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Inhibitory effects of polyphenols from grape pomace extract on collagenase and elastase activity

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Caftaric acid (PubChem CID: 6440397)
Quercetin 3-*O*-glucoside (PubChem CID: 5280804)
Quercetin 3-*O*-glucuronid (PubChem CID: 12004528)
Procyanidin B1 (PubChem CID: 11250133)
Procyanidin B2 (PubChem CID: 122738)
Trans-resveratrol (PubChem CID: 445154)

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ABSTRACT

Breakdown and disorganization of extracellular matrix proteins like collagen, fibronectin and elastin are main characteristics of skin aging due to the enhanced activation of proteolytic enzymes such as collagenases and elastases. Inhibition of their enzymatic activities by natural plant compounds might be a promising approach to prevent extrinsic skin aging. Especially polyphenols are supposed to interact with those enzymes due to their molecular nature. In our investigation, extracts of pomace from Riesling grapes were analyzed for their inhibitory properties on collagenase as well as elastase. Crude grape pomace extract showed a dose-dependent inhibitory activity against both enzymes with IC_{50}-values of 20.3 μg/ml and 14.7 μg/ml for collagenase and elastase activity, respectively. The extracts were fractionated into four fractions containing phenolic compounds differing in chemical structure and polarity. Except for the stilbene containing fraction, all other fractions showed inhibitory effects on both enzyme activities. The most pronounced impact was found for the hydrophilic low molecular weight polyphenols containing the free phenolic acids. In particular, gallic acid showed considerable inhibition values. EGCG was used as a positive control and showed a dose-dependent inhibition of collagenase activity (IC_{50} = 0.9 mM).

1. Introduction

Skin aging is a complex biological phenomenon influenced by several factors including genetics, environmental exposure, hormonal changes, and metabolic processes. As the skin is the only organ directly exposed to the environment, aging processes resulting from environmental damage are of considerable relevance. Particularly solar UV radiation adversely impacts skin health, due to, amongst others, generation of reactive oxygen species (ROS) [1–3]. ROS are able to initiate complex molecular pathways including the activation of enzymes that degrade extracellular matrix (ECM) proteins in the dermis such as collagen and elastin ensuring the skin's three-dimensional integrity [1,2]. As a consequence of protease activation, breakdown, fragmentation and disorganisation of

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the ECM proteins occur, visibly manifested by the typical UV induced skin alterations such as deep wrinkles, loss of skin tone and resilience [3].

Matrix metalloproteinases (MMPs), a family of multi-domain zinc-containing endopeptidases with a broad range of substrate specificities, constitute the major proteolytic enzyme group involved in the degradation of dermal connective tissue components. Under normal physiological condition, MMP activities are precisely regulated at the level of transcription and by endogenous protein inhibitors. In addition, MMPs are secreted from cells as an inactive precursor requiring cleavage by extracellular proteinases to be activated. These regulation processes enable the remodeling of connective tissue under physiological and pathophysiological circumstances like wound healing, inflammation and cancer. The impairment of this balance, due to oxidative stress, results in an excess of MMPs being a key feature of premature aging of the skin as well as various inflammatory and degenerative diseases.

In addition, serine proteases such as elastases are involved in ECM degradation. Elastolytic enzymes are released from neutrophils and dermal fibroblasts in response to UV induced skin inflammation [4]. Due to their active side catalytic triad, elastases are multi-specific and cleave several proteins within the extracellular matrix including collagen, fibronectin and elastin. Elastin is an important ECM protein having the unique property of elastic recoil, which is vital for providing elasticity to skin and other tissues like arteries, lungs and ligaments. Several studies have shown that human neutrophil elastase is also involved in the degradation of ECM connective tissue through the activation of inactive MMP-1 and MMP-2 precursors [5–7].

Inhibiting the activity of ECM degrading proteins like collagenases and elastases may be a useful approach to prevent UV induced skin alterations and premature skin aging. Scavenging of ROS by natural antioxidants might be one option to inhibit such skin deteriorative enzymes, as ROS play an important role in the activation of these enzymes. Phenolic compounds are an important group of natural antioxidants. They belong to diverse subclasses of secondary plant metabolites classified as phenolic acids, flavonoids, stilbenes and lignans and are ubiquitously found in the plant kingdom. In particular, red and white grapes contain high amounts of phenolic acids and flavonoids such as gallic acid and catechin [8]. Due to their chemical structure, polyphenols have powerful antioxidant activities being able to scavenge a wide range of ROS such as hydroxyl radicals and superoxide radicals [9]. In addition, polyphenols may inhibit the activity of proteolytic enzymes *in vitro* by acting as complexing or precipitating agents as indicated in literature [10,11]. Especially, green tea polyphenols such as catechin and epigallocatechin gallate, commonly used as ingredients in anti-aging skin care formulations, have been shown to exhibit moderate inhibitory effects against collagenase and elastase activity, presumably through non-covalent binding [12–14]. However, the mechanism of action of polyphenols is not fully understood, and studies about the inhibitory effects of natural active ingredients on skin degrading enzymes are scarce. Most of the studies have been performed with isolated compounds, thus not considering the complex nature of crude extracts. Additionally, data about inhibitiory properties of further polyphenolic subclasses are scarce. However, in particular, for the systematic development of natural formulations exerting skin health promoting properties, such investigations would be of basic interest.

In the present study, the *in vitro* inhibitory potential of phenolic compounds obtained from white grape pomace against the activity of *Clostridium histolyticum* collagenase (ChC) and porcine pancreatic elastase (PPE) was investigated. The polyphenolic profile of the grape pomace extract was determined, moreover, different fractions of the extract as well as individual polyphenols were assayed for their inhibitory activity against ChC and PPE, respectively.

2. Materials and methods

2.1. Materials

Grape pomace from white wine (*Vitis vinifera* L. cv. 'Weisser Riesling') of vintage 2010 was kindly provided by the Baden-Badener Winzergenossenschaft (Baden-Baden-Neuweier, Germany). Pomace samples (voucher specimen #600385.1 as deposited at the Fraunhofer IVV, Freising, Germany) were collected after pressing the mash, freeze dried, sealed in polyethylene bags, and kept at 5 °C until further use.

C18 reversed-phase cartridges (Chromabond, 2000 mg) were obtained from Macherey-Nagel (Düren, Germany). *C. histolyticum* collagenase type IA (ChC), N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA) and porcine pancreas elastase type III (PPE) were purchased from Sigma (St. Louis, MO). N-Succ-Ala-Ala-Ala-p-nitroanilide (AAAPVN) was obtained from Serva Electrophoresis (Heidelberg, Germany). Polyphenols used as standards for the inhibition studies were gallic acid (≥97%), catechin (≥99%), epigallocatechin gallate (≥95%) (Sigma, St. Louis, MO); caftaric acid (≥97%), quercetin 3-*O*-glucoside (≥98%), quercetin 3-*O*-glucuronid (≥95%), procyanidin B1 (≥90%), procyanidin B2 (≥90%) (Fluka, Buchs, Switzerland), and *trans*-resveratrol (98%) (ABCR, Karlsruhe, Germany).

All reagents and chemicals used were of analytical grade and purchased from VWR (Darmstadt, Germany).

2.2. Preparation of polyphenol rich extracts

2.2.1. Crude extract

Extraction of polyphenols from grape pomace was carried out according to Kammerer, Claus, Carle and Schieber [8]. Briefly, lyophilised grape pomace was ground using a ZM 100 centrifugal mill with a 1 mm ring sieve (Retsch, Haan, Germany). Aliquots of 2 g of the powdered samples were weighted into Erlenmeyer flasks, flushed with nitrogen and extracted with 50 ml of methanol for 60 min under shaking, using a KS 500 laboratory shaker (Janke & Kunkel, IKA Labortechnik, Staufen, Germany). Extracts were filtered through a paper filter (Munktell, grade 3hw); the residue was re-extracted with 50 ml of the solvent for 30 min to ensure an exhaustive extraction. Supernatants were combined, and the organic solvent was removed by evaporation *in vacuo* at 40 °C. Residues obtained were dissolved in 10 ml water and filtered through a 0.2 μm syringe filter (Sartorius, Göttingen, Germany), and the pH was adjusted to 7.0 with sodium hydroxide solution (1 M).