 of lactose
Umami	0.10 g of L-glutamic acid monosodium salt
Almond	10 µl of almond aroma IT-10M-1C-FA0128
Ananas	10 µl of ananas aroma IT-10M-1C-FA0061
Butter	1 g of butter ghee
Cooked egg yolk	10 µl of cooked egg yolk aroma IT-10M-1C-FA0404
Cooked milk	1 ml of boiled milk
Fresh milk	10 µl of milk aroma IT-10M-1C-FA0268
Fresh cream	3 ml of fresh cow cream
Hazelnut	10 µl of hazelnut aroma IT-10M-1C-FA0002
Honey	10 µl of honey aroma IT-10M-1C-FA0263
Mushroom	10 µl of mushroom aroma IT-10M-1C-FA0486
Olive oil	10 µl of olive oil aroma IT-10M-1C-FA0322
Cooked ham	10 µl of cooked ham aroma IT-10M-1C-FA0454
Raw ham	10 µl of raw ham aroma IT-10M-1C-FA0455
Smoked	10 µl of smoked aroma IT-10M-1C-FA0406
Yogurt	6 ml of whey by low-fat yogurt

¹ For 100 g of cheese-like mix.

3.3. Sensory evaluation

Among the 240 obtained evaluation sheets, only for 27 of these the sensory descriptor was not identified. The results indicate that the tasters had difficulty identifying some descriptors such as ‘Umami’, ‘Almond’, ‘Cooked egg yolk’ and ‘Mushroom’, which were correctly identified by only 2 out of 12 tasters (Table 2). Contrarily, other descriptors, such as ‘Acid’, ‘Bitter’, ‘Fresh milk’ and ‘Fresh cream’ were correctly identified by about two-thirds of the tasters since these descriptors are more known and largely used for the sensory analysis of cheese. The low number of correct identification can also be attributed to the lack of a list of the sensory descriptors supplied with the samples to simplify the identification.

Regarding the intensity for some descriptors, such as ‘Salty’, ‘Sweet’, ‘Umami’, ‘Almond’, ‘Ananas’, ‘Butter’, ‘Hazelnut’, ‘Honey’ and ‘Raw ham’, the reported values were very narrow, whereas the other descriptors showed a very high variability (Table 2).

Table 2. Results of the sensory analysis conducted on the cheese-like standards.

Attributes	Identification		Perceived intensity	
	Positive	Negative	Minimum	Maximum
Acid	8	4	2.7	8.7
Bitter	7	5	4.9	9.9
Salty	3	9	10.1	12.0
Sweet	3	9	5.3	6.2

Umami	2	10	8.3	11.8
Almond	2	10	4.6	6.5
Ananas	4	8	8.0	11.0
Butter	5	7	3.5	7.8
Cooked egg yolk	2	10	5.1	8.5
Cooked milk	4	8	7.0	12.4
Fresh milk	8	4	2.7	7.3
Fresh cream	8	4	4.2	9.9
Hazelnut	6	6	7.9	11.5
Honey	3	9	5.0	7.8
Mushroom	2	10	3.5	10.2
Olive oil	6	6	4.0	9.9
Cooked ham	3	9	6.6	10.8
Raw ham	4	8	3.4	5.9
Smoked	5	7	5.0	11.6
Yogurt	3	9	3.8	8.3

Actually, it is impossible to define an intensity value for the reported attributes and concentration then it is necessary to modify the concentration for each attribute and train the panellist in order to define the extreme values for each of it as required by the ISO 11035:1994 [11]. A direct comparison of the performance of these new reference standards with that of the traditional ones (commercial products and pure chemicals) will be useful for a definitive validation and to improve the formulation. The potential approaches for this purpose are the ones followed by Meilgaard et al. [56] and Noble et al. [36] for beer and wine as well as also by Hunter and McEwan [57] and Nielsen and Zannoni [58] for different sensory panels, even across different countries.

4. Conclusions

The results of this study indicated that the developed CM can be used for panellist training on odour/aroma and taste attributes. The use of standardised and very simple ingredients as well as the easy method for production make it simple to obtain. The absence in this product of taste, odour and aroma but the presence of a structure similar to a fresh cheese simplifies the training of panellist.

Also the products used in this study in order to reproduce odour/aroma and taste attributes of cheeses are very simple and standardized then could be the starting point of a list for panellist training. In the future, it is necessary to evaluate new products/ingredients in order to define other attributes.

Similarly, the quantity of each product/ingredient used in this study determined very different perceived sensations in the panellists then are needed new researches in order to define well the range of intensity for each attribute, to define the standardised intensity values and above all to define the extreme values of each.

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