Sensory evaluation as a tool in assessing the quality of new fermented products

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ABSTRACT

Ten starter cultures of lactic acid bacteria were used to ferment five mixtures of milk and pea protein (0%, 10%, 20%, 30% and 40% of pea) to select the cocktail that can lead to products similar to traditional yogurt. Product quality evaluation was performed by comparing the sensory profile of 49 formulated products with the profile of a milk fermented by commercial lactic ferments. The sensory profiles were analyzed by means of three-way ANOVAs and a principal component analysis (PCA). Substitution of cow milk protein with 40% of pea proteins reduce starter cultures effects and decrease product quality. In contrast, until 30% of pea protein, starter cultures show positive and negative effects. For example, products fermented by Streptococcus thermophilus + Lactobacillus acidophilus with 30% pea protein have positive characters like creamy and smooth, but Lactobacillus delbrueckii subsp. Bulgaricus + Lactobacillus rhamnosus caused bad quality and negative characters like bitter and astringent even with 100% cow milk.

Keywords: sensory profile, new fermented product, quality evaluation, pea protein

1. INTRODUCTION

The increase in global demand of animal proteins in recent years may become a major issue in a close future. In order to initiate the reduction in the use of animal proteins in European diet, it might be interesting to combine dairy proteins with vegetable proteins in products already known by consumers. Tu, et al. [1] have shown that consumers can accept dairy-like products combining cow milk and soybean proteins if the ratio of soybean protein does not exceed 50%. However, soybean might not be the best substitution protein as consumers tend to have negative attitudes towards this source of protein [1,2]. Zare, et al. [3] have suggested lentil flour as an alternative protein substitution. They showed that supplementation with 1-3% of lentil flour did not affect much sensory properties and overall acceptance compared to traditional yogurt. However, this rate of substitution is not high enough to initiate
a change in food habits towards an animal/vegetal balanced diet.

In this paper, we propose pea protein as a good substitute for cow milk protein in dairy products, because of its balanced amino acid profile, low level of allergy, functional properties and availability at an affordable price. Pea could be a better substitute for milk protein than soybean because of its high digestibility level, the absence of phytoestrogens and its environmentally friendly and local agriculture. Although pea protein has positive characteristics and has been used in sports foods and in meat based products, its use is almost absent in fermented products due to its intense flavor and odor.

The objective of this research was to determine the combination of starter culture and pea concentration that will give a dairy product close to traditional yogurt. A standard descriptive analysis approach was used to compare the sensory profiles of fermented products obtained by fermenting five ratios of cow/pea milks with 10 starters with that of a cow milk yogurt fermented with a commercial milk ferment.

2. MATERIALS AND METHODS

2.1 Products

Figure 1 illustrates the yogurt preparation. Starting from skim milk powder purchased from Régilait (Saint-Martin-Belle-Roche, France) and pea protein isolate Nutralys® S85F supplied by Roquette (Lestrem, France), two different milks were prepared with the same concentration of protein (45 g/L), lactose, calcium and citrate. Different mixtures of the two milks were prepared with five concentrations of pea milk (0%, 10%, 20%, 30% and 40%). These five mixtures were inoculated with 10 different starter cultures and incubated at 37°C for 24 hours to obtain 50 fermented products (Table 1). The products were prepared in jars, stirred for 30 seconds and placed in sealed plastic cups coded with three digit numbers. Samples were stored at 4°C and kept at room temperature (22°C) to equilibrate, one hour before serving. Panelists evaluated the products in standard sensory booths under green light to hide color variance in the products.

| Preparation of cow milk | Preparation of pea milk |
|-------------------------|-------------------------|
| Rehydrating 128.6 g of skim milk with tap water qs 1 liter | Hydrating 253 g of Pea protein isolate with tap water qs 2 liters |
| Adding 1.75 g sodium citrate | Adding 66.5 g lactose 1.6 g monocalcium phosphate and 1.75 g sodium citrate for 1 litre of the supernatant|
| Stirring for 5-10 minutes | Stirring for 3 hours |
| Adjusting the pH of each milk at 6.5 with HCl and NaOH | Centrifuging at 12000g for 50 minutes |
| Heating in water bath 10 minutes after reaching 90°C | Autoclaving for 15 minutes at 121°C |
| Inoculating the mix of milks with 10⁷ CFU/mL of each lactic acid bacteria | Incubating the inoculated milks at 37°C for 24 hours |

Figure 1. Yogurt preparation
Table 1. List of products

| Pea Concentration (%) | Starter culture                                                                 |
|-----------------------|----------------------------------------------------------------------------------|
| A                     | A00                                | Alsa (Streptococcus thermophilus + Lactobacillus delbrueckii subsp. Bulgaricus) |
| A10                   |                                    |                                                                                 |
| A20                   |                                    |                                                                                 |
| A30                   |                                    |                                                                                 |
| A40                   |                                    |                                                                                 |
| B00                   | B10                                | Streptococcus thermophilus + Lactobacillus delbrueckii subsp. bulgaricus        |
| B20                   | B30                                | Streptococcus thermophilus + Lactobacillus helveticus                          |
| B40                   |                                    | Streptococcus thermophilus + Lactobacillus rhamnosus                           |
| C00                   | C10                                | Lactobacillus delbrueckii subsp. Bulgaricus + Lactobacillus helveticus          |
| C20                   | C30                                | Lactobacillus delbrueckii subsp. Bulgaricus + Lactobacillus acidophilus         |
| D00                   | D10                                | Lactobacillus delbrueckii subsp. Bulgaricus + Lactobacillus fermentum           |
| D20                   | D30                                | Lactobacillus delbrueckii subsp. Bulgaricus + Lactobacillus rhamnosus           |
| E00                   | E10                                | Lactobacillus delbrueckii subsp. Bulgaricus + Lactobacillus rhamnosus           |
| E20                   | E30                                |                                                                                 |
| E40                   |                                    |                                                                                 |
| F00                   | F10                                | Streptococcus thermophilus + Lactobacillus acidophilus                          |
| F20                   | F30                                |                                                                                 |
| F40                   |                                    |                                                                                 |
| G00                   | G10                                | Streptococcus thermophilus + Lactobacillus casei subsp. casei                   |
| G20                   | G30                                |                                                                                 |
| G40                   |                                    |                                                                                 |
| H00                   | H10                                |                                                                                 |
| H20                   | H30                                |                                                                                 |
| H40                   |                                    |                                                                                 |
| I00                   | I10                                |                                                                                 |
| I20                   | I30                                |                                                                                 |
| I40                   |                                    |                                                                                 |
| J00                   | J10                                |                                                                                 |
| J20                   | J30                                |                                                                                 |
| J40                   |                                    |                                                                                 |

2.2 Panels

The panel was composed of 10 women between the ages of 22 and 50. All panelists were recruited amongst the students and staff of AgroSup Dijon, France. They attended 10 one-hour training sessions, one per week one session for selection, two sessions for vocabulary generation, four sessions for training and three sessions for panel performance evaluation. After that, the trained panelists evaluated in duplicate the 50 products (two one-hour sessions each week for 4 weeks).

2.3 Procedure

2.3.1 Selection

One selection session was carried out for testing the panelists’ ability to detect tastes (i.e., bitter, acid) and odors (butter, herbs, peas, and earth) on pea “yogurt” as well as their verbal fluency and ability to describe products. The 12 panelists with the highest detection performance, verbal fluency and ability to describe a product were selected. Among those 12 panelists, two abandoned the panel due to availability problem.

2.3.2 Generation of attributes

Attribute generation was conducted in two sessions. In the first session, panelists were asked to describe with their own words five samples selected among the 50 possible products so as to span as much as possible the product sensory space. Generated attributes were compiled to form a preliminary list. In the second session, panelists were presented five new products which they had not been exposed to before, and were asked to rate every attributes on a 6-point intensity scale (from 0 to 5) using the preliminary list of attributes. Panelists were free to add attributes to the list if necessary. A reduction of the list of attributes was then performed following the ISO 11035:1994 standard.

2.3.3 Training procedure

During training, panelists agreed upon definitions, references and procedures for each attribute and were trained to rank different water and yogurt solutions containing substances that give the required attributes (e.g. acid lactic for the acid attribute or caffeine for the bitter attribute). Finally, 10 new products were presented in duplicate to determine whether the panel was homogeneous, discriminant and repeatable.

2.3.4 Final profiling

The final profiling consisted of eight one-hour sessions (two sessions a week). Fifty products were evaluated in duplicate on a structured interval scale going from 1 (low) to 10 (high).
2.4 Data analysis

2.4.1 Panel performance

A three-way ANOVA was carried out for each attribute with the following model:

\[
\text{score} = \text{assessor} + \text{product} + \\text{assessor} \times \text{product} + \text{session} + \text{product} \times \text{session}.
\]

Both assessor and product were considered as fixed factors. When a significant product \(\times\) assessor interaction was found, a principal component analysis (PCA) was performed to evaluate the consensus between panelists. Data were analyzed using SPAD version 7.4.

2.4.2 Product description

Intensity scores obtained for each attribute were averaged across repetition and submitted to three-way ANOVAs with assessor, starter culture and pea concentration as within subject factors. Assessor was considered as a random factor and both starter culture and pea concentration as fixed factors. Attributes with a significant effect of either starter culture or pea concentration were then submitted to a normalized principal component analysis (PCA) and a hierarchical cluster analysis (HCA). ANOVA were performed using SAS 9.3, and PCA and HCA with the SPAD 7.4.

Table 2. List of descriptors and references generated by the panel to describe yogurts

| French  | Descriptor | English | Attribute classification | References                               |
|---------|------------|---------|--------------------------|------------------------------------------|
| Texture| Astringent | Astringent | -                        | Oak tannins                              |
|         | Fluid      | Fluid   | -                        | Whole milk (Carrefour)                    |
|         | Lisse      | Smooth  | +                        | Faisselle (Carrefour)                     |
|         | Crémeux    | Creamy  | +                        | Fresh cream 30% fat (Carrefour)           |
| Taste   | Sucré      | Sweet   | +                        | Lactose                                  |
|         | Amer       | Bitter  | -                        | Caffeine - Burdock [4]                    |
|         | Acide      | Acid    | -                        | Lactic acid - Contis, et al. [5]          |
| Aroma   | Vinaigre   | Vinegar | -                        | Acetic acid - Burdock [4]                |
|         | Terre      | Earth   | -                        | Beet juice                               |
|         | Végétale   | Vegetable | -                  | cis-3-Hexen-1-ol - Burdock [4]          |
|         | Fumé       | Smoked  | -                        | Barbecue sauce (Carrefour)               |
|         | Laitage    | Dairy   | +                        | Cow milk (Carrefour)                      |
|         | Pois       | Pea     | -                        | Pea flour                                |

3. RESULTS AND DISCUSSION

3.1 Attributes generated by the panels

To describe the products, the panelists used 13 attributes including four of texture (astringent, fluid, smooth, creamy) three of taste (sweet, bitter, acid) and six of aroma (vinegar, earth, vegetable, smoked, dairy, pea). The 13 attributes were classified as positive or negative attributes based both on the literature [6] and on a preliminary study (Table 2).

3.2 Panel performance

The product effect was significant for all the attributes at the 5% level. Therefore, the panelists were able to discriminate between the 50 products. The repetition effect was significant at the 5% level for six descriptors (fluid, creamy, smooth, astringent, bitter and acid). This repetition effect can however be due in part to differences in the products rather than...
in the panelists. A significant interaction assessor x product was found for all attributes. However, the PCA performed on each descriptor with assessors as variables, showed a good consensus between assessors except for the attribute bitter.

3.3 Product description and comparison with the standard

3.3.1. ANOVA: product description

The three-way ANOVA (Table 3) showed a significant effect of starter cultures for all descriptors except smoked. Globally, we found Alsa, Lactobacillus delbrueckii subsp. Bulgaricus + Lactobacillus fermentum, Streptococcus thermophilus + Lactobacillus rhamnosus and Lactobacillus rhamnosus to have higher intensity for positive descriptors such as creamy, dairy and sweet, and lower intensity for negative descriptors such as vegetable, earth and vinegar. On the other hand, starter cultures of Lactobacillus delbrueckii subsp. Bulgaricus + Lactobacillus helveticus, Lactobacillus delbrueckii subsp. Bulgaricus + Lactobacillus rhamnosus and Streptococcus thermophilus + Lactobacillus helveticus have higher intensity for negative descriptors such as acid and astringent but rather low intensity for pea and earth. Starter cultures, which lead to the highest intensity in negative descriptors such as pea or vegetal like Alsa, Streptococcus thermophilus + Lactobacillus rhamnosus and Lactobacillus rhamnosus have also high positive effect leading to descriptors as smooth and creamy.

A significant effect of pea concentration was also found for eight descriptors (vinegar, earth, fluid, creamy, acid, smoked, dairy and pea). Among those descriptors, as expected, the intensity of negative descriptors pea, earth, fluid, vinegar, and smoked increased with pea concentration, whereas the intensity of positive descriptors creamy, acid and dairy decreased with pea concentration.

Beside the main effects, significant interactions between starter culture and pea concentration were found for all descriptors except dairy and vegetable. An effect of pea concentration was observed for five negative descriptors (earth, smoked, pea, acid and fluid) as well as for two positive descriptors (smooth and creamy) for most starter cultures. For the other descriptors (vinegar, bitter, sweet and astringent), we observed a pea concentration effect for only a small number of starter cultures thus indicating that only a small number of starter cultures are able to counterbalanced the negative effect of pea proteins.

### Table 3. Results of three-way ANOVA

|                      | Starter culture | Pea concentration | Starter culture & pea |
|----------------------|-----------------|-------------------|-----------------------|
|                      | F Value         | Pr > F            | F Value               | Pr > F               | F Value              | Pr > F               |
| Odor vinegar         | 5.54            | <.0001            | 2.92                  | 0.0343               | 1.73                 | 0.0076               |
| Odor earth           | 2.57            | 0.0121            | 8.66                  | <.0001               | 1.73                 | 0.0074               |
| Odor vegetable       | 5.32            | <.0001            | 2.41                  | 0.0675               | 1.42                 | 0.0615               |
| Texture fluid        | 3.61            | 0.0008            | 26.70                 | <.0001               | 8.89                 | <.0001               |
| texture creamy       | 7.96            | <.0001            | 10.12                 | <.0001               | 5.49                 | <.0001               |
| texture smooth       | 10.49           | <.0001            | 2.72                  | 0.0446               | 9.31                 | <.0001               |
| sensation astringent | 4.73            | <.0001            | 1.05                  | 0.3950               | 1.50                 | 0.0387               |
| Taste sweet          | 7.48            | <.0001            | 1.16                  | 0.3443               | 2.77                 | <.0001               |
| Taste bitter         | 3.21            | 0.0023            | 2.66                  | 0.0485               | 1.50                 | 0.0386               |
| Taste acid           | 29.14           | <.0001            | 4.42                  | 0.0052               | 4.39                 | <.0001               |
| Aroma smoked         | 1.20            | 0.3041            | 24.31                 | <.0001               | 1.56                 | 0.0249               |
| Aroma dairy          | 2.88            | 0.0055            | 17.28                 | <.0001               | 1.17                 | 0.2377               |
| Aroma pea            | 6.03            | <.0001            | 36.17                 | <.0001               | 1.68                 | 0.0113               |

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3.3.2 PCA and HCA: comparison with the standard

Figure 2a and b represents the first two PCA dimensions that explain 61.36% of the total variance. The first dimension, that explains 35.67% of the total variance opposes the negative aroma attributes pea, earth and smoked as well as the negative texture attribute fluid to the positive aroma attribute dairy. It represents a gradient in pea concentration going from 0% to 40%. Negative attributes are mostly associated with the 40% pea concentration yogurts and the positive one to 0% pea concentration yogurts. The second dimension that explains 25.69% of variance opposes the positive attributes: sweet and creamy to the negative attributes: vinegar, astringent, acid and bitter. It opposes starter cultures yielding negative attributes such as Lactobacillus delbrueckii subsp. Bulgaricus + Lactobacillus helveticus and Lactobacillus delbrueckii subsp. Bulgaricus + Lactobacillus rhamnosus to starter cultures yielding positive attributes as Alsa (Streptococcus thermophilus + Lactobacillus delbrueckii subsp. Bulgaricus) and Streptococcus thermophilus + Lactobacillus acidophilus independently of pea concentration.

Figure 2b shows that with smaller concentrations of pea protein, an effect of starter cultures is observed. Some starter cultures seem to attenuate the negative effect of pea proteins whereas some others seem to exhaust it. For example, with 10% of pea protein, Alsa (Streptococcus thermophilus + Lactobacillus delbrueckii subsp. Bulgaricus) gave dairy and creamy characteristics whereas other ones like Streptococcus thermophilus + Lactobacillus helveticus and Lactobacillus delbrueckii subsp. Bulgaricus + Lactobacillus helveticus gave bitter, astringent and acid characteristics. The comparison between all formulated yogurts and the standard, situated in the bottom right corner, showed that one starter culture Streptococcus thermophilus + Lactobacillus casei subsp. casei gave rise to yogurts with up to 40% of pea protein. On the other hand, two starter cultures Streptococcus thermophilus + Lactobacillus acidophilus and Streptococcus thermophilus + Lactobacillus delbrueckii subsp. bulgaricus gave rise to yogurts with up to 30% of pea protein with sweet, smooth and creamy attributes close to those of a yogurt made with commercial starter culture and 100% of cow milk.

**Figure 2.** First two dimensions of the principal component analysis performed on the attribute by product matrix a) correlation circle, b) projections of the yogurts. Colors represent product groups yielded by the hierarchical cluster analysis.
The HCA performed on the first two PCA dimensions showed that the 50 products could be divided into four classes (Table 4). The first class includes eight products, seven of them without pea protein. It has been described with positive attributes close to the attributes of traditional yogurts usually consumed by the panel. Tu, et al. [6]. Moreover, products fermented by traditional starter culture Streptococcus thermophilus + Lactobacillus delbrueckii subsp. Bulgaricus have good evaluation (neither acid nor astringent). The second class includes 10 products; two of them have been described with negative descriptors as bitter, astringent and acid despite the absence of pea protein. This may be caused in “I” starter culture by the high ability of acidification of Lactobacillus rhamnosus that could decrease sensory characteristics, [7], or by the high esterase activities of the two strains in “E” starter culture Lactobacillus delbrueckii subsp. Bulgaricus + Lactobacillus helveticus [8]. The third group includes promising products, which were not associated with negative characteristics such as astringent, acid or bitter and have received high scores for positive descriptors like sweet, smooth and creamy. The metabolic activity of some microorganisms such as Lactobacillus acidophilus [9], and Lactobacillus casei [10], results in production of flavor, and aroma that cause good organoleptic properties. These organoleptic properties could be cumulated to those of traditional strains used in fermented products (Streptococcus thermophilus and Lactobacillus delbrueckii subsp. Bulgaricus), when they were used together as starter cultures. The main quality caveat of this group of products is the presence of pea-derived aromas (pea, vegetal and earth) which might be reduced using aromatization processes. The fourth class includes products with 40% of pea protein, which are characterized by high intensity of pea aromas. With this level of pea protein, no starter culture was able to decrease the negative characteristics of pea protein.

Table 4. Four yogurt classes yielded by the hierarchical cluster analysis performed on the projection of the yogurts on the first two dimensions of the principal component analysis. The plus and minus sign indicate the positive and negative descriptor that are more significantly present in the class than in the whole set of products (t-test, α=0.05)

| Class 1          | Class 2          | Class 3          | Class 4          |
|------------------|------------------|------------------|------------------|
| A00 A10 B00 C00 D00 F00 G00 H00 | B10 C10 E00 E10 E20 E30 G10 I00 I20 I30 | A20 A30 B20 B30 C20 D10 F10 F20 F30 G20 H10 H20 H30 H40 I10 J00 J10 | A40 B40 C30 C40 D20 D30 D40 E40 F40 G30 G40 I40 J30 J40 |
| +                | -                | +                | -                |
| Dairy Creamy     | Vegetable Vinegar Fluid Earth Pea Smoked | Acid Astringent Bitter | Pea Creamy Smooth Sweet |
| Sweet Smooth Creamy | Astringent Acid Bitter | Earth Pea Smoked Vegetable Fluid Sweet Vinegar | Dairy Acid Creamy |
physico-chemical properties the current results will enable us to select the starter cultures and highest pea protein concentration possible that give products with the closest quality to the traditional yoghurt.

Đánh giá cảm quan: một công cụ để đánh giá chất lượng các sản phẩm lên men

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TÓM TẮT

Mười chế phẩm canh trường giống vi khuẩn lactic (i.e., lactobacillales) được sử dụng để lên men 5 hỗn hợp sữa và protein đậu (với tỷ lệ tính theo protein đậu lần lượt là 0%, 10%, 20%, 30% và 40%) nhằm xác định tỷ lệ phù hợp mà sản phẩm tạo ra có tính chất cảm quan tương đồng với sản phẩm yaourt truyền thống. Chất lượng sản phẩm được đánh giá bằng cách so sánh cường độ các tính chất cảm quan (sensory profile) của 49 công thức được tạo ra với cường độ các tính chất cảm quan của sản phẩm sữa được lên men bởi giống lactic thương phẩm.

Số liệu thu nhận được phân tích bằng phương pháp phân tích phương sai (ANOVA) 3 yếu tố và phương pháp phân tích thành phần chính (PCA). Kết quả cho ra rằng: ở tỷ lệ thay thế protein sữa bò bằng protein đậu 40%, sản phẩm lên men sẽ giảm (mất) đi các tính chất cảm quan tốt và làm giảm chất lượng sản phẩm. Ngược lại, ở tỷ lệ thay thế bằng hoặc dưới 30%, canh trường giống có ảnh hưởng tích cực lên tiêu cực. Ví dụ, các sản phẩm được lên men bằng chủng Streptococcus thermophilus + Lactobacillus acidophilus ở tỷ lệ thay thế 30% protein đậu sẽ sở hữu các tính chất cảm quan tốt như creamy (sệt) và smooth (mịn), nhưng nếu lên men bằng chủng Lactobacillus delbrueckii subsp. Bulgaricus + Lactobacillus rhamnosus, sản phẩm tạo ra sẽ có các tính chất cảm quan không mong muốn như vị đắng và chất cho dù sản phẩm đó được lên men từ hỗn hợp có tỷ lệ sữa bò bằng 100%.
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