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Food Chemistry 89 (2005) 175–179
Food Chemistry
www.elsevier.com/locate/foodchem

Cells immobilized in a starch–gluten–milk matrix usable for food production

S. Plessas, A. Bekatorou, M. Kanellaki, C. Psarianos *, A. Koutinas

Food Biotechnology Group, Department of Chemistry, University of Patras, GR-26500, Patras, Greece

Received 24 November 2003; received in revised form 5 February 2004; accepted 5 February 2004

Abstract

A mixture of wheat flour and sour milk was treated according to the method of the traditional Greek fermented food "*trahanas*", and was used as model cereal-based support (starch–gluten–milk matrix) for co-immobilization of lactic bacteria and yeast for potential use in food production. Cell immobilization was proved by scanning electron microscopy and by the efficiency of the immobilized biocatalyst for alcoholic and lactic repeated fermentations at various temperatures (5–30 °C). The stability of the system was good at all studied temperatures, revealing suitability for industrial applications. Finally, respectable amounts of lactic acid and volatile by-products were produced, revealing potential application of the immobilized biocatalyst in fermented food production or use as a food additive, to improve nutritional value, flavour formation or preservation time.

Keywords: *Cell immobilization*; *Baker's yeast*; *Food production*; *Lactic acid*

1. Introduction

Cell immobilization when compared to the free cell systems, has a wide research interest because of its various advantages (Margaritis & Merchant, 1984). Although the immobilized cells achieve high rates of operation, in general, the applied supports used for cell immobilization in food production must be materials of food grade purity and also cheap and abundant. Inorganic materials are usually excluded in the fermentation process, as supports for cell immobilization, because they are characterized as unsuitable materials for human or animal nutrition. Usually, for the above purpose, various organic supports are used, such as polystyrene fibres (Yoshioka & Shimamura, 1986), gelatins (Parascandola, DeAlteriis, Farris, Budroni, & Scardi, 1992), polyvinyl gels (Lozinsky & Plieva, 1998) and alginate (Sheoran, Yadav, Nigam, & Singh, 1998). In fermentation technology related to wine making, brewing and whey fermentation, supports such as delignified cellulosic material, figs and apple pieces were proposed because they are of food grade purity (Bardi & Koutinas, 1994; Bardi, Bakoyanis, Koutinas, & Kanellaki, 1996; Bekatorou et al., 2002; Kourkoutas, Komaitis, Koutinas, & Kanellaki, 2001). All these materials contain non-digestible carbohydrates, which constitute the base for cell immobilization. Likewise, materials such as cereals, also containing non-digestible carbohydrates, could be applied as supports for cell immobilization. Furthermore, they can be used, either as food additives or as fermentation substrates, for the production of novel foods with functional properties (Charalampopoulos, Wang, Pandiella, & Webb, 2002; Charalampopoulos, Pandiella, & Webb, 2003).

On the other hand, there is a recent great interest in foods with probiotic properties, better flavour and extended shelf-life that has led to the use of defined mixed starter cultures, containing lactic acid bacteria, for the production of a large number of foodstuffs, commercial or traditional, in which yeasts are the predominant species, such as in commercial bread-making (Linko, Javanainen, & Linko, 1997; Messens & De Vuyst, 2002).

* Corresponding author. Tel.: +30-2610-997175; fax: +30-2610-997105.
E-mail address: cps@upatras.gr (C. Psarianos).

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doi:10.1016/j.foodchem.2004.02.017

Therefore, it would be very interesting to exploit materials with non-digestible carbohydrates of food grade purity for mixed cell immobilization and to investigate its application in food production, such as baking.

The aim of this research is to prove that a traditional Greek food "*trahanas*", produced from cereals, impregnated with sour milk and baker's yeast is a suitable system for mixed cell immobilization and to evaluate the produced biocatalyst for its efficiency in food production and for high rates of alcoholic and lactic fermentations.

2. Materials and methods

2.1. Materials

A novel support, of starch–gluten–milk matrix, was used for yeast and lactic bacteria immobilization prepared from wheat flour and sour sheep's milk containing viable lactic bacteria according to the method of preparing the traditional Greek cereal-milk fermented food "*trahanas*". Baker's yeast was a commercial *Saccharomyces cerevisiae* strain commonly used in the Greek baking industry, in the form of pressed blocks (70% w/w moisture). The synthetic culture medium used for the fermentation experiments contained 132 g/l of glucose monohydrate, 1 g/l of $(NH_4)_2SO_4$, 1 g/l of KH_2PO_4, 5 g/l of $MgSO_4$ and 4 g/l of yeast extract (7.5–8° Be initial density), and it was sterilized by autoclaving at 130 °C for 15 min.

2.2. Preparation of immobilization support

3.5 kg of commercial flour from wheat (hard type, 70%) was thoroughly mixed with 2.5 kg sour sheep's milk, and the thick mixture was boiled for 15 min. After that it was impregnated for 30 min with fresh sour milk and was left on sterilized absorbent paper for four days at 30 °C to allow bacterial growth (maturation). The easily grated material obtained was cut into small cubes of about 1 cm^3 and was used as carrier for yeast cell immobilization.

2.3. Yeast cell immobilization

Two hundred grams of the starch–gluten–milk material, prepared as described above, were added to 800 ml of synthetic culture medium wherein 16 g of pressed baker's yeast had been suspended. The system was allowed to ferment at 30 °C to a final density of 0.1° Be (about 6 h). The fermented liquid was then decanted and the solid material was washed twice with 400 ml of fresh culture medium and was used as a biocatalyst for glucose fermentations.

2.4. Fermentations

Fermentations of glucose were carried out, anaerobically, using cells immobilized in the starch–gluten–milk matrix, as described above. Specifically, 400 ml of synthetic culture medium and 200 g of immobilized biocatalyst were added to a glass conical flask and repeated fermentations (batches) were carried out, at successively decreasing temperatures (30, 20, 15, 10, and 5 °C). After each fermentation batch was completed, a sample was collected for analysis and the fermented liquid was decanted. The biocatalyst was washed twice with 200 ml of fresh culture medium and used for the next fermentation batch.

Additional fermentations were carried out in parallel and in the same manner at 20 and 15 °C using 10 g of free (pressed baker's yeast) cells instead of biocatalyst. Fermentation kinetics were assessed by measuring the °Be density in both systems at various time intervals.

2.5. Assays

Ethanol and residual sugar were determined by high performance liquid chromatography, on a Shimadzu HPLC system consisting of a SCR-101N stainless steel column, a LC-9A pump, a CTO-10A oven at 60 °C and a RID-6A refractive index detector. Thrice distilled and filtered water was used as mobile phase with a flow rate of 0.8 ml/min and butan-1-ol (0.1% v/v) as internal standard. Samples were filtered through 0.2 μm microfilters, before injection. Lactic acid analysis was also performed by HPLC, in a Shimadzu HPLC system, consisting of a Shim-pack IC-A1 column, a CTO-10A oven, a LC-10AD pump, and a CDD-6A detector. A solution of 2.5 mM phthalic acid and 2.4 mM Tris (hydroxymethyl) aminomethane was used as mobile phase at a flow rate of 1.2 ml/min. The column temperature was 40 °C.

Volatile by-products (acetaldehyde, ethyl acetate, propan-1-ol, isobutyl alcohol and amyl alcohols) were determined by means of gas chromatography on a Shimadzu GC system, consisting of a stainless steel column packed with Escarto-5905 (squalene 5%, Carbowax-300 90% and di-ethyl-hexyl sebacate 5%). Nitrogen was used as carrier gas (20 ml/min). Injection port and FID detector temperatures were 210 °C. The column temperature was 70 °C. Butan-1-ol was used as internal standard (0.01% v/v). Samples of 4 μl were directly injected into the column. Methanol was determined by GC on a similar Shimadzu system with a commercial column packed with Porapac-S and nitrogen as carrier gas (40 ml/min). The column temperature was programmed at 120–170 °C (2 °C/min), and butan-ol-1 was also used as internal standard (0.01% v/v). Samples of 2 μl were injected directly into the column. Determinations in both HPLC and GC assays were done by means of standard curves, and all values were the means of three repetitions.

2.6. Scanning electron microscopy

A pellet of the immobilized biocatalyst was washed and dried overnight at room temperature. Then, it was coated with gold in a Jeol JFC-1100 ion sputter for 10 min, to increase electron conductivity, and examined in a Jeol JSM-6300 scanning electron microscope.

3. Results and discussion

Electron microscopy examination (Fig. 1) clearly showed the presence of cells on the surface of the immobilization support. Table 1 shows that the formation of lactic acid was in respectable amounts (8.4–14.4 g/l), proving the presence of lactic bacteria among the

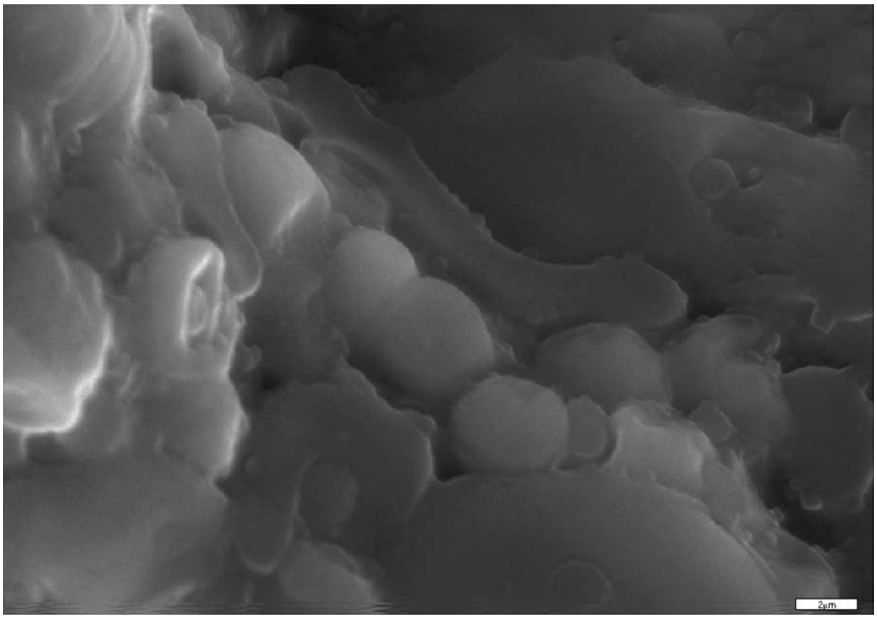

Fig. 1. Electron micrographs at 3300×, showing immobilized cells on the surface of the support.

Table 1
Kinetic parameters of the repeated batch fermentations of glucose monohydrate (132 g/l) using immobilized yeast cells on the starch–gluten–milk matrix, at various temperatures

Temperature (°C)	Batch	Fermentation time (h)	Initial sugar (g/l)	Residual sugar (g/l)	Conversion (%)	Ethanol (% v/v)	Ethanol productivity (g/l/d)	Lactic acid (g/l)
30	1	84	139	25.2	81.7	6.4	14.4	9.9
30	2	36	136	14.3	89.5	7.5	39.5	9.6
30	3	26	136	12.1	91.1	7.7	56.2	10.4
30	4	22	128	10.1	92.1	7.3	62.9	11.3
30	5	22	128	11.1	91.3	6.7	57.7	10.5
25	6	24	128	13.1	89.8	7.4	58.5	8.9
25	7	22	136	16.2	88.1	7.7	66.4	11.3
25	8	21	128	14.6	88.6	6.9	62.3	8.4
25	9	21	128	6.7	94.8	8.7	78.5	8.5
25	10	21	134	12.2	90.9	7.9	71.5	8.8
20	11	19	138	8.6	93.8	8.3	82.8	9.5
20	12	19	139	8.3	94.0	8.3	82.3	9.6
20	13	19	141	6.6	95.3	8.4	83.8	8.6
20	14	22	140	6.8	95.1	8.5	74.6	11.1
20	15	21	139	6.3	95.5	8.4	73.3	10.4
15	16	27	140	8.8	93.7	7.9	55.5	14.4
15	17	27	140	15.4	89.0	8.0	56.2	12.2
15	18	27	136	37.5	72.4	6.4	44.9	10.3
15	19	26	127	20.1	84.2	6.8	49.6	10.1
15	20	27	136	17.4	87.2	7.5	52.7	9.5
10	21	200	128	7.4	94.2	7.7	7.3	12.1
10	22	195	128	9.7	92.4	7.5	7.3	11.2
10	23	182	125	11.8	90.6	6.9	7.2	9.9
5	24	385	136	34.7	74.5	6.6	3.3	8.8

immobilized cells in the matrix of starch–gluten–milk. As shown in Table 1, the fermentation times and ethanol productivities were stable for the first 20 batches, except the first one (probably due the adaptation of the biocatalyst to the fermentation environment), while the sugar conversion, as calculated from initial and residual sugar, was constant, even at low temperatures. The same stability also shown by the products, ethanol and lactic acid. All the above prove that the efficiency of immobilized cells was stable from batch to batch and they were therefore suitable for industrial fermentations (Bakoyianis & Koutinas, 1996; Bekatorou, Koutinas, Kaliafas, & Kanellaki, 2001; Loukatos, Kanellaki, Komaitis, Athanasiadis, & Koutinas, 2003). The operational stability of the biocatalyst, even at low temperatures, shows its suitability at low temperatures. The formation of lactic acid, leading to reduction of pH, would be useful in baking, increasing the preservation time of the bread, as the low pH of sourdough is a major factor influencing the inhibitory effect of lactic bacteria on the growth of rope-forming bacillus (Katina, Sauri, Alakomi, & Mattila-Sandholm, 2002; Oscroft, Banks, & McPhee, 1990). Here the model support could be considered as a novel baker's yeast. The fermentation kinetics of biocatalyst, as compared to free cells (Fig. 2), show that the immobilized cells increase the fermentation rate, even at low temperatures. This could make the use of biocatalyst for baking more attractive than the traditional process due to the decreased time and amount of cells needed for dough fermentation.

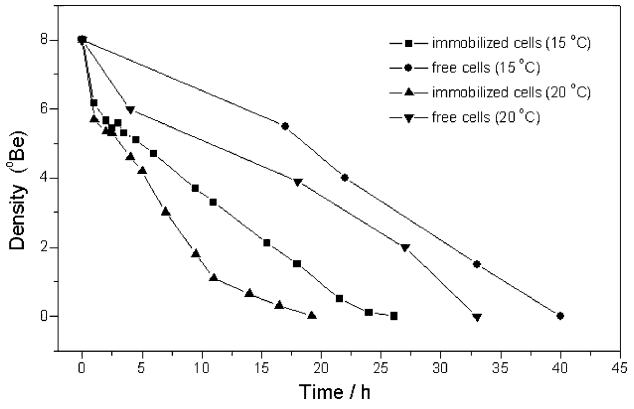

Fig. 2. Fermentation kinetics observed at 15 and 20 °C of the synthetic medium containing glucose (8 °Be) by immobilized cells and free cells.

Regarding the effect of the proposed biocatalyst on the aroma, volatile by-products were examined. Table 2 shows the effects of temperatures (30, 25, 20, 15, 10 and 5 °C) on the formation of the main volatile compounds in all batch fermentations. Methanol, ethyl acetate, acetaldehyde, propan-ol-1, and isobutyl alcohol concentrations were determined in expected amounts (Bekatorou et al., 2002), while amyl alcohols were present at higher concentrations. At low temperatures, below 15 °C, the concentrations of amyl alcohols, as much as isobutyl alcohol and propan-ol-1, decreased with temperature drop. This is in accordance with other works (Bekatorou et al., 2002) and desirable, since this produces fine quality

Table 2
Volatile by-products formed during repeated batch fermentations of glucose monohydrate (132 g/l) using immobilized yeast cells on the starch–gluten–milk matrix, at various temperatures

Temperature (°C)	Batch	Ethyl acetate (mg/l)	Acetaldehyde (mg/l)	Propan-ol-1 (mg/l)	Amyl alcohols (mg/l)	Isobutyl alcohol (mg/l)	Methanol (mg/l)
30	1	23	12	8	113	139	45
30	2	62	9	14	181	97	11
30	3	40	13	16	190	55	23
30	4	49	21	14	169	40	29
30	5	45	4	14	152	29	32
25	6	51	4	18	267	41	17
25	7	23	5	7	106	18	27
25	8	36	5	12	186	37	25
25	9	42	4	11	179	38	26
25	10	35	5	13	145	43	14
20	11	25	7	15	173	34	41
20	12	30	5	18	244	49	26
20	13	36	4	17	239	50	40
20	14	34	6	15	256	57	41
20	15	38	6	15	230	55	28
15	16	50	11	15	183	33	35
15	17	61	18	11	166	22	37
15	18	44	30	9	124	21	42
15	19	23	4	11	111	22	34
15	20	32	3	7	121	21	31
10	21	27	4	10	109	20	25
10	22	23	3	5	105	19	32
10	23	13	12	6	75	14	21
5	24	12	17	4	46	15	42

products as the above compounds are considered to be responsible for off-flavours.

4. Conclusion

The above results demonstrate that the proposed model is an effective support for mixed cell immobilization, with three discrete advantages.

(1) It has a high operational rate, due to the achieved cell immobilization, and industrial application due to its stability, over a range of temperatures, 5–30 °C.

(2) It presents the properties of a food additive because (i) it consists of high nutritive value materials, such as starch and gluten, (ii) it produces lactic acid, which is important for extension of shelf-life of fermented foods, (iii) it leads to the formation of respectable amounts of volatile by-products, showing potential effects on food flavour.

(3) It has a potential healthy effect on the human organism because the formation of lactic acid proves that cell growth of lactic bacteria was induced by the starch–gluten–milk matrix. Some of these bacteria are considered to have probiotic properties and others a significant effect on human health due to their ability to produce antimicrobial compounds called bacteriocins (Soomro, Masud, & Anwaar, 2002).

The above proposed novel product, having the described properties, could be applied in baking as a biocatalyst for the production of a new type of bread on an industrial scale. It is faster and cheaper than traditional processes and gives products with new aroma and taste, with longer shelf-life and probably with healthy and probiotic properties. Therefore, it is very important to investigate the use of this novel biocatalyst in baking as well as its effects on dough fermentation, taste quality, flavour formation and shelf-life.

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