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Oak kombucha protects against oxidative stress and inflammatory processes

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ABSTRACT

Black tea infusion is the common substrate for preparing kombucha; however other sources such as oak leaves infusions can be used for the same purpose. Almost any white oak species have been used for medicinal applications by some ethnic groups in Mexico and could be also suitable for preparing kombucha analogues from oak (KAO). The objective of this research was to investigate the antioxidant activity and anti-inflammatory effects of KAO by examining its modulation ability on macrophage-derived TNF-alpha and IL-6. Herbal infusions from oak and black tea were fermented by kombucha consortium during seven days at 28 °C. Chemical composition was determined by LC-ESI-MS/MS. The antioxidant activity of samples against oxidative damage caused by H_2O_2 in monocytes activated (macrophages) was explored. Additionally, it was determined the anti-inflammatory activity using lipopolysaccharide (LPS) - stimulated macrophages; in particular, the nitric oxide (NO), TNF-alpha, and IL-6 production was assessed. Levels of pro-inflammatory cytokines IL-6 and TNF-alpha were significantly reduced by the sample treatment. Likewise, NO production was lower in treatment with kombucha and KAO compared with LPS-stimulated macrophages. Fermented beverages of oak effectively down-regulated the production of NO, while pro-inflammatory cytokines (TNF-alpha and IL-6) in macrophages were stimulated with LPS. Additionally, phytochemical compounds present in KAO decrease oxidative stress.

1. Introduction

Some fermented foods have transcended their sources to become everyday products in more than one continent; fermentations involved in these foods are of enormous complexity, and their study has provided us a wealth of biotechnology knowledge. An attractive bioprocess consists on the degradation of glucose and fructose through the fermentation action of a bacterial and yeast consortium called Kombucha [6]. This Kombucha is a fermented beverage that has been traditionally consumed in China for over 2200 years. This ancient beverage is composed of two portions: a floating biofilm of cellulose and the sour liquid broth [4]. Several positive effects have been reported, including gastro protective effect of the culture broth and probiotic potential of the Kombucha microbiome [1,13]. In particular, in the culture broth the main metabolites identified are gluconic and glucuronic acids, glycerol, phenolic acids and caffeine; some are associated with beneficial effects on health. The two main classes of involved polyphenols are flavonoids and phenolic acids. Their chemical and structural modifications are due to biotransformation and metabolism by the kombucha consortium action, and have not been taken into account in previous studies of kombucha analogues obtained from other sources. The biotransformation of flavonoids has been a topic of research due to the interest in explaining the correlation between the beneficial properties of flavonoids and the structures of the active compounds. In Kombucha obtained from black tea, the

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epigallocatechin-3-gallate biotransformation to epigallocatechin, epicatechin-3-gallate and epicatechin by enzymes excreted by kombucha microbiome has been demonstrated [9].

Concordantly, the development of functional beverages fermented from new sources that not only mitigates the consumer's thirst, but also contains phytochemicals that provide protective effects to health, is currently considered an innovative area with high value-added potential. Fermentation with the consortium Kombucha of infusions made from waste defoliation of oak has proven to be a viable alternative to obtain beverages rich in bioactive metabolites and increased bioavailability, which gives the advantage of consuming more bio-effective products [25]. Thus, using fermentation technology for the development of analogues of Kombucha may help to propose healthier food alternatives to the population.

Most biological activities *in vitro* have been tested using the aglycone forms of polyphenols. However in nature, flavonoids are conjugated with sugars, which can affect the antioxidant properties of compounds. Furthermore, recent studies have demonstrated the limited bioavailability of most polyphenols and the role of conjugated species such as glycosides or glucuronides in the absorption and circulating forms in the body. Although deglycosylation is likely to occur either in pre- or post-absorption, metabolism *in vivo* of these compounds may lead to neo-conjugation of one or more hydroxyl groups with sulphate and glucuronic acids [14]. The antioxidant properties of polyphenols are generally associated with the presence of *ortho* phenolic groups and the nature and position of these substitutions affect subsequent biological activities, possibly reducing or suppressing activities detected in the aglycone forms.

It is difficult to distinguish the effect of oak leaves infusion composition over the development of kombucha consortium, because chemical compounds present in infusion have demonstrated antimicrobial activity [22]. Until now, there are few reports describing the use of oak as a substrate for Kombucha analogues [25]. Moreover, there are no reports in the literature describing the metabolic profiles produced by the consortium kombucha after consumption of phenolic compounds. Since polyphenols present in this source are important from their antioxidant, antihypertensive and anti-inflammatory properties, it is therefore necessary to study the content and fate of bioactive polyphenols in fermented beverages.

Polyphenols, as a group of secondary metabolites broadly distributed in natural products, are in general considered as health promoters by their antioxidant activity [16]. Some polyphenols from oak leaves infusions include catechin, quercetin, kaempferol, naringin, naringenin and ellagic acid [19] among other compounds. In *Quercus* species, have also been reported hydrolysable tannins as vescalagin and castalagin [18] as well as bioactive proanthocyanidins [21]. These sources have proven to exhibit antimicrobial activity against some pathogens [22], anticarcinogenic and antioxidant potential [15]. However, infusions obtained from oak cause astringent or bitter taste [20]. Most polyphenolic compounds exhibit astringency and to be consumed with pleasure is necessary to mask their taste [12]. Therefore, the biological activity of polyphenols is complex, suggesting further investigations on these metabolites and their properties [23].

In this study, some white species such as *Quercus resinosa*, *Q. arizonica* and *Q. convallata* (formerly classified as *Q. obtusata*) were explored as potential sources for obtaining Kombucha analogues from oaks (KAO), evaluating changes on their phenolic compounds, sugar and organic acids by LC-ESI-MS/MS, and their antioxidant capacity and anti-inflammatory potential in a cellular model of macrophages.

2. Materials and methods

2.1. Reagent and biological materials

Starter Kombucha consortium (Healthy, Natural Life, Tlaquepaque, Jal., Mexico). THP-1 human monocytic cells were obtained from American Type Culture Collection (ATCC). Catechin, epicatechin, gallocatechin, gallocatechin gallate, epicatechin-gallate, rutin, myricetin, kaempferol, kaempferol 3-O-glycoside, quercetin, quercetin glucuronide, naringin, phloridzin, gallic acid, 3,4-dihydroxybenzoic acid, chlorogenic acid, 4-hydroxybenzoic acid, 4-O-caffeoylquinic acid, caffeic acid, 2,4,6, trihydroxybenzaldehyde, coumaric acid, ferulic acid, 3,4-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, salicylic acid, succinic acid, acetic acid, glucuronic acid, gluconic acid, fructose, glucose, sucrose, RPMI 1640 medium, fetal bovine serum, L-glutamine, sodium pyruvate, penicillin, streptomycin, phorbol-12-mirystate-13-acetate,3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, lipopolysaccharide, commercial ELISA kit (TNF-alpha and IL-6), hydrogen peroxide, 2,3-diaminophthalene, dichlorofluoroscein-diacetate were obtained from Sigma Chemical (St. Louis, MO, USA), acetonitrile LC-MS grade (J.Baker).

Quercus resinosa leaves were obtained from trees located at 9.2–9.4 km in Mezquital-Charcos Road in Southern Durango, Mexico, *Q. arizonica* leaves and *Q. convallata* leaves were collected from trees located at 53–54 km in El Tecuán – Regocijo road in Durango, Mexico. The leaves were air dried in the shade at 25 °C followed by milling to a particle size of 0.7–100 mm.

2.2. Preparation of herb infusions

Infusions were prepared by adding 1 g of ground material to 100 mL of water and allowed to stand for 10 min at 80 °C, followed by centrifugation at 4500 rpm for 10 min and then filtered using a 0.5 mm pore size filter.

2.3. Started cultures and fermentation

The started Kombucha was maintained in sweetened (sucrose 10%) black tea at 25 °C. The freshly cultured Kombucha was used for further subcultures of fresh fermentation batches.

The fermentation conditions were previously established with *Quercus resinosa* species according to [25]: fermentation time (7 days), sugar concentration (10%), starter culture (10%), inoculum of consortium (2.5%) and temperature (25 °C). The study was extended to fermenting infusions of *Quercus* spp. from white species (*Q. resinosa*, *Q. arizonica*, and *Q. convallata*).

2.4. Chemical characterization

Detection and quantification of major compounds was achieved using electrospray ionization/tandem spectrometry in multiple reaction-monitoring mode (MRM) to follow transitions of molecules into their specific fragmentation ions. Calibration curves for each compound were created by plotting standard concentrations (*x*-axis) and peak area ratios (*y*-axis) using linear regression and the concentration of 28 reference compounds in the sample calculated according to the slope from their standard curves.

2.5. Sugar, gluconic and glucuronic acid content in fermented beverages

The LC system consisted of a sample manager (5 °C) and a binary solvent manager coupled with a tandem Xevo TQ-S triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA). The