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Control of Food Yeast Spoilage Using Natural Compounds

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Abstract

Foods and beverages are nutrient-rich ecosystems in which most microorganisms are able to grow. The yeast population has been well-characterized in fresh and processed fruit and vegetables, dairy products and beverages, among others. Some species are agents of alteration in different foods and beverages. Food contamination can be due to natural yeast populations in raw ingredients and environmental contamination in the manufacturing industries. This review addresses the role of yeasts in foods and beverages degradation by considering the modes of contamination and colonization by yeasts, the yeast population diversity, and the analytical techniques for their identification, primarily molecular methods. In recent years, interest in the use of biologically active compounds from natural sources increased, because consumers are looking for safer and healthier food without addition of chemicals. In nature there are many different types of antimicrobial compounds. Phenolic compounds may affect growth and viability of microorganisms and the use of phenolic compounds as antimicrobial agents would provide an additional benefits, including dual-function effects of both preservation and delivery health benefits. Polyphenols present in different vegetables could be feasible natural and non-toxic alternative to prevent growth of spoilage yeasts and improve the microbiological quality of foods.

Keywords: spoilage yeasts; food and beverage; identification methods; sources of contamination, phenolic compounds.

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1. INTRODUCTION

Yeasts are a big and heterogeneous group of eukaryotic microorganisms. A general definition is that yeast is a fungus that divides by budding or fission and that the sexual state, if known, is not enclosed in a fruiting body [1].

They are of great interest to the food industry, since they can be used as starters in the production of various foods and beverages such as bread, wine, beer, kefir, koumiss, or table olives [2], and participate in the maturation of some cheeses [3]. Nevertheless, many yeast species are also commonly presented in numerous foods as contaminants that can cause food spoilage [4].

The yeasts have certain particular characteristics that allow them to grow and contaminate in foods of dairy origin, among them the fermentation or assimilation of lactose, production of extracellular proteolytic enzymes, for example: lipases, assimilation of lactic and citric acid, growth at low temperatures and halo [5].

Yeasts predominate in foods and beverages with high acidity, water activity or high sugar content and, consequently, it is in these products that yeasts play a central role in the spoilage. Several works have been published, describing the incidence of yeast species involved in food and beverage degradation, such as "Fungi and Food Spoilage" [6], and the "Handbook of Food Spoilage Yeasts" [7], and "Control of *Hanseniaspora osmophila* and *Starmerella bacillaris* in strawberry juice using blueberry polyphenols" [4].

The analysis in published researches indicates that equipment, manufacturing facilities, raw ingredients, air and water are the main niche for spoilage yeast contamination of processed foods and beverages. Knowledge and understanding about the source of contamination and the yeast species involved can contribute to greater control over the development of undesirable species.

From the molecularly identified strains, certain potential spoilage yeasts are relatively ubiquitous species, with strains isolated worldwide from sea water, tree exudates, insects, soil, and foods [1], while others appear to be restricted to certain habitats.

Food preservatives are substances added to food to slow or prevent food spoilage caused by microorganisms. Different additives have been used in food industry for centuries. Initially, their application was meant only for food preservation by pickling, salting, or by adding sulfur dioxide. Later on, certain additives and supplements have been applied to improve the taste and the appearance of food.

Some yeast are extraordinarily resistant to chemical preservatives such as sodium benzoate and potassium sorbate commonly used in the food industry, which reduces the shelf-life of them, producing large economic losses in the food industry and/or health risks to consumers.

These health risks include allergic reactions, gastrointestinal disorders and cancer [8; 9]. For example, the use of the food preservative furylfuramide has been banned since 1974 when it has been proven to be carcinogenic for experimental animals [10] and for humans [11].

More than 10 years ago, interest in the use of biologically active compounds from natural sources increased, because consumers are looking for safer and healthier food without addition of chemicals and without the use of thermal treatment. In nature there are many different types of antimicrobial compounds; natural products of higher plants provide a variety of antimicrobial agents, probably demonstrating novel mechanisms of action [12].

Phenolic compounds are secondary metabolites which are synthesized by plants and play important structural roles in the cell wall, act as a defense against UV light, protect against pathogen ingress and are involved in repair of injury. Many foods and beverages contain high levels of phenolic compounds, which often provide colour, taste, astringency and other sensory characteristics [13]. Derived from the basic structure of phenol (hydroxybenzene), the term "phenolic" refers to any compound with a phenol-type structure. Interest in phenolic compounds has increased in recent years because of their potential beneficial effects on human health. Phenolic compounds are well documented for their biological effects, which led to the belief that diet rich in fruit and vegetables contributes to good health [13]. Phenolic compounds may affect growth and viability of microorganisms and the use of phenolic compounds as antimicrobial agents would provide an additional benefits, including dual-function effects of both preservation and delivery health benefits.

Berries are rich in phenolic compounds, and several researchers have reported the content and antimicrobial activity of phenolic compounds in berries [14; 15]. Blueberry (*Vaccinium spp.*) contains relatively high amounts of acids and phenolic compounds [16] that display potential health benefits such as protection against cancer and cardiovascular diseases [17; 18; 19].

Bioactive compounds are considered as molecules with therapeutic potential that can exert actions on energy intake, while decreasing excessive oxidative stress, pro-inflammatory state and metabolic disorders [20]. A growing interest is brought to the antioxidant micronutrients owing to the diverse biological properties that they would be able to exert. For decades, these compounds have been correlated to a risk reduction of chronic diseases such as cardiovascular disease, cancer, diabetes, Alzheimer disease, cataracts and age-related functional decline. Antioxidants in fruits and vegetables are mainly phytochemicals, and more specifically phenolic compounds and carotenoids. The polyphenols are the most abundant antioxidants in the human diet compared to vitamin C, vitamin E, and vitamin A and its precursor's carotenoids [21]. These compounds constitute a heterogeneous group of compounds. More than 8000 molecules were identified and classified into five main classes according to their chemical structure: flavonoids, phenolic acids, stilbenes, lignans, and curcuminoids [22]. The considerable diversity of their structures put polyphenols apart from other antioxidants. Their bioavailability and biologic properties vary to a great extent and are affected by their chemical structure [23].

For several years much interest has been attributed to plant products with high polyphenols content due to their extensive biological potential, including their antioxidant, hypoglycemic, hypolipidemic and anti-inflammatory properties [24; 25; 21; 26].

The possible use of phenolic compounds from vegetable as a natural antifungal would be of great importance in the food industry.

2. EXPERIMENTAL SETUP, RESULTS AND DISCUSSION

2.1 Food spoilage yeast

Some authors isolated and identified spoilage yeast in food. In fermented foods*, Candida* and *Pichia,* capable of tolerating up to 20% moisture (aw = 0.845), have been reported as edible mushroom contaminants conserved in brine [27; 28; 4]. The mayonnaise sauces and the ketchup (pH 3.5 - 4.5) can be contaminated with yeasts of the species *Zygosaccharomyces bailli* and *Pichia membranensis*, known as tolerant acid yeasts [29; 30]. In potato chips and commercial pasta salads, the predominant species are *Rhodototrula glutinis, Rhodotorula mucilaginous, Rhodotorula minuta, Cryptococcus albidus* and *Cryptococcus laurentii*, most of which are psychrophilic and possess lipolytic and proteolytic activity [31]. Rice grains are infected with yeasts, among the polluting groups, the genera *Candida, Cryptcoccus, Pichia, Hanseniaspora, Rhodotorula* and *Sporobolomyces* predominate [32].

In milk, yeast contamination can take place after pasteurization, in which participate *Cryptococcus flavus, Cryptococcus diffluens, Debaryomyces hansenii* and *Kluyveromyces marxianus* [31]. One of the dairy products most altered by the action of yeasts is yogurt, due to the addition of fruits and flavorings derived from fruits. The pollutants with the highest incidence are *Debaryomyces hansenii, Kluyveromyces marxianus, Saccharomyces cerevisiae, Rhodotorula mucilaginous, Kluyveromyces lactis, Clavaria versatilis*; on a smaller scale the genera *Rhodotorula, Soporobolomyces* and *Debaryomyces* [33; 34; 35].

Correct identification of the different yeast genera is required to determine the microbial species responsible for food spoilage and their sources in the processing environment. When food spoilage is detected, manufacturers must resolve the problem quickly. The first issue is to determine the cause of spoilage, which can often be determined from microscopic examination of the product. If yeasts are the source of spoilage, a preliminary identification can sometimes be made from appearance of the cells. For example, cell division by fission suggests *Schizosaccharomyces* or *Trichosporon* [1].

Vallejo et al., [4] isolated and identified yeasts from spoiled commercial strawberry juice in Argentina. Authors isolated yeast by placing a sample of deteriorated juices onto YMPG agar medium, pH 5.5 and supplemented with chloramphenicol (YMPG-C). Presumptive identification of isolates was performed based on morpho-physiological criteria [36]. Then, the authors carried out the genotypic identification of yeasts using chromosomal DNA isolated according to the protocol described by [37]. Molecular identification of the selected isolated yeasts was carried out by amplification and sequence analysis of the fragment containing the genes encoding ribosomal RNA (rRNA): 18S, 5.8S and 26S. Universal primers ITS1 and ITS4 were used to amplify the internal transcribed spacer 1 (ITS1), 5.8S rRNA, and internal transcribed spacer 2 (ITS2) sequences, while primers NL-1 and NL-4 were used to amplify the 26S rRNA D1/D2 domain. PCR amplifications of a fragment containing ITS1, 5.8S rRNA, and ITS2 sequences were performed according to [38] while that of 26S rRNA D1/D2 domain were carried out according to [39]. DNA sequencing of both strands was performed using the dideoxy chain termination method with an ABI Prism 3730 DNA analyzer, at the DNA sequencing facility at Macrogen Inc. Sequence comparisons were performed using the BLAST tool available within the GenBank database. ClustalW software [40] was used for local alignment of multiple sequences. Phylogenetic and molecular evolutionary analyses were carried out with MEGA 5.2 [41] by using neighbor-joining analysis [42]. For construction of phylogenetic trees, only sequences belonging to type strains of closely related species, whose names have been validly published in public databases, were considered.

Results of the morpho-phenotypic characterization of 100 yeasts isolated from deteriorated juice based on morpho-physiological criteria were shown in Table 2.1. The authors reported that 49 of the 100 isolates seem to be the same yeast (Group I) and 51 isolates share the same/similar characteristics. Isolates of group I and II were presumptively identified as *Hanseniaspora* and *Starmerella* genus, respectively.

Table 2.1. Phenotypic characterization of isolated yeasts from deteriorated strawberry juice.

	Group I	***Group II***

Number of isolated yeasts	49 isolates	51 isolates
Macro-morphology	White and glossy colonies with raised center and flat periphery.	Butyrous colonies with well delimited periphery.
Micro-morphology	Lemon-shaped; occur single or in pairs.	Cells are ellipsoidal; occur single or in pairs.
Classification	non-*Saccharomyces*	non-*Saccharomyces*
Sporulation	Yes	Yes
Ascospores/asci	1-2	1-2
Pseudomycelium	Yes	No
Growth at different temperatures	20 °C (v) 28 °C (+) 37 °C (-)	20 °C (v) 28 °C (+) 37 °C (v)
ACF	No	No
D-Glucose **fermentation**	+	+
Sucrose	-	+
Raffinose	-	+
Trehalose	-	+
Maltose **assimilation**	+	-
Cellobiose	+	-
D-Mannitol assimilation	-	+
Raffinose assimilation	-	+

ACF: Amylaceous compound formation - v: variable
From: Vallejo et al., [4].

Partial sequence analysis of rRNA of the isolates showed that Group I bore a remarkably close relationship, sharing 100% similarity with strains of the genus *Hanseniaspora* and Group II shared 95% identity with the genus *Starmerella* (Fig. 2.1 a,b). Partial rRNA sequences of *H. osmophila* and *S. bacillaris* were submitted to the public Genbank database under access numbers KJ880968 and KJ880972, respectively [43; 44]. Phylogenetic trees of isolates from Group I and Group II revealed that isolates from Group I showed a close phylogenetic relationship with reference strains of *H. osmophila* validated at GenBank, whereas isolates from Group II were closely related to reference strains of *S. bacillaris*.

Fig. 2.1. Phylogenetic trees of the yeast *H. osmophila* (a) and *S. bacillaris* (b) based on rRNA partial sequencing

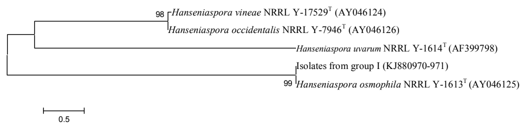


Fig. 2.1a

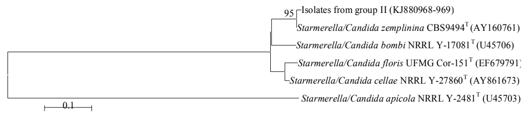


Fig. 2.1b

From: Vallejo et al., [4].

The authors demonstrated presence of *Starmerella bacillaris* and *Hanseniaspora osmophila* in spoiled strawberry juice from the Northwest of Argentina and said that a deeper knowledge of spoilage yeasts is the first step to find a solution.

2.3 Effects of yeasts on food spoilage

The main effects of yeast growth on foods are turbidity, swelling, slime formation, discoloration, and off-flavours. In wine and juice industry, several kinds of alterations have been associated with contaminant yeasts, including biofilm formation, sediments, turbidity, gas production, and/or off-flavours and unembellished tastes [45; 4]. In wines, numerous studies have clarified the main role of *Dekkera bruxellensis* [46]. This species synthesizes the volatile phenols 4-ethylphenol and 4-ethylguaiacol, from the hydroxycinnamic acids naturally present on grapes, the off-flavours are identified as 'horse stable'. Other species involved in the generation of ethylphenols include *Pichia guilliermondii* [47], *Peronospora manshurica* [48], *Candida wickerhamii* [49], or *Candida ishiwadae* [50], and *Trigonopsis cantarellii* [51]. Increases in acetic acid, mousy flavour compounds or biogenic amine production are other defects associated with contaminant yeast, particularly *Brettanomyces*. Recently, Vallejo et al., [4] reported that the main spoilage yeasts of damaged strawberry juice are *Starmerella bacillaris* and *Hanseniaspora osmophila*; the authors reported that these species produce changes in the color, turbidity, flavor and odor of strawberry juice.

Although there is still some controversy about the role of yeasts [2] in olive fermentation, some studies suggest yeasts have a key role in the sensorial properties of the final product [52]. Nonetheless, various negative qualities can be conferred by yeasts during or after fermentation, such as carbon dioxide production, fruit softening due to pectinolytic and proteolytic activity, clouding of brines, biofilm production, and, probably, off-flavour production [53].

2.4 Preservatives used in the food industry

Food preservatives are usually synthetic chemicals, such as sorbates, benzoates, nitrates and nitrites [54; 55]. In recent times have begun to shed light on the non negligible health risks of consuming these synthetic food additives. These health risks include allergic reactions, gastrointestinal disorders and cancer [56; 57].

However, food additives are still used regardless the harmful effects. Saccharin and its sodium salts, are extensively used sweeteners primarily because of their value to diabetes patients, although sodium saccharin has manifested carcinogenic effect in experimental animals [58]. Remarkably, some additives and pharmaceuticals can possess minor toxicity but in low concentrations may induce certain genotoxicity [59]. They can lead to cell death or can potentiate carcinogenic processes [60].

There is therefore much ongoing interest in more "natural" sources of food preservatives, which can be plant extracts, essential oils or purified secondary metabolites [61; 62].

2.5 Bioactive compounds as antifungal compounds

Fruits and vegetables rich diet is well established for its efficiency to promote human health [63; 64]. Consumption of fruits and vegetables is considered by many organizations (World Health Organization-WHO, Food and Agriculture Organization- FAO, United States Department of Agriculture-USDA and European Food Safety Authority-EFSA) as a major public health issue and is the subject of nutritional recommendations worldwide [65; 66].

Bioactive compounds in vegetables are numerous and chemically diverse [67; 68; 69]. In fruits and vegetables, molecules of nutritional interest are fibers, vitamins, minerals, phenolic compounds including flavonoids, phytoestrogens, sulfur compounds, monoterpenes and bioactive peptides. Their level in fruits and vegetables is a function of varieties, maturity and agronomical practices such as nitrogen addition and irrigation.

Plants produce a wide array of both primary and secondary metabolites that impact human health and nutrition. The carbohydrates, proteins, lipids and vitamins, made by plants as primary metabolites, are crucial to human nutrition. Secondary metabolites often play important roles in plant defense against biotic and abiotic stresses. Generally, they can be categorized as phenolics, terpenoids, nitrogen-containing alkaloids and sulfur-containing compounds. Phenolics and carotenoides are the main phytochemicals that help maintain human health [70].

The widespread occurrence of phenolic compounds in plants naturally extends to the human diet as well. Daily total consumption of phenolic compounds may exceed 1 g [71], with major contributing sources being fruits and vegetables, and beverages, such as tea, cocoa, wine and beer. Commercial applications of phenolic compounds include food additives, nutraceuticals, pharmaceuticals, cosmetics and others.

The interest in the composition of berry fruits has been also intensified because of an increased awareness of their possible health benefits, as they are rich sources of micronutrients and phytochemicals such as polyphenols. Some of these phenolic compounds, which can act as antioxidants and antimicrobials, have been identified by different authors [72; 73; 74; 4].

The berries are commonly consumed in soups, as porridge with rice and added to numerous meat and vegetable dishes [75], eaten raw, as a juice, wine or in tea preparations, as also processed as tinctures, powders, and tablets [75].

Blueberries are known for their rich bioactive compounds, including flavonoids, phenolic acids, tannins, and anthocyanins, which individually or synergistically help protect against cardiovascular disease, cancer, inflammation, obesity, diabetes, and other chronic diseases [76; 77]. Some studies have demonstrated that leaves of berry plants extracts, such as strawberry, cranberry and blueberry have antileukemic activity *in vitro* [78]. The aqueous extract of wild strawberry leaves is a direct, endothelium dependent vasodilator, and its action is mediated by nitrogen oxide and cyclooxygenase products with the potency similar to that of the hawthorn aqueous extracts [79].

The province of Tucumán in Argentine is the leading producer of strawberries and blueberries in northern Argentina and consumption of strawberry juice is high [4]. Blueberry (*Vaccinium spp*.) is rich in phenolic compounds, and several researchers have reported the content and antimicrobial activity of phenolic compounds in berries [15]. Blueberry contains relatively high amounts of acids and phenolic compounds [16], that display potential health benefits such as protection against cancer and cardiovascular diseases [17; 18; 19].

Another widely studied species is *Lycium barbarum L.*, which has several vernacular names, being "goji" the most common one [72]. Since the beginning of the 21th century, goji products have been introduced in Europe and North America and their consumption has increased rapidly due to their claimed beneficial properties for wellbeing and longevity [80]. Recent studies also suggest that *L. barbarum* leaves have shown a broad development and application prospects in the food industry due to the rich nutrients, biological active ingredients and trace elements [81].

Vallejo et al., [4] studied and identified the main phenolic compounds with antifungal activity present in four cultivars of blueberries grown in Argentina (Table 2.2). The authors reported that the major compounds in the four blueberry cultivars were chlorogenic acid and quercetin.

Table 2.2 Phenolic compounds identified in blueberry cultivars.

Phenolic Compounds identified in blueberries
Gallic acid - Methyl gallate - Caftaric acid
Cutaric acid – Procyanidin – Catechin
Chlorogenic acid - Trans-caffeic acid - Ferulic acid
Trans-ρ-coumaric acid - Ellagic acid
Quercetin-3-glucoside - Laricitrin 3-galactoside
Isorhamnetin-3-galactoside - Kaempferol-3-glucoside
Laricitrin-3-glucoside - Isorhamnetin-3-glucoside

From: Vallejo et al. [4].

Results reported by Vallejo et al., [4] in relation with the phenolic profile of Argentinean blueberries are coincident with those informed by other authors, who reported chlorogenic acid as the major phenolic acid in blueberries cultivated in Spain and Greece, among other countries [82; 83; 84; 85]. Dudonné et al., [86] reported that ρ-coumaric, caffeic and ferulic acid in all blueberry cultivars are in higher concentrations than gallic and protocatechuic acids. In addition, *Ribes nigrum*, a blueberry cultivar grown in Europe, contained mainly ρ-coumaric, ferulic and caffeic acid, with caffeoyl glucoside and flavonols such as myricetin, quercetin, isorhamnetin and kaempferol as the most important compounds [87; 88].

Then, the authors demonstrated that all blueberries phenolic extracts and several individual phenolic compounds present in blueberry inhibited the growth of two spoiled yeast, *Hanseniaspora osmophila* and *Starmerella bacillaris* (Table 2.3). Quercetin, kaempferol and ρ-coumaric, ellagic and chlorogenic acid showed highest antifungal activity, and they are probably responsible for the antifungal activity of blueberries.

Table 2.3 Antifungal activity of extracts of four blueberry cultivars and individual phenolic compounds

	Starmerella bacillaris	***Hanseniaspora osmophila***

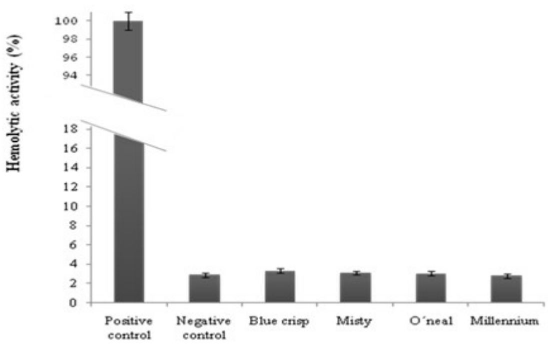
Misty	w	+
Millennium	+	+
Blue Crisp	+	++
O´Neal	w	+
Gallic acid	+	+
ρ-coumaric acid	++	++
Ferulic acid	+	+
Caffeic acid	+	+
Chlorogenic acid	++	++
Ellagic acid	++	++
Catechin	+	+
Quercetin	++	++
Kaempferol	++	++
Control (+)	+++	+++
Control (-)	-	-

Antifungal activity: Inhibition zone <1 mm, nil (-); Inhibition zone 1-5 mm, weak (w); Inhibition zone 6-11 mm, moderate (+); Inhibition zone 12-19 mm, high (++); inhibition zone >19 mm, strong (+++).
From: Vallejo et al., [4].

The antifungal activity of phenolic compounds of different natural matrices is widely studied. Berries are rich in phenolics and have been reported to have antimicrobial activity [17; 18; 19; 4]. Studies have been conducted to determine the content and antimicrobial activity of phenolic compounds in berries [89]. Yang et al., [90] reported that the phenolic compounds responsible for the antifungal activity of phenolic extracts of blueberry leaves and fruits from China, were gallic, caffeic, syringic, ρ-coumaric and ferulic acids.

The cytotoxicity assay demonstrated that the blueberries were not toxic to humans and that they did not modify the sensorial qualities of strawberry juice [4]. The authors reported that blueberry extracts could be a good natural and non-toxic alternative to prevent growth of spoilage yeasts of juice strawberry. These results are coincident with those reported by Miceli et al., [91] and they were similar to observations in a hydroalcoholic extract of *Ocimum sanctum* [92].

Fig. 2.2. Hemolytic activity of blueberry polyphenols using human red blood cells.



Source: Vallejo et al. [4].

3. CONCLUSION

Polyphenols present in different vegetables could be feasible natural and non-toxic alternative to prevent growth of spoilage yeasts and improve the microbiological quality of foods.

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