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Exploring Alternative Potentialities of Portuguese and Spanish Craft Beers: Antioxidant and Photoprotective Activities

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Abstract: Craft beer has gained popularity due to its unique sensory characteristics and complex matrix with nutritional and potential health benefits. Studies linking beer consumption to skin conditions are limited, however, the high content of bioactive compounds is related to biological activities such as antibacterial, anti-inflammatory, anti-oxidative, and anti-carcinogenic. This study aims to evaluate the antioxidant, photoprotective and metabolic activity in human keratinocytes (HaCaT). Eighteen craft and four industrial beers were analyzed after dealcoholizing, degassing and freeze-drying. Total phenolic content (TPC) and antioxidant activity were determined. The most promising craft beer was studied for its photoprotective and metabolic activity. An India Pale Ale beer (ALM-IPA) presented the second best TPC (8.96 ± 0.64 mg of GAE/g) and promising antioxidant activity by ABTS (IC_{50} = 55.21 ± 4.68 µg/mL), H_2O_2 (IC_{50} = 23.54 ± 1.53 µg/mL) and FRAP (53.74 ± 1.27 µmol TE/g) assays. Regarding photoprotective activity, a solar photoprotection factor of 48.85 ± 0.39 was obtained. ALM-IPA showed no cytotoxicity up to a concentration of 250 µg/mL after 24 and 48 h of incubation. The potential benefits of beer extracts on skin can be seen, but further studies are essential to corroborate the findings and guarantee the safety of the extracts.

Keywords: craft beer; antioxidant; photoprotection; keratinocytes; metabolic activity

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1. Introduction

Beer represents the most widely consumed alcoholic drink worldwide [1]. In 2022, around 1.89 billion hectoliters of beer were brewed, with beer consumption in the European Union at around 313 million hectoliters. Germany stands out as the largest European producer with a total of 87,832 hectoliters [2]. Portugal appears in 13th place with a production of 7787 hectoliters [2]. In the same year, Portugal and Spain had a per capita consumption of 53 and 58 liters, respectively. The main distributors of craft beer are the United States of America (USA) and Europe, accounting for 46% and 43% of the market, respectively [3].

Craft beer is characterized by various authors as beer produced in small quantities (e.g., <50 thousand hectoliters/year in Spain; <6 million barrels/year in the USA; <200 thousand hectoliters/year in Italy), using traditional methods and raw materials

(water, malt, hops and yeast) to which can be added innovative raw materials such as extracts of aromatic and/or medicinal plants and probiotics that give different aromas and flavors. This beverage can also be considered as having health-promoting capacities, due to its promising nutritional and biological properties [1,3–12]. Craft beers differ from industrial ones as most of them skip the processes of pasteurization and microfiltration, which are crucial to avoid microbiological contamination [1,3,8,13]. However, as craft beers are often not filtered and not pasteurized, the phenolic compounds total content and antioxidant activity can be improved [3]. The craft beer production is also determined by its innovative aspects in a constant search for differentiating organoleptic characteristics and biological activities and the attention to population niches [8,10].

Beer's nutritional and biological potential comes from its heterogeneous composition of active ingredients, which include hydroxycinnamic acid derivatives (e.g., ferulic acid), hydroxybenzoic acids (e.g., gallic acid), flavanols (e.g., catechin), flavonol esters (e.g., galatocatechin), flavanol glycosides (e.g., kaempferol-3-*O*-glucoside) and prenylated flavonoids (e.g., xanthohumol) [6,8,14]. Several of these compounds are antioxidants, with renowned health benefits as they protect our body of the exacerbated production of reactive oxygen species, that culminates in oxidative stress, triggering internal damage to mitochondria, lipids and proteins, which can be reflected in skin damage such as dermatitis, acne, rosacea, photoaging and cancer [13,15].

Despite the lack of studies regarding the potential benefits of craft beer extracts on the skin, some effects observed may be related to the beverage's antioxidant potential and its raw materials, documented in several studies [6,13,15]. Therefore, the raw materials and breweries by-products have been associated with promising activities such as photoprotective, antibacterial, anti-inflammatory, anti-elastase, anti-tyrosinase, anti-proliferative and anti-cancer [16–21]. Such activities can result in skin damage prevention, which can affect social interaction, and it is one reason for seeking cosmetic products and/or aesthetic procedures to reverse it [22,23].

This experimental work aims to characterize Portuguese and Spanish craft beers in terms of their physicochemical characteristics, determine their phenolic compound content, assess their antioxidant activity, estimate their photoprotective potential and as well as assessing the metabolic activity of HaCaT cells (human keratinocytes) in the presence of beer.

2. Materials and Methods

2.1. Chemicals

Gallic acid (GA), Folin-Ciocalteu reagent, and hydrochloric acid were purchased from Merck (Darmstadt, Germany). Absolute ethanol, glacial acetic acid, sodium hydroxide (NaOH), iron (II) sulfate, disodium phosphate, iron (III) chloride, sodium acetate, and ethylenediaminetetraacetic acid (EDTA) were purchased from VWR (Radnor, PA, USA). Quercetin, 3,5-dinitrosalicylic acid (DNS), sodium potassium tartrate, tert-butyl hydroperoxide (*t*-BOOH), and monobasic potassium phosphate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Potassium persulfate was obtained from Biochem (Cosne Sur Loire, France). Ferrozine, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and 1% antibiotic and antimycotic solution were purchased from Thermo Scientific (Karlsruhe, Germany). Monosodium phosphate was obtained from J. T. Baker (Amsterdam, The Netherlands). Dimethyl sulfoxide (DMSO), sodium chloride, and ascorbic acid were purchased from Fisher Scientific (Loughborough, UK). Also, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were purchased from the company Acros Organics (Beel, Belgium). Sodium carbonate was obtained from Atom Scientific (Hyde, UK). Glucose was obtained from Labkem (Barcelona,

Spain). Hydrogen peroxide was obtained from PanReac AppliChem (Barcelona, Spain). Trypsin, phosphate-buffered saline (PBS), and RPMI were purchased from Corning (Glendale, CA, USA). Fetal bovine serum (FBS) was purchased from the company Biochrom KG (Darmstadt, Germany). Finally, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from TCI (Zwijndrecht, Belgium).

2.2. Beer Samples

A total of 18 craft beers were purchased in supermarkets or provided by breweries (ten Portuguese and eight Spanish craft beers). Four Portuguese industrial beers, used for comparison purposes, were also obtained in supermarkets. All beers were coded with letters and the brand names were omitted (Table 1).

Table 1. Characteristics of the beers (style, packaging type and volume, and source).

Type	Style	Code	Packing	Packing Volume (mL)	Country	Craft vs. Industrial
Ale	Milk Stout	EL-MS	Glass bottle	330	Spain	Craft
	Imperial Stout	EME-IS	Glass bottle	330	Spain	Craft
		IS-ES	Glass bottle	330	Spain	Craft
		IS-N	Glass bottle	330	Portugal	Craft
	Oatmeal Stout	ALM-OS	Glass bottle	330	Portugal	Craft
	Sweet Stout	SS-SB #	Can	330	Portugal	Industrial
	Brown Ale	BA-ET	Glass bottle	330	Spain	Craft
	Dubble Belga	DB-ET	Glass bottle	330	Spain	Craft
	Brown Porter	BP-N	Glass bottle	330	Portugal	Craft
	India Pale Ale	EL-IPA	Glass bottle	330	Spain	Craft
		ALM-IPA	Glass bottle	330	Portugal	Craft
		DC-IPA	Glass bottle	330	Portugal	Craft
		IPA-N	Glass bottle	330	Portugal	Craft
	Pale Ale	PA-ET	Glass bottle	330	Spain	Craft
Lager	Pilsner	S-P #	Can	330	Portugal	Industrial
		EL-P	Glass bottle	330	Spain	Craft
		M-P	Glass bottle	330	Portugal	Craft
		P-L	Glass bottle	330	Portugal	Craft
		P-SB #	Glass bottle	200	Portugal	Industrial
	Munich Dunkel	S-MD #	Glass bottle	250	Portugal	Industrial
		B-MD	Glass bottle	330	Portugal	Craft
	Lager	L-N	Glass bottle	330	Portugal	Craft

#: Industrial Beer; information regarding pasteurization and/or filtration of the beers was not provided by the producers.

2.3. Beer Preparation for Analyses

The preparation of beers was carried out as described by Silva et al. [15], Labrado et al. [24] and Censi et al. [13], with slight modifications. The content of each bottle was homogenized for 10 s, and then degassed by sonication (Bandelin Sonorex®, Bandelin, Berlin, Germany) for 40 min at 35 kHz, at room temperature. Samples were then dealcoholized using a rotary evaporator (IKA®, VWR, Lisbon, Portugal) at 40 °C, 60 rpm and 900 mbar for 1 h. Finally, all samples were freeze-dried (Labconco®, VWR, Lisbon, Portugal) under freeze-drying conditions of 0.07 mbar, with a condenser surface temperature of −72 °C, and storage at −80 °C until analysis.

2.4. Chemical Analysis of Beer Samples

All beers (craft and industrial) were characterized by determining pH, total acidity (TA) and reducing sugar content (RSC). The parameters relating to alcohol content, color and bitterness were obtained from the packaging or provided by the breweries.

2.4.1. pH Determination

The determination of the pH was carried out according to Silva et al. [15], with slight modifications. The pH was measured in 15 mL of the degassed and dealcoholized beer using a calibrated pH meter (BANTE instruments 900®, Shanghai, China).

2.4.2. Total Acidity (TA)

The determination of the TA was carried out by potentiometric titration, as described by Silva et al. [15]. Therefore, a 0.1 M NaOH solution was added to 15 mL of the degassed and dealcoholized beer, until the pH equaled 8.2. The results were calculated using the formula: TA (as Lactic Acid %) = (Volume (0.1 M NaOH) × 0.9)/15.

2.4.3. Reducing Sugar Content (RSC)

RSC was analysed using the DNS colorimetric method described by Silva et al. [15], with slight modifications. The 1% (*w*/*v*) DNS solution was first prepared by dissolving 1 g of DNS in 20 mL of 2 M NaOH, to which 30 g of sodium potassium tartrate was added, and the mixture was diluted in 1 L of distilled water. Next, 1 mL of DNS solution was added to 1 mL of sample (beer or glucose-positive control). The mixture was vortexed vigorously (VWR® ZX3), incubated in a water bath at 100 °C for 5 min (Memmert® w-270), and then cooled in an ice bath for 5 min. Different concentrations of glucose were used (50, 100, 200, 400, 600, 800 and 1000 mg/L) as a standard solution. The absorbance was read at 540 nm on a UV-Vis spectrophotometer (Model VWR UV-1600PC, VWR, Lisbon, Portugal)). The RSC of the samples was expressed in mg of glucose equivalents/L of sample (mg GE/L).

2.5. Total Phenolic Content (TPC) of Beer Samples

TPC was determined using the Folin-Ciocalteu spectrophotometric assay as described by Alves et al. [25] and Silva et al. [15], with some modifications. Protected from light, 1.25 mL of 0.2 M Folin-Ciocalteu reagent was added to 250 µL of the sample (beer or gallic acid-positive control), left to stand for 5 min and then 2 mL of 75 mg/L sodium carbonate and distilled water were added to make 5 mL. After being incubated for 1 h at room temperature and protected from light, the absorbance was read at a wavelength of 760 nm on a UV-Vis spectrophotometer (Model VWR UV-1600PC). Different concentrations of GA were used (5, 10, 20, 40, 60, 80, 100 µg/mL) as a standard solution. The results were expressed as gallic acid equivalents per gram of extract (mg GAE/g).

2.6. Antioxidant Capacity of Beer Samples

The antioxidant capacity of all beer samples was determined using the following assays: ABTS, H_2O_2, metal chelating activity and FRAP.

2.6.1. ABTS Radical Scavenging Activity

The ABTS assay was carried out as described by Petrón et al. [26] and Censi et al. [13], with slight modifications. The ABTS· radical was formed by reacting 2.45 mM potassium persulphate with 7 mM ABTS reagent (diluted in water), protected from light, for 16 h. The ABTS solution was then diluted with PBS to obtain an absorbance of 0.7 ± 0.05 at 734 nm. Then, 2700 µL of ABTS· was added to 300 µL of sample (concentration of 1, 5, 10, 25, 50, 100, 250, 500 and 1000 µg/mL) (beer or trolox-positive control). After incubating

for 30 min at room temperature and protected from light, the absorbance was measured at 734 nm using a UV-Vis spectrophotometer (Model VWR UV-1600PC). ABTS scavenging ability was expressed as IC_{50} (µg/mL) and the inhibition percentage calculated using the following formula: ABTS· inhibition (%) = (Blank absorbance − Sample absorbance)/(Blank absorbance) × 100.

2.6.2. Hydrogen Peroxide (H_2O_2) Scavenging Activity

The H_2O_2 assay was carried out as described by Jayaprakasha et al. [27] and Bhatti et al. [28] with some modifications. The 40 mM H_2O_2 solution was prepared in a 0.2 M phosphate buffer solution (pH = 7.4). Protected from light, 1 mL of sample (1, 5, 10, 25, 50, 100, 250, 500 and 1000 µg/mL) (beer or ascorbic acid-positive control) was mixed with 3 mL of 0.2 M phosphate buffer and 1 mL of H_2O_2. After incubating for 10 min at room temperature, the absorbance was read at 230 nm using a UV-Vis spectrophotometer (Model VWR UV-1600PC) and the H_2O_2 scavenging ability was expressed as IC_{50} (µg/mL). The inhibition percentage was calculated using the following formula: Scavenging de H_2O_2 (%) = (Blank absorbance − Sample absorbance)/(Blank absorbance) × 100.

2.6.3. Metal Chelating Activity (Ferrozine Assay)

The metal chelating activity or ferrozine assay was carried out as described by Silva et al. [15]. Briefly, 50 µL of the sample (beer or EDTA-positive control) was added to 0.15 mM ferrous sulphate solution. After resting for 5 min, protected from light, 50 µL of 0.5 mM ferrozine was added. The mixture was vigorously stirred and left for 10 min at room temperature and protected from light. Absorbance was measured at 562 nm in a microplate reader (Thermoscientific® MULTISKA FC). Metal chelating activity was expressed as IC_{50} (µg/mL) and the inhibition percentage calculated using the following formula: Chelating Activity (%) = (Control absorbance − Samples absorbance)/(Control absorbance) × 100.

2.6.4. Ferrous-Reducing Antioxidant Power Assay (FRAP)

The FRAP colorimetric assay was carried out as described by Censi et al. [13] and Ulloa et al. [29], with modifications. Briefly, FRAP reagent was prepared by mixing 15 mL of 0.3 M acetate buffer solution (pH = 3.6) with 1.5 mL of 10 mM TPTZ solution (dissolved in 40 mM HCl) and 1.5 mL of 20 mM $FeCl_3$ solution. Then, 80 µL (10 mg/mL) of sample (beer or trolox-positive control) was added to 2400 µL of FRAP reagent. After incubating for 15 min in an incubator (Stuart® SI500) at 37 °C and 5 min at room temperature (protected from light), the absorbance was measured at 593 nm using a UV-Vis spectrophotometer (Model VWR UV-1600PC). The results were estimated by extrapolation using a calibration curve with trolox (25, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900 µmol) and expressed as µmol of trolox equivalents per gram of extract (µmol TE/g).

2.7. Assays to Determine Photoprotective Potential

The craft beer with the most promising antioxidant activity was selected for the determination of SPF (Sun Protection Factor) and UV absorption capacity, in vitro.

2.7.1. Determination of SPF

The SPF determination was carried out as described by Priyanka et al. [30]. Briefly, aqueous solutions (1 mg/mL) of beer sample were prepared. As a positive control, a commercial sunscreen (SPF = 50+) was used, which was prepared as described by Dutra et al. [31]. Therefore, 1 g of the beer sample was diluted in 100 mL of absolute ethanol and the solution subjected to an ultrasonic bath (Bandelin Sonorex®) for 5 min. The mixture was filtered, and 5 mL of the filtrate was transferred to a 50 mL volumetric

flask and topped up with absolute ethanol. Then, 5 mL of the previously prepared solution was transferred to a 25 mL volumetric flask and topped up with absolute ethanol. Each solution (beer and positive control) was measured using a UV-Vis spectrophotometer (Model VWR UV-1600PC) in the wavelength range of 290–320 nm (with increments of 5 nm). The results were expressed using the Mansur et al. [32] equation [30,32]:

$$SPF = CF \times \sum_{320}^{290} EE\ (\lambda) \times I(\lambda) \times Abs(\lambda)$$

where CF = correction factor; EE = erimatogenic effect at wavelength (λ), I = radiation intensity at length λ, Abs (λ) = absorbance obtained experimentally at each wavelength.

2.7.2. UV Absorption Capacity

The UV absorption capacity was determined as described by Mejía-Giraldo et al. [33] and Priyanka et al. [30]. Briefly, 1 mg/mL of sample (beer or commercial sunscreen-positive control) was measured for absorbance over a range of wavelengths between 200–450 nm in a UV-Vis spectrophotometer (Model VWR UV-1600PC), with increments of 50 nm. The results were expressed according to the following formula: UV absorption coefficient = Absorbance/(Sample concentration (mg/mL)) × 100.

2.8. Cellular Assays

To evaluate the protective capacity of the samples at a cellular level, a model of human keratinocytes, HaCaT cells, was used. These cells are derived from immortal, non-tumourogenic human keratinocytes [34].

2.8.1. Cell Line Maintenance

The maintenance of the HaCaT cells followed the procedures described by Wilson et al. [34] and Alnuqaydan et al. [35], with some modifications. HaCaT cells were cultured in RPMI 1640 medium supplemented with 10% (V/V) FBS, 1% (V/V) antibiotic (ampicillin and streptomycin) and incubated at 37 °C with 5% CO_2 (Advantage®-Lab AL01-01-100) [34–36]. The HaCaT cells grew in culture medium at a ratio of 1.0×10^6 cells/mL, and culture medium changed every two days. Subcultures were carried out when 60/80% confluence was reached.

2.8.2. Metabolic Activity Assessment Assay

The metabolic activity of HaCaT cells was evaluated using the MTT assay, following the procedure described by Censi et al. [13] and Oliveira et al. [37] with some modifications. HaCaT cells were incubated into a 96-well plate (2.0×10^4 cells/mL) for 40 h, after which the beer sample was added at concentrations of 1–500 µg/mL. After 24 h and 48 h of incubation, cell viability was estimated using the MTT assay, as described by Mosmann [38] and De la Cruz-Concepción et al. [39], with some modifications. Briefly, 10 µL of MTT (5 mg/mL) was added to 100 µL of culture medium with beer sample. After incubation at 37 °C for 4 h, the formazan crystals formed were dissolved in DMSO with absolute ethanol (1:1). The absorbance was measured at a wavelength of 570 nm in a microplate reader (Thermoscientific® MULTISKA FC). The percentage of cells with metabolic activity was calculated using the following formula: Cell viability % = (Samples absorbance)/(Blank absorbance) × 100.

2.9. Statistical Analysis

All assays were done in triplicates, and results are expressed as the mean ± standard deviation. Statistical analysis was performed using GraphPad Prism® 8.0 software. Statistical differences in the TPC and antioxidant assays were analyzed by Welche's *t* test, and by

one-way analysis of variance (ANOVA) with Sidak's multiple comparison test. In cellular assays, the one-way ANOVA test with Dunnett's multiple comparison was used to analyze and compare the data. Correlation coefficients (r) were calculated using Pearson Product Moment Correlation, to determine the correlations among means. Differences with a $p < 0.05$ were considered significant.

3. Results and Discussion

3.1. Physicochemical Parameters of Beers

The analysis of beers physicochemical parameters is fundamental, as they are considered important factors in the classification and characterization of different beer styles, also interfering with the organoleptic properties and quality of this beverage [15,40,41]. Therefore, Table 2 shows the parameters analyzed in sample beers related to alcohol content, color, bitterness, pH, total acidity (TA) and reducing sugar content (RSC).

Table 2. Physicochemical parameters of beer samples.

Type	Style	Code	ABV (% V/V)	Color (EBC)	Bitterness (IBU)	pH	Total Acidity (Lactic Acid %)	RSC (mg GE/L)
Ale	Milk Stout	EL-MS	6.20	93	22	4.51 ± 0.01 ^{a}	0.25 ± 0.02 ^{a}	18,548.75 ± 68.75
	Imperial Stout	EME-IS	8.00	ND	98	4.39 ± 0.01 ^{b}	0.28 ± 0.00 ^{b}	22,255.00 ± 25.00
		IS-ES	9.10	70	68	4.10 ± 0.01 ^{c}	0.26 ± 0.00 ^{a,b,c}	13,553.15 ± 239.00 ^{a}
		IS-N	8.50	124	30 ^{a}	4.60 ± 0.01 ^{d}	0.26 ± 0.01 ^{a,b,c,d}	16,480.00 ± 156.62
	Oatmeal Stout	ALM-OS	6.50 ^{a}	98.2	39.6	4.49 ± 0.01 ^{a,e}	0.44 ± 0.01	21,646.67 ± 125.83
	Sweet Stout	SS-SB $^{\#}$	5.00 ^{b}	185	17	4.07 ± 0.00 ^{c,f}	0.25 ± 0.01 ^{a,c,d,e}	15,767.50 ± 200.00
	Brown Ale	BA-ET	5.50	21	25	4.10 ± 0.00	0.22 ± 0.00 ^{a,e,f}	5011.25 ± 68.75 ^{b}
	Dubble Belga	DB-ET	6.80 ^{c}	ND	24 ^{b}	4.57 ± 0.00 ^{d}	0.20 ± 0.00 ^{g}	6772.13 ± 38.95
	Brown Porter	BP-N	4.80	59	30 ^{a}	4.03 ± 0.00 ^{f,g}	0.14 ± 0.01 ^{h}	7276.88 ± 65.62
	India Pale Ale	EL-IPA	6.80 ^{c}	7 ^{a}	24 ^{b,c}	4.63 ± 0.00 ^{d}	0.33 ± 0.00 ^{g,i}	2767.50 ± 25.00 ^{c}
		ALM-IPA	6.50 ^{a}	17.5	67.9	4.69 ± 0.03	0.18 ± 0.00	21,088.33 ± 143.80
		DC-IPA	4.50 ^{d}	ND	ND	4.12 ± 0.00 ^{c,h}	0.18 ± 0.00 ^{g,i,j}	3948.75 ± 118.75 ^{d}
		IPA-N	6.00 ^{e}	11	50	4.26 ± 0.00	0.19 ± 0.01 ^{g,i,j,k}	5836.25 ± 43.75 ^{e}
	Pale Ale	PA-ET	6.00 ^{e}	ND	54	4.48 ± 0.03 ^{a,e,i}	0.18 ± 0.00 ^{g,i,j,l}	5448.75 ± 93.75 ^{b,e}
Lager	Pilsner	S-P $^{\#}$	5.00 ^{b,f}	ND	ND	4.43 ± 0.01 b	0.13 ± 0.01 ^{h,m}	1917.50 ± 75.00 ^{f}
		EL-P	5.20 ^{g}	7 ^{a,b}	24 ^{b,c}	4.74 ± 0.01 ^{j}	0.19 ± 0.01 ^{g,i,j,k,n}	2967.50 ± 62.50 ^{g}
		M-P	4.50 ^{d}	ND	39	4.35 ± 0.00 ^{b}	0.20 ± 0.00 ^{f,g,j,k,l,n,o}	3259.17 ± 36.08 ^{c,g}
		P-L	5.00 ^{b,f,h}	7 ^{a,b}	26	4.03 ± 0.00 ^{f,g}	0.20 ± 0.00 ^{g,j,k,l,n,o}	3913.33 ± 85.09 ^{d}
		P-SB $^{\#}$	5.20 ^{g,i}	8	18 ^{d}	4.11 ± 0.00 ^{c,f,h}	0.17 ± 0.01 ^{i,j,k,l,n,p}	1567.50 ± 12.50 ^{f}
	Munich Dunkel	S-MD $^{\#}$	4.10	ND	ND	3.89 ± 0.00	0.13 ± 0.01 ^{h,m}	13,346.67 ± 220.20 ^{a}
		B-MD	5.20 ^{g,i}	40	20	4.78 ± 0.05 ^{j}	0.37 ± 0.00	15,000.83 ± 470.43
	Lager	L-N	5.00 ^{b,f,h}	10	18 ^{d}	4.49 ± 0.00 ^{a,e,i}	0.16 ± 0.01 ^{h,i,l,p}	2475.83 ± 26.02 ^{c}

The pH, TA and RSC values are the mean ± standard deviation of three independent samples (n = 3), in triplicate. $^{\#}$: Industrial Beer. ND: No Data. %: Percentage. ABV: Alcohol by Volume. EBC: European Breweries Convention. IBU: International Bitterness Units. GE: Glucose Equivalents. RSC: Reducing Sugar Content. The different letters indicate statistically significant differences ($p < 0.05$).

3.1.1. Alcohol (ABV%)

Alcohol content is important for sensory perception and the physiological effects of beer consumption. The alcohol content, expressed in ABV, usually ranges from 4–6% (V/V), and in some beers it can reach 14% (V/V). There are also no-alcohol or low-alcohol beers (up to 1.2% (V/V) [42–44]. In the present study the ABV ranged from

4.10 (S-MD, Munich Dunkel) to 9.10% (IS-ES, Imperial Stout). Since alcohol content depends on the extraction of carbohydrates from malt and the fermentation process, differences observed in beers may be related to the use of different raw materials and production methods [45].

3.1.2. Color (European Brewery Convention—EBC)

Beer color, according to Hughes et al. [46] ranges from 4.5–1550 EBC, with each color value giving a flavor attribute to the beer [46]. In the present study, this parameter ranged from 7 (EL-IPA, India Pale Ale; EL-P and P-L, Pilsner) to 185 EBC (SS-SB, Sweet Stout). Therefore, beers can vary greatly in color due to the use of different types of malt and ingredients (fruits or spices), and the temperature and roasting time of malted and unmalted cereals (the higher the roasting level of cereals, the higher the EBC of beer) [6,45].

3.1.3. Bitterness

The bitterness of beers was expressed in IBU, which can range from 0–100 IBU, providing an approximate value of iso-α-acids present in milligrams of iso-α-acid per liter of beer [47,48]. Thus, the intensity of bitterness is correlated with the quality of the raw materials, the fermentation process, and the brewing techniques [49]. Among the raw materials, the polyphenols present in malt contribute to bitterness, but the main responsible is hops (type and quality of hops, level of plant development, time, temperature, extraction methods and addition time), due to the presence of alpha-acids [49]. These compounds are isomerized during the must boiling, creating iso-alpha-acids, which are the main compounds responsible for bitterness. The fermentation process can also influence bitterness, especially when hops are added during or after fermentation, a process known as dry-hopping [43,50]. This step can alter the extraction of alpha acids and, consequently, the final bitterness of the drink. Furthermore, brewing techniques also play a crucial role, particularly in the wort boiling stage, since it is during boiling that both the extraction and isomerization of the alpha-acids present in hops occur, defining the bitterness characteristics of the final product [51,52].

In this study, the bitterness of craft beers ranged from 18 (L-N, Lager) to 98 (EME-IS, Imperial Stout), while in industrial beers it was equal to 17 (SS-SB, Sweet Stout) and 18 (P-SB, Pilsner). The higher content of bitter compounds in craft beers may be due to the higher concentration of isohumulone (the most bitter compound), while in industrial beers isohumulone (the least bitter compound) predominates [53,54].

3.1.4. pH and Total Acidity (TA)

The pH and TA of beer is important, affecting the organoleptic characteristics and biological and chemical stability of the beverage [55]. In general, beers pH can range from 4.3–4.6 [56]. In the present study, values varied between 3.89 ± 0.00 (S-MD, Munich Dunkel) to 4.78 ± 0.05 (B-MD, Munich Dunkel). TA content in beer is expected to vary between 0.1% and 0.3% lactic acid equivalents. The total acidity of the beer samples ranged from 0.13 ± 0.01% (S-P, Pilsner; S-MD, Munich Dunckel) and 0.44 ± 0.01% lactic acid equivalents (ALM-OS, Oatmeal Stout) [57].

3.1.5. Reducing Sugar Content (RSC)

RSC provides important information for the optimization of the fermentation process, allowing an increase in the final product's yield and quality [58]. In this study, the RSC ranged from 1567.50 ± 12.50 (P-SB, Pilsner) to 22,255.00 ± 25.00 mg GE/L (EME-IS, Imperial Stout). The differences observed in RSC are related to different brands, beer styles, raw materials, and production processes [45]. In addition, yeasts can only metabolize low

molecular weight sugars, namely fructose, maltose, sucrose, glucose and maltotriose. Therefore, if fermentation is interrupted beer will have higher RSC [55].

The greater variability in the chemical parameters of craft beer may be associated with the lower standardization of the brewing process compared to industrial beers, which consumers perceive as higher quality [59,60].

3.2. Total Phenolic Content (TPC) of Beer Samples

In craft beers, TPC ranged from 3.51 ± 0.16 mg GAE/g (DC-IPA, India Pale Ale) to 9.54 ± 0.32 mg GAE/g (BP-N, Brown-Porter), and in industrial beers ranged from 5.02 ± 0.08 mg GAE/g (P-SB, Pilsner) to 7.58 ± 0.09 mg GAE/g (SS-SB, Sweet Stout) (Table 3).

Table 3. Results of total phenolic content (TPC) in beers.

Type	Style	Code	TPC (mg de GAE/g)
Ale	Milk Stout	EL-MS	7.95 ± 0.69^{a}
	Imperial Stout	EME-IS	4.62 ± 0.16^{b}
		IS-ES	$6.79 \pm 0.64^{a,c}$
		IS-N	$8.27 \pm 0.16^{a,d}$
	Oatmeal Stout	ALM-OS	$7.24 \pm 0.40^{a,c,d,e}$
	Sweet Stout	SS-SB #	$7.58 \pm 0.09^{a,c,d,e,f}$
	Brown Ale	BA-ET	$5.49 \pm 0.08^{b,c,g}$
	Dubble Belga	DB-ET	$5.97 \pm 0.08^{b,c,e,g,h}$
	Brown Porter	BP-N	$9.54 \pm 0.32^{a,d,i}$
	India Pale Ale	EL-IPA	$7.32 \pm 0.48^{a,c,d,e,f,h,j}$
		ALM-IPA	$8.96 \pm 0.64^{a,d,h,i,k}$
		DC-IPA	$3.51 \pm 0.16^{b,h,l}$
		IPA-N	$6.60 \pm 0.24^{a,c,e,f,g,h,j,m}$
	Pale Ale	PA-ET	$4.06 \pm 0.40^{b,g,h,l,n}$
Lager	Pilsner	S-P #	$5.17 \pm 0.08^{b,g,h,o}$
		EL-P	$6.68 \pm 0.00^{a,c,e,f,g,h,j,m,p}$
		M-P	$6.92 \pm 0.24^{a,c,d,e,f,g,h,j,m,p,q}$
		P-L	$5.49 \pm 0.08^{b,c,g,h,m,n,o,p,q,r}$
		P-SB #	$5.02 \pm 0.08^{b,g,h,n,o,r,s}$
	Munich Dunkel	S-MD #	$6.29 \pm 0.08^{c,e,f,g,h,j,m,p,q,r,s,t}$
		B-MD	$8.06 \pm 0.56^{a,c,d,e,f,i,j,k,p,q}$
	Lager	L-N	$6.37 \pm 0.16^{c,e,g,h,j,m,o,p,q,r,s,t}$

Industrial Beer. The different letters indicate statistically significant differences ($p < 0.05$).

The results, in craft beers, are in accordance with the study performed by Marques et al. [61], where a dark craft beer (Brown Porter), showed a higher TPC (48.5 ± 0.19 mg GAE/L) [61]. Also, in the study performed by Silva et al. [15] the craft beer with the highest TPC was a dark Imperial Stout (2172.5 ± 170.1 mg GAE/L) [15]. On the other hand, in the study performed by Breda et al. [6], the highest TPC was found in a light beer (1.614 ± 0.280 g GAE/L), which may justify the second highest TPC found in the present study (8.96 ± 0.64 mg GAE/L) with an India Pale Ale beer [6].

In general, TPC observed in the present study are in accordance with the literature, where according to Scioli et al. [62] and Jastrzebski et al. [63], varied between 3.21 ± 0.07 and 9.33 ± 0.33 mg of GAE/g, respectively [62,63]. Censi et al. [13], demonstrated that TPC of Italian craft beers ranged from 18.961 ± 1.082 mg GAE/g to 35.822 ± 0.147 mg GAE/g, higher than the values obtained [13]. The differences observed can be explained by the use of different brands or styles, raw materials and brewing techniques [1,64].

Several studies have analyzed craft beers regarding their phenolic composition, with the compounds being gallic acid, gallocatechin, catechin, caffeic acid, *p*-coumaric acid,

ferulic acid, luteolin, epicatechin, kaempferol-3-o-glucoside, quercetin and trans-cinnamic acid of the most commonly identified [6,10,61,65–67].

3.3. Antioxidant Capacity of Beer Samples

Beer is a heterogeneous matrix with antioxidants which act differently [68,69]. The antioxidant activity of beer samples was determined using four antioxidant assays: ABTS, H_2O_2, FRAP and metal chelating activity (Table 4).

Table 4. Antioxidant activity of beers according to ABTS, H_2O_2, ferrozine and FRAP assays.

Type	Style	Code	ABTS (IC_{50} µg/mL)	H_2O_2 (IC_{50} µg/mL)	Ferrozine (IC_{50} µg/mL)	FRAP (µmol TE/g)
Ale	Milk Stout	EL-MS	74.20 ± 4.37 ^{a}	33.79 ± 0.82 ^{a}	ND	43.16 ± 3.37 ^{a}
	Imperial Stout	EME-IS	108.98 ± 5.44 ^{b}	36.04 ± 2.57 ^{a,b}	ND	33,98 ± 2.21 ^{a,b}
		IS-ES	115.61 ± 7.17 ^{c}	35.00 ± 0.63 ^{b,c}	ND	43.64 ± 0.01 ^{c}
		IS-N	80.09 ± 1.11 ^{a,d}	26.96 ± 1.49 ^{d}	ND	44.44 ± 0.01 ^{c,d}
	Oatmeal Stout	ALM-OS	87.94 ± 3.82 ^{d,e}	34.28 ± 2.24 ^{a,b,c,f}	ND	50.91 ± 0.70 ^{a,b,e}
	Sweet Stout	SS-SB #	76.08 ± 1.14 ^{a,d,e,f}	20.15 ± 1.10 ^{g}	ND	32.74 ± 0.01 ^{f}
	Brown Ale	BA-ET	93.66 ± 1.41 ^{e,g}	36.04 ± 0.34 ^{a,b,c,f,h}	ND	34.47 ± 0.01 ^{f,g}
	Dubble Belga	DB-ET	123.64 ± 4.73 ^{c,h}	47.48 ± 0.34 ^{i}	ND	29.31 ± 0.01
	Brown Porter	BP-N	89.23 ± 1.81 ^{d,e,g,i}	31.89 ± 0.98 ^{a,b,c,d,f,h,j}	ND	47.21 ± 0.00
	India Pale Ale	EL-IPA	83.27 ± 0,21 ^{a,c,d,e,f,g,i,j}	31.60 ± 1.05 ^{a,b,c,d,f,h,j,k}	ND	40.56 ± 3.07 ^{a,b,e,h}
		ALM-IPA	55.21 ± 4.68	23.54 ± 1.53 ^{d,g,l}	ND	53.74 ± 1.27 ^{a,e,h,i}
		DC-IPA	38.74 ± 0.47	13.85 ± 1.30	ND	47.77 ± 1.63 ^{a,b,e,h,i,j}
		IPA-N	105.97 ± 1.15 ^{b,k}	37.49 ± 0.13 ^{a,b,c,f,h,m}	ND	32.50 ± 0.00 ^{a,b,e,h,j,k}
	Pale Ale	PA-ET	99.09 ± 0.71 ^{b,e,g,i,k,l}	43.37 ± 1.87 ^{i,n}	ND	58.61 ± 0.01
Lager	Pilsner	S-P #	114,56 ± 5,47 ^{b,c,h,k,m}	50,92 ± 3,24 ^{i,o}	ND	27,57 ± 1,57 ^{a,b,h,k,l}
		EL-P	84.30 ± 4.27 ^{a,d,e,f,g,i,j,n}	40.92 ± 1.34 ^{b,h,m,n,p}	ND	34.24 ± 0.77 ^{a,b,e,h,j,k,l,m}
		M-P	101.71 ± 0.90 ^{b,g,k,l}	39.80 ± 0.99 ^{b,c,h,m,n,p,q}	ND	41.74 ± 0.13 ^{a,b,e,h,i,j,k,l,m,n}
		P-L	117.86 ± 0.82 ^{b,c,h,k,m,o}	27.53 ± 0.32 ^{d,j,k,l}	ND	43.57 ± 0.01 ^{c,d}
		P-SB #	86.01 ± 6.41 ^{a,e,f,g,i,j,n,p}	46.67 ± 0.42 ^{i,n,o,r}	ND	20.27 ± 0.01
	Munich Dunkel	S-MD #	123.85 ± 6.81 ^{c,h,m,o}	50.65 ± 5.04 ^{i,o,r}	ND	46.67 ± 0.13 ^{a,b,e,h,i,j,k,m,n,o}
		B-MD	80.66 ± 4.11 ^{a,d,e,f,i,j,n,p}	37.82 ± 1.23 ^{a,b,c,f,h,m,p,q,s}	ND	48.94 ± 1.13 ^{a,b,e,h,i,j,k,m,n,o}
	Lager	L-N	68.33 ± 2.80 ^{a,d,f}	37.16 ± 1.49 ^{a,b,c,f,h,j,m,p,q,s}	ND	33.41 ± 0.01 ^{f,g}

Industrial Beer. ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid assay. H_2O_2: Hydrogen peroxide assay. FRAP: Ferric Reducing Antioxidant Power Assay. IC_{50}: Concentration able to inhibit by 50%. TE: Trolox Equivalents. The different letters indicate statistically significant differences ($p < 0.05$). ND—Not determined

The ABTS assay is a colorimetric assay that assesses the antioxidant capacity of an extract through its ability to neutralize the ABTS· radical by transferring electrons or hydrogen atoms [70,71].

According to Kuete & Efferth [72], an extract has high antioxidant activity when the IC_{50} values are less than 50 µg/mL, moderate antioxidant activity when the IC_{50} values range from 50–100 µg/mL and low antioxidant activity when the IC_{50} values are greater than 100 µg/mL [72].

In the ABTS assay, the IC_{50} values of craft beers ranged from 38.74 ± 0.47 (DC-IPA, India Pale Ale) to 123.64 ± 4.73 µg/mL (DB-ET, Belgian Dubble). An India Pale Ale beer (ALM-IPA) showed the second best IC_{50} value (IC_{50} = 55.21 ± 4.68 µg/mL), and the second highest TPC. The study performed by Breda et al. [6] corroborates these results, as the best antioxidant capacity using the ABTS assay was found in a light beer [6]. In this study, 5.6% of craft beers (n = 18) have a high antioxidant capacity, 61.1% have a moderate antioxidant capacity and 33.3% have a low antioxidant capacity. Also, 50% of the industrial beers (n = 4) showed moderate antioxidant capacity and the other half had low antioxidant capac-

ity. The IC_{50} values of the industrial beers ranged from 76.08 ± 1.14 (SS-SB, Sweet Stout) to 123.85 ± 6.81 µg/mL (S-MD, Munich Dunkel).

In the study performed by Breda et al. [6], with craft beers, ABTS values (1142 ± 0.025 to 10,913 ± 0.305 mmol TE/L), were higher than those obtained for industrial beers by Zhao et al. [73] (0.55 ± 0.04 mmol TE/L to 1.95 ± 0.05 mmol TE/L) and Zhao et al. [74] (0.16 ± 0.09 mmol TE/L to 2.23 ± 0.04 mmol TE/L) [6,73,74]. These results are in accordance with our study, since the best ABTS scavenging activity was observed in an India Pale Ale style craft beer (DC-IPA). This can be attributed to the different raw materials used, rich in antioxidants such as phenolic compounds, and the brewing process, which influences the extraction and stability of these antioxidant compounds [73].

The H_2O_2 assay is based on the ability of the antioxidants in the sample to neutralize H_2O_2 molecules, preventing the formation of ·OH free radicals, and the initiation of the oxidative chain [70]. For craft beers, IC_{50} values ranged from 13.85 ± 1.30 (DC-IPA, India Pale Ale) to 47.48 ± 0.34 µg/mL (DB-ET, Belgian Dubble). An Indian Pale Ale beer (ALM-IPA) showed the second best IC_{50} value (23.54 ± 1.53 ug/mL). In turn, for industrial beers, IC_{50} values ranged from 20.15 ± 1.10 (SS-SB, Sweet Stout) to 50.92 ± 3.24 µg/mL (S-P, Pilsner).

All the beers showed high antioxidant activity according to the H_2O_2 assay (IC_{50} < 50 µg/mL), except for the industrial beers S-P (Pilsner) and S-MD (Munich Dunkel), which showed moderate antioxidant activity (50 < IC_{50} < 100 µg/mL) [72].

To date, literature is scarce on how to carry out the H_2O_2 neutralization assay to determine the antioxidant capacity of beer. In the study performed by Okechukwu et al. [75], an industrial beer (German Pilsner) showed an H_2O_2 scavenging activity of 88.86 ± 0.13% [75]. However, this assay is important because it makes it possible to determine the elimination capacity of a physiologically relevant radical, responsible to initiate the oxidative chain [76,77].

DC-IPA corresponds to the beer with the highest antioxidant activity with ABTS and H_2O_2 assays ($p < 0.05$). However, this beer presented the lowest TPC. This results can be explained by the presence of antioxidant compounds, other than phenolics, such as carotenoids, tocopherols, melanoidins, iso-α-acids, and ascorbic acid [74,78].

The metal chelating activity assay, also known as ferrozine assay, is based on the antioxidant agent's ability to chelate Fe^{2+} (ferrous ion), preventing its oxidation to Fe^{3+} (ferric ion) and, consequently, the synthesis of the hydroxyl radical ($H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH\cdot$) [79,80]. Therefore, an extract with the ability to chelate Fe^{2+} ions prevent it from forming complexes with the reddish-coloured ferrozine [79].

In the ferrozine assay, it was not possible to determine the IC_{50} value in the range of concentrations tested (1–1000 µg/mL), that is the concentration of sample that has the amount of antioxidants capable of reducing the initial concentration of Fe^{2+} ions by 50%. Therefore, with this assay, beer samples presented low antioxidant capacity (IC_{50} > 100 µg/mL) [72]. In the study performed by Silva et al. [15], the metal ion chelation capacity of craft beers ranged from 1.6 ± 0.4% to 113.4 ± 15.8% [15].

The FRAP assay is a colorimetric method based on the ability of the antioxidants in the sample to reduce the Fe^{3+} ion, present in the TPTZ complex, to Fe^{2+} by electron transfer. When this reduction occurs, the color of the solution changes from light purple to deep purple [81].

In FRAP assay, a Pale Ale craft beer (PA-ET) showed the highest Fe^{3+} ion reduction value (58.61 ± 0.01 µmol TE/g), which was significantly higher comparing with other beers ($p < 0.05$). Also, an Indian Pale Ale beer (ALM-IPA) showed the second-best value in this assay (53.74 ± 1.27 µmol TE/g). An Industrial Pilsner beer (P-SB) had the lowest FRAP value (20.3 ± 0.0 µmol TE/g). In the study performed by Censi et al. [13], FRAP assay

values ranged from 104.046 ± 7.680 µmol TE/g (Triple malt) to 125.159 ± 1.237 µmol TE/g (Black), which were higher than those observed in the present study [13]. These results also suggest that the use of different raw materials, manufacturing processes, and samples can influence the antioxidant power of Fe^{3+} ion reduction [82].

The results of antioxidant assays vary considerably, even when analyzing the same sample using different methods, as each antioxidant activity assay is based on different chemical backgrounds and reaction mechanisms [69]. In short, an Indian Pale Ale craft beer (ALM-IPA) showed the second highest TPC (8.96 ± 0.64 mg GAE/g), antioxidant activity with ABTS (IC_{50} = 55.21 ± 4.68 µg/mL), H_2O_2 (IC_{50} = 23.54 ± 1.53 µg/mL) and FRAP (53.74 ± 1.27 µmol TE/g) assays, and was subjected to the study of photoprotective and metabolic activity in HaCaT cells.

3.4. Correlation Between Physical-Chemical Characteristics, TPC and Antioxidant Assays

In this study, alcohol content was positively correlated with IBU and RSC (Table 5). EBC, IBU and TA presented a positive correlation with RSC. On the other hand: EBC correlated negatively with H_2O_2 assay; pH correlated with TA; TPC correlated positively with RSC; and ABTS assay correlated positively with the H_2O_2 assay.

Table 5. Correlation between physicochemical parameters, TPC and antioxidant activity.

	ABV	EBC	IBU	pH	TA	RSC	TPC	ABTS	H_2O_2	FRAP
ABV	1.000	0.213	0.570 *	0.293	0.320	0.430 *	0.139	0.150	−0.108	0.229
EBC	-	1.000	−0.077	−0.150	0.378	0.657 **	0.422	−0.157	−0.554 *	0.256
IBU	-	-	1.000	−0.003	0.290	0.475 *	−0.209	0.266	−0.053	−0.113
pH	-	-	-	1.000	0430 *	0.176	0.284	−0.271	0.074	−0.287
TA	-	-	-	-	1.000	0.768 **	0.367	−0.244	−0.323	−0.217
RSC	-	-	-	-	-	1.000	0.431 *	−0.113	−0.281	−0.154
TPC	-	-	-	-	-	-	1.000	−0.185	−0.214	0.016
ABTS	-	-	-	-	-	-	-	1.000	0.681 **	0.165
H_2O_2	-	-	-	-	-	-	-	-	1.000	−0.100
FRAP	-	-	-	-	-	-	-	-	-	1.000

ABV: Alcohol by volume. EBC: European Brewery Convention. IBU: International Bitterness Units. TA: Total Acidity. RSC: Reducing Sugar Content. TPC: Total Phenolic Content. ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid assay. H_2O_2: Hydrogen peroxide assay. FRAP: Ferric Reducing Antioxidant Power Assay. * Correlation is significant at 0.05 level (two-tailed); ** Correlation is significant at 0.01 level (two-tailed).

The lack of correlation between TPC and the antioxidant assays could be attributed to the presence of other antioxidant compounds, which also have the ability neutralize free radicals and chelate metal ions [74,78]. The study of Silva et al. [15] corroborates the results obtained, having observed no correlation between TPC and antioxidant assays [15]. However, there are several studies that do not corroborate the findings, mentioning the existence of a correlation between TPC and the antioxidant power of beers, that is, a positive correlation between TPC and the values of the FRAP and ABTS assays [6,55,73,74,82–86]. In addition, the literature mentions the existence of a positive correlation between antioxidant assays, namely ABTS and FRAP [82,85].

3.5. Photoprotection

The spectrophotometric determination of sun protection factor (SPF) is an inexpensive, easy to perform, reproducible and accurate in vitro method [87]. According to European Regulation 647/2006 of 22nd September, only compounds with an SPF greater than 6 provide protection against UV radiation. In this sense, ALM-IPA beer presents photoprotective potential (48.85 ± 0.39) (Table 6) [88].

Table 6. Sun protection factor (SPF) for ALM-IPA beer (India Pale Ale).

Type	Style	Code	SPF
Positive Control		PC	134.21 ± 0.45
Ale	India Pale Ale	ALM-IPA	48.85 ± 0.39 *

SPF: Sun Protection Factor. * Statistically significant differences ($p < 0.05$) compared to the positive control.

The beer's SPF value is significantly lower than the positive control (commercial sunscreen 50+) ($p < 0.05$), which would be expected given that it is the result of a combination of organic filters guaranteeing protection against UVB and UVA.

The UV absorption capacity assay makes it possible to determine the photoprotective capacity of the extracts in the UVB and UVA regions. Table 7 shows the UV absorption capacity of ALM-IPA beer, showing that PC outperforms beer at wavelengths of 300 and 350 nm ($p < 0.05$). This is to be expected, as PC is a commercial sunscreen with UVB and UVA protection, so its protection range is between 290 and 400 nm. It should be noted that at wavelengths of 250, 400 and 450 nm, ALM-IPA beer has a higher UV absorption capacity than PC ($p < 0.05$). The 250 and 450 nm wavelengths are outside the protection spectrum of PC, and these results are predictable, which is not the case with the 400 nm wavelength [89].

Table 7. UV absortion capacity of ALM-IPA beer (India Pale Ale style).

ƛ (nm)	PC	ALM-IPA
200	ND	
250	50.00 ± 0.26	131.90 ± 0.98 *
300	175.77 ± 1.37	73.73 ± 0.64 *
350	140.50 ± 1.15	26.87 ± 0.06 *
400	2.30 ± 0.17	9.33 ± 0.25 *
450	0.23 ± 0.06	5.43 ± 0.32 *

ƛ: wavelength. PC: Positive Control. ND: No Data. * Statistically significant differences ($p < 0.05$) compared to the positive control.

As craft beer contains phenolic compounds, namely flavonoids (e.g., quercetin, isoflavones), phenolic acids (e.g., gallic and cinnamic acid) and polyphenols (e.g., resveratrol), it can be a source of bioactive compounds with photoprotective activity [90,91].

This is also and as far as we know, the first report of a combined determination of the SPF and UV absorption capacity of beer, so it is only possible to make comparisons with the PC, highlighting the need for further studies in order to increase the robustness of the information.

Kurzawa et al. [19] studied the photoprotective potential of hop extracts (aqueous, aqueous-glycolic and oily) and found that the SPF was higher in the oily extract (21.05 at a concentration of 1.6 mg/mL) compared to the aqueous extract (5.58 at a concentration of 0.8 mg/mL) and the aqueous-glycolic extract (10.18 at a concentration of 0.4 mg/mL) [19]. Therefore, it is possible that beer has a greater photoprotective potential than hops (raw material), so the complexity of the matrix and the manufacturing process may be relevant variables in terms of photoprotection.

In short, the results suggest that beer may have synergistic effects with commercial sunscreens and may be able to enhance the photoprotective effect by reducing the concentration of synthetic filters in the formulation. In this way, the disadvantages associated with synthetic filters can be minimised, namely photoinstability, the possibility of triggering

allergic reactions, environmental impact and human toxicity (given the detection of filters in marine beings and in human biological samples) [90,92,93].

In addition, beer has shown anti-inflammatory potential, so its SPF can increase in vivo, similar to rutin (also present in beer) which, when incorporated into a formulation with avobenzone and aminobenzoic acid, showed reduced SPF in vitro, but due to its anti-inflammatory potential, SPF increased in vivo [94,95]. In addition, freeze-dried beer extract showed potential anti-tyrosinase action (55.16 ± 0.52 mg of kojic acid equivalent/g), so beer not only has photoprotective potential but can also minimize hyperpigmentation resulting from exposure to UV radiation [62].

3.6. Metabolic Activity

The determination of cell viability aims to quantify the number of viable cells, after incubation with a compound or exposure to a certain condition, allowing the ideal concentration for the objective of the assay to be determined [96]. Therefore, HaCaT cells (human keratinocytes), a model widely used in studies in vitro, were used to investigate the effect of beer extract on the skin.

The MTT assay is one of the assays that can be used to determine viable cells, based on the ability of metabolically active cells to reduce the reagent (MTT), originating purple-colored formazan crystals (insoluble in water) [96,97]. Therefore, the amount of crystals and, consequently, the intensity of the purple color establishes proportionality with the number of metabolically active cells [96].

HaCaT cells were incubated with ALM-IPA beer extract for 24 h and 48 h (Figure 1). Incubation at two different times provides the possibility of comparing the effects of the extract under study with different contact times. Allowing the identification of safe concentrations that can increase cell viability.

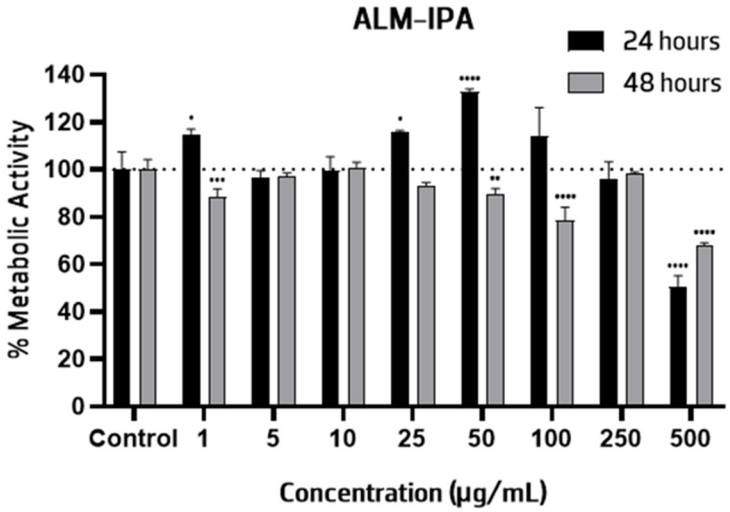

Figure 1. Metabolic activity determined by MTT assay after 24 and 48 h incubation of HaCaT cells with ALM-IPA beer. Results are expressed in comparison with the negative control. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

In this sense, a cell viability equal to or greater than 60% indicates weak/non-existent cytotoxicity and less than 60% indicates moderate/strong cytotoxicity [98]. Therefore, only at a concentration of 500 µg/mL in a 24 h incubation presented moderate/strong cytotoxicity. At concentrations of 1 µg/mL ($p < 0.05$), 25 µg/mL ($p < 0.05$) and 50 µg/mL ($p < 0.0001$) there was observed a significant increase in cell viability in the 24 h incubation, compared to the control. In the 48 h incubation, cell viability remained close to control at concentrations of 5 µg/mL, 10 µg/mL and 250 µg/mL, with a decrease at other concentra-

tions. The 25 µg/mL concentration presents itself as promising as it significantly increases cell viability in the first 24 h ($p < 0.05$), after which it decreases to values no different from the control. Thus, ALM-IPA beer not only allows the maintenance of cell viability, but also promotes its increase, potentially contributing to the re-epithelialization of damaged tissues, contributing to the restoration of the skin barrier, due to its ability to mediate the wound healing, inflammatory and proliferative response [99].

To our knowledge, no study has previously investigated the effect of beer extracts on HaCaT cells. However, in the study by Merinas-Amo et al. [100], industrial lager beer extracts when inoculated with rat fibroblasts (NIH3T3) were cytotoxic at a concentration of 25 mg/mL (IC_{50}) and when inoculated with cells derived from human leukemia (HL-60) at a concentration of 125 mg/mL [100]. The same extracts were tested in in vivo models (Drosophila) at concentrations from 3.125–50 mg/mL, verifying the absence of toxicity (100% survival), increased average life expectancy and protection against pro-oxidative damage induced by H_2O_2, in a dose dependent manner [100].

4. Conclusions

Craft beer, despite its harmful effects when consumed in excess, is, effectively, a functional drink that, due to the complexity of its composition, brings together numerous properties beneficial to health, with potential benefits in the cosmetics area. However, most studies only evaluate the potential benefits of raw materials and by-products from their manufacture, which reiterates the relevance of the present study. In terms of physicochemical parameters, the beers included are in accordance with those described in the literature, which is advantageous given their influence on sensory characteristics, biological and chemical stability, and the fermentation process. The beers analyzed revealed promising antioxidant activity, which was not correlated with the TPC value. Additionally, ALM-IPA beer demonstrated photoprotective potential and lack of cytotoxicity in HaCaT cells, highlighting potential skin benefits. This study highlights new a promising potential of craft beers, with a skin application that has yet to be further developed, but which can be exploited by breweries as a new niche application for implementation in the pharmaceutical and cosmetics industries.

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