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DOI:
10.1111/1750-3841.14775

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Document Version
Peer reviewed version

Citation for published version (Harvard):
Igoumenidis, P, Iosifidis, S, Lopez-Quiroga, E, Bakalis, S & Karathanos, V 2019, 'Absorption of phenolic acids in rice kernels after boiling in spearmint aqueous extracts of different concentrations: a diffusion study', *Journal of Food Science*, vol. 84, no. 10, pp. 2859-2867. https://doi.org/10.1111/1750-3841.14775

Link to publication on Research at Birmingham portal

Publisher Rights Statement:
Checked for eligibility: 05/09/2019

This is the accepted manuscript for a forthcoming publication in Journal of Food Science.

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Absorption of phenolic acids in rice kernels after boiling in spearmint aqueous extracts of different concentrations. A diffusion study

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Word count of text: 6314
Short version of title: Diffusion of phenolic acids in rice
Choice of journal/section: Food Engineering, Materials Science, and Nanotechnology

ABSTRACT

In this study, an attempt was made to fortify white milled rice grains with phenolic compounds using a hydrothermal process and spearmint aqueous extracts of different % w/v concentrations. In addition, a mathematical model was acquired in order to simulate the diffusion of specific phenolic acids in rice kernels during boiling inside the extracts. Results showed that the amount of phenolic acids in rice, the potential equilibrium concentration values, as well as the diffusivity of these compounds in rice material were positively affected by the increase in % w/v bulk concentration of the aqueous extract. It was also shown that the diffusion process could be sufficiently described by a Fickian model and the estimated diffusion coefficients ranged from 6.86×10^{-12} to 3.56×10^{-11} m^2/s, with the p-coumaric acid presenting the highest average diffusivity in boiling rice material among all examined compounds. The chemical affinity of each phenolic acid to rice macromolecules was believed to play the most important role concerning their diffusivity in rice during fortification process.

Keywords: hydrothermal process, diffusivity, fortification, Fickian, phytochemicals

Practical Application

Consumer’s interest for functional food products is constantly growing during the last decades. This study may act as preliminary for the production of fortified rice products, possessing adjusted bioactive content, in industrial scale. The proposed methodology for the production of quick-cooking or ready-to-eat fortified rice may be adopted by rice industries and applied by only making slight modifications in their existing parboiling units.

1. Introduction

White milled rice is a staple food which could either be cooked prior to its consumption or undergo some treatment in order to get rich in health-promoting compounds that could enhance its nutritional value. Our recent studies (Igoumenidis, Lekka, & Karathanos, 2016; Igoumenidis & Karathanos, 2016) have shown that white rice has the ability to absorb and maintain a significant amount of antioxidants, as well as colors and aromas, after being boiled in *Mentha spicata* aqueous extracts. Therefore, it could be considered a promising carrier of useful compounds after fortification. Literature during the last couple of decades has mainly focused on white rice fortification with nutrients coming from the outer layers of rough rice kernels (bran, husk, etc) which has been extensively applied using the well known parboiling process (Elbert, Tolaba, & Suarez, 2001; Sridhar & Manohar, 2003; Miah, Haque, Douglass, & Clarke, 2002; Oli, Ward, Adhikari, & Torley, 2014). Most of these studies aimed to clarify the phenomena occurring inside rice grains during parboiling, among which are water diffusion and starch gelatinization within the grain, in order to optimize the quality of parboiled rice by predicting the soaking behavior of the paddy. There are also a number of other studies that concern rice enrichment with minerals, such as Fe, Zn and iodine, either through germination or hydrothermal processes (Wei et al., 2012; Wei et al., 2013; Tulyathan, Laokuldilok, & Jongkaewwattana, 2007) and rice fortification with vitamins and aromatic substances by spraying and hydrothermal processes (Kyritsi, Tzia, & Karathanos, 2009; Yahya, Fryer, & Bakalis, 2011). However, none of them is dealing with the calculation of diffusivity of microconstituents inside rice grains during fortification process.

Moisture absorption and modeling of its diffusion in rice kernels during hydration using hydrothermal treatments have been studied extensively in the past.

Many researchers have applied either analytical (Ahromit, Ledward, & Niranjan, 2006; Cheevitsopon & Noomhorm, 2011; Bello, Tolaba, & Suarez, 2004) or numerical techniques (Balbinoti, Jorge, & Jorge, 2018; Perez, Tanaka, & Uchino, 2011; Bakalis, Kyritsi, Karathanos, & Yanniotis, 2009), showing that the hydration of rice during soaking in hot water or cooking at several different temperatures follows Fick's second law for diffusion. A number of studies dealing with the diffusion of constituents, such as sugars, salts, phenols and vitamins in foodstuffs other than rice are also available in literature (Telis, Murari, & Yamashita, 2004; Lombardi & Zaritzky, 1996; Rozek, Achaerandio, Guell, Lopez, & Ferrando, 2009; Rastogi & Raghavarao, 1998). In particular, most of these studies focus on the mass transfer of these compounds in food matrices and the calculation of their diffusion coefficients during osmotic dehydration processes.

Phenolic acids constitute a major part of total phenolics contained in plant tissues and can be classified in many different categories, the most important of which are cinnamic, benzoic and phenylacetic acid derivatives, detected in plant and plant-derived foods like fruits, vegetables, teas, spices and grains. Their presence inside plant material has been associated with a variety of functions such as protein synthesis, nutrient uptake and enzymatic action, as well as structural and protective roles concerning plant cells (Stalikas, 2007). Phenolic acids have been found to exhibit antioxidant properties, the strength of which depends on their structural features, especially on the number and position of hydroxyl groups in the phenolic ring, as studies on hydroxycinnamic, hydroxybenzoic and hydroxyphenylacetic acids revealed (Rice-Evans, Miller, & Paganga, 1996). In addition, most of phenolic acids present a good absorbance rate when ingested by human, particularly when they are under their aglycone-free form, and except for having the ability to scavenge free radicals and prevent oxidative stress, they can also prevent the appearance of chronic diseases (Kroon & Williamson, 1999; Lafay, Morand, Manach, Besson, & Scalbert, 2006; Zhao & Moghadasian, 2010; Lafay & Gil-Izquierdo, 2008). For this reason, phenolic acids could contribute to the enhancement of the nutritional value of a food product, such as white milled rice, when they are used for fortification purposes.

The main aim of the present study was to examine the correlation between the concentration of specific phenolic acids, contained in an herbal extract medium, and their rate of absorption in boiling rice grains. Diffusion phenomena in rice kernels during hydration were modeled by using Fick's second law, and the diffusion coefficients of each compound were estimated by fitting the model to experimental absorption data. In addition, potential equilibrium concentrations for each compound were also obtained from fortified rice flour samples. Finally, an attempt was made to correlate the calculated diffusivity of the examined compounds with their chemical structure and affinity to boiling rice material.

2. Materials and methods

2.1. Materials

White milled long grain rice of type "nychaki" of Greek origin and dried spearmint leaves of Egyptian origin, in coarse powder form, were purchased from a local market. Folin-Ciocalteu reagent and methanol (analytical grade) were purchased from Merck (Darmstadt, Germany). Bis-(trimethylsilyl)-trifluoroacetamide reagent (BSTFA) and 3-(4-hydroxyphenyl)-1-propanol were obtained from Aldrich Chemie GmbH (Steinheim, Germany). Caffeic, protocatechuic and 3,4-dihydroxyphenylacetic acids were purchased from Fluka (Steinheim, Germany). P-coumaric and gallic acids were obtained from Sigma (Steinheim, Germany).

2.2. Preparation of spearmint aqueous extracts

Deionized water (15 L) was used for the preparation of spearmint aqueous extract. After water was heated up to its boiling point, it was removed from the heating source and dry spearmint leaves' powder (2 kg) was soaked and remained fully immersed in it for 10 min. The aqueous herbal extract produced following the above procedure was then infiltrated, collected and cooled down to room temperature. Three samples (10 ml) of aqueous extract were stored in plastic tubes till freeze-drying for further analysis. Afterwards, the extract was properly diluted using deionized water and 4 aqueous solutions were produced, each one containing a different concentration of phytochemical components. The final content in dry spearmint extract of the 4 aqueous solutions was 0.33% w/v, 0.66% w/v, 1% w/v and 2% w/v.

2.3. White milled rice and rice flour boiling in aqueous herbal extracts of different concentrations.

A specific volume (2 L) of each of the 4 spearmint aqueous extracts was heated up using a conventional cooker and when it reached boiling temperature white milled rice (50 g) was added in order to be cooked. The initial moisture content of rice was 0.11 kg water/kg dry matter and the dimensions of 20 white milled rice grains were measured by using a calliper. The initial length of rice grains was found to be 6.98±0.29 mm, while their initial two diameters of the kernels, considered as ellipse, were found to be 1.93±0.06 and 1.78±0.06 mm, respectively. The cooking process lasted 20 min and took place under continuous stirring. The final moisture content of rice was 2.92 kg water/kg dry matter. Samples of cooked rice (approx. 10 g) were collected at 4 min time intervals (t_4, t_8, t_{12}, t_{16}, t_{20}) and then freeze dried before undergoing extraction with methanol and further analysis.

The above experiments were repeated for white rice flour for much longer time (90 min, to simulate a fortifying process of infinite duration), in order to achieve equilibrium which may not be achieved with rice kernels. Thus, white rice flour granules, produced by grinding the same rice source (′′nychaki′′), were boiled in spearmint aqueous extracts containing same phytochemical component concentrations by following the technique used for rice kernels. Cooked rice flour samples were removed at the following time steps (subscripts indicate minutes): t_{30}, t_{60}, t_{70}, t_{80}, t_{90} and then freeze-dried. All rice samples' cooking processes and analyses were performed in triplicate.

2.4. Analyses of individual phenolic compounds and total polyphenol content

After freeze drying, the samples of the initial pure aqueous herbal extract were accurately weighed (5±0.1 mg) and diluted in methanol (5 mL) in order to be analyzed by GC/MS. The techniques used for the extraction of phenolic compounds from fortified rice and rice flour samples have been previously described in Igoumenidis et al. (2016). In particular, a total of 20 mL MeOH/g of freeze-dried sample was used for the extraction procedure. Dried MeOH extracts of each fortified rice and rice flour sample were diluted in 1 mL of MeOH and led to further analyses.

The detection of 4 common phenolic acids in aqueous spearmint extract, as well as in methanolic extracts of cooked fortified rice and rice flour samples was performed by GC/MS analysis, as described by Kalogeropoulos, Konteles, Troullidou, Mourtzinos, & Karathanos (2009). Briefly, a Selective Ion Monitoring (SIM) method was employed for the analysis of 4 target phenolic acids, which were transformed in their respective trimethyl-silyl-ether forms. In addition, the quantification of all examined components in every sample was made by using 3-(4-hydroxyphenyl)-1-propanol as internal standard. Results were expressed in μg of phenolic acid/g dry rice or dry rice flour and in μg of phenolic acid/g of dry aqueous spearmint extract.

The total polyphenol content of fortified rice flour methanolic extracts was also determined by applying the Folin-Ciocalteu photometric method as being adapted to microscale by Arnous, Makris, and Kefalas (2002). The results concerning total polyphenol content were presented as mg of Gallic Acid Equivalents (GAE)/g of dry rice flour.

2.5. The diffusion model

A mathematical model was used to simulate the diffusion of the investigated phenolic acids in rice kernels during their boiling inside spearmint aqueous extract media of 4 different concentrations in diluted solids (w/w). The agitation of the boiling medium during rice fortification process was natural, sufficient though, so surface resistance to phenolic mass transport was considered to be small and only the internal resistance of rice grains was taken into consideration. In addition, phenolic acid concentration gradient between the surface and the center of rice kernels was supposed to be the driving force for the diffusion of the compounds in rice and also Fickian diffusion was assumed to be the predominant mechanism of phenolic mass transfer inside the kernels. The geometry of rice grain has been simplified to an equivalent sphere with equivalent radius $R_{eq} = 2\text{x}10^{-3}$ m. The simplification was done by following the same assumptions, equations and procedures as in the study of Chapwanya and Misra (2015) for the transformation of the elliptical geometry of a grain to an equivalent spherical geometry. According to Gastón et al. (2004) the geometry deviation in diffusion coefficients for the above transformation is expected to be about 15%. The equivalent radius was calculated by using data and assumptions corresponding to hydration of rice grains (including expansion parameter during boiling) from a previous study (Bakalis et al., 2009).

Diffusion of phenolic acids through the rice grain was described using Fick's second law:

$$\frac{\partial C_i}{\partial t} = \frac{1}{r^2}\frac{\partial}{\partial r}\left[D_i r^2 \frac{\partial C_i}{\partial r}\right] , \forall r \in \ (0, R) \qquad \text{Eq. 1}$$

where C_i (µg/g dry rice) and D_i (m^2/s) are the concentration and the effective mass diffusivity, respectively, of each phenolic acid, r (m) is the radial coordinate and t (s) is the boiling time.

The boundary condition imposed at $r = 0$ (rice kernel center) followed from the symmetry of the system:

$$\frac{\partial C_i}{\partial r}(0, t) = 0 \qquad \text{Eq. 2}$$

while at the external boundary $r = R$ (= R_{eq}, grain surface) the compound concentration was defined as the equilibrium one $C_{i,\infty}$ (µg/g dry rice flour):

$$C_i(R, t) = C_{i,\infty} \qquad \text{Eq.3}$$

The equilibrium concentration was estimated by taking the mean experimental concentrations of phenolic acids in rice flour during the last time steps of the fortification process.

The experimental concentrations of phenolic acids in the rice prior to its fortification $C_{i,0}$ (µg/g dry rice) were used to define the corresponding initial conditions for each phenolic compound:

$$C_i(r, 0) = C_{i,0} \qquad \text{Eq. 4}$$

The predicted concentrations of phenolics were obtained as volume average according to the following equation (Chapwanya and Misra, 2015; Ruiz et al 2008):

$$C_i(t) = \frac{3}{R_{eq}^3}\int_0^{R_{eq}} r^2 c(x, t) dr \qquad \text{Eq. 5}$$

where $C_i(t)$ is the volume averaged concentration of phenolic compound *i* and $c(x,t)$ is 224 the spatially distributed concentration, given as output of the numerical model.

2.5.1. Dimensionless transformations

The dimensionless concentration of each compound was defined as:

$$\breve{C}_i = \frac{C_i - C_{i,0}}{C_{i,\infty} - C_{i,0}} \qquad \text{Eq. 6}$$

The time scale of the diffusion phenomena for each compound is given by the Fourier number:

$$\tau = \frac{tD_i}{R^2} \qquad \text{Eq. 7}$$

while the dimensionless independent spatial variable is defined as:

$$\check{r} = \frac{r}{R} \qquad \text{Eq. 8}$$

2.5.2. Dimensionless formulation

The dimensionless form of the governing equation is given by:

$$\frac{\partial \breve{C}_i}{\partial \tau} = \frac{1}{\check{r}^2}\frac{\partial}{\partial \check{r}}\left(\check{r}^2 \frac{\partial \breve{C}_i}{\partial \check{r}}\right), \forall \check{r} \in (0,1) \qquad \text{Eq. 9}$$

with boundary:

$$\frac{\partial \breve{C}_i}{\partial \check{r}}(0,\tau) = 0 \qquad \text{Eq. 10}$$

$$\breve{C}_i(1,\tau) = 1 \qquad \text{Eq. 11}$$

and initial conditions:

$$\breve{C}_i(\check{r},0) = 0 \qquad \text{Eq. 12}$$

2.5.3. Numerical simulations

The model formed by Eqs. 6-12 has been solved using the Finite Element (FE) method (commercial software COMSOL Multiphysics 4.3b®). A uniform spatial mesh consisting of 121 elements has been employed in all the numerical simulations.

2.5.4. Mass diffusivity estimation

The effective mass diffusivity coefficient D_i (m^2/s) for each compound and for each extract concentration was estimated by minimizing the sum squared of the difference between experimental and simulated concentrations (Least Square method implemented in MATLAB 2013). The goodness of the fitting has been evaluated using the value of the coefficient of determination R^2, while the 95% Confidence Intervals (CI) for the diffusivity constants were calculated using the Bootstrap method (Efron & Tibshirani, 1993).

3. Results & Discussion

The content of the spearmint aqueous extract in four major phenolic acids (i.e. caffeic, 3-4 di-OH-phenylacetic, protocatechuic and p-coumaric**)** detected by GC/MS is presented in Table 1. Caffeic acid seems to be the predominant one as its concentration surpasses the levels of 3000 μg/g of dry extract, while most of the other examined compounds present much lower concentrations in dry extract (see Table 1). As shown in a previous study (Igoumenidis et al., 2016) the concentration of phenolic acids in a similar type of spearmint extract (*Mentha spicata*) did not present significant changes after being boiled for at least 40 minutes, so the values of concentrations listed in Table 1 could be considered as being quantitatively stable throughout the whole rice fortification process.

Experiemental results for rice fortification with four different phenolic acids contained in spearmint aqueous extract are presented in Figure 1. All the investigated compounds seem to follow an increase in their content in rice grains with boiling time, as well as an increase with the % w/v concentration of spearmint extract used in the hydrothermal process. Figure 1 also shows that by increasing the concentration of phenolic acids in the aqueous extract, this is, their availability at the surroundings of rice grains during boiling, their content inside fortified rice kernels keeps on increasing from the beginning until the end of the hydrothermal process (20 min). However, there is no indication of approaching equilibrium levels (saturation) in their concentrations in rice grains. In fact, these equilibrium levels of phenolic acid concentrations could probably be reached under different experimental conditions, i.e. by changing boiling time, temperature, pressure, concentration of aqueous extract medium or even size and shape of rice grains. That is why a number of experiments was repeated at the same conditions in rice flour (instead of rice kernels) of much smaller size so that the potential equilibrium content of each phenolic acid in rice material could be estimated. In this way, it would be easier to understand the diffusion mechanism of phenolic acids in rice grains during fortification and also to clarify whether or not Fick's second law for diffusion is followed in this case, as it happens in the case of water diffusion in rice, according to a number of studies (Balbinoti et al., 2018; Bakalis et al., 2009; Bello et al., 2004).

Regarding the total polyphenol content of fortified rice flour, Figure 2 shows that it approaches equilibrium levels after being boiled at different % w/v aqueous extract concentrations for an equivalent of infinite boiling time (90 min). The same pattern was followed (data not shown) when the concentrations of the four specific phenolic acids in fortified rice flour were measured as a function of boiling time. In particular, the equilibrium levels of the examined phenolic compounds in rice material appear to be depending on the % w/v concentration of the aqueous extract boiling medium, as data in Table 2 suggest.

Data in Table 3 correspond to the fitted diffusion coefficient values of the examined compounds in rice kernels during the fortification process. According to these results, the diffusivity coefficient of phenolic acids in rice during boiling seems to be almost 2 levels of magnitude lower than the diffusivity of water in rice (7.5x10^{-}

^{10} m^2/s, according to Bakalis et al., 2009) after following a similar hydrothermal process. This could be attributed to the much larger molecular size of the examined phenolic acids, in comparison to the size of water molecules, which could probably cause a relative decrease in their ability to penetrate rice material and/or a decrease in their molecular mobility inside rice grains. In addition, Table 3 also shows a noticeable increase in the diffusivity of the examined phenolic acids in rice kernels with the concentration of spearmint aqueous extract. Similar dependence of the diffusivity coefficient on concentration can be found in studies looking at moisture diffusivity in gelatinized foods or air drying processes. In such cases the effective diffusivity of water has been found to present increased values at high moisture contents, compared to the diffusivity at lower moisture contents, during dehydration (Zogzas, Maroulis, & Marinos-Kouris, 1996; Maroulis, Kiranoudis, & Marinos-Kouris, 1995). Moreover, there are studies that correlate moisture diffusivity with starch gelatinization rate and moisture content in starchy foods during hydrothermal treatment. In general, gelatinization is considered to be a first-order reaction that normally restricts the transport of water in foods, while the degree of gelatinization has been found to have a significant effect on water diffusion during soaking processes (Gomi, Fukuoka, Mihori, & Watanabe, 1998; Elbert et al., 2001). Dependence of penetrant diffusivity on its concentration has also been noted in studies related to materials other than foods. In particular, this phenomenon is quite common in polymer science, e.g. solvent absorption by a polymer in glassy state and subsequent swelling (Danner, 2014; Vrentas & Vrentas, 1998; Thomas & Windle, 1980).

One of our recent studies (Igoumenidis, Zoumpoulakis, & Karathanos, 2018) showed that caffeic acid presents the ability to interact with rice starch after applying a hydrothermal treatment similar to that followed in our current study. In particular, the addition of the phenolic acid in rice starch – water matrix, during heating, seemed to have a significant effect on gelatinization properties, with stronger effects as the concentrations of caffeic acid in the mixture increase. Similar results have been also reported in literature (Karunaratne & Zhu, 2016; Zhu, 2015; Wu, Lin, Chen, & Xiao, 2011), showing that phenolic compounds interact with starch molecules during gelatinization, reduce its degree and alter its thermal properties probably by interfering with the starch - water matrix. The potential interaction of phenolic extract constituents with the rice starch - water matrix during boiling may be a possible explanation for the concentration dependent apparent diffusivity of the examined phenolic acids in rice kernels being observed in Table 3.

According to the results listed in Table 3, the average effective diffusivity of the examined compounds in all experimental conditions (0.33%, 0.66%, 1% and 2% w/v of aqueous extract media) follows the order: $D_{p\text{-Coumaric}} > D_{\text{Caffeic}} > D_{\text{Protocatechuic}} > D_{3\text{-}4\ \text{diOH Phenylacetic}}$. During fortification process the spherical rice grains of our study could be considered as porous networks of low porosity that absorb water molecules quickly and maintain a rubbery state until the end of hydrothermal processing (Biliaderis, Page, Maurice, & Juliano, 1986; Thuc, Fukai, Truong, & Bhandari, 2010; Bertotto, Gaston, Batiller, & Calello, 2018). Given that the main components of these membranes are amylopectin and water molecules (>70% w/w), the diffusivity of phenolic acids through them is therefore affected by the nature of the porous network, as well as by the nature of the penetrating molecules, e.g. the size and shape of penetrating molecules, and also the chemical affinity between the components contained in the membranes and the diffusing molecules. Regarding the chemical affinity factor, it has been reported that the greater the attraction is between the penetrants and the constituents of the porous network, the lower is the penetrating ability and the diffusivity of the former through the polymeric membrane. (George & Thomas, 2001; Cu & Saltzman, 2009; Dury-Brun, Chalier, Desobry, & Voilley, 2007)

In this study, the difference found in diffusivities of four phenolic acids in rice grains (Table 3) may not be explained by the difference in molecular size and shape of the examined molecules, as they could be considered to be of similar MW (between 154 and 180) and structure (1 benzene ring per molecule). In addition, the permeability through a porous network (i.e. rice material) is greatly affected by the size and shape of the penetrating molecules only in case that the membrane is in its glassy state (George & Thomas, 2001). In our case, the structural characteristics of phenolic acids may not have a significant effect on their diffusivity as the boiling rice grains are mostly found in their rubbery state during fortification treatment.

The diffusivity of the examined compounds can also depend on their chemical affinity to rice main constituents, which could be expressed by chemical properties such as polarity and hydrophobicity. In particular, hydrophobicity of a compound is defined as its liquid-liquid partition coefficient (P) between octanol and water and it is expressed on log scale as logP. Hydrophobic molecules are generally characterized by positive values of logP while negative values of logP are characteristic of hydrophilic compounds (Dury-Brun et al., 2007). The results in Table 3 show that p-coumaric acid presents the highest average diffusivity in rice grains during fortification process, followed by caffeic, protocatechuic and 3,4-diOH phenylacetic acids. The same trend is also followed by the estimated logP values of the investigated phenolic acids (Supplementary Table 1). Assuming that hydrated rice mainly consists of amylopectin molecules - which have an estimated logP value of -10.6 - and water, boiled rice could be considered as a rather hydrophilic material. This indicates that the diffusion of more hydrophobic compounds such as p-coumaric acid was favored, while more hydrophilic ones (3,4-diOH phenylacetic acid) may present a stronger attraction to rice matrix components, chemically interact with them and result in lower diffusion coefficients.

The goodness of fit of the Fickian model, together with the estimated D_i values, is illustrated in Table 3 (columns presenting R^2 values) and Figure 1, where the predicted evolution of the phenolic acids concentrations along time is compared to the experimental results. Overall, there is a good agreement between model and measurements ($R^2 > 0.9$), with the exception of the p-coumaric acid at the lowest extract concentrations. This suggests that the diffusion of the investigated phenolic acids during hydrothermal fortification processes can be overall well described by a Fickian model.

To confirm Fickian diffusion of the phenolic acids during the fortification process, the dimensionless concentrations of acids $\check{C}_i$ were plotted against the square root of Fourier time $\sqrt{\tau}$ in Figure 3a. This revealed a linear relationship between phenolic acid concentrations ($\check{C}_i$) at much lower levels than $C_{i,\infty}$ (i.e. $\check{C}_i<0.35<<1$) and small Fourier times τ (far away from the equilibrium state). According to Peppas and Brannon-Peppas (1994), a linear correlation between dimensionless concentration of the diffusive species and $\sqrt{\tau}$ during the first stages of an absorption process is a reliable indicator of Fickian diffusion. Finally, Figure 3b presents the correlation between the dimensionless concentration ($\check{C}_i$) of phenolic acids in fortified rice and Fourier times τ. As shown, experimental results from all phenolic compounds and concentrations collapse onto a characteristic Fickian master curve.

4. Final remarks

In general, our study showed that the proposed methodology could result in the production of fortified rice grains presenting the ability to achieve an adjusted-predicted concentration of particular nutrients in their mass. This could be based on the selection of a combination of aqueous herbal extract bulk concentration, time of rice hydrothermal processing and source of specific herb. For example, it is well known that prolonged boiling times, as well as the need for drying of the fortified product, usually have a detrimental effect on the sensory attributes and the structure of the final product. So the production of a ready-to-eat fortified rice product of a desirable phytochemical content, which does not require a drying step, may be preferably done by applying a standard % w/v bulk concentration of herbal aqueous extract together with relatively high boiling times. On the other hand, the production of a quick-cooking fortified rice product, of same phenolic acid content as the ready-to-eat one, would probably require the application of a much higher % w/v bulk concentration of herbal aqueous extract together with a shorter boiling time, as the production of such products includes a drying as well as a rehydration step which usually act at the expense of the sensory quality of the final product. The rice industry could possibly adopt the proposed technique and select appropriate herbal extracts of relatively stable phytochemical content to produce optimal fortified rice products, concerning phytochemical content and sensory characteristics, according to consumers' needs. In addition, the launch of such products in the market could promote both the nutritional and commercial value of white milled rice.

5. Conclusions

The use of various % w/v concentrations of aqueous herbal extract for the enrichment of rice grains with phenolic acids during boiling (20 min) showed that there was a significant increment in the amount of phenolic acids absorbed in rice for higher % w/v concentrations of extract. In addition, the relative diffusivities of the examined phenolic acids in rice during boiling were found to be dependent on the chemical affinity of each compound to rice material. The proposed application of rice fortification through hydrothermal process, described in this study, seems to be quite interesting for the food industry, as it may offer the opportunity to produce fortified rice of either quick-cooking or ready-to-eat type which could contain adjusted portions of phenolic acids, together with an optimum final product appearance. This could probably be achieved by utilizing existing parboiling units of rice industries, after making slight modifications, and selecting the proper aqueous herbal extracts, as sources of phytochemicals or bioactive compounds of known diffusivities in rice material. However, extra work would be needed to predict the diffusivities of other health-promoting compounds (i.e. flavonoids, trace elements, vitamins, natural pigments) in rice, as well as their stability during hydrothermal processing.

Author Contributions

S. Bakalis and V. T. Karathanos designed the study, contributed to the interpretation of the results and reviewed the manuscript. P. E. Igoumenidis conducted the experiments, analyzed data, interpreted the results and wrote the manuscript. S. V. Iosifidis carried out the experiments and participated in data analysis. E. Lopez-Quiroga did the mathematical modeling and contributed to the revision of the final manuscript. V. T. Karathanos supervised the whole project.

Nomenclature

t	boiling time (s)
D_i	mass diffusivity of phenolic acids in rice kernels (m^2/s)
R	radius of fortified rice grain sphere (m)
R_{eq}	equivalent radius of rice grain (m)
r	radial coordinate (m)
C_i	phenolic acid concentration in rice grains (µg/g dry rice)
$C_{i,0}$	initial concentration of specific phenolic acids in rice (µg/g dry rice)
$C_{i,\infty}$	potential equilibrium concentration of phenolic acids in rice grains (µg/g dry rice flour)
$c(x,t)$	spatially distributed concentration of phenolic acids in rice grains (µg/g dry rice)
$C_i(t)$	volume averaged concentration of phenolic compound i (µg/g dry rice)
$\check{C}_i$	dimensionless concentration of individual phenolic acids in spherical rice grains
$\check{r}$	dimensionless radius of rice grain
τ	dimensionless Fourier number

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Table 1: Concentration of examined phenolic acids in spearmint aqueous extract at the beginning (t=0) of boiling process (μg/g of dry extract). All measurements are presented in mean values ± Standard Deviation (n=3)

Major phenolic acids in spearmint dry extract (μg/g)			
Caffeic	**3-4 di-OH-Phenylacetic**	**Protocatechuic**	**p-Coumaric**
3373.1±159.3	1983.3±166.2	180.8±14.1	171.3±3.9

Table 2: Estimated equilibrium concentration of major phenolic acids in fortified rice flour (μg/g dry rice flour) as a function of spearmint aqueous extract concentration (% w/v). All concentrations are presented in mean values ± Standard Deviation (n = 3).

Spearmint aqueous extract concentration	**Equilibrium phenolic concentrations in fortified rice flour (μg/g dry rice flour)**			
	3,4 di-OH-Phenylacetic	**Caffeic**	**Protocatechuic**	**p-Coumaric**
0.33% w/v	144.1±26.3	85.7±8.0	19.1±2.7	5.9±1.2
0.66% w/v	255.7±31.7	112.9±6.5	25.4±2.2	9.1±0.6
1% w/v	337.3±29.9	149.8±17.1	27.5±2.8	11.2±0.8
2% w/v	388.3±34.0	200.8±18.6	33.8±4.4	14.6±1.2

Table 3: Fitted values of the diffusion coefficient (Di) of the examined phenolic acids in rice grains during boiling in spearmint extract media. Correlation coefficient R^2 shows how good the fitting of simulated results with experimental data is. CI are the 95% confidence intervals.

Phenolic Acids	**Spearmint aqueous extract concentration**											
	0.33% w/v			**0.66% w/v**			**1% w/v**			**2% w/v**		
	D (m^2/s)	**CI**	**R^2**	**D (m^2/s)**	**CI**	**R^2**	**D (m^2/s)**	**CI**	**R^2**	**D (m^2/s)**	**CI**	**R^2**
3,4 di-OH Phenylacetic	7.7298E-12	7.7289E-12, 7.7554E-12	0.99	8.87E-12	8.8329E-12, 8.8785E-12	0.96	1.57E-11	1.5664E-11, 1.5699E-11	0.93	3.42E-11	3.3368E-11, 3.4232E-11	0.92
Caffeic	1.1443E-11	1.1442E-11, 1.1446E-11	0.98	1.81E-11	1.8145E-11, 1.8174E-11	0.97	2.55E-11	2.4967E-11, 2.5542E-11	0.92	3.56E-11	3.5420E-11, 3.5641E-11	0.98
Protocatechuic	6.859E-12	6.8433E-12, 6.8616E-12	0.97	9.84E-12	9.5958E-12, 9.8571E-12	0.91	1.95E-11	1.9407E-11, 1.9584E-11	0.95	3.32E-11	3.1216E-11, 3.3217E-11	0.91
p-Coumaric	1.2229E-11	1.2224E-11, 1.2230E-11	0.88	2.06E-11	2.0141E-11, 2.0599E-11	0.86	2.60E-11	2.5618E-11, 2.6086E-11	0.97	3.50E-11	3.4624E-11, 3.5100E-11	0.96

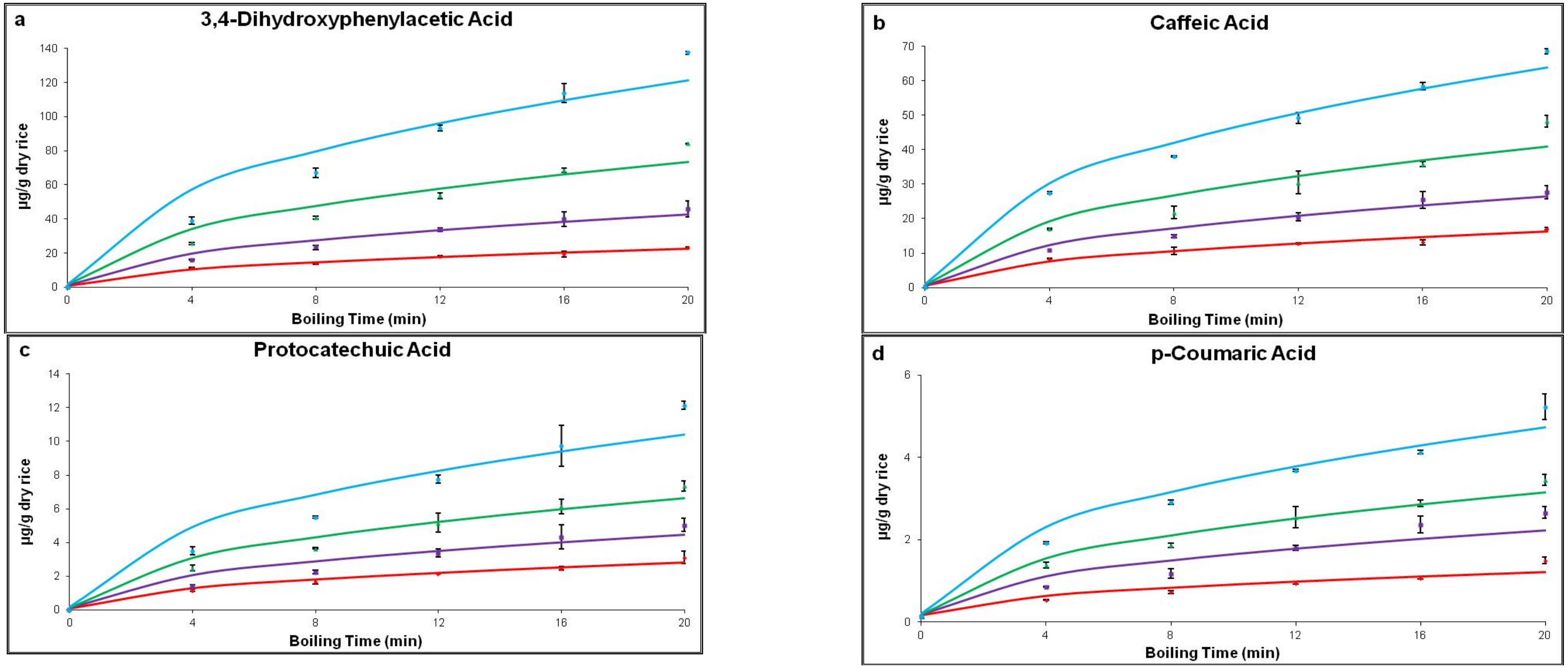


Figure 1: Comparison of the experimental concentrations of 3,4-dihydroxyphenylacetic (a), caffeic (b), protocatechuic (c) and p-coumaric (d) acids in rice grains (dots) with the simulation results (solid lines) obtained using the fitted Di values. Dots and lines corresponding to boiling in 0.33% w/v concentration of spearmint extract are in red, while results concerning 0.66%, 1% and 2% w/v concentrations of extract are presented in purple, green and light blue, respectively. Error bars represent the standard deviation of the samples (n=3).

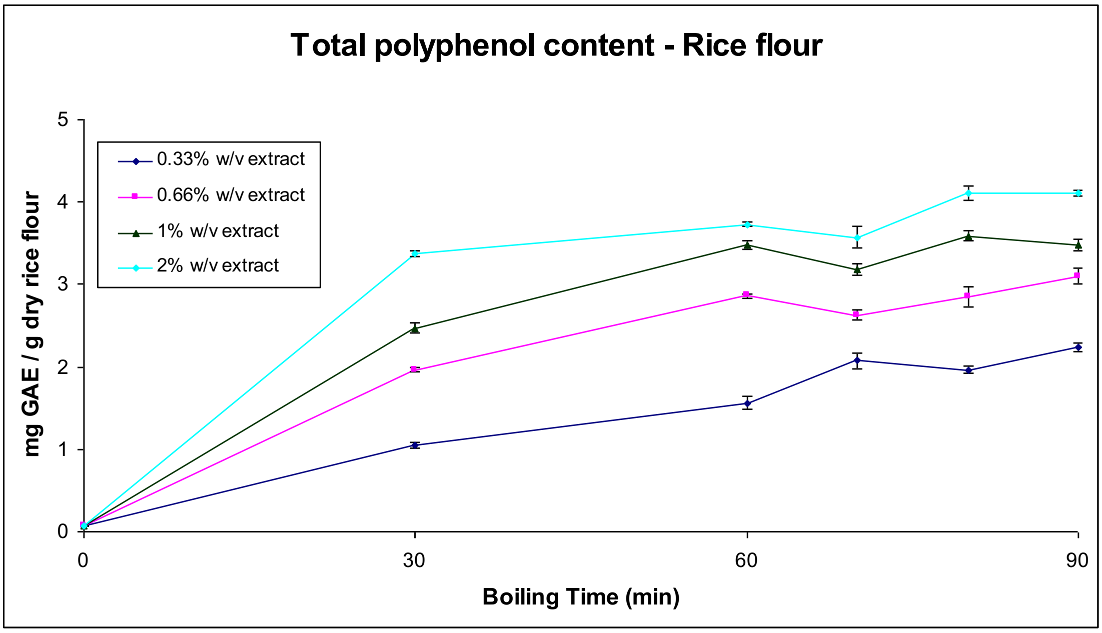


Figure 2: Total polyphenol content of rice flour as a function of boiling time and initial concentration of spearmint extract. All values are expressed as mean values of mg GAE/g dry rice flour and error bars represent the standard deviation of samples (n=3).

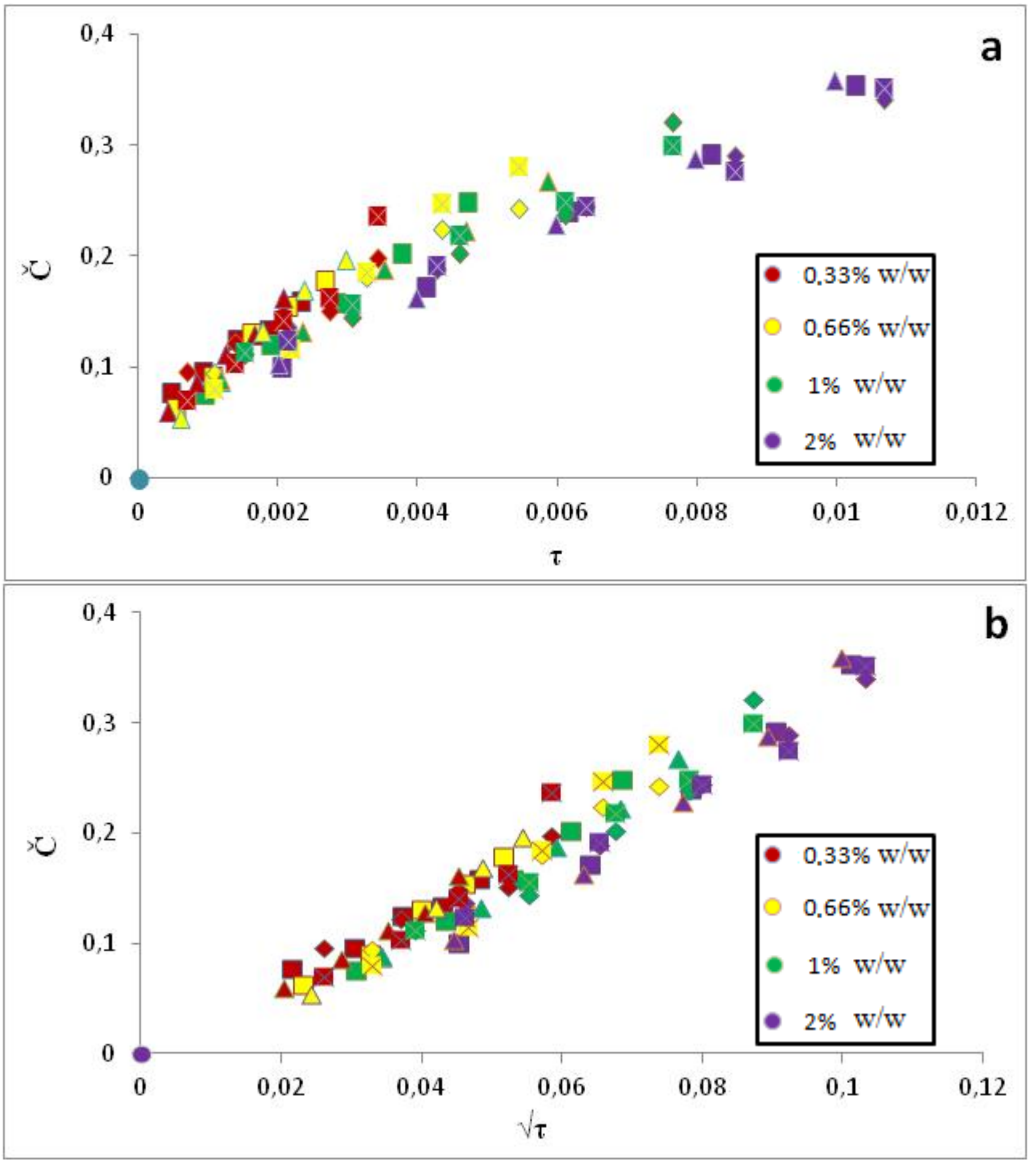
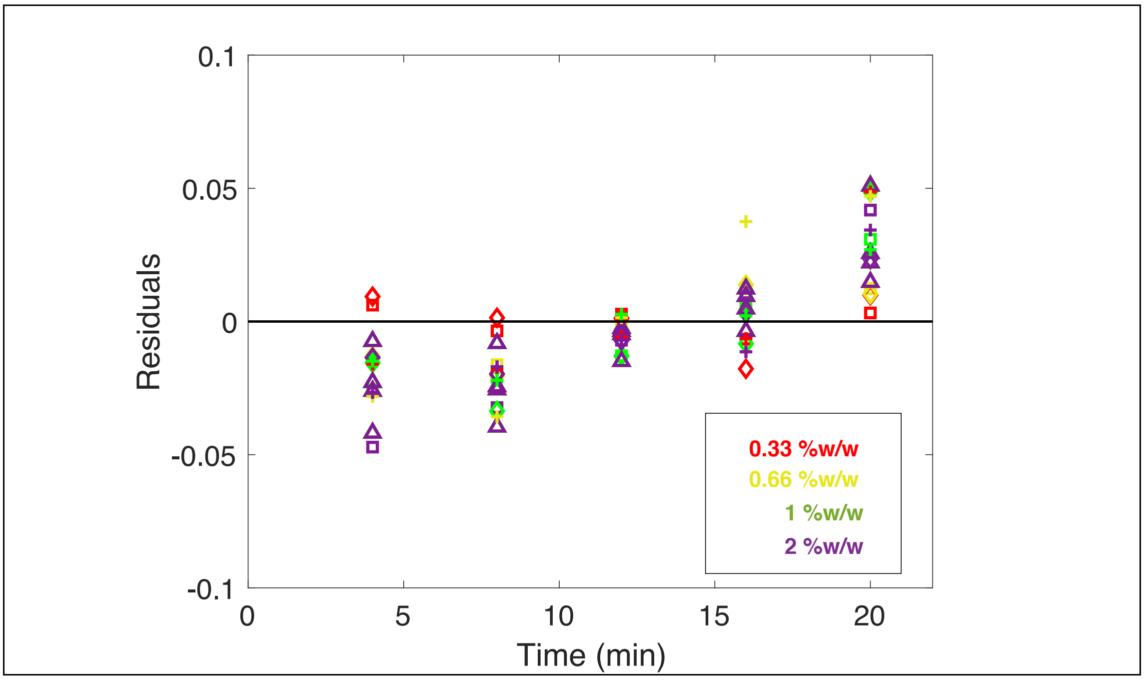


Figure 3: Master curves indicating the correlation between dimensionless concentration (Č) of examined compounds in rice grains during hydrothermal treatment and dimensionless time of the process (either τ (a) or √τ (b)). Symbols correspond to: 3,4-DiOH phenylacetic acid (squares), Caffeic acid (rhombus), Protocatechuic acid (triangles) and p-Coumaric acid (squares with X). Points corresponding to the 0.33% w/v herbal extract concentrations are in red. Yellow represents the 0.66% w/v data, while green and purple are for the 1% w/v and 2% w/v concentrations, respectively.

Supplementary Table 1: Molecular weight and hydrophobicity factor (log*P*) of major phenolic acids adsorbed in rice grains (Source: PUBCHEM). By *P* is symbolized the partition coefficient of each compound in a biphase of liquid solvents, one polar solvent (water) and a non-polar solvent (*n*-octanol).

Phenolic acids	**Molecular Weight**	**Estimated value of log*P***
Caffeic	180	1,2
3,4 di-OH Phenylacetic	168	0,5
p-Coumaric	164	1,5
Protocatechuic	154	1,1



Supplementary Figure 1: Residuals of the fitted data along time, for all the concentrations and compounds.

Legend: 3,4-DiOH phenylacetic acid (squares), Caffeic acid (rhombus), Protocatechuic acid (triangles) and p-Coumaric acid (crosses).