Development of Fermented Dairy Ingredients as Flavor Enhancers for Bread^{1,2}

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ABSTRACT

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Optimal conditions for the preparation of concentrated fermented dairy ingredients for bread are presented. The inoculation of an equal mixture of milk and whey (20% dairy solids) with *Lactobacillus casei* subsp. *rhamnosus* gave good flavor development, without off-flavor, after 16 hr of fermentation, but best results were obtained after 24 hr. Incorporation of second patent wheat flour in the growth medium had a marked buffering effect and led to a higher total titratable acidity (TTA). Best results were obtained with whole wheat flour (30% w/v), which further increased TTA as well as flavor formation, especially at 38°C. Partially replacing L.

casei with L. helveticus and Streptococcus thermophilus (4% for each culture), followed by growth at 38 or 42° C, gave higher TTA and higher levels of lactic acid and diacetyl (only at 42° C). Compared to bread made without dairy solids, bread prepared with fermented dairy ingredients was mostly characterized by its higher content in lactic acid, ethanol, and diacetyl. Its aroma was described as pleasant (cheese-type) and more intense than that of the control. Fermented dairy ingredients can be dried and used as flavor enhancers (1-2%, db) in breadmaking processes with short fermentation periods or used as sourdough bases (up to ~10%, db).

Dairy products are traditional ingredients for breadmaking. Skimmed milk and sweet whey help to improve bread quality (Vetter 1984). Besides nutrition aspects, the main benefits from the use of dairy ingredients in bread formulations include higher water absorption, better fermentation time tolerance, and nicer crumb grain as well as crust color formation (Pyler 1988). Doerry (1989) has also stressed the positive effect of dairy ingredients on bread flavor. However, milk replacers are becoming increasingly popular and cost less than skimmed milk. Thus there is a need for improved dairy ingredients for breadmaking.

Fermentation is an effective process in developing new dairy ingredients. Main (1991) described four types of fermented dairy products as food ingredients: traditional fermented dairy products (cheese or yogurt); form-modified fermented dairy ingredients (spray-dried yogurt); flavor-modified fermented dairy ingredients (enzyme-modified cheese); and functionally engineered fermented ingredients (cultured whey). The definition of cultured whey is rather nonspecific and has been applied to fermented whey containing propionic acid as an antimycotic agent.

Information on the use of fermented dairy ingredients in bread is scarce. Yogurt may be used in bread formulations (Hill 1974), and the subsequent product certainly benefits from the positive image of this dairy ingredient. Lehmann and Dreese (1981) have shown that yogurt has a positive effect on the flavor of bread, but it decreases loaf volume. Shenkenberg et al (1972) proposed to incorporate acid whey in a bread recipe, this dairy ingredient being the main by-product from cottage cheese manufacturing. Bread flavor is markedly changed by this process, which produces a sourdough-type bread. Other fermented dairy ingredients may be tailored to enhance bread flavor to speed up sourdough processing. In addition, these flavorants may be important in shortened breadmaking systems, such as the no-time dough process, where fermentation times are kept at minimum and cannot significantly contribute to flavor development.

In fermented milk technology, the choice of microbial cultures is of major importance. According to their optimal growth temperature, two classes of lactic acid bacteria are normally used for the preparation of fermented milks: mesophilics and thermophilics. Most mesophilics are used in cheese manufacturing and for the preparation of some fermented milks such as cultured buttermilk. Adding citrate to some species produces diacetyl, a highly flavored component important in butter flavor. Mesophilics are good aroma producers, but not very acidifying. Thermophilics, the other group of lactic acid bacteria offering some potential, are used for the production of fermented milks such as yogurt. Depending on their ability to produce mainly lactic acid, these cultures are considered either homofermentative or heterofermentative. They are good acid producers and develop flavorful compounds such as acetaldehyde and, possibly, diacetyl (Dellagio 1988).

In modern sourdough technology, the choice of microbial starters is also of great importance (Brümmer 1991). Homofermentative (*L. plantarum, L. acidophilus*) and heterofermentative (*L. brevis, L. fermenti*) lactic acid bacteria, as well as yeasts, are representative groups of microorganisms naturally found in sourdoughs or used as starters. Therefore, it would be possible to design specific fermented dairy products to be incorporated as ingredients for breadmaking, considering that milk is a very good growth medium for lactic acid bacteria. These formulations must be as concentrated as possible and have no off-flavor, which is of major concern.

In this article, we report on the development of fermented dairy ingredients to be used in breadmaking. The outline of this process was disclosed in Gélinas et al (1992). The effects of strain selection and growth conditions on pleasant flavor development are presented. Flavorants were evaluated at a high concentration in a no-time dough recipe and as a sourdough base. In addition, our intention was also to use fermented dairy ingredients at low concentrations as bread flavor enhancers.

MATERIALS AND METHODS

Bacterial Cultures

Four types of lyophilized cultures were tested (Institut Rosell, Montreal): 1) a mesophilic culture for buttermilk composed of Leuconostoc cremoris, Streptococcus lactis subsp. lactis, S. lactis subsp. diacetylactis and S. lactis subsp. cremoris; 2) a thermophilic yogurt culture composed of Lactobacillus bulgaricus and S. thermophilus; 3) a thermophilic facultative heterofermentative culture, L. casei subsp. rhamnosus; 4) a thermophilic homofermentative culture composed of L. helveticus and S. thermophilus.

Dairy Ingredient Preparation

A volume of 1 L of dairy ingredient was prepared with variable proportions of reconstituted high-heat skimmed milk and sweet whey. The medium was supplemented with 1% sodium citrate and was inoculated with 2.5% (4 × 10⁸ cells/g of dairy preparation) of lyophilized starter. Second patent flour (0-30 g/100 ml of reconstituted dairy product) was added to the dairy preparation.

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Temperature, time, and shaking conditions had been established in preliminary studies. General fermentation conditions were 38° C, 24 hr, and 140 rpm in 500-ml Erlenmeyer flasks containing about 350 ml of dairy preparation.

Breadmaking Process

White pan bread was produced using a no-time dough procedure with: 100% flour (14% moisture), 4% sugar, 3% compressed yeast (30% solids), 3% shortening, 2% salt, 100 ppm ascorbic acid, 60 ppm potassium bromate, water (variable), and 26% fermented dairy ingredient in the liquid form (giving 10% total solids, considering that specific liquid ingredient had 30% whole wheat flour added to it).

Four replicates of each batch were produced from 2 kg of flour with a Hobart mixer (A-200T), giving nine doughs scaled to 330 g. Doughs were rounded, bench-rested for 10 min, molded, and proofed at 40°C and 100% rh to constant height (2.5 cm above rim). Doughs were baked at 193°C for 20 min. Loaf volume was measured by rapeseed displacement after the breads had cooled for 1 hr. Bread prepared without dairy solids was used as a control.

Total Titratable Acidity

TTA and pH were determined on 9 g of dairy ingredient supplemented with 18 g of water. TTA was measured in duplicate with NaOH N/9 to pH 8.6. TTA of bread samples was measured using a modified procedure where 15 g of bread crumb (from the center of the loaf) was shaken for 30 min in 100 ml of water and TTA was measured by titration with NaOH N/9 to pH 6.6 (Sutherland 1989). TTA was expressed as meq mol of lactic acid per gram, calculated as:

> Volume NaOH (ml) \times Normality (meq mol ml⁻¹) /weight of sample (g)

Organic Acids Analysis

Fermented milk samples (50 g) were diluted with 100 ml of water, centrifuged for 15 min at $20,000 \times g$ and filtered through a 0.22 μ m membrane filter before injection for high-performance liquid chromatography. Bread samples (20 g) were extracted with 80 g of water then processed as described by Lönner and Preve-Åkesson (1988). Samples were homogenized, heated for 5 min at 60°C, cooled at room temperature, and adjusted to pH 7 with NaOH (1N). The mixture was stirred for 30 min, centrifuged, and filtered through a 0.22 μ m membrane filter. Separation was performed by high-performance liquid chromatography (Bio-Rad) using a 300 mm \times 7.8 mm i.d. interaction cation-exchange ION-300 polymer resin column (Mandel Scientific Co. Ltd., Rockwood, ON, Canada), at 25° C with H₂SO₄ (0.01N) as mobile phase at 0.4 ml min⁻¹. All analyses were performed in duplicate from two repetitions (two different batches of fermented dairy ingredient or bread).

Volatile Compounds Analysis

Volatile compounds were extracted by steam distillation, according to Lin and Jeon (1985), with slight modifications: 20 g of liquid fermented dairy ingredient was diluted with 30 ml of

TABLE I
Influence of Milk-Whey Ratio on pH and Total Titratable
Acidity (TTA) of Fermented Dairy Ingredient ^a

Ingredient Composition	рН	TTA (× 10 ⁻² meq mol g ⁻¹)
Milk 100%	4.44	20.8
Milk 75%/whey 25%	4.48	18.4
Milk 50%/whey 50%	4.40	18.0
Milk 25%/whey 75%	4.52	14.9
Whey 100%	4.66	12.8

^aData are means of two repetitions. Ingredients were prepared with *Lactobacillus casei* subsp. *rhamnosus* grown at 38°C for 24 hr and shaken at 140 rpm.

Separation was performed by gas-liquid chromatography (Hewlett-Packard, model HP-8590A) using a Supelco-Wax column (30 m length \times 0.75 mm i.d.). After 12 min, the oven temperature was raised from 35 to 150°C at a rate of 10°C/ min, with a 10-min hold at the end. Injection port and flame ionization detector were maintained at 200°C. All analyses were performed in duplicate from two repetitions (two different batches of fermented dairy ingredients or breads). As described by Lin and Jeon (1985), standard curves (acetaldehyde, methyl sulfide, acetone, 2-butanone, 2-pentanone, and 2-heptanone) were used to quantify peaks.

RESULTS AND DISCUSSION

Selection of Microorganisms

Three types of lactic bacteria were tested for their acidification potential in milk, without producing off-flavors, over long fermentation periods. We were also looking for a microorganism capable of producing dairy product flavors that were as tasty as possible.

In milk (20% solids, 2.5% inoculum [w/v] giving $\sim 4 \times 10^8$ cells/g of solution), both the yogurt culture (L. bulgaricus and S. thermophilus) and L. casei subsp. rhamnosus developed higher acidification after 24 hr at 38°C (2.38 and 2.41%, respectively) than did the buttermilk culture grown at 24 or 29°C (1.66%), even though the latter was more aromatic. In fact, acidity of the long-fermented yogurt was about three times higher than that of commercial yogurt. Unlike L. casei, the yoghurt culture gave off-flavors to the final product; this was probably due to proteolysis and to the rather extended fermentation period (24 hr vs. 4-6 hr for the regular yogurt process). Thus, we chose L. casei subsp. rhamnosus, a representative heterofermentative lactic acid bacteria, for use in the rest of this study. No difference in TTA was observed when 5% inoculum was used instead of 2.5%, corresponding to 4×10^8 cells/g of growth medium (data not shown).

Effect of Medium Composition

The effect of the dairy solids content (%) of milk on TTA of *L. casei* subsp. *rhamnosus* was important below 16%, but no difference was observed between 20 and 24% (data not shown). The effect of substituting whey for milk in the growth medium is presented in Table I. As a by-product of cheese manufacturing, whey is cheaper than milk and can easily support fermentation by lactobacilli. As milk was replaced by whey, TTA markedly dropped and off-flavors appeared. Equal mixtures of milk and whey (20% total solids) gave high TTA without perceptible off-flavors in the dairy preparation. This was determined twice by a difference test (multiple comparisons) with a panel of four experts in the field. These conditions (20% total solids, equal mixture of whey and milk) were kept constant for the rest of the study.

Flour was added to the dairy preparation to study its effect on fermentation (Table II). At 10% (w/v) of reconstituted dairy preparation, flour had some buffering effect and probably had a stimulating effect on the activity of the lactic acid bacteria added to the dairy preparation. Final pH lowered to 3.9 compared to 4.6 when no flour was used. Above 20% white flour, acidity production stabilized to about 27×10^{-2} meq mol g⁻¹. Using whole wheat flour to replace regular white flour led to slightly higher acidity development. For the rest of the study, the incorporation of 30% (w/v) whole wheat flour was chosen as the upper limit concentration because it led to high development of acids and flavors without causing too many sedimentation problems.

With 15% dairy solids, incorporating white flour into the dairy preparation at 10, 5, or 0% had no effect on TTA. This is contrary

TABLE II
Influence of Flour Concentration on pH and Total Titratable
Acidity (TTA) of Fermented Dairy Ingredient ^a

Flour Type	Concentration (g/100 g pre-ferment)	pН	TTA (× 10 ⁻² meq mol g ⁻¹)
No flour	0	4.63	16.4
White	10	4.23	21.4
	20	3.97	26.1
	30	3.92	27.0
	40	3.92	26.1
Whole wheat	10	4.12	23.7
	20	4.12	28.0
	30	3.89	28.0
	40	3 88	28.1

TABLE III
Influence of Starter and Growth Temperature on pH and Total
Titratable Acidity (TTA) of Fermented Dairy Ingredient and Bread

	Temperature	Dairy Ingredient		Bread	
Starter	(°C)	pН	TTA ^b	pН	TTA
Standard (no inoculation)				5.32	1.9
L. casei subsp. rhamnosus	38	3.99	2.6	4.55	5.0
L. casei subsp. rhamnosus +					
L. helveticus/S. thermophilus	38	3.99	2.7	4.56	5.2
L. casei subsp. rhamnosus + L. helveticus/S. thermophilus	42	3.95	2.8	4.48	5.6

^aData are means of two repetitions. Ingredients were prepared with *Lactobacillus casei* subsp. *rhamnosus* grown at 38°C for 24 hr and shaken at 140 rpm.

^aData are means of four repetitions. Ingredients were prepared with 30% whole wheat flour, fermented for 24 hr and shaken at 140 rpm.

^bCalculated as $\times 10^{-2}$ meq mol g⁻¹.

TABLE IV	
Influence of Starter and Growth Temperature on Organic Acids (mg/g) of Fermented Dairy Ingred	ient [*]

	-	-				
Starter	Growth Temperature (°C)	Lactic Acid	Citric Acid	Formic Acid	Propionic Acid	Acetic Acid
L. casei subsp. rhamnosus	38	82.88	19.50	11.08	6.03	3.73
L. casei subsp. rhamnosus + L. helveticus/S. thermophilus	38	93.98	19.28	0.80	6.55	4.29
L. casei subsp. rhamnosus + L. helveticus/S. thermophilus	42	112.43	19.43	1.60	6.54	3.97

^aData are means of four repetitions. Ingredients were prepared with 30% whole wheat flour, fermented for 24 hr and shaken at 140 rpm.

to what was obtained with 20% dairy solids. Dairy solids content (20% of the growth medium) had a more decisive effect on TTA than the flour content. Because of sedimentation problems from the use of flour in the growth medium, it was necessary to shake the flasks during fermentation. Flasks shaken at 140 rpm gave excellent results; higher rates such as 200 rpm reduced TTA development (2.37-1.86%). Cells were probably inhibited by the formation of oxygen peroxyde when oxygen was incorporated in growth medium (Driessen and Puhan 1988).

Adding sodium citrate (up to 1%, w/v) to the growth medium did not affect TTA, but it did slightly improve the flavor of the resulting dairy product; at 2%, TTA dropped markedly (data not shown). Citrate is a precursor for diacetyl; it is metabolized by mesophilic lactic acid bacteria and, in some cases, by heterofermentative thermophilic lactobacilli such as *L. casei* subsp. *rhamnosus* and possibly the microbial flora of the flour added to the dairy base. Flour added to the dairy base was a probable source of bacteria and yeast. Both groups contributed, to some extent, to flavor production.

Effect of Temperature and Time

Fermentation temperature had a marked effect on TTA formation, which was higher at 38° C than it was at 34 or 42° C (data not shown). All previous fermentations had been performed at 38° C, which is the optimal growth temperature for *L. casei* subsp. *rhamnosus*. At 42° C, TTA was slightly higher than it was at 34° C. In all cases, pH never dropped below 3.8, the lowest pH attainable by these lactobacilli under the tested conditions. After 16 hr, the acidification rate was lowered, and flavor development peaked after about 24 hr. In less than 8 hr, pH dropped to 4.4.

Combination of Starters: Effect on TTA and Loaf Volume

To get higher TTA in the fermented dairy products, a mixture of homofermentative lactic acid bacteria (L. helveticus and S. thermophilus, both at a concentration of 4% of the total inoculum) was added to the heterofermentative culture (L. casei subsp. rhamnosus) and fermented at 38 or 42°C. With mixed cultures, higher titratable acidity was formed in fermented milks and in the corresponding breads (Table III). No off-flavor was detected in either the dairy ingredients or the breads.

With such a high concentration of fermented dairy ingredient

 TABLE V

 Influence of Starter and Growth Temperature on Volatile

 Compounds (mg/kg) of Fermented Dairy Ingredient⁴

	Starter						
Volatile Compound	L. casei (38°C)	L. casei + L. helveticus/ S. thermophilus (38° C)	L. casei + L. helveticus/ S. thermophilus (42°C)				
Diacetyl	161	137	269				
Acetoin	163	89	149				
Ethanol	6	8	12				
Acetone	3	3	3				
Acetaldehyde	2	2	1				
Ethyl levulinate	0.2	1.2	1.3				
2-Heptanone	0.01	0.04	0.04				
Ethyl caproate	0.002	0.060	0.040				
Ethyl caprylate	0.03	0.03	0.01				
Ethyl acetate	0.010	0.004	0.001				

^aData are means of four repetitions. Ingredients were prepared with 30% whole wheat flour, fermented for 24 hr and shaken at 140 rpm.

containing 30% whole wheat flour (26%, which corresponded to 10% total solids), specific volume of bread was typical of sourdough bread, being lower ($4.00-4.25 \text{ cm}^3/\text{g}$) than the milk bread control ($5.0 \text{ cm}^3/\text{g}$); it did not vary according to starter combination. The ingredient could be dried without losing too much flavor, and using lower concentrations in the recipe (1-2% instead of 10%) slightly enhanced the flavor of bread prepared by the notime dough process (Gélinas et al 1992). The specific effects of adding fermented dairy ingredients on dough rheology and bread characteristics are being investigated.

Combination of Starters: Effect on Organic Acids and Volatile Compounds

Fermented dairy ingredients. Lactic acid was the main organic acid in fermented dairy ingredients (Table IV). Pre-ferment, prepared at a higher temperature (42°C) with mixed cultures, contained the highest amount of lactic acid. Acetic acid production was low and constant for all dairy ingredients. The use of mixed cultures reduced formic acid production at either 38 or 42°C.

Table V presents the profiles of the main volatile compounds

Of Bread"							
Starter	Growth Temperature (° C)	Lactic Acid	Citric Acid	Formic Acid	Propionic Acid	Acetic Acid	
Standard (no inoculation)	•••	6.18	1.49	12.08	42.00	5 70	
L. casei subsp. rhamnosus	38	28.75	5.25	13.58	36.17	5 30	
L. casei subsp. rhamnosus +						0.00	
L. helveticus/S. thermophilus	38	29.63	5.75	10.10	28.89	5 73	
L. casei subsp. rhamnosus +						0.10	
L. helveticus/S. thermophilus	42	34.50	5.63	11.25	40.56	6.48	

TABLE VI

^aData are means of four repetitions. Ingredients were prepared with 30% whole wheat flour, fermented for 24 hr and shaken at 140 rpm.

TABLE VII Influence of Starter and Growth Temperature on Volatile Compounds (mg/kg) of Bread^a

Influence of Charles 1.0

		Starter				
Volatile Compound	Control Bread	L. casei (38°C)	L. casei + L. helveticus/ S. thermophilus (38°C)	L. casei + L. helveticus/ S. thermophilus (42°C)		
Ethanol	90	250	211	332		
Acetoin	1	4	5	9		
Acetaldehyde	1	2	2	3		
Acetone	0.3	0.4	0.4	0.6		
Diacetyl	0.03	0.04	0.20	0.70		
Ethyl lactate	Trace	0.3	0.2	Trace		
Ethyl levulinate	0.20	0.07	0.01	0.03		
2-Heptanone	0.005	0.004	0.003	0.005		
Ethyl caproate	0.006	0.010	0.020	0.020		
Ethyl caprylate	0.006	0.004	0.020	0.002		
Propionaldehyde	0.004	Trace	0.001	0.004		
Ethyl acetate	Trace	Trace	Trace	0.003		

^aData are means of four repetitions. Ingredients were prepared with 30% whole wheat flour, fermented for 24 hr and shaken at 140 rpm.

of the three fermented dairy ingredients. Diacetyl was the most concentrated volatile compound, followed by its precursor acetoin (flavorless). At 42° C, more diacetyl was produced. All fermented dairy ingredients also contained 1–10 mg/kg of ethanol, acetone, or acetaldehyde. Acetone is not considered a bacterial metabolite (Marsili 1981), and a small fraction of the ethanol probably came from wild yeasts in the flour. Ethyl levulinate was the most prevalent ester, but other ethyl esters might also contribute to flavor. Ethyl esters are quite common in fermented milks, especially those containing high levels of ethanol (Marshall 1984).

Breads. As shown in Table VI, breads prepared with fermented dairy ingredients contained more lactic acid compared to the control bread. The use of mixed cultures slightly enhanced lactic acid content of bread, but the baking process reduced some of the differences observed in the dairy ingredients. Citric acid was already present in the fermented dairy ingredients (from the addition of sodium citrate) and was thus found in breads prepared from it. Acetic acid and formic acid contents of all breads, including the control, were similar. This means that lactic acid was the main organic acid enhancing the flavor of breads prepared from fermented dairy ingredients. However, other acids, present at concentrations lower than that of lactic acid, probably contributed much to flavor development. Among breads prepared with fermented dairy ingredients, the profile of the volatile compounds was quite similar, except for ethanol, which was much higher at 42°C (Table VII). Otherwise, it was difficult to distinguish the breads on the sole basis of their volatile composition. Compared to the control, breads made with fermented dairy ingredients had two to four times more ethanol and slightly more diacetyl. It is also possible that the presence of lactic acid bacteria in the dough or the acidity of the fermented dairy ingredient stimulated the yeast activity during dough fermentation. Other minor volatile compounds and acids probably contributed much to flavor development. Compared to the control, the aroma of bread prepared

with mixed cultures was described as more intense and pleasant, recalling the aroma of specialty cheese (at high concentrations).

CONCLUSION

Results show the effects of fermentation conditions (dairy solids content, milk-whey proportion, time, temperature, starter, addition of flour, addition of sodium citrate) on TTA of fermented dairy ingredients for breadmaking. The most interesting dairy ingredients were briefly characterized according to their contents in acids and volatile compounds. Combining homofermentative cultures with Lactobacillus casei subsp. rhamnosus mainly increased lactic acid production. With combined cultures, fermentation at 42°C instead of 38°C gave higher concentrations of lactic acid and diacetyl; the resulting product had a more pronounced taste, reminiscent of specialty cheese. Compared to the control breads, breads prepared with fermented dairy ingredients had a lower volume, typical of sourdough bread, and were mainly characterized by higher lactic acid and ethanol contents and, to a lesser extent, diacetyl. Fermented dairy ingredients can be dried and used at high concentrations (up to 10%, dwb) as sourdough bases or at low concentrations (1-2%) to slightly enhance bread flavor (Gélinas et al 1992).

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