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Using microbial systems in order to obtain fermentation flavorings

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Abstract

Food has complex flavor difficult to elucidate qualitatively and quantitatively, mainly due to the large number and diverse of the flavor components with high volatility and of the sensitivity techniques of isolation and concentration. Research concernings to flavorings obtained by biotechnological methods provides information on the microbial systems used for fermentation of natural media, optimized in order to accelerate the production of flavorings.

This paper is an extended study on the main flavorings, such as butter, Cheddar and Roquefort cheese, with multiple possibilities for use in food processing.

Keywords: flavorings, biotechnological way, Cheddar chesse, Roquefort chesse

Introduction

Preference for the early special flavor was influenced by lack of food during in the round year, which led to the need for storage and conservation through thermal processes (cold, boiling, roasting, salting, smoking) or by using herbs and spices. Effect of flavor from these treatments is constituted as a "bonus" food quality [1]. It should provide satisfaction and joy.

The desire to produce such food has been possible to obtain and use flavoring, initially in the form of natural flavor extracts, and then in the form of synthesis. Such products have been used to flavor sugar confectionery products, beverages, canned products or flavoring preparations. Technical progress of the food industry in the last century has influenced the development of outstanding research aromas, especially in the last 4 decades. Entering chromatography techniques since 1955 in research aromas, has meant, mainly, to identify a large number of flavor components [2, 3, 4]. Were published lists of ~10,000 aroma substances by S. van Straten and FJ of Vrier (1973), and Maarit H. C.A. Visscher (1984, 1985) [5,6,7].

Interest in the field of flavors, using modern instrumental techniques, such as flamionizing detection (1957), by electronic capture (1960), flamfotometryc (1966) and coupling analysis by gas chromatography-mass spectrophotometry (1964) [8, 9] resulted in the rapid development of techniques for separating and identifying the components of flavor, which is usually in very low concentrations of 10^{-5} – 10^{-9}, and by the discovery of "key" substances in multicomponent system.

Realization of unconventional products, such semipreparations, those instantinizated, simulated-based proteins and dietetics was faced with negative the decrease in flavor and/or their quality.

Alcoholic beverages and milk products obtained by fermentation processes are time consuming and space. Because a true technological revolution they have been subjected to modern processing techniques,

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simplified mechanized or partially automated, streamlined considered, which had negative effects on flavor [10, 11, 12].

Under these conditions, research of aromas has focused to find solutions potentiation or replacement of traditional flavor. One of them was obtaining flavorings (flavoring concentrates) from natural sources, containing the singular flavor component such as menthol from peppermint, citral of citrus peel, benzaldehyd of bitter almonds, eugenol of cloves, etc., through traditional processes (pressing, extraction, distillation). These food flavorings gives a uniform flavor, different by overall flavor, who is complex and nuanced.

Biological systems forming the aroma

Therefore, research attention has been given intensive use of the biological systems flavor forming, and driven the development of biotechnology, as modern interdisciplinary science. Biotechnology encompasses all those areas, using practical operating biological principles, such as molecular biology, biochemistry, genetics, microbiology, physical chemistry, etc.

Biotechnology uses microorganisms (bacteria, yeasts, moulds, "in vitro" cultures of plant and animal cells), for production of specific substances and economical manner. Biotechnological processes of synthesis of flavor compounds utilized microorganisms due of the complex biochemical potential, rapidly multiplying, adapt to changing environment, the possible use of single or combined cultures, the possibility of phenotypic and genotypic handling application in „situ" (aging or refining of food matter and products) or "per se" (growing on culture complex media, natural or synthetic) [13, 14].

Flavoured concentrates obtained by biotechnological way used as flavorings or in optimizing of aging processes of foodstuffs, with implications in improving sensory quality and increase their nutritional value.

- On the international front, potentiation, manufacture or maintain the flavor of food products by biotechnology techniques has imposed in the following main directions, namely:
- the use of enzymes for the production of protein hydrolysates and fat lipolysed;
- using microorganisms to obtain: flavor potentiators (sodium glutamate and 5'-ribonucleotide), fermented milk products, flavorings of cheese (Roquefort, Cheddar), butter, fruit, bread, wine (Mossela), mushrooms, fermented foods of plant origin, raw dry sausages;
- cultivation „in vitro"of plant cells tissues, to achieve obtaining and multiplication of valuable plant genotypes for;
- -the production of various useful substances nourishment, pharmaceutics, cosmetics, chemical industry, etc.

Bioindustry is a top-level domain in the future of the most developed countries, where is national programs, carried out by research institutions or eminent collective scientists, which determinate system of goverment act to for essential to investment and guidance policy of the research in this regard. Markets flavorings industry represents tens of millions of dollars, which explains the competition between laboratories rush to patent and secrecy who wrapped these inquiries. Currently, eight concerns have 67,4% from global flavors market [15].

Flavour-flavoring, dilemma of identity

Flavor, flavoring, savour are ,,key'' terms used by specialists in research flavorings that, for a clear understanding, must speak the same language. Of all the terms currently use in worldwide and known in most languages, the word ,,flavor'' is the oldest.

Origin comes from ancient Greek and has been identified with the sense of smell caused by spices. Later, he was pleasant sensations associated with the odor produced by flavored plants and other aging products. Meaning of the term was extended to the smell of essential oils, and then was synonymous with the perception of odor produced during food consume.

Subsequently, the aroma was defined by pleasant smell and taste of food. Documentary information, related to concepts and methods involved in researching aromas, has, in turn, the definitions of flavor compounds and different classification criteria. For example, the term „savour''describes the total sensory impression, including sensations of smell, taste and texture, except for appearance, a fact important in sensory perception. Taste substances were designated only by sensations of sweet, sour,salty,bitter.

Flavor substances are classified into three groups, according to FAO / FWO Codex Committee on Food Additives (1978), universally accepted and matched in different amendments of laws, such as Codex Alimentarius and the "Flavors" / EC Countries Europe Gras (Generally recognized as Safe) in the U.S. [16]:

- natural flavor substances produced solely by physical methods from vegetable or animal raw matters for human consumption;
- flavorings identical with natural ones - substances isolated by the chemical way from raw matters or obtained by synthesis; they are chemically identical to substances present in natural products for human consumption;
- artificial flavorings - substances which are not binding obligatory in natural products for human consumption.

Spectrum of natural flavorings was enriched by detecting the aroma substances obtained by enzymatic or biotechnological techniques which are not found in previous regulations.

Flavors Directive 88 (388) EEC of 11.04.1991 defining the concept of flavoring and presents a classification into four groups of substances flavor [23]: "*Flavoring means any substance added to food to give a smell, taste, or smell and taste, except those who have only a taste - sweet,salty,acid*."

Edible products are not flavorings, excluding that so, aromatic plants, spices, etc.

Biotechnology and flavorings production

Of the many species of microorganisms eucaryote and procaryote type known until now, a limited number is used make useful products, which may include food and drink with pleasant flavor. Over time, the art of using microorganisms has been scientifically substantiated, for the purpose realized of the food, that provide satisfaction and pleasure for consumer. Modern biotechnology and bioindustry uses microorganisms (bacteria, yeasts, moulds) for the formation of flavor components [17, 18, 19, 20, 21].

The main ways of flavors and flavorings producing synthesized by biotechnological are the following: :

- formation of secondary flavors during alcoholic fermentation, aging of cheese and raw dry sausages [22, 23, 24];

- biotechnological production of traditional flavors as concentrates flavor: flavorings Roquefort and Cheddar cheese type, obtained by the cultivation of species of *Penicillium roqueforti*, respectively, *Micrococcus sp*. on natural media [22, 25, 26];
- "novo" synthesis of the singular flavor compounds (lactones, esters, pirazine, diacetil) by microorganisms grown on selected and appropriate media [17, 27];
- microbiological transformation by the bioconversion of complex molecules in flavor compounds (aroma of apple, tomato, cucumber etc.) [17, 28].

Practical aspects about to obtain flavor concentrates

While modern flavors industry produces many of the singular flavor components (eg, isolated from essential oils or chemical synthesis), the recombinant full of complex flavor of these food components is still a goal, primarily because of the special "key" components. Thus, the component "key" although has a complex role in the aroma, her perception in the total flavor is subject by the amount and other substances from the flavor spectrum. It is known that the aroma of a singular isolated component may be totally different from the impression given to the entire complex. For example, sulfur compounds (tioli, furfuril-

difsulfid), present in coffee, or meat and who have unpleasant odor notes in pure form, contributes positively to the complex flavor. The main current application of the components of flavor remains singular use as intensification of natural flavor complexes.

In this way, the most important approach in flavorings research is to obtain flavor concentrates on various ways, the most important being following:

- isolation of the complex aroma from natural sources;
- production of the flavor complex on the biological way;
- production of flavor complex by heat treatment;
- isolation of singular flavor components from natural sources;
- synthesis of singular flavor components.

Application of thermal processing (eg. controlled Maillard reaction) and biotechnological fermentation, for production of flavor concentrated resulted, mainly, in obtaining meat flavoring [29, 30], respectively, meat chicken flavoring, or fish [31, 32] and flavoring cheese of Roquefort [14, 33]. Flavor concentrates produced using biotechnological techniques pose a number of additional problems (quality, technological, health and toxicological).

Of these aspects, which are considered a barrier to development and their use are: choice support substances, liquids or solids that will provide them with efficiency and stability and the legislation related to the toxicological considerations, in terms the new food with composition changed.

Flavorings fermentation obtained by biotechnology

Documentary information investigated on obtaining fermentation flavorings presented by biotechnological research in this area, focusing on some systems for microbial fermentation of natural media, optimized in order to accelerate the production of flavorings. Flavorings fermentation obtained in this way are: of butter and cheese (Cheddar, Roquefort), flavored with fruit, bread, mushrooms.

Butter flavorings

There are a variety of food that flavor butter gives them expected sensory qualities by the consumer. Synthetic flavorings use for this purpose, even if they have some advantages (price low cost, effectiveness, purity) did not satisfy the need for quality and flavor of the finished product and ar subject to suspicion about safety and toxicological aspects.

Butter flavorings produced in a controlled fermentation system were among the first flavor concentrates obtained and used for:

- potentiation dairy taste (fresh cream, ice cream); -aromatizing of bakery products and sugar confectionery products (dietetic products, cookies, snack products);
- flavoring industry products fats and oils (margarine, granular fat, powder, and baking fat for fried);
- flavoring cereal products (food pastes instant meals.

Microbial biosynthesis of aroma of butter is made by inoculation culture medium (skimmed dairy products or whey) with microorganisms traditionally used in the milk (mixture of acidifying strains and flavoring), often with a microbial lipase or animal origin, followed by a fermentation process in optimal conditions [17, 20]. Possible variants technological processing fermented medium by drying, distillation and spray drying and distillation is economically acceptable, while freeze drying, reverse osmosis, criodesicated, although it ensures a high retention of aroma components, are considered expensive. The fermentation medium with bacterial culture was realized at pH =4,3-4,5...5,0-5,5 and 28^0C temperature.

Bacterial cultures used contain two species of microorganisms (*Leuconostoc and Lactococcus lactis ssp lactis biovar. diacethyilactis*), in mixed starter cultures of LD type, they were considered lactic bacteria producing flavor. *Leuconostoccus*

sp. and Lactococcus ssp lactis biovar. diacethylactis produce diacethyl and acetoine of citrate, and lactic and acetic acid by fermentation of lactose and glucose.

The amount of diacethyl considered "key" component of the flavor of butter and acetoine produced by the mentioned bacteria, depending on cultivation conditions (discontinuous, continuous), the composition of themedium, temperature, pH, addition, citrus. Thus, an medium of milk in terms of a culture obtained discontinuously, the amount of diacethyl and acetoine produced by *Lactococcus lactis ssp. lactis biovar. diacethylactis and Leuconostoc mesenteroides ssp cremoris* is 4 mg/l, respectively, 500 mg/l, and in the presence of a lactose - peptone and sodium citrate 1%, production diacethyl and acetoine increase 3 times [34]. To obtain flavor of butter in the products that use the mentioned flavoring is sufficient to ensure a level of 1 mg diacethyl/Kg.

Flavoring cheddar cheese

Flavoringh Cheddar cheese (Chester Cheshire) are seasoned cheese, with paste, hard, compact, with goals of fermentation, white to yellow, with delicate flavor, well expressed, the nut, tart-tasting, slightly bitter.

Paste formation and development of characteristic aroma and taste is based on the degradation of more or less advanced partial degradation of casein and a lipides made by starter cultures of lactic bacteria mixed (*Lactococcus lactis ssp cremoris, Lactococcus lactis spp. lactis biovar . diacethylactis, L. helveticus, L. casei*) well-chosen, with power acidification, strong and active at postheated temperatures. Selection of culture to be used is essential for achieving a good quality cheese, especially the texture, which determines its identity, and a balanced flavor, in which no single component can not give a characteristic flavor.

Streptococcus sp., Lactococcus lactis and Lactococcus lactis ssp cremoris are acidifying bacteria, but exhibit proteolytic properties and *Lactococcus lactis ssp cremoris* is often responsible for bitter taste. *Lactobacillus sp*. have proteolytic influence decreased, but add in mixed cultures enhances flavor and accelerates aging (*L. casei*). *Micrococus sp*. were controversial action, in that it enhances the flavor, but gives a bitter taste, undesirable.

Flavor formation by lipase activity contributes both own enzymes and bacterial enzymes: milk lipase, bacterial endoenzyme with role in the formation of fatty acids. Compounds responsible for the characteristic flavor of Cheddar cheese are methyl ketones obtained by β-oxidation of the fatty acids. Increasing amounts of the fatty acids (C_2 and C_8) and H_2S, influenced intensity of flavor [25, 35].

Obtaining a flavoring of Cheddar cheese involves inoculation of a mixture of butterfat, protein (casein or other suitable protein) and lactose with lactic bacteria cultures. The curd formed is suspended in water and inoculated with *Micrococcus sp*. The period of fermentation inoculated medium is 1-2 weeks.

Flavoring Cheddar cheese has a flavor intensity 20 times higher than a Cheddar cheese matured for 1 year and by adding in fresh Cheddar cheese were a development of characteristic flavor intensity compared to that induced by natural after 3 months.

Flavorings type Roquefort cheese
Roquefort cheese type are soft consistency, greasy, with mould in paste, presenting a design characteristic "erborinatura", given by the blue or green stems throughout the mass, with typical intense flavor, with pleasant notes of pungent, oxidized, mushrooms, easily mould.

Origin of Roquefort cheese (blue cheese) is the famous French Roquefort cheese made from sheep's milk. In many countries, the assortment of this type of cheese is obtained from cow's milk or mixture of ewes and cows, such as Gorgonzola, Castelmagno (Italy), Stilton, Dorset Blue (England), Danish Blue (Denmark) Sarazzin (Switzerland), Gammelast (Norway). In Romania, Roquefort cheese type (Bucegi) are manufactured from sheep's

milk, cow's milk, or mix the two, the mixture of milk, buffalos, sheep, cows and cheese Homorod obtained from milk buffaloes.
Sorts of different countries or even regional require different technological parameters: type of raw matter, temperature, humidity, duration aging. Special strains of *Penicillium roqueforti* acti through lipoliytc and proteolytic processes in a normal period of aging of 5-8 weeks, at low temperature of 8-12°C and relative high humidity of air (95-100%).

Preparation of an flavoring Roquefort cheese by reassembly singular components of flavor, which supplement the loss of flavor, to shorten the period of maturation or to be used to flavor other products, has proved difficult to achieve given the complexity of interrelation between the components of flavor and between them and substrate. Also, to identify gas-chromatography of the components are "beingartefacts", compounds that do not belong to the complex flavor itself, but that influence overall flavor profile. Flavoring composition simulated in which the acids (acetic, butyric, capronyc, caprylic), acetone, methyl ketones (2-C_5, C_7, C_9, C_{11}), alcohols (2-C_5, C_7, C_9), esters (ethyl butanoat, ethyl hexanoat) presented only a flavor similar to "Roquefort", missing, in particular, note the taste conferred by mould and components that are found in extremely small quantities (0001 - 0.1 ppm).

In the last 20 years tried to obtain flavoring of "Roquefort" type through biotechnological processes using the medium as milk fermentation, the same microorganisms and enzymes that act normally during aging Roquefort cheese. Flavorings of this kind are natural products with sensory properties superior to those of synthetic.
Information documentary presents various technological production flavoring Roquefort cheese type. From their analysis can detach the following steps, of which some may be missing, take place successively or simultaneously: preparation of a fermentation medium, containing milk (full, concentrated skimmed), animal fats and / or vegetable (butter, vegetable oils containing medium chain triglycerides of carbon atoms (-C_4-C_{14}-), protein (whey powder, soy protein, sodium caseinate, etc.);

- obtaining a sporifer inocul *Penicillium roqueforti* on the diverse media;
- microbial fermentation of culture medium by cultivating spores of *Penicillium roqueforty*;
- adding a lipase or lipolyzate fat prepared earlier in the fermentation medium, prior to cultivation with spores or during fermentation, to stimulate or accelerate the production of methyl ketones;
- -inactivation of lipase at the end of fermentation, by pasteurization or sterilization;
- flavor isolation obtained.

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