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Biogeneration of 2-(1-hydroxyethyl)-4,5-dihydrothiazole as precursor of roasted and popcorn-like aroma for bakery products

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Abstract

Bread aroma is one of the most important criteria of the quality of bakery product. Molecules exhibiting roasted notes largely contribute to this flavour and 2-acetyl-2-thiazoline (2-AT) was identified as one of the strongest. We report here the formation of its precursor 2-(1-hydroxyethyl)-4,5-dihydrothiazole via the bioconversion of cysteamine, ethyl-L-lactate and D-glucose with baker's yeast as biocatalyst. The microbial reduction of the carbonyl group of 2-acetyl-2-thiazoline using the same microorganism is an alternative to produce the target precursor in good yield (up to 60%). Heat treatment of 2-(1-hydroxyethyl)-4,5-dihydrothiazole generated attractive and intensely roasted, popcorn-like and bread crust-like notes. High amounts of 2-acetyl-2-thiazoline were detected in the heated samples. Finally, the addition of the precursor significantly enhanced the aroma of pizza bread when mixed into the dough.

Keywords: Aroma; Precursor; Baker's yeast; Roasted; Bakery

1. Introduction

Flavours are generally produced by conventional routes, such as organic synthesis or extraction from plants. However, microbial processes seam to be the most promising ways to prepare pure aroma compounds or complex mixtures thereof [1].

Bread aroma is one of the most important criteria of the quality of chilled and shelf-stable bakery products, such as refrigerated and frozen pizzas. Actually, it reflects the freshness of the product. This subtle flavour is composed of a complex mixture of odorants as identified in the crust of baguette [2], a typical French white bread. In the present work, our interest was focused on roasted notes. In this area, 2-acetyl-1-pyrroline (2-AP) is incontestably the main key compound, but it is highly unstable and consequently very difficult to control. Then 2-acetyl-2-thiazoline (2-AT) was selected because it has a strong roasted and popcorn-like aroma and is more stable than 2-AP. Hofmann et al. [3] proposed a reaction pathway involving cysteamine and methylglyoxal as precursors of 2-(1-hydroxyethyl)-4,5-dihydrothiazole (HDT), which was then transformed to 2-AT via Maillard reaction.

The aim of the present study was to prepare HDT using baker's yeast as biocatalyst, then to evaluate its potential as precursor of 2-AT upon heat treatment or baking. For this, bioconversion from cysteamine and ethyl-L-lactate was performed with baker's yeast. The

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microbial reduction of the carbonyl group of 2-AT was also conducted using the same microorganism to get HDT in high yield. The obtained HDT was then heated in different media to verify the generation of the target odorant 2-AT. Finally, an application trial in a pizza model demonstrated the feasibility of the approach in food.

2. Experimental

2.1. Chemicals

Cysteamine, ethyl-L-lactate and D-glucose were supplied by Merck (Germany) as well as dichloromethane and Lichrosolv isopropanol and *n*-hexane. Baker's yeast was purchased from Hefe Schweiz (Switzerland) and 2-acetyl-2-thiazoline was from Aldrich (USA). As HDT was not commercially available, it was purified by chromatography (details not described). Its purity was determined by NMR before use as standard.

Acetonitrile was ultra gradient HPLC grade from J.T. Baker (Holland) and trifluoroacetic acid was sequanal grade from Pierce (USA).

2.2. Bioconversion based on cysteamine and ethyl-lactate

Cysteamine (5 mmol), ethyl-L-lactate (5 mmol) and D-glucose (10 and 5 g, respectively, added after 4 and 24 h) were incubated with commercial baker's yeast suspension (150 ml, 20% of dry matter). The temperature was controlled at 35 °C and pH was automatically maintained at 9.8, by addition of 2 M sodium hydroxide, throughout the bioconversion (48 h). Centrifugation was used by the end of the reaction, to remove the yeast cells and the supernatant was ready for further analyses.

2.3. Biotransformation of 2-AT

Melted 2-AT (1.8 mmol) was incubated for 3.5 h with baker's yeast suspension (100 ml, 20% of dry matter) under controlled temperature at 30 °C and pH 6.5. As previously, the yeast cells were removed by centrifugation and the supernatant was used in further treatments, analyses or application trials.

2.4. Generation of 2-AT upon heat treatment

Supernatant from the biotransformation of 2-AT was saturated with sodium chloride before liquid–liquid extraction using a same volume of dichloromethane. The organic phase was then dried over anhydrous sodium sulfate. After determination of the HDT content of the organic extract, an aliquot containing 1.7 mg of HDT was introduced in a 4 ml glass vial and solvent was evaporated under a stream of nitrogen to give the so called "HDT in dried form". Another aliquot of organic phase was incorporated into sunflower oil and the solvent was removed by rotative evaporation.

Four millilitre glass vials containing 1 ml of aqueous or oily samples (about 1.5 mg of HDT each) or the equivalent amount of "HDT in dried form", were sealed and placed in a heating dry-block, preheated at 100 °C or into an oil bath preheated at 205 °C. Periodically, one vial was removed and rapidly cooled in ice for 1 min before HPLC analysis.

2.5. Analytical methods

Supernatant from incubation with baker's yeast was filtered through a 0.2 μm filter and 5 μl were analyzed by HPLC using a Nucleosil 100, RP-18 column (250 mm × 4 mm, 5 μm particle size). Elution was carried out at 1 ml/min with a linear gradient from 10 to 50% acetonitrile in 0.1% trifluoroacetic acid over 10 min. 2-AT and HDT were, respectively, detected at 306 and 262 nm.

Samples in sunflower oil or in dried form were diluted in *n*-hexane, filtered through a 0.2 μm filter and 5 μl were injected into a normal phase, Nucleosil 100-OH HPLC column (250 mm × 4 mm, 7 μm particle size). Elution was carried out isocratically using 10% isopropanol in *n*-hexane at a flow rate of 1.5 ml/min. As previously mentioned, UV-detection was achieved simultaneously at 262 and 306 nm.

2.6. Application trials and sensory evaluation

Pizza samples were prepared as food model, with 50 g of fresh dough per piece. Spiked samples were obtained by the addition of the supernatant coming from the biotransformation of 2-AT, partially replacing the water involved in the recipe. The final level of HDT

corresponded to 5 mg per 50 g of fresh dough. Sensory evaluation was conducted on freshly baked pizza samples. Thirty panelists participated to the triangle tests comparing spiked samples versus the reference.

3. Results and discussion

The aim of the study was to prepare HDT using baker's yeast as biocatalyst and to evaluate its potential as precursor of 2-AT upon heat treatment. The latter molecule is one of the most important heterocyclic compound with an intense roasted aroma. The formation of HDT, as presented in Fig. 1, consisted in the aerobic incubation of cysteamine, ethyl-L-lactate and D-glucose with baker's yeast.

Chemical analysis of the reaction mixture after removal of yeast cells, indicated not only the presence of HDT (5%) but that of 2-AT (0.1%) too. The formation of the key aroma compound itself (2-AT) was not desired at this stage of the study. Actually, for bakery applications, the addition of 2-AT in dough, will result at least in the release or in a worse case, in the degradation of 2-AT during the baking step. In addition, off-notes such as sulfury, putrid and amine-like were also present in our bioconversion mixture, probably due to the prolonged incubation of the yeast at high pH.

To avoid all these problems, the microbial reduction of the carbonyl group of 2-AT using baker's yeast as biocatalyst was selected as the most appropriate way to produce HDT in one step and under mild conditions (Fig. 2).

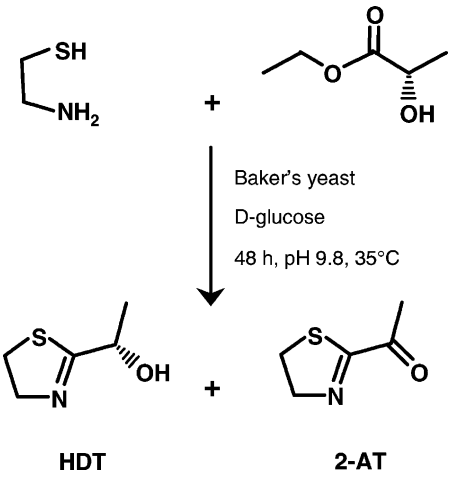

Fig. 1. Bioconversion based on cysteamine and ethyl-L-lactate.

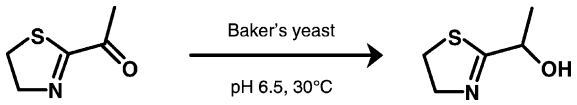

Fig. 2. Biotransformation of 2-AT into HDT.

As shown in Fig. 3, incubation for 3.5 h was sufficient to transform 2-AT into HDT.

The yield was estimated at about 60%, considering only HDT present in the supernatant at the concentration of 1.66 ± 0.04 mg/ml. HDT left in the discarded yeast cells was not recovered to avoid the dilution of the HDT solution destined for bakery application.

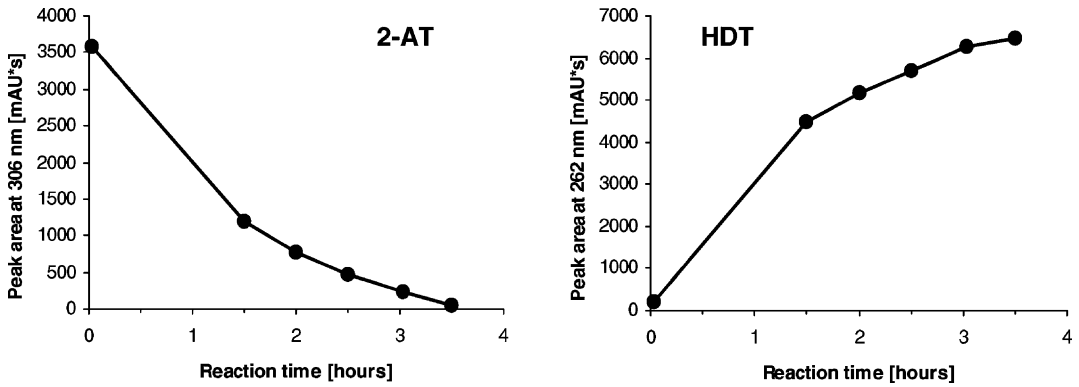

Fig. 3. Kinetic study of the biotransformation of 2-AT into HDT.

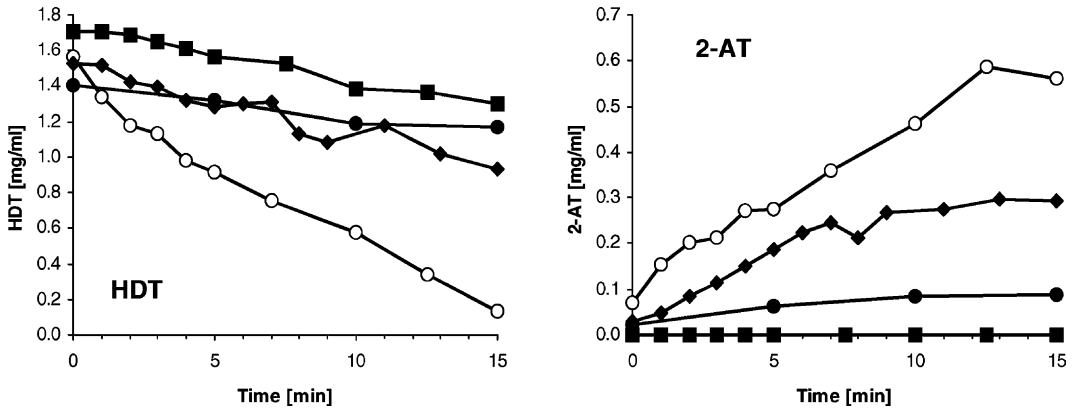

Fig. 4. Formation of 2-AT by heat treatment of HDT: (○) in oil at 205 °C; (●) in oil at 100 °C; (■) in dried form at 100 °C; (◆) in aqueous medium at 100 °C.

In order to evaluate the potential of HDT as precursor of 2-AT upon heat treatment, different media were tested. The supernatant of the biotransformation was considered as aqueous solution. HDT was extracted with dichloromethane and the organic solvent was evaporated to give "HDT in dried form" or the organic extract was incorporated in sunflower oil before removal of the solvent. The amount of HDT ranged from 1.4 to 1.7 mg, corresponding to 1 ml in the case of aqueous or oily solutions. Samples were placed into sealed vials and heated in dry-block set at 100 °C or in an oil bath at 205 °C. Results are represented in Fig. 4.

On one hand regarding HDT, the effect of the temperature was considerable, as the decrease was much faster at 205 °C than at 100 °C. The medium had only a minor influence. On the other hand, the temperature gave a higher level of 2-AT at 205 °C than at 100 °C, the yield was about 35% after 15 min at 205 °C. This value was certainly underestimated due to the release of 2-AT into the head-space. At 100 °C, no 2-AT was formed in the aqueous medium but we observed the degradation of HDT by the opening of the thiazole ring. This phenomena also occurred when the biotransformation of 2-AT was performed in acidic conditions as reported by Bel Rhlid et al. [4]. Better results were obtained with HDT in sunflower oil. Finally, the highest amount of 2-AT was reached from "HDT in dried form". This last result was very encouraging because dough based products containing up to 25% water, dry rapidly during baking.

As it was demonstrated that HDT was the precursor of 2-AT upon heat treatment, some application trials were performed to confirm the feasibility of using it in a food product. The model chosen was pizza. Samples were prepared by mixing the supernatant of the biotransformation of 2-AT into HDT with other ingredients of the dough. Actually, it replaced partially the water involved in the recipe, in order to keep the moisture content constant. The level of the aroma precursor corresponded to 5 mg of pure HDT per 50 g of fresh dough. In this study, no topping was added on the pizza, such as tomato or cheese, to optimally perceive the effect of HDT on the bread aroma. After baking, spiked samples were immediately compared to the reference in triangle test. Thirty panelists participated to this sensory evaluation session, which resulted in a significant difference between spiked sample and reference with a confidence level of 99.9%.

4. Conclusions

HDT was generated in good yields (up to 60%), either by the bioconversion of cysteamine, ethyl-L-lactate and D-glucose or by the microbial reduction of 2-AT using baker's yeast as biocatalyst in both approaches. However, the second way was preferred as the first one generated a mixture of both 2-AT and HDT and exhibited off-notes probably due to the

prolonged incubation with yeast at higher pH. Heat treatment of HDT in different media resulted in attractive and intense roasted, popcorn-like and bread crust-like notes. High amounts of 2-AT were detected in heated samples. Finally, the addition of HDT into the dough resulted in an enhanced aroma of pizza bread.

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References

[1] R.G. Berger, Biotechnology of Aroma Compounds, in: Advances in Biochemical Engineering/Biotechnology, vol. 55, Springer, Berlin, 1997, 274 pp.
[2] G. Zehentbauer, W. Grosch, J. Cereal Sci. 28 (1998) 81–92.
[3] T. Hofmann, P. Schieberle, J. Agric. Food Chem. 43 (1995) 2946–2950.
[4] R. Bel Rhlid, Y. Fleury, I. Blank, L.B. Fay, D.H. Welti, F. Arce Vera, M.A. Juillerat, J. Agric. Food Chem. 50 (2002) 2350–2355.