

## Fermented Dairy Product for a Low-Fat Bakery Product Application: Chemical and Sensory Analysis

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### Abstract

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Whey was fermented using different lactic acid bacteria in order to obtain an ingredient as a substitute for butter in pastry products. *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis* was selected based on its capacity to produce buttery flavours like diacetyl. Fermentation parameters such as temperature, aeration, citrate addition, and the duration of each fermentation step were selected in order to boost diacetyl production. Fermented whey was then added as an ingredient to white sandwich bread which did not initially contain any butter in the recipe. Sensory analysis brought to light that the buttery aroma was under the perception threshold in the bread containing the mixture even though the fermented whey contained diacetyl.

**Keywords:** *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*; buttery flavour; diacetyl, butter; pastry product

Over recent decades, campaigns in European and the other Western countries have underlined the nutritional impacts of modern food on consumer health. Indeed, world organisations, such as WHO or FAO, and scientific groups, have recommended the setting up of nutritional strategies to improve the overall health of a country's population. One important programme set up in France in 2001 is the Programme National Nutrition Santé – PNNS (national nutrition and health programme) (HERCBERG *et al.* 2008). In this context, several food companies signed an agreement with the government to certify, for instance, their engagement to reduce the energy content of their foodstuffs.

In pastry products, butter is one of the most important ingredients, generating pleasant organoleptic characteristics. Besides having the high lipid content, butter also contains a lot of aroma compounds (BENNION & BAMFORD 1997). Consumers may be put off choosing alternative low-fat products without butter because of the alteration this implies to their organoleptic characteristics: they simply do

not recognise the product they have purchased. In order to prevent the deterioration of organoleptic characteristics as a consequence of butter reduction in the recipe, food producers supplement their formulas with flavourings such as diacetyl, a molecule known to be a key compound in the butter aroma. But too frequently, this artificial diacetyl flavouring fails to restore a pleasant nutty-creamy buttery aroma (BUHLER *et al.* 1972). In addition, negative publicity over the years has given flavourings a bad name, but they must nonetheless appear on all foodstuff packaging in line with the European Regulation EU/1334/2008. To overcome these difficulties, the addition of fermented ingredients to the recipe could be an alternative to reducing butter. These fermented products are thought to have the capacity to improve the buttery aroma thanks to the action of microorganisms. Diacetyl is produced for instance by *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* (but also by many *Leuconostoc* strains, yeast, and even many Gram-negative bacteria) and results from the citrate metabolism. Besides diacetyl,

other aroma compounds originating from the bacterial metabolism contribute to the pleasant buttery aroma (LONGO & SANROMÁN 2006). This solution could prevent aroma degradation during the different steps of the baking process, especially cooking, thanks to the formation of interactions between the components of the matrix and the buttery aroma molecules – namely diacetyl.

The aim of this work was to study the effect on the aroma characteristics of white sandwich bread (also called in France pain de mie) of adding a buttery aroma mixture – resulting from the citrate fermentation of a food matrix by two *Lactococcus* strains – to the recipe.

## MATERIAL AND METHODS

**Bacterial strains.** Two *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* strains were used. One of the two lactococci was selected from a pool of citrate positive *Lactococcus* strains picked up in traditional fermented dairy products and the other was a citrate positive selected starter. The first strain, named F2.3, was isolated from raw goat whey, phenotypically characterised and identified by 16S rRNA gene sequencing. The second strain, named Dx1, was a commercial strain used for producing buttery aroma in dairy fermented products. This strain was used as a control. The strains were kept frozen at  $-80^{\circ}\text{C}$  (Thermo Fisher Scientific, Waltham, USA) in a mixture of culture medium (M17 broth; Biokar, Beauvais, France) and glycerol 30% (Sigma-Aldrich, St-Quentin Fallavier, France).

***Lactococcus* growth conditions, pH analysis, and diacetyl production.** The food matrix, composed of a sweet whey solution (10%, w/v; Euroserum, St Martin Belle Roche, France), was prepared and aliquoted in 1 l flasks (500 ml/flask). This volume enabled to optimise the surface-to-volume ratio, thus maximising the oxygenation of the whey solution during the later step of fermentation. This solution was sterilised at  $110^{\circ}\text{C}$  during 10 minutes. Two flasks were each seeded with one strain of *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, F2.3 or Dx1, at the level of 6 log (CFU)/ml (Figure 1). Pre-fermentations were performed aerobically during 16 h at  $25^{\circ}\text{C}$  by shaking the whey solution vigorously with a magnet bar rotating at 1100 rpm (RCT Basic magnetic stirrer; IKA, Staufen, Germany). At the end of the pre-fermentation step, the pH was adjusted

to 4.5 (PHM210 standard, MeterLab®, Villeurbanne, France) and anhydrous trisodium citrate (1%, w/v; Quimdis, Levallois-Perret, France) was added to the pre-fermented whey solution. During the second step of citrate fermentation, the whey mixture was again vigorously shaken for 24 h at  $20^{\circ}\text{C}$ . During this second step, it was assumed that large amounts of diacetyl and other flavourings were produced via the citrate metabolism. In this article, the fermented whey mixtures are referred to as diacetyl.F2.3 and diacetyl.Dx1 for the products obtained with the strains F2.3 and Dx1, respectively. These cultures were then quickly cooled down in frozen water and the pH was recorded. Each culture was split into two parts: one used for the extraction of volatile components, the other for preparing loaves of white sandwich bread. Each fermentation was performed in triplicate.

**General procedure of diacetyl measurement.** The extraction of diacetyl was performed using acetone. One millilitre of the diacetyl mixture sample was mixed with 1 ml of acetone and shaken vigorously for 30 s at 3000 rpm (Vortex agitator; IKA, Staufen, Germany). After centrifugation at 4000 g for 5 min (5417R microcentrifuge; Eppendorf, Montesson, France), the supernatant was removed for specific diacetyl quantification. This fraction was kept at  $-80^{\circ}\text{C}$  for less than 7 days. After filtration on a cellulose acetate filter (0.2  $\mu\text{m}$ ), the supernatant was directly injected into the gas chromatography apparatus.

Gas chromatography analysis of diacetyl was performed using a gas chromatograph equipped with a flame ionisation detector (GC-FID) (Perkin Elmer Autosystem GC, Waltham, USA) (MACCIOLA *et al.* 2008). A capillary column (30 m  $\times$  0.32 mm, 0.25  $\mu\text{m}$  film thickness) with a 100% polyethylene glycol phase (ZB-Wax column; Phenomenex, Torrance, USA) was used. Gas chromatography parameters were as follows: helium carrier gas (1.5 ml/min), injected amount 5  $\mu\text{l}$ , split mode injection at 1 : 10 splitting ratio, injector and detector temperatures 240 and  $255^{\circ}\text{C}$ , respectively; the oven temperature ran from  $50^{\circ}\text{C}$  to  $240^{\circ}\text{C}$  at  $7^{\circ}\text{C}/\text{min}$  with three isotherms: 2 min at  $91^{\circ}\text{C}$ , 3 min at  $107^{\circ}\text{C}$ , and 3 min at  $186^{\circ}\text{C}$ . The amount of detected molecules was expressed in surface area units (mV/min) calculated with the Azur Version 5.0.10.0 software (Launaguet, France). The means and the standard deviations were calculated with the data obtained from three replications with the same strain culture.

**White sandwich bread preparation.** The control white sandwich bread (named thereafter bread.Ctrl)

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was composed of 1000 g of white flour (T450), 250 g of water, 50 g of sugar, 30 g of fresh yeast, 30 g of neutral colza oil, and 10 g of salt. Test breads were made following the same recipe except that 100 g of water were replaced by the equivalent volume of the diacetyl.F2.3 and diacetyl.Dx1 preparations. These breads will be named bread.F.2.3 and bread.Dx1 hereafter.

The ingredients were mixed prior to kneading (SPI 11; VMI Vendée Mécanique Industrie, Montaigu, France) for 15 min at 120 rpm. The dough was left to rest in bulk for 20 min, scaled into 450 g portions, moulded in a small Exoglass® mould (base dimensions: 250 × 80 × 85 mm, lid dimensions: 290 × 95 mm) (Machinefabriek Holtkamp BV, Almelo, Netherlands), and placed in the proofer (BFA; Bongard, Strasbourg, France) set to  $28 \pm 1^\circ\text{C}$  and 75% relative humidity for 105 minutes. Baking was carried out at  $170^\circ\text{C}$  for 30 min in a deck oven (2000; Jolivet, Gommegnies, France). The core temperature of the loaf at the end of the baking stood at  $99^\circ\text{C}$  (digital probe thermometer, Kat. Nr. 30.1018; TFA, Düsseldorf, Germany). The loaves were removed from the pan and allowed to cool down for 120 min on cooling racks at room temperature. Individual loaves were stored at  $25^\circ\text{C}$  for 24 hours.

**Sensory analysis.** The loaves were cut with a bread slicer (Industrade, Strasbourg, France) into 10 mm thick pieces. Triangle tests were carried out to assess differences in the characteristic aroma present in the loaves made by the two different recipes. Sessions were carried out in a specific sensory room 1 h after the sample preparation. For each triangle test, samples were given to 30 untrained judges, spanning an age range from 20 to 50, the two genders being present in equal numbers. During the session, judges were asked to taste 3 samples. Panellists were forced to answer the question (forced-choice method). Samples were presented to the judges in a random order. A significant taste difference for a risk of 5% between two breads was obtained when it was perceived by more than 15 judges according to the binomial distribution B ( $n, 1/3$ ),  $n$  being the group size (NF EN ISO 4120:2007 – Sensory analysis Methodology – Triangular test).

## RESULTS AND DISCUSSION

**pH analysis and diacetyl quantification.** During incubation, the two strains F2.3 and Dx1 raised

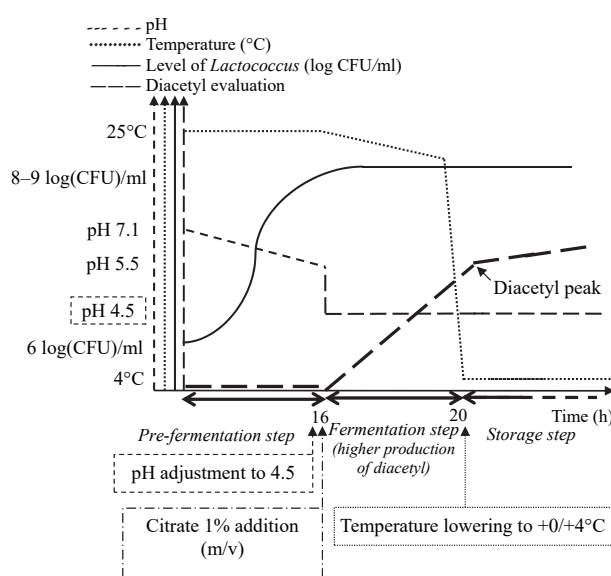


Figure 1. Monitoring of the pre-fermentation and the citrate fermentation steps for the diacetyl production by citrate positive *Lactococcus* strains (F2.3 and Dx1); the characters in a frame represent the actions led for the pilotage of the fermentation

the pH of their medium from 4.5 at the end of the pre-fermentation step to 6.1 and 5.7, respectively, at the end of the fermentation step (Figure 1). The pH measurement was used as an indicator of citrate metabolism efficiency and the correlative production of flavourings. Indeed, the citrate metabolism is generally assumed to lead to the re-alkalisation of the medium (GEMELAS *et al.* 2014). The pH increase of the whey mixture therefore suggests that diacetyl production has occurred. The analysis of diacetyl by a chromatographic method (GC-FID) confirmed this assumption: diacetyl was present in the two bacterial cultures, F2.3 or Dx1. The two samples released a pleasant creamy-buttery odour, slightly more pronounced in the case of the Dx1 starter. According to the chromatographic analysis, the areas corresponding to the diacetyl peak were equal to  $1.5 \pm 0.5$  and  $23.8 \pm 8.3$  mV/min for strains F2.3 and Dx1, respectively, i.e. a 1 : 16 ratio. This latter strain is habitually used to improve the buttery aroma of dairy fermented products. This great difference in a diacetyl amount between the two strains was expected: in an industrial fermentation process, the starter strains are selected for their technological properties, bringing optimum acidity and/or flavour to the product (WOUTERS *et al.* 2002). The perception threshold of diacetyl in butter is low. Concentrations ranging from 0.0045 ppm to 0.01 ppm and between

0.01 and 0.055 ppm of diacetyl are perceptible by orthonasal and retronasal olfaction, respectively (SCHIEBERLE *et al.* 1993; MALLIA *et al.* 2008). Thus, even the F2.3 strain, a low diacetyl-producing strain compared with the Dx1 strain, was expected to flavour the end product. In addition, the wild strain F2.3, selected from a pool of citrate positive strains originating from natural whey starters, could prove very useful for the industrial production of a diacetyl mixture. These wild rustic microbes display a lot of interesting features, among them a broad spectrum of resistance to phages. This allows to select variants facilitating rotation, thereby helping to prevent microbial cultures from phage attacks (MOINEAU 1999). For these reasons, the strain F2.3 was the one retained hereafter to test the effect of adding a microbial culture to a bakery product to guarantee, or even to enforce, a buttery aroma.

**Sensory analysis.** The two microbial cultures (diacetyl.F2.3 and diacetyl.Dx1) were added to the recipe of white sandwich bread to evaluate if and how the buttery aroma would be perceived in this bakery product. Triangle tests were performed to find out if there were differences in aroma between bread.Ctrl (i.e. without any creamy or buttery aroma addition) and bread.F2.3 and bread.Dx1. Results showed that bread.Ctrl was not significantly different from bread containing the diacetyl.F2.3 culture. We can then consider that the buttery aroma was under the perception threshold in the bread.F2.3. The low level of diacetyl in this mixture, and thus inside the bread (owing to the rate of dilution induced by dough preparation), could explain this absence of buttery aroma in the white sandwich bread. However, the same result was obtained for the bread.Dx1: the buttery aroma was therefore assumed to be under the threshold of perception in the bread.Dx1. In the culture medium, as observed above, the strain Dx1 produced 16 times more diacetyl than the strain F2.3. These results complement a previous study undertaken with another citrate positive strain: the heterofermentative *Lactobacillus casei* subsp. *rhannosus*. GELINAS *et al.* (1995) developed dairy-flour mixtures fermented by a pure culture of a citrate positive *Lactobacillus* strain. These fermented ingredients were then used in milk bread recipes and sensory analyses were performed. Cheese-like aroma and acidic flavours were recorded by the panellists but without any buttery note, just like in the present study. The buttery aroma is imperceptible regardless of the microbial culture used and the bakery product tested.

The absence of the buttery aroma in the bread made with the citrate positive cultures used in this experiment could result from the evaporation of aroma compounds during the bread-making process, and especially during cooking. The relative volatility – characterising the capacity of a compound to reach a gaseous phase – influences the rate at which the volatile compound dissipates. This parameter is influenced by three factors. Firstly, compounds with low molecular weight have a greater ability to diffuse throughout the matrix during cooking, i.e. small molecules are retained less than the largest ones (DE ROOS *et al.* 2003). Secondly, polar molecules are retained less than nonpolar compounds. Indeed, polar molecules – soluble in water – can spread easily throughout the matrix by diffusion (ROSENBERG *et al.* 1990). Thirdly, the lower the boiling point, the greater the relative volatility of a compound is. If we consider diacetyl, it matches all three of these characteristics: it is a polar diketone with four carbon atoms (and so a small molecule) and a low boiling point (88°C). In light of the core temperature of the bread at the end of baking – 99°C, it would seem that all the diacetyl had been evaporated during the bread-making process. In the same way, GASSENMEIER and SCHIEBERLE (1994) showed that 90% of the diacetyl present in puff-pastry evaporated during the cooking step (12 min at 180°C). Therefore, our postulate concerning the preservation of diacetyl produced by a fermentation process turned out to be inaccurate. Contrary to a fresh milk product, dough is baked and the buttery aroma could have evaporated during the baking step. Therefore, the way to give bread a natural buttery aroma without using butter is still to be found. Relative volatility also depends on the interaction between the aroma compounds and the matrix (BANGS & REINECCIUS 1982). This way could be explored to preserve diacetyl from evaporation during the baking process. The possible entrapment of diacetyl in a macromolecular network of exopolysaccharides produced by another lactic acid bacterium, *Leuconostoc*, will be investigated in a future article.

## CONCLUSION

In white sandwich bread, the diacetyl produced by a citrate positive lactic acid bacterium and added to the recipe, was not perceptible by the panellists of a triangle test. We can wonder whether panellists are ‘really’ able to perceive butter in a pastry product or



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whether butter contributes in other ways to the quality of the final product – texture for instance – but not only to aroma. But we can also postulate that the diacetyl evaporated during the bread-making process and that the residual concentration was below its threshold value of perception. To circumvent this latter problem, the next steps of our research will aim at the use of exopolysaccharides produced by *Leuconostoc* strains for the entrapment of diacetyl. This work will be the subject of an upcoming article.

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