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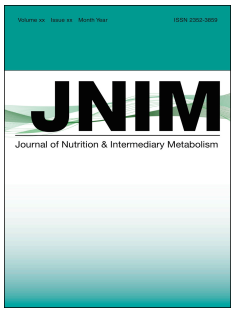

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Chelating properties of beer: implications on calcium homeostasis in PE/CA-PJ15 cells

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ABSTRACT

Beer contains a large variety of different molecules, of which some can chelate Ca^{2+}. Here, we show that in PE/CA-PJ15 cells, an experimental model previously employed to investigate Ca^{2+} homeostasis, chemical entities with Ca^{2+}-chelating properties can markedly impact intra- and extra-cellular levels of the metal ion. This was consistently shown by commercial beers, as well as aliquots of unfinished product taken during the last step (clarification) of the brewing process. Our data suggest that a large number of molecules with chelating properties, can cross the cell membrane and, thus, potentially lead to important biological effects associated with changes in Ca^{2+} homeostasis. In this regard, when PE/CA-PJ15 cells were exposed to H_2O_2 in order to experimentally prompt an oxidative stress, beer antagonized a non-capacitative Ca^{2+} entry that was otherwise elicited by H_2O_2 in its absence. Higher Ca^{2+}-chelating activity of stout, as compared to lager beer, suggest that melanoidins, which are generally present in dark malts, are major chelating agents in dark beers. Further, based on dose-response determinations, we report, for the first time, that Ferulic Acid can bind both intra- and extra-cellular Ca^{2+} and, as one of the most abundant hydroxycinnamic acids in malted barley, largely account for Ca^{2+} chelation.

These results support the notion that beer may be considered a natural source of chemical entities that, based on their binding activity to Ca^{2+} and, possibly, other metal ions, might be considered as nutritional supplements, detoxification agents, or antibiotics.

Keywords: Cytosolic calcium; Beer; FURA 2AM method; Chelating activity; Ferulic acid; Reactive oxygen species (ROS).

INTRODUCTION

Beer is a worldwide consumed and popular drink that, from a nutritional standpoint, can be considered an effective vehicle of natural products beneficial to human health [1]. This beverage is essentially a hydro-alcoholic solution, with a pH display in the 4.0-4.5 range, which generally contains hundreds of different molecules. The components of fresh, bottled beer are not in a chemical equilibrium [2], which in turn may potentially prompt a variety of reactions during storage and, thus, influence the type and characteristics of the aged product.

Oxygen, in particular, is a major factor affecting a rapid deterioration of the beer's taste, in that it triggers oxidation reactions, catalyzed by metal ions present in trace amounts, followed by the formation of reactive oxygen species (ROS). In aging beer, ROS correlate with increasing levels of divalent ions and oxygen, and storage temperature [3]. ROS react with many types of organic molecules present in beer and, consequently, may cause significant alterations of the sensory features of the beverage.

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The superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) are widely involved in physiological and pathophysiological processes associated with oxidized proteins, lipids and nucleotides [4], while a large body of literature underscores the notion that Ca^{2+} and ROS signaling are intimately integrated. [5]. Cytosolic calcium $[Ca^{2+}]_c$, which is relevant to signal transduction in most cells, is maintained in the nM range by various mechanisms, whereas the ion accumulates in the endoplasmic reticulum (ER) [6]. It is also widely accepted that exogenous ROS can induce dynamic changes in $[Ca^{2+}]_c$ in a variety of cell types [5]. This effect might be due to mobilization of intracellular Ca^{2+} stores and influx of extracellular Ca^{2+} [5, 7, 8].

The antioxidant properties of beer have been correlated with the presence of polyphenols and melanoidins, as well as chelating agents, which reduce the formation of ROS by preventing the bioavailability of bivalent ions [9, 10, 11]. Beer is also rich in amino acids, phytic acid, polyphenols and melanoidins, with the latter being formed through a Maillard reaction during kilning and roasting of malt [12]. In this regard, melanoidins,

which may impact flavor and color of beer [13, 14], display anionic groups, and can be considered potent chelating agents. In beer, there is an equilibrium between free and chelated metal ions which, depending on type of chelator, ultimately impacts ROS formation [15]. Among phenolic compounds, hydroxycinnamic acids occur in malted barley, the main raw material in beer production. Within this class of aromatic acids, Ferulic acid [16] was previously reported for its beneficial effects on human health as an anti-inflammatory and a free radical scavenger [17, 18].

Here, to better characterize the properties of beer as a calcium-chelator, and assess whether such properties would impact calcium homeostasis, we assessed its chelating properties in both the cytosol and extracellular space of the human cell line PE/CA-PJ15 by the fluorescent probe FURA 2AM [19]. To minimize the incidence of confounding variables, measurements were conducted in beer samples obtained during the production phases of brewing (Brewing Process, BP) and in different styles of commercial beers (Commercial Beer, CB). Further, we evaluated the effects of beer in calcium homeostasis following stimulation of oxidative stress in cells. Our findings strongly suggest that Ferulic acid present in beer largely contributes to its Ca^{2+}-chelating properties.

MATERIALS and METHODS

Reagents

FURA 2-AM (FURA-2-*pentakis* (acetoxymethyl) ester, Triton X-100 (t-octylphenoxypolyethoxyethanol), EGTA (ethylene glycol-*bis* (β-aminoethyl ether)-N,N,N',N'-tetracetic acid), Ferulic acid, Trypan blue, and Iscove's modified Dulbecco Medium were purchased from Sigma-Aldrich corporation (St. Louis, Missouri, USA). Other reagents (reagent grade) were obtained from common commercial sources.

Beer samples

CERB (Italian Brewing Research Center) pilot plant beer samples were collected during different brewing steps, such as: 1st (not saccharified mash), 2nd (saccharified mash), 3rd (boiler full), 4th (boiled wort), 5th (cold wort), 6th (finished product). Aliquots of samples were centrifuged at 5,000 x g for 10 min prior to being used for the determination of cytosolic and extracellular Ca^{2+}. Commercial beers used were: Trappistes Rochefort (A), Budejovichy Budvar (B), Schneider Weisse (C), O'Hara's (D), and Old Tom (E).

Cell Preparations

PE/CA-PJ15 cells [20, 21] were maintained (37℃, 5% CO_2) in Iscove's Modified Dulbecco medium (IMDM) supplemented with 1% L-glutamine, penicillin/streptomycin (100 U/mL each), and 10% inactivated fetal calf serum. Following a 72 hour incubation, cells were washed 3 times in phosphate buffered saline (PBS) to remove the excess of serum. After a 10-min trypsinazation step using 0.05% trypsin in 0.02% EDTA (Euroclone) at 37^{o}C, 10% fetal calf serum in IMDM was dispensed to inactivate trypsin. Cells were subsequently harvested by centrifugation at 400g for 5 min and resuspended in a volume of medium to obtain 1x10^{6} cells/mL. Cell viability was monitored in a Burker chamber by Trypan blue (2.22 mg/mL in PBS) exclusion staining.

Calcium measurements**:**

Extracellular

Determinations were performed using an aliquot of cell suspension (1 mL, 10^{6} cells) diluted with 3 mL of HBSS and then lysed with 1% Triton X-100 prior to treatment with FURA-2 AM (2 µL of a 2 mM solution in DMSO). Extracellular calcium was adjusted to 1mM to reflect physiological levels. Cells were lysed to release cellular esterases required for FURA-2AM activation, that is, a prerequisite step to form a fluorometrically sensitive a Ca^{2+} / FURA complex. Beer samples (20 µL) or, for comparison purposes, a same volume of HBSS, were added 250s after the 1% Triton X-100 treatment. Fluorometric readings were taken after 300-350 s (Fig. 1).

Cytosolic

FURA-2 AM (2 µL of a 2 mM solution in DMSO) was added to 1 mL of cell suspension (10^{6} cells) and incubated for 60 min at 37℃ in the dark . Cells were harvested by centrifugation at 800 x g x 10 min and resuspended in 1 mL Ca^{2+}-free HBSS buffer (120 mM NaCl, 5.0 mM KCl, $MgCl_2$ 1mM, 5 mM glucose, 25 mM Hepes, pH 7.4). This sample was then centrifuged at 800 × g × 5 min, after which cells were harvested and resuspended in 3 mL of Ca^{2+}-free HBSS containing 0.1 mM EGTA, that was included to rule out or *at least minimize a background signal due to contaminating ions.*

Fluorescence was measured in a Perkin-Elmer LS 50 B spectrophotofluorometer equipped with a double excitation system (ex. 340 and 380 nm, em. 510 nm). Slit widths were set at 10 nm and 7.5 nm for excitation and emission, respectively. Fluorometric readings were normally taken after 300-350 s (Fig. 1). Cytosolic calcium concentrations ($[Ca^{2+}]_c$) were calculated as previously reported [19]. When required, samples of beer, Ca^{2+} and H_2O_2

were added for specific purposes, as described in the Results section. In all experimental instances described later in the text, cell viability was consistently >95%.

Statistics and EC50 determinations.

Results were normalized Analyses were routinely conducted using Prism 7 from GraphPad Software Inc.

RESULTS

Ca^{2+}-chelating properties of beer during specific steps of the brewing process (BP) and commercial beers (finished product, CB).

Extracellular space

Aliquots of finished products and samples taken during the brewing process were added (20 µL) to PE/CA-PJ15 cell lysates obtained as described in the Materials and Methods section.

At first, we performed pilot tests in order to optimize assay conditions, in particular i) the amount of beer in relation to a standard concentration of EGTA used as a reference for the comparison of chelating properties, and ii) a suitable incubation time of the fluorometric probe prior to the measurements. In this regard, using a 50-1500 µM concentration range, we determined that the EC50 of EGTA concerning Ca^{2+} chelating activity in the presence of 1mM $CaCl_2$, was 650 µM (data not shown). As shown in Figure 1, we then compared

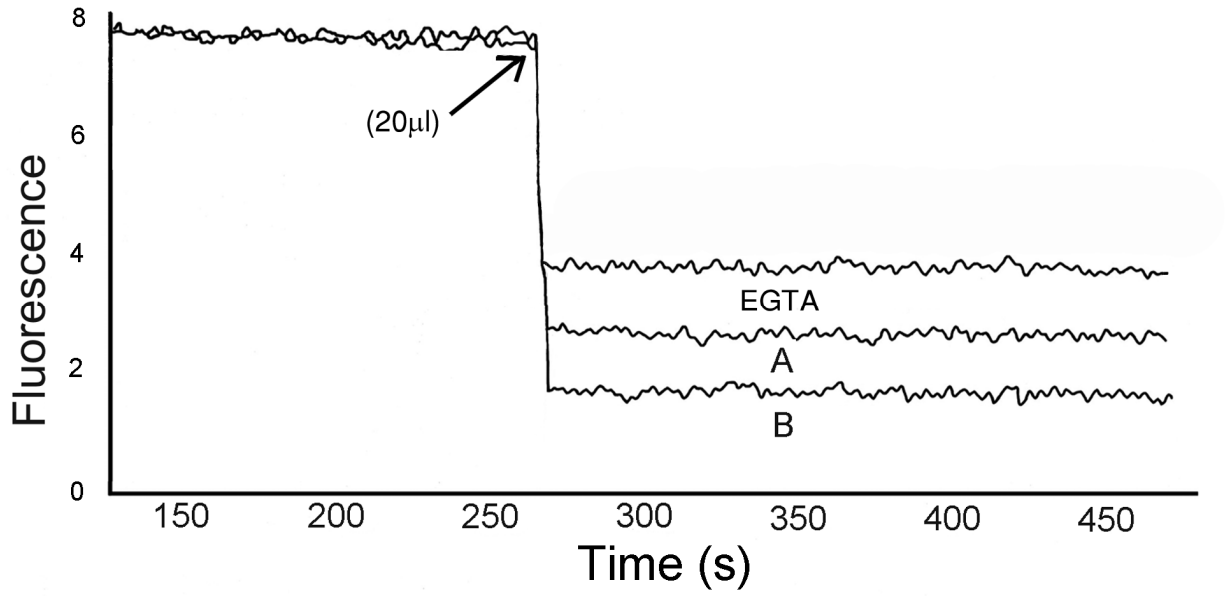

Figure 1.

the effect of commercial beers A and B (20 µL) in relation to 650 µM EGTA, and found

that a 300-350 s window was ideal for correct data capture. As shown in Fig. 1, we treated cells with beer 250s after the start of fluorometric measurements. Under these conditions, beers A and B were found to cause a decrease of FURA fluorescence of approximately 60-75 %, which is strictly comparable to that displayed by 650 μM EGTA (Fig. 1).

TABLE 1:

BPs	**1**	**2**	**3**	**4**	**5**	**6**
Dry residue (mg/20 μL)	2.27 ± 0.20	3.16 ± 0.25	1.90 ± 0.15	1.93 ± 0.16	1.84 ± 015	0.56 ± 0.06
CBs	**A**	**B**	**C**	**D**	**E**	
Dry residue (mg/20 μL)	1.03 ± 0.08	0.78 ± 0.05	0.84 ± 0.06	0.63 ± 002	1.19 ± 0.06	

In Figure 2, which shows the effect of both CBs (panel A) and BPs (panel B) under optimal, standardized conditions, fluorometric readouts (F_X) were normalized to 650 μM EGTA (F_{EGTA}) according to the equation $\%F_X / \%F_{EGTA}$ = fluorometric decrease by the sample over fluorometric decrease by 650 μM EGTA. Results were further normalized in relation to the dry residue contained in a 20 μL volume of each sample (Table 1). We found that BPs (Fig. 2, panel A) reduced the fluorescence of the complex Ca^{2+}/FURA, but to a lower extent than EGTA, throughout the initial five phases of the production process. However, fluorescence was found to markedly decrease during the 6^{th} step of the brewing process, in parallel with a large reduction of the dry residue. Instead, the entire set of CBs displayed a marked reduction of the complex Ca^{2+}/FURA fluorescence (Fig. 2, panel B), which was similar to sample 6 of the production process (final beer) and, overall, higher than that shown by 650 μM EGTA.

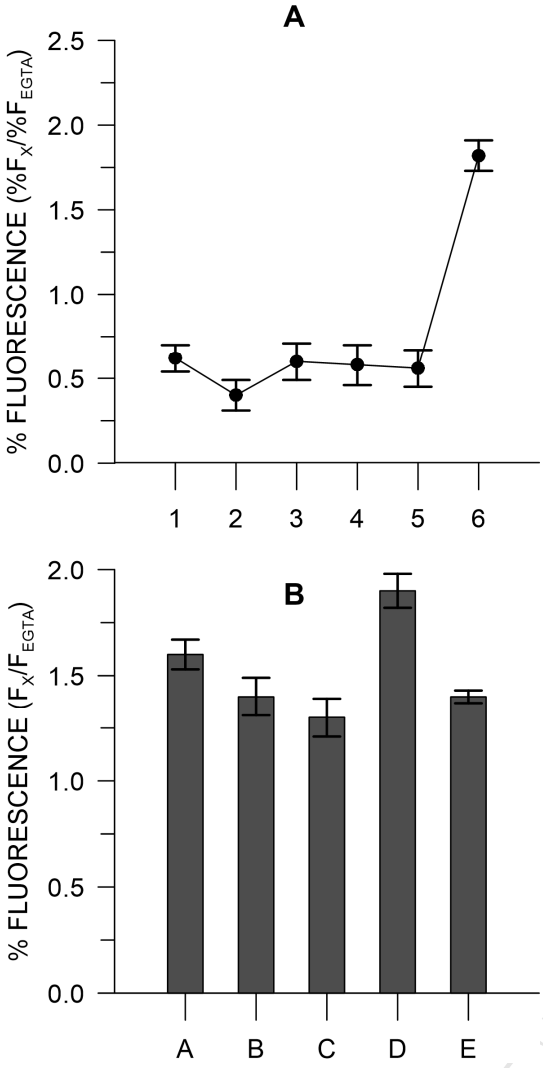

Figure 2.

Cytosol

PE/CA-PJ15 cells were treated with FURA-2, washed, and transferred to Ca^{2+}-free medium, as previously shown by Grynkiewicz *et al.* in 1985 to assess cytosolic calcium $(Ca^{2+})_c$. For testing, BP and CB samples were routinely added in a volume of 20 μL. In each sample, the dry residue varied as shown in Table 1. Changes in $[Ca^{2+}]_c$ were expressed as $\Delta[Ca^{2+}]_c$ (nM) based on a 300 s exposure to the detector following treatment. Under these experimental conditions, we observed, once again, different trends in the Ca^{2+}-chelating impact of BP as compared to CB. In particular, consistent with the results observed in the extracellular space, BP samples showed a slight decrease of $[Ca^{2+}]_c$ in the first five steps of the brewing process, with values ranging between 5-10 $\Delta[Ca^{2+}]_c$(nM), whereas samples from the 6th processing step displayed a remarkable increase of 29 $\Delta[Ca^{2+}]_c$(nM). On the other hand, CBs exhibited a consistently high $[Ca^{2+}]_c$-chelating ability comparable to that of the 6th-step PB samples (Figure 3, panel B). Within the commercially

brewed beers, the highest chelating capacity was shown by sample D. Taken together, these results indicate that the chemical entity(ies) with the ability to chelate extracellular Ca^{2+}, was also capable of moving across the cell membrane of PE/CA–PJ15 cells, thereby suggesting a likely impact on Ca^{2+} homeostasis.

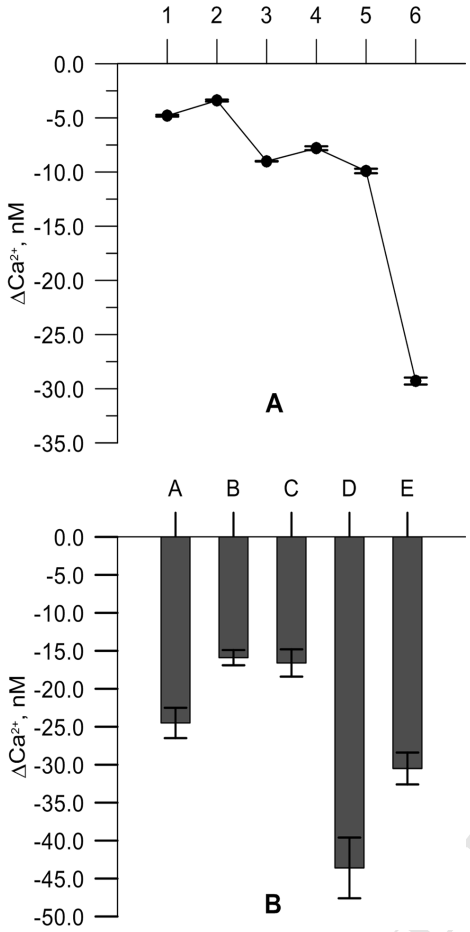

Figure 3.

Correlation between Ca^{2+}-chelating ability of Ferulic acid and that of a 6^{th}-step BP sample.
In an attempt to identify Ca^{2+}-chelating entities present in beer, we tested whether Ferulic acid, a hydroxycinnamic acid, could be a relevant player. Our rationale was based on both the marked abundance of hydroxycinnamic acids in beer [18], and the antioxidant activity of Ferulic acid [17] that might be attributable to its combination with Ca^{2+} within an interplay between Ca^{2+} levels and ROS [5-7].
Thus, we performed dose-response curves to assess, in parallel, Ca^{2+}-chelating properties for both beer (ie. a 6^{th}-step BP aliquot) and Ferulic acid. In this regard, Fig. 4 shows that both curves were highly comparable in relation to the measurement of $\Delta[Ca^{2+}]_c$ (nM)

changes in both the cytosol and extracellular space, with EC_{50} values of approximately 1 µmol (Ferulic acid) and 1.4 mg dry residue (6^{th}-step PB). These results suggest that Ferulic acid and, most likely, the entire pool of hydroxycinnamic acids present in beer largely contribute to the Ca^{2+}-chelating properties displayed by beer samples.

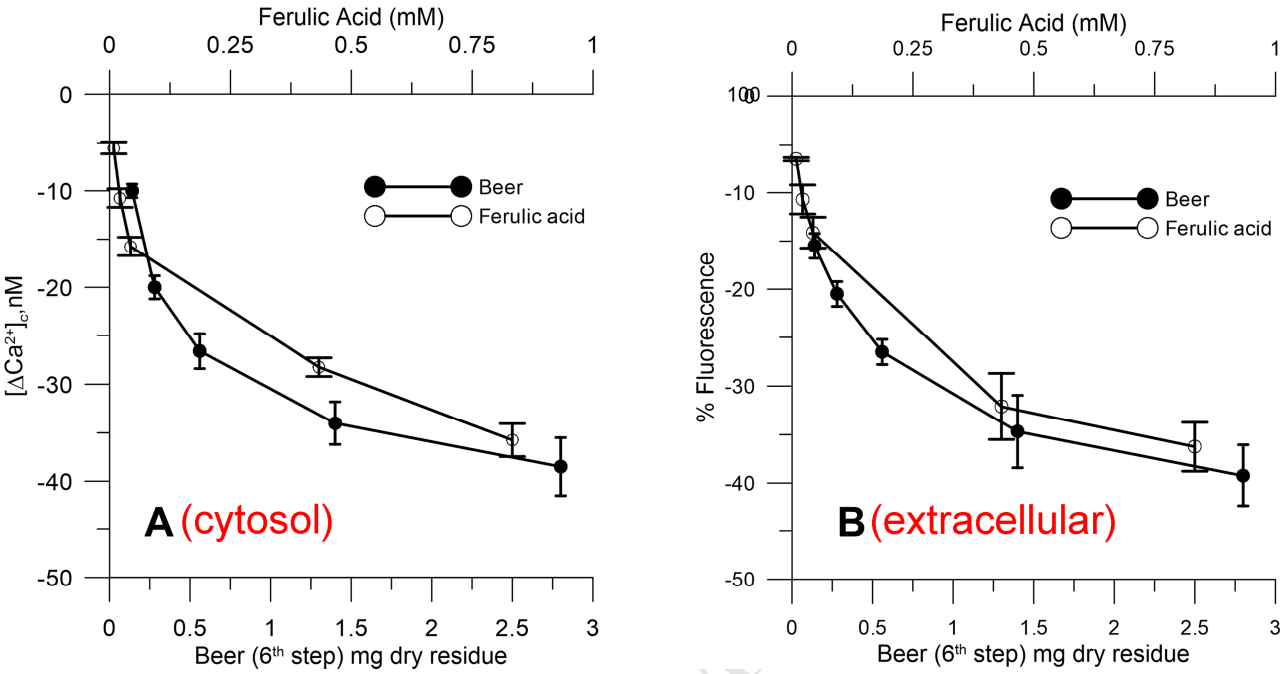

Figure 4.

Chelating ability of beer in the presence of H_2O_2.

Finally, to preliminarily determine the impact of beer under oxidative stress, we treated cells with H_2O_2 as an exogenous ROS, and then monitored the spatial distribution of Ca^{2+} in PE/CA PJ-15 cells. To this end, we employed a "Ca^{2+} add-back" approach to study the effects of H_2O_2 on Ca^{2+}-store depletion and Ca^{2+} entry into the cells. H_2O_2 application at both 200 µM and 500 µM did not cause an increase in cytosolic $[Ca^{2+}]_c$ levels in the absence of extracellular Ca^{2+}, suggesting that despite its relatively high concentrations, H_2O_2 did not lead to Ca^{2+} store depletion. However, in the presence of extracellular Ca^{2+} (1mM $CaCl_2$), we observed a $[Ca^{2+}]_c$ increase, that was indicative of a positive impact of H_2O_2 on Ca^{2+} entry in the cell (Figure 5, inset)

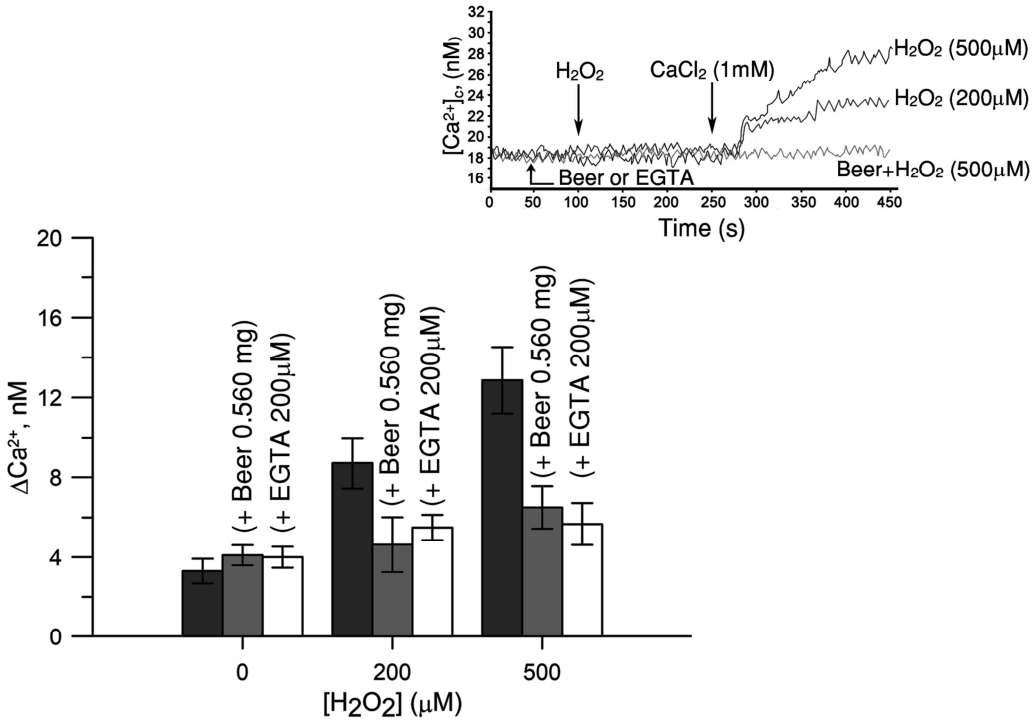

FIGURE 5.

When a 20 μL sample of a 6th-step BP preparation (with a 0.56 mg dry residue,Table 1), was added to the cells in the presence of H_2O_2, virtually no $[Ca^{2+}]_c$ change was observed with 200 μM H_2O_2, whereas only a slight increase in $[Ca^{2+}]_c$ could be measured in the presence of a 500 μM preparation. Interestingly, the same results were also obtained with 200 μM EGTA, thereby confirming that the effect of beer was due to its Ca^{2+}-chelating properties.

DISCUSSION

Beer is a hydro-alcoholic solution composed of hundreds of different molecules, of which some can play a role in the direct sequestration of metal ions dissolved in the beverage [1].

Here, we show that commercial beers contains molecules that may have an impact on Ca^{2+} intra- and extra-cellular levels, and that these entities appear in the finished product during the last step of the brewing process. To this end, it was employed a cell-based model (ie. PE/CA-PJ15 cells) that we previously explored to study alterations of Ca^{2+} homeostasis [22]. The PE/CA-PJ15 cell line is obtained from a squamous, cell tongue carcinoma that accounts for about 90% of head-and-neck tumors. While a cancer phenotype does not clearly reflect normal physiological events, however these cells

overcome technical problems associated with isolation and culture of healthy, primary cells and, compared to normal epithelial cells, express similar levels of laminin and cytocheratins [20, 21], thus representing, at least in part, molecular features of the oral cavity, namely the most proximal area exposed to food and drinks. Despite the fact that beer contains substantial amounts of calcium, ranging between 35-40 mg/L [23], we developed and optimized assay conditions to include a large excess of $CaCl_2$ (1mM in 3 ml total volume) in relation to the amount which is endogeneously present in 20 μL, that is, the volume of beer samples typically tested in the experiments (extracellular Ca^{2+} tests). In addition, 0.1mM EGTA was included in the tests (cytosolic Ca^{2+} determinations) to minimize possible interference caused by contaminating ions.

Our findings indicate that, in beer, entities with the ability to chelate extracellular Ca^{2+} are also capable to cross the cell membrane of PE/CA-PJ15 and, thus, affect Ca^{2+} homeostasis by reducing levels of $[Ca^{2+}]_c$.

Extracellular calcium was chelated by both CBs and BPs. We found that the process of saccharification, spent grain removal, addition of hops, boiling step, and clarification in whirlpool (trub removal), did not influence the ability of PBs to sequester extracellular Ca^{2+}. However, during the last phase of the brewing process, namely step 6, which turns the boiled wort into finished beer, the Ca^{2+}-chelating capacity, assessed in relation to a standard dose (650 μM) of EGTA, increased markedly to achieve levels comparable to commercial beers. In particular, the 6^{th} step includes pitching, fermentation, diacetyl break, maturation and removal of particulate matter, suggesting that the a 3.25-fold increase in Ca^{2+}-chelating activity (Figure 2, panel A)is most likely due to a comparable decrease in dry residue, as shown during the brewing progression from step 5 (cold wort) to step 6 (3.28-fold, Table 1). This inverse correlation, in turn, appears to rule out the possibility that the enhanced chelating activity is directly associated with products of the yeast metabolism. This notion, however, should be investigated in a greater detail, considering that the yeast autolysate process can generate dipeptides subject to Maillard reaction and, subsequently, products with antioxidant features (24) that might also be due, at least in part, to Ca^{2+}-chelating properties.

Within the different types of CB (samples A, B, C, E), activities were generally comparable to BP, except for sample D (an Irish stout), that exhibited a much greater ability to sequester Ca^{2+}. Despite the relatively small number of samples tested, this finding appears to highlight a correlation between malt color (as well as beer color) and chelating activity. Dark malts are prepared based on certain kilning conditions, such as

high temperatures, high moisture levels, and high concentrations of amino acids and sugars, ultimately leading to the formation of melanoidins [25], that is, high molecular weight compounds with an intense color and high anionic charge [9]. In this regard, it is known that crystal malt and roasted cereal grain used in stout beers are richer in melanoidins than pale ale malts used in lager beers [26]. Melanoidins, which can be generated through Maillard reactions, display antioxidant properties via the ability to sequester divalent ions. For example, the inhibitory effect of melanoidins on autoxidation was shown to be much stronger than buthylhydroxyanisole (BHA) and propyl gallate [27].

Hydroxycinnamic acids may also have a primary role in metal ion chelation. Here, we report for the first time that one of these, Ferulic acid, can bind intra- and extracellular Ca^{2+}. Based on dose-response determinations performed in parallel with both beer (6th-step BP) and a preparation of commercially available Ferulic acid (Fig.4), our results suggest that such chelating activity could account for a significant proportion of beer's ability to impact Ca^{2+} homeostasis in PE/CA-PJ15 cells. In support of this notion, Ferulic acid is one of the most abundant hydroxycinnamic acids of barley malt; in addition, this class of aromatic acids is largely represented in beer [18]. Fig. 4 shows that similar levels of Ca^{2+}-chelating activity are achieved with 1 µM Ferulic acid (194 µg/L) and 1.4 mg dry residue of 6^{th} step BP aliquots. Since the actual amount of Ferulic acid present in beer is within a 3-8 mg/L range [28], our findings suggest that, due to its poor solubility in water, Ferulic acid is probably associated with more soluble, abundantly distributed entities such as, for example, dextrins.. This, in turn, complicates the analytical identification of Ferulic acid in beer samples.

Oxygen, a major cause for the rapid deterioration of the beer taste, triggers oxidation reactions catalyzed by metal ions, leading to ROS formation. ROS may react with a large variety of organic molecules dissolved in beer, thus causing marked, generally deleterious changes of its sensory profile. ROS, such as superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), are widely involved in physiological and pathological processes following modification of proteins and lipids. Ca^{2+} and ROS constitute the most important intracellular signaling molecules participating in the regulation and integration of different cellular functions [4, 29]. It is well known that H_2O_2 induces apoptosis but also necrosis through the mobilization of intracellular Ca^{2+} stores and influx of extracellular Ca^{2+} in different cell types. [5].

We observed that relatively high doses of H_2O_2 (200 and 500 µM), used to experimentally raise oxidative stress, did not release Ca^{2+} from intracellular stores, while significantly

stimulating extracellular Ca^{2+} entry into PE/CA-PJ15 cells. These results indicate that H_2O_2 increases $[Ca^{2+}]_c$ by stimulating Ca^{2+} entry without causing depletion of Ca^{2+} stores, thereby providing an example of non-capacitative calcium entry, that is, independent of the levels of Ca^{2+} stored [7, 8]. Within this context, our results show that beer has an antagonistic effect by preventing the entry of extracellular Ca^{2+}, and, consequently, a $[Ca^{2+}]_c$ increase. This effect was also produced, in a parallel test, by EGTA, which is typically used as an exogenous Ca^{2+} buffer in order to experimentally reproduce the intracellular environment [30].

Finally, we should also acknowledge that the chelating properties of beer might also extend beyond Ca^{2+} and, as for other chelating agents such as EDTA, EGTA, and BAPTA, involve other ions, for example iron. This may indeed be included in Fenton and/or Haber-Weiss reactions and, consequently, lead to oxidative stress [31].

The results shown here, although being limited to Ca^{2+}only, suggest however further research efforts aimed at investigating beer as a natural source of chemical entities that may counteract multiple sources of oxidative stress. Based on common applications of chelators in the biomedical and healthcare fields, these molecules could also be investigated as possible nutritional supplements [32, 33], detoxification agents [34], or antibiotics [35].

Figure & table captions

Table 1. Dry residue (mg) contained in 20μL BP and CB samples used for Ca^{2+}-chelating comparisons described in the text. Results shown in both panels report the mean values obtained from 10 samples ± SEM.

Figura 1 Optimization of FURA-2 assay conditions.
PE/CA-PJ15 cells were treated with FURA-2, washed, transferred in HBSS buffer and then lysed with 1% Triton X-100. Commercial beers A or B (20 μL) were added to 1 mL cell suspension, after which measurements were immediately performed for up to 450 s. EGTA (650 μM, a dose corresponding to the EC_{50} for Ca^{2+} binding) is also included for comparison purposes. An arrow indicates the time when beer was added to the system.

Figure 2. Chelating ability of BP and CB on extracellular Ca^{2+}.
Aliquots of 20 μL taken from different steps of the brewing process (BP, panel A) and a variety of commercial beers (CB, panel B) were tested for their chelating abilities on extracellular Ca^{2+} as described in the Materials and Methods (MM). Brewing steps (1- 6, panel A) and the identity of the commercial beers (A-E) are also reported in the MM section. Results are expressed as the fluorometric decrease by the sample (F_x) normalized to the fluorometric decrease caused by 650 μM EGTA (F_{EGTA}). In all instances, values were further normalized in relation to the dry residue in each sample.
Data shown in both panels correspond to the means obtained from 10 experiments (biological replicates) ± SEM.

Figure 3. Chelating ability of PBs and CBs on cytosolic Ca^{2+}.
PE/CA-PJ15 cells were cultured and treated with beer aliquots (panel A: BPs, steps 1-6; panel B: CBs, samples A-E) as described in the Materials and Methods. In all instances, beer aliquots were 20 μL . Results are expressed as changes of cytosolic Ca^{2+} concentration (nM). Data shown in both panels correspond to the means obtained from 10 experiments (biological replicates) ± SEM.

Figure 4. Comparison of Ca^{2+}-chelating activities between beer samples (taken from the 6th-step of the brewing process) and Ferulic acid.
PE/CA-PJ15 cells were treated with serially-diluted samples of beer (aliquots from the 6th-step of the brewing process) and Ferulic acid prior to measurement. The graphs display changes in the concentration (nM units) of cytosolic (panel A) and extracellular (panel B) Ca^{2+} using doses of beer and Ferulic acid reported as mg of dry residue and μmol, respectively.
Data shown in both panels correspond to the means obtained from 10 experiments (biological replicates) ± SEM.

FIGURE 5. Ca^{2+}-chelating ability of beer in the presence of H_2O_2.
Inset: PE/CA-PJ15 cells were treated with H_2O_2 alone (200 μM and 500 μM) and with H_2O_2 (500 μM) in the presence of beer (0.56 mg [dry residue] aliquots from the 6th-step of the brewing process) to assess entry of extracellular Ca^{2+} (in nM units) into the cells. To this end, tissue culture media was supplemented with 1mM $CaCl_2$. H_2O_2, beer or EGTA, and $CaCl_2$ were added to the cultures at specific time points, as shown by arrows.

Bar graph: shown is the relative increase of $[Ca^{2+}]_c$ based on the data reported in the inset. Results are expressed as the means obtained from 10 experiments (biological replicates) ± SEM.

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Highlights

- Chelating activity of commercial beer (CBs) and production phases of brewing (PBs)
- Binding of extracellular and cytosolic calcium in PE/CA-PJ15 cells of CBs and PBs
- Effects of EGTA in extracellular Ca^{2+}
- Effects of ferulic acid in cytosolic calcium in PE/CA-PJ15 cells