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Research Article

CREATION OF FUNCTIONAL SOLID FOOD MODEL ENRICHED WITH *CALAMINTHA NEPETA* (L.) SAVI.: KINETIC GAIN OF PHENOLICS COMPOUND

Abdelaziz Merouane[1,2]*, Sara Fellag[3], Abdallah Noui[1,2]

[1] *Hassiba Benbouali University, Laboratory of Natural Bio-Resources, Algeria*

[2]*Hassiba Benbouali University, Faculty of Natural and Life Sciences, Algeria*

[3]*Ahmed Benbella University, Faculty of Medicine, Department of Pharmacy, Algeria*

**Corresponding Author:*
E-mail: a.merouane@univ-chlef.dz

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ABSTRACT. The current research looks to benefit from *Calamintha nepeta* (L.) Savi. in food enrichment by using osmotic dehydratation (OD) for supplementing a solid foodstuff model. The kinetic gain of food model in terms of individual phenolics using HPLC-DAD, total phenolics (TPC), total flavonoids contents (TFC) as well as antioxidant properties were examined at different impregnation times. The food model gained the same phenolics profile of osmotic solution dominated by acacetin and Cryptochlorogenic acid. The enrichment process created a rich osmo-dehydrated food in TPC and TFC reaching the bioactive features of most of rich-in-phenolics human foods. The food model exhibited a potent antioxidant activity, even at a short impregnation time (4h), attaining 92.07±3.63% as β-carotene bleaching potency and 47.64±0.59% (at 10 mg/mL) as DPPH scavenging capacity. The kinetic gain of individual phenolics compounds depends on their concentration. This investigation supports valorization of *Calamintha nepeta* for the production of functional foods.

Keywords: *Osmotic dehydratation, antioxidant properties, functional food, bioactive content, acacetin.*

INTRODUCTION

Medicinal plants (MPs) were explored as remedies since the antique civilizations and are still nowadays used by humanity, especially as primary healthcare in spite of the domination of synthetic drugs [1]. These bioressources constitute an immense reservoir of secondary metabolites that can found applications against various ailments and symptoms through a wide range of beneficial biological potencies, mainly, the antioxidant activity preventing the incidence of chronic diseases related to oxidative stress such as diabetes [2], cancer, cardiovascular and neurodegenerative disorders [2], [3] by adsorbing and neutralizing the excess of endogenic and exogenic free radical compounds of the organism including reactive oxygen species (ROS) and reactive nitrogen species [4].

Among the chemical mixture of MPs, phenolic compounds appear as the main candidates contributing to the claimed beneficial effects associated with these plants [5]

and the most phytochemical group studied by the scientific community [6]. In addition to their powerful antioxidant activity, these bioactive compounds participate potentially in a rich array of health benefits characterizing herbal extracts [7]. The scientific knowledge about these properties has been increased the importance of phenolics in pharmaceutical, cosmetic and food industries [8].

Initially, the food fortification approach was innovated to correct micronutrient deficiencies in monotone alimentation dominated by starchy food crops such as cereals and tubers containing low micronutrient amounts [9]. The modern lifestyle and recent growing interest toward phenolics compounds, exerting improved bioactivity associated with health benefits, integrated this food processing in the production of new phenolics-enriched products. For this purpose, diverse approaches have been adopted to fulfill the demand such as the addition of fruits to staple foods (juices and yogurts) [2], mixing plant powders with flour [10], direct adding of phenolics extracts to beverages and dairy products [11], and use of osmotic dehydratation (OD) strategy [12].

The OD method is a simple food technology developed in the 1960s permitting the removal of water from the food and modification of their properties by impregnation in a specific aqueous concentrated solution [12]. This process is based on the natural and non-destructive phenomenon of osmosis defined into two simultaneous countercurrent movements, namely, water outflow from the food into the osmotic solution accompanied with solutes diffusion from the osmotic solution into the food [13]. The food fortification by OD requires anaerobic conditions (total immersion) to avoid the oxidative and thermal degradation of sensible constituents in osmotic solution and food product. This technique has been applied mostly on fruits due to their diverse advantages: inhibition of enzymatic browning; preservation of natural color and flavor without additives supplementation; and characterized with less energy costs [14,15].

MPs could be an economic and reachable natural source of antioxidants due to their richness in phenolic compounds, their beneficial values have been clearly avowed by the traditional medicines and modern scientific investigations. The biological activities of MPs have been scientifically researched and widely published. The current study looks differently to these natural bioressources, which can constitute an ideal source for the enrichment of foodstuffs with functional compounds to enhance their nutritional qualities and to develop new bioactivities in foods.

Since no earlier reports are available regarding the supplementation of foodstuffs by OD using MPs extracts as osmotic solution, the purpose of this research is to enhance the bioactive content and antioxidant properties of a food model using OD technique and *Calamintha nepeta* (L.) Savi. extract as osmotic solution. Moreover, the phenolics compounds in the osmotic solution and food model, at different impregnation intervals, were identified and quantized by high-performance liquid chromatography with diode-array detection (HPLC–DAD), and the kinetic gain of individual phenolics by the food model was discussed.

MATERIALS AND METHODS

Preparation of osmotic solution

The aerial parts of *Calamintha nepeta* (L.) Savi. were collected in April 2021 from Beni-Bouaziza district, Beni-Haoua, Chlef province, Algeria. The plant material was identified by Dr. A. Merouane. The aerial parts were immediately rinsed and shade dried

at room temperature for 7 days, then finely powdered and conserved in glass airtight bottles at 4 °C until further experimentation.

The osmotic solution was prepared according to the habitual procedure of traditional tea's making. 200 mL of distilled water was brought to rolling boil then added to 5 g of plant powder representing the solid/liquid ratio of 1/40 (w/v). The mixture was kept to diffuse in darkness at room temperature until cooling, with manual stirring, and then used instantly for the enrichment step.

Preparation of food model

The food model was created following the protocol described in the previous research [12] with modifications. The agar-agar gel was served as a food model and prepared in an Erlenmeyer flask; 2 g of agar-agar, 5 g of sucrose and 100 mL of distilled water were mixed in the bottle and sterilized in autoclave apparatus at 120 ˚C for 20 min. The mixture was cooled, at liquid state, in cube moulds giving agar-agar cubes characterized with similar dimensions (25 mm × 22 mm × 10 mm) and weight (3.67±0.04 g).

Osmotic dehydration procedure

The enrichment of food model was guaranteed by total immersion of agar-agar cubes in the osmotic solution. About 18 g of food model cubes was submerged in 300 mL of osmotic solution with continuous back-and-forth stirring. The solution/gel rate was 16:1 (v/w). The procedure was processed for 4, 8, 12 and 24h under atmospheric pressure in darkness condition at 4 ˚C to prevent phenolics against oxidation. At each interval time, three gel cubes were removed from the solution, carefully sponged with tissue paper and crushed to give a homogeneous paste. A negative control paste was prepared from non-enriched cubes. The pastes were entered directly in the extraction step. pH of the osmotic solution was controlled at the beginning and end of enrichment procedure.

Extraction of bioactive compounds from food model

To obtain the food extracts, a sample of crushed gel (10 g) was extracted with 100 mL of distilled water at room temperature under continuous shaking (WIS-10, Daihan Scientific co. Ltd., Korea) for 2 h. The sample was then filtered by Whatman No. 1 filter paper. The residue was extracted twice again with 25 mL of distilled water and the extracts were combined and stored in darkness at 4 ˚C until analysis.

HPLC-DAD analysis

The qualitative and quantitative features of phenolic compounds were screened using high-performance liquid chromatography (HPLC) adapted from conditions previously described by Gonçalves et al. [5] with modifications. Food and plant extracts were prepared in methanol then filtered through a 0.45 µm membrane. 20 µL from each extract was injected into the HPLC Gilson unit using Spherisorb ODS2 column (Phenomenex Inc., Torrance, CA, USA) characterized with 5 µm particle size and 250 mm × 4.6 mm length. The absorbance was monitored at 280, 320 and 350 nm and the mobile phases consisted of A: 3% acetic acid (diluted in distilled water) and B: methanol. The gradient program adopted was: 0-15 min 5-15% B, 15-30 min 15-35% B, 30-40 min 35-55% B, 40-45 min 55-75% B, 45-60 min 75-100% B. The flow rate was 0.8 mL/min. Identification of phenolics was performed by comparing their retention times and MS

spectra with those of separately injected authentic standards. Phenolic compounds were quantized in reference to external calibration curves of authentic standards.

Determination of total phenolics

The total phenolics content (TPC) was determined by the spectrophotometric method given by Singleton et al. [16], involving Folin-Ciocalteu's phenol reagent and gallic acid as standard. The reaction mixture was prepared by mixing 0.1 mL of each extract prepared at 2 mg mL^{-1} and 2.5 mL of 10% Folin-Ciocalteu's reagent in water. After 10 min, 2.5 mL of 7.5% $NaHCO_3$ was added. The mixture was incubated in the dark at room temperature for 45 min and the absorbance was read at 765 nm using UV-Vis spectrophotometer (Optizen 2120, Mecasys Co. Ltd., Korea) against blank contemporaneously prepared by replacing extract with methanol. All tests were carried out in triplicate. TPC was expressed as mg gallic acid equivalents /100 g (mg AGE/100g food) for food and as mg gallic acid equivalents /g dry matter of plant (mg AGE/g DM) for *Calamintha nepeta* (L.) Savi.infusion based on gallic acid calibration curve. The phenolics gain rate (PGR) of foodstuff model at a given impregnation period was calculated using the following formula "*Eqn. 1*":

$$PGR = TPC/t$$

Eqn. 1

Where, *PGR* means the phenolics gain rate expressed in µg/min/100g of food, *TPC* is the total phenolics content (µg) at a given impregnation time and *t* represents the impregnation time (min).

Determination of total flavonoids

The total flavonoid content (TFC) was determined using the method described by Tepe et al. [17] with slight modifications. In brief, 0.5 mL of each extract prepared at 2 mg. mL^{-1} was mixed with 1 mL of 2% aluminum chloride ($AlCl_3$) dissolved in methanol. The mixture was allowed to stand for 1 h in a dark condition at room temperature and measured spectrophotometrically (Optizen 2120, Mecasys Co. Ltd., Korea) at 420 nm against the blank prepared by replacing the extract with methanol. TFC was calculated from the standard calibration curve constructed with various concentrations of quercetin and expressed as mg quercetin equivalents/100g (mg QE/100g food) for food and as mg quercetin equivalents/g dry matter of plant (mg AGE/g DM) for *Calamintha nepeta* (L.) Savi. extract.

The flavonoids gain rate (FGR) of foodstuff model at a given impregnation period was calculated using the following formula "*Eqn. 2*":

$$FGR = TFC/t$$

Eqn. 2

Where, *FGR* means the phenolics gain rate expressed in µg/min/100g of food, *TFC* is the flavonoids content (µg) at a given impregnation interval and *t* represents the impregnation time (min).

Determination of DPPH free radical scavenging activity

The antiradical potency of initial osmotic solution and food model extracts was measured by using DPPH test following the procedure of Muid et al. [18]. 500µL of food model extract (10 mg mL^{-1}) was added to 1 mL of freshly methanolic DPPH solution prepared at 0.1 mM. The mixtures were homogenized vigorously and stored in the dark at room temperature for 30 min. The same process was repeated for the BHT. Uv-vis readings were performed using a spectrophotometer Optizen 2120 at 517 nm. Inhibition rate of DPPH free radicals expressed in percent (I %) was calculated by using the following equation "*Eqn. 3*":

$$I(\%) = \left(\frac{A0 - As}{A0}\right) \times 100$$

Eqn. 3

Where A_0 is the absorbance of the freshly prepared DPPH solution without extract and A_S is the absorbance of food samples. The antiradical activity of *Calamintha nepeta* (L.) Savi. infusion was evaluated, in parallel, in terms of IC_{50}, defined as the concentration (µg DM. mL^{-1}) which neutralizes 50% of the initial DPPH.

Determination of β-Carotene–linoleic acid bleaching

β-carotene–linoleic acid method is an indirect approach which measures the ability of extracts to inhibit β-carotene bleaching by products regenerated from linoleic acid oxidation [19]. One ml of β-carotene solution dissolved in chloroform (0.5 mg in 10 mL) was mixed with 25 µL of linoleic acid and 200 mg of Tween 20. After complete evaporation of the chloroform using a vacuum evaporator at 40 ˚C, 50 mL of distilled water (aerated with oxygen for 1 h) was added with vigorous shaking. 2.5 mL of this emulsion was dispersed in test tubes and 350 µL of each food model extract or synthetic antioxidant BHT prepared at 2 mg mL^{-1} was added. The mixture was stored in the dark at room temperature during 72 h. Uv-vis readings were performed against blank (consisting of 350 µL methanol in 2.5 mL of emulsion) using a spectrophotometer Optizen 2120 at 490 nm. Total antioxidant activity was expressed in terms of percentage inhibition relative to the synthetic antioxidant BHT calculated following the "*Eqn. 4*":

$$\%\ \text{Inibition} = \frac{As}{Ac} \times 100$$

Eqn. 4

Where A_S is the absorbance of test sample after incubation time and A_C is the absorbance of the freshly prepared BHT.

Statistical analysis

The measurements were carried out in triplicate. The data were subjected to analysis of variance and appropriate mean separation was conducted using Tukey's significant differences post hoc test in SPSS 16.0 software for Windows (SPSS Inc., Chicago, IL, USA). A statistical difference at $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

pH of the osmotic solution

The pH of the osmotic solution was measured at the beginning and end of the enrichment process to verify any possible changes due to a possible fungal growth and chemical alteration. During the impregnation period of food model in the osmotic solution, values of pH varied from 6.18 to 6.15, this interval indicates a stability of pH parameter as recommended for the OD technique.

The increase of pH during the OD operation is one of the causes of phenolic's oxidation in addition to the thermal degradation, air contact and intensive agitation [12], [20]. Therefore, prevention of the bioactive chemical mixture of herbal extracts from oxidizing by controlling the operating conditions is a key point to conserve the bioactive compounds of foods enriched with MPs extracts.

Phenolic profiles in plant and foodstuff model

The phenolic features of *C. nepeta* (L.) Savi. and food model were verified by HPLC-DAD technique and reported in Fig. 1. The analysis showed six phenolic acids in all extracts with clear dominance of cryptochlorogenic and rosmarinic acids, whereas the flavonoids were represented by acacetin and rutin.

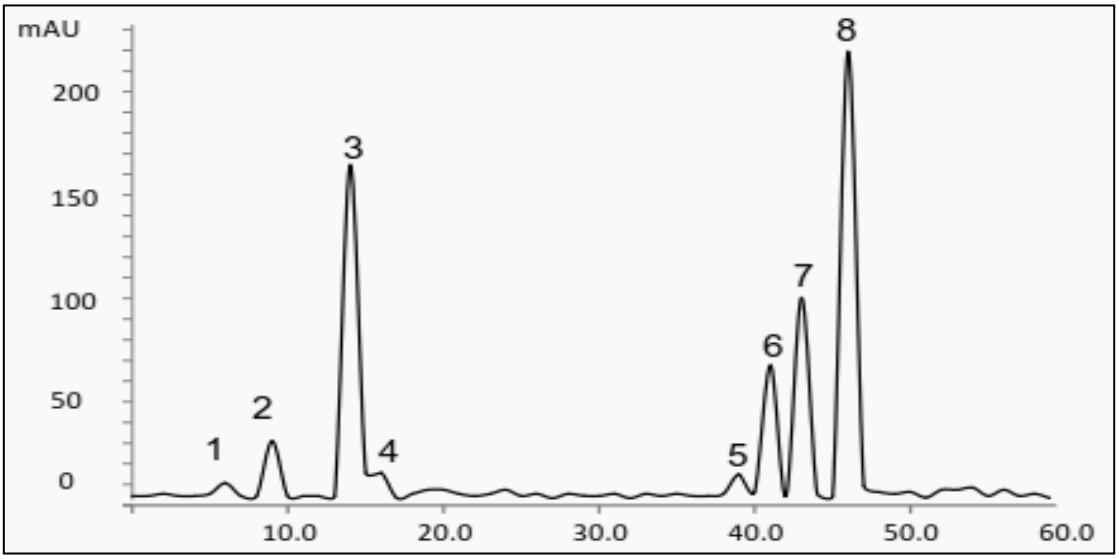

Fig 1. *HPLC–DAD chromatogram of C. nepeta (L.) Savi. [1: quinic acid, 2: chlorogenic acid, 3: cryptochlorogenic acid, 4: caffeic acid, 5: rosmarinic acid, 6: rutin, 7: salvianolic acid, 8: acacetin]*

The osmotic solution prepared from *C. nepeta* (L.) Savi. presented high content of acacetin mentioned previously as taxonomic marker in *Calamintha* genus [21] and strong anti-inflammatory natural agent explaining the traditional uses of this medicinal species [22]. In the other hand, a moderate amount of rosmarinic acid (3.24 mg/g of dry extract), regarded as a taxonomic marker of *Lamiaceae* family [23], was observed in the extracts. The phenolic mixture of *C. nepeta* (L.) Savi. is less complex (number of phenolic compounds) but quite variable (content of phenolic compounds) dependently on the region of collection as demonstrated from South Portugal [5] and South Italy [22]. The current research from North Algeria validates this observation.

The analysis of phenolics gain by the food model demonstrated the presence of all compounds recorded in the osmotic solution during diverse impregnation intervals. However, the rate of gain was variable (Fig. 2). After 4h of impregnation, both flavonoids (acacetin and rutin) represent the most dominant compounds in the osmo-dehydrated food with 51.28% of the phenolic's mixture. The phenolic acids quinic, chlorogenic, rosmarinic and caffeic acids followed the same behavior characterized with weak initial penetration in agar-agar cubes followed by stationary phase (Fig. 2). In contrast, the dominant compounds (acacetin, rutin, cryptochlorogenic acid and salvianolic acid) penetrate with high quantities during the first phase (0-12 h) then followed with slowness diffusion in the second phase (12-24 h) except for the major component acacetin that continued to flow with a same manner. The penetration rate of phenolics compounds in the agar-agar cubes is attributed to their concentrations that control the osmotic pressure of the osmotic solution.

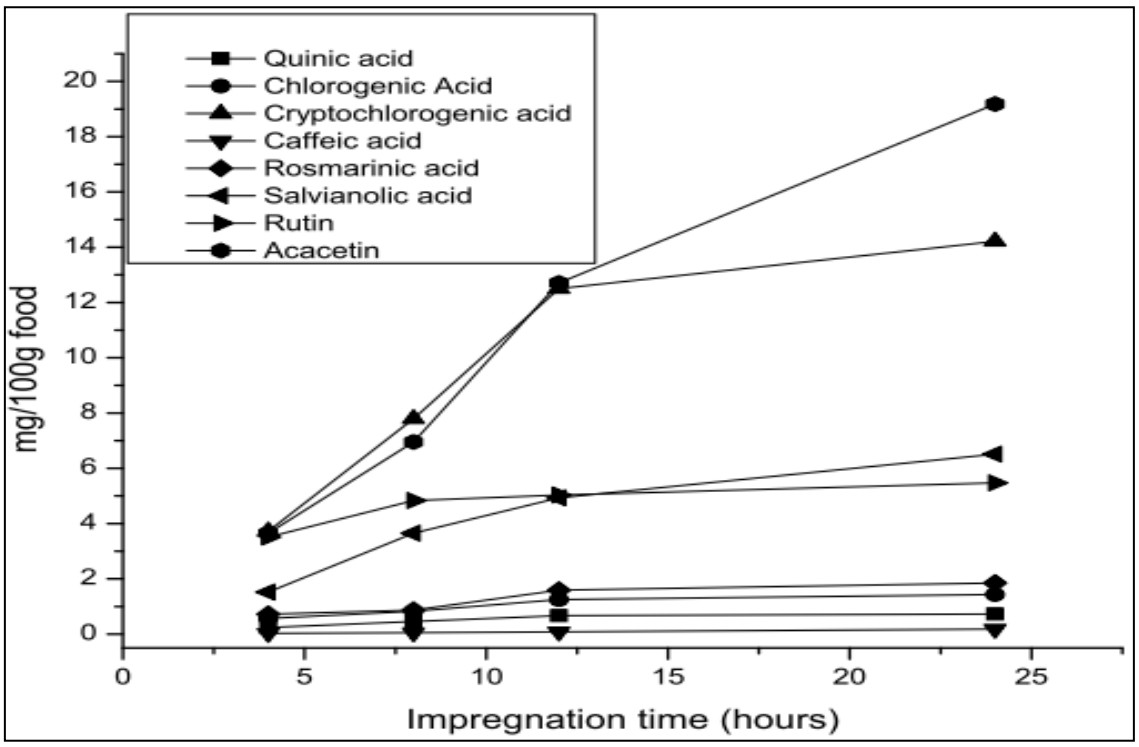

Fig 2. *Kinetic gain of phenolics compounds by the agar-agar food model*

Bioactive contents

The evaluation of bioactive content of MPs to explore in preparation of osmotic solution is a decisive factor for their choice as a natural source in food enrichment procedure. In the current investigation, the TPC and TFC of the osmotic solution and foodstuff model were evaluated. The results obtained are given in Table 1.

The herbal tea obtained from *Calamintha nepeta* (L.) Savi. showed 35.87±2.81 mg GAE/g DM and 5.32±0.29 mg QE/g DM of phenolic compounds and flavonoids amounts respectively. The present and previous researches [5, 22] revealed richness of *C. nepeta* in phenolic compounds exerting diverse beneficial bioactivities. Acacetin is characterized with various therapeutic virtues in cancer, inflammation, infections, diabetes and

inhibition of microbial/ viral infections, lipid accumulation (obesity) and rheumatoid arthritis [24]. Salvianolic acid is recognized for their anti-fibrosis and anti-cancer effects [25], whereas the rosmarinic acid exhibits diverse therapeutic potencies such as anti-inflammatory, antiviral, antibacterial, antidepressant, anticarcionogenic, and chemopreventive properties [26]. These potencies suggest *C. nepeta* as an excellent bioresource of natural agent that can found application in naturally processed foods to promote consumer's health.

The intake of phenolic compounds, known for their antioxidant and free radical scavenging activities [6], has been associated with beneficial roles in human health such as prevention/cure of diseases related to oxidative stress such as cancer, diabetes and degenerative diseases [27]. The MPs constitute a palatable source of phenolic compounds that can give a chance to produce phenolic-enriched foods with a specific mixture to target-specific health effect [3].

Table 1. *Bioactive content of food model at different interval times.*

Interval time (hours)	Phenolics		Flavonoids		Flavonoids/ Phenolics
	Content (mg GAE/100g)	PGR	Content (mg QE/100g)	FGR	
4	39.37±1.79^{a}	164.04	26.88±0.55^{a,c}	112.00	0.68
8	42.21±1.33^{a,b}	87.94	26.58±2.28^{a,b}	55.38	0.62
12	46.98±3.35^{b}	65.25	22.86±1.21^{b}	31.75	0.48
24	56.68±0.69^{c}	39.36	30.66±1.18^{c}	21.29	0.54

a-c: Values (mean ± standard deviation, n=3) in the same column sharing different letters are significantly different ($P < 0.05$). GAE: Gallic acid equivalents; QE: Quercetin equivalents, PGR: Phenolics gain rate expressed in µg/min/100 g of food model, FGR: Flavonoids gain rate in µg/min/100 g of food model

The impregnation of agar-agar cubes in *Calamintha nepeta* (L.) Savi. infusion evolutes their phenolic status from free-phenolics to rich-phenolics food. Phenolics and flavonoids amounts recovered by food model from osmotic solution as well as gain rates (PGR and FGR) variation are shown in Table 1. After 4h of OD, the food model showed 39.37±1.79 mg GAE/100g; this amount means a high supplementation rate attaining 164 µg GAE/min (100g of food) (Table1) due to the vast difference in phenolics concentration between the food model product and the surrounding osmotic solution. At 8h, the osmo-dehydrated food retains the same TPC ($p > 0.05$).

As can be seen from the Table 1, the intervals 12h and 24h generated considerable and variable TPC ($p < 0.05$), the highest amount of (poly)phenols was remarked at the end of enrichment process (56.68±0.69 µg GAE/100 g). The quantity of (poly)phenols introduced in the foodstuff model by osmosis during the last 20h of impregnation didn't exceed 17.31 mg, whereas the phenolic gain during the initial 4h was more than twofold higher than that obtained between 4-24h. The molecular weight of phenolic compounds attaining the food may explain the difference of phenolic gain between both intervals 0-4h and 4-24h.

With respect to TFC, the amount followed the same behavior of TPC at 4 and 8h of impregnation time ($p > 0.05$). However, these bioactive phenolics fraction showed a

significant decline at 12h (comparatively to the 4h interval at $p < 0.05$) before re-increasing the gain to reach the highest quantity (30.66±1.18 µg QE/100 g) at 24h interval and equal to the initial content obtained at 4h of the fortification process.

During the initial interval of enrichment (4h), the flavonoid gain of food model reaches approximately 112 µg QE/min (for 100 g of food) and the TFC represented about 68% of the phenolic's mixture, this fraction diminished to 54% of the TPC at the 24h interval (Table 1). These ratios indicate superiority of flavonoids in the bioactive mixture of the osmo-dehydrated food, which attributed to their low molecular weights and high concentration in osmotic solution. These results are in agreement with HPLC-DAD analysis.

According to Rózek et al. [12], flavonoids and phenolics acids with low molecular weight (≤ 610 g/mol) are the main phenolics in the osmo-dehydrated food enriched with a concentrated red grape jus, our results are in accordance with these finding where the molecular weight of phenolics range from 180.16 (caffeic acid) to 610.51 g/mol (rutin) . The presence of these bioactive compounds in foods can promote the protective effects in biological systems against the oxidative stress, flavonoids show a great interest by their important potencies to scavenge free radicals [28], activate antioxidant enzymes [29] and inhibit oxidases [30].

The osmo-dehydrated food model enriched with *Calamintha nepeta* (L.) Savi. infusion displayed highest phenolic amount than major human food sources of (poly)phenols foods (except berries regarded as a palatable phenolic's source) including fruits (grapefruit, peach, kiwi fruit, pear, avocado, and pineapple), beverages (green tea, black tea and white wine), vegetables (lettuce, basil, white cabbage, spinach, parsley, cauliflower, broccoli, carrot, red beet, potato, tomato and aubergine) and cereals (rice, wheat's flour and pasta) [31].

The food model enriched with *Calamintha nepeta* (L.) Savi. displayed higher flavonoids amount than wide range of fruits, including raspberry, Sweet cherry, peach, fig [32], Mandarin orange, avocado [33] and date palm fruits [34], as well as widely consumed vegetables such as Carrot, Parsley, Tomato, Red cabbage and Broccoli [32]. Findings of the current study open a new approach to employ MPs in the production of foods with specific phenolic composition.

The data on the beneficial effects of flavonoids on human health and their amounts in common fruits, vegetables and foods for human nutrition are listed in a database (https://data.nal.usda.gov) at the US Department of Agriculture, Agricultural Research Service [35].

DPPH free radical scavenging

The osmotic solution and food model extracts obtained at 4, 8, 12 and 24h were screened for their antioxidant (β-carotene-linoleic acid) and antiradical (DPPH free radical scavenging) potencies. Fig. 3 displays the antiradical results exerted by the food model.

The DPPH free radical scavenging activity of *Calamintha nepeta* (L.) Savi. infusion showed a noticeable IC_{50} equal to 12.32 µg/mL, this efficacious antiradical power indicates the importance of this species as a prosperous bio-source of antioxidants and merit its valorization in food technology.

As shown in Fig. 3, all the food model extracts prepared at 10 mg/mL reduced DPPH free radical. The results revealed that the ability of food to quench DPPH radicals is proportionally correlated with the impregnation time, where the highest scavenging

efficacy (47.64±0.59%) was reached at the 24h interval. This appreciable potential is attributed to the presence of known antiradical agents (rosmarinic acid, chlorogenic acid, rutin…etc.) in the phenolics profile of food model.

Our findings are in line with the data obtained for food model enriched with grape jus concentrated at 40, 50 and 60% (soluble/solids), where the highest antiradical properties of food model were reached at the end of OD enrichment process [12].

Scavenging of free radicals inhibits and/or prevents oxidation of cellular components (sugars, amino acids, lipids, and nucleotides) resulting in the equilibrium of free radicals in living systems. From the results obtained in these OD conditions, the osmo-dehydrated model food showed a potential antiradical capability that surpasses or equal potencies of citrus juices [36].

The correlation analysis between antiradical properties and phytochemical amounts of food model at different intervals (Table 2) revealed high impact (r^2= 0.925, $p < 0.01$) of TPC on the potencies of food model to neutralize DPPH free radical. On the other hand, a moderate correlation was found between DPPH data and TFC (r^2= 0.401).

β-carotene bleaching

To better appreciate the antioxidant capacities of the osmo-dehydrated food model, their capacity to inhibit β-carotene rapid discoloration was measured relatively to BHT at identical concentration (2 mg mL^{-1}). Fig. 3 displays the results obtained at different impregnation intervals.

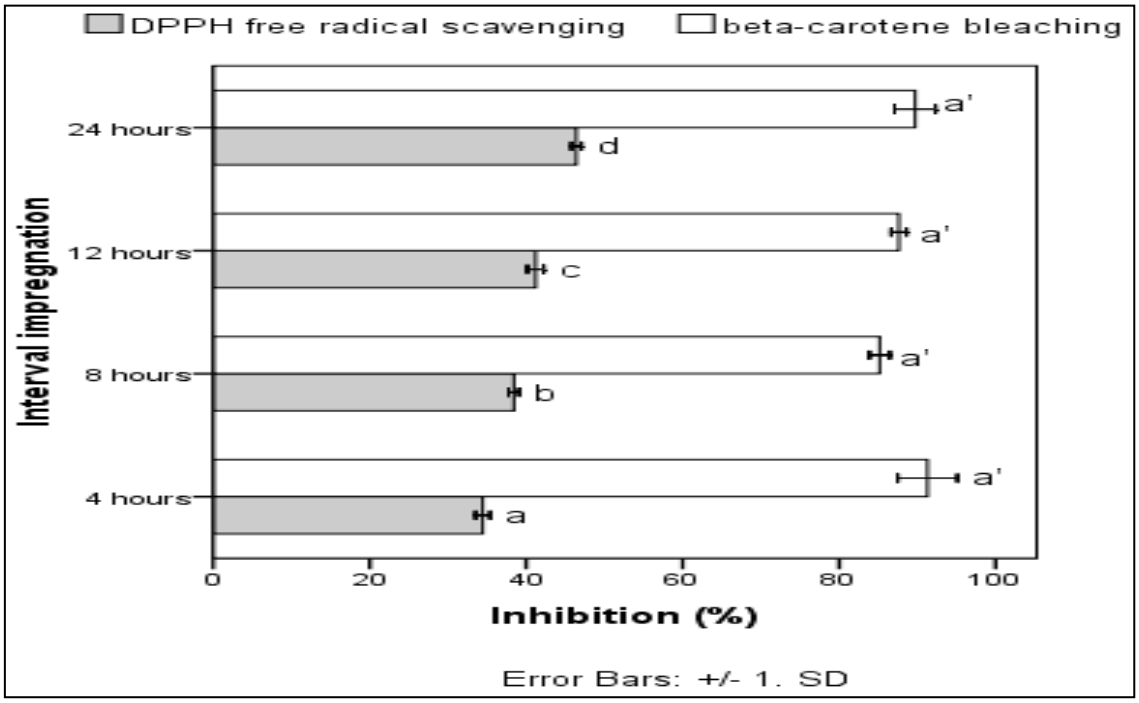

Fig 3. *Antioxidant activities of food model as assessed by DPPH free radical scavenging and β-carotene bleaching tests. Different letters in the same assay indicate significant difference at $p < 0.05$.*

As can be seen from Fig. 3, there is no significance impact of the impregnation time on the relative antioxidant capacity of agar food model. The shorter processing time (4h)

was sufficient to reach the maximum of activity (92.07±3.63%), this potency is comparable to the synthetic antioxidant BHT (94.17±0.51%) widely used as food conservative agent.

Table 2. *Pearson correlation coefficients between assays of food model.*

Parameters	TPC	DPPH	β -carotene	TFC
TPC	1			
DPPH	0.925*	1		
β-carotene	0.090	-0.014	1	
TFC	0.412	0.401	0.263	1

TPC: total phenolic content; TFC: total flavonoids content; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; *Correlation is significant at the 0.01 level (2-tailed).

Contrarily to DPPH assay, no correlation was found between β-carotene inhibition and bioactive content (Table 2). However, the strong potential of food model could be accredited to the synergistic effects of the bioactive chemical matrix of *Calamintha nepeta* (L.) Savi. and other phyto-constituents of this resource such as terpenoids [1] that can attain the agar-agar food model and giving it the specific plant fragrance.
The strongest β-carotene inhibition activity of osmo-dehydrated food model, obtained in these OD conditions, encourage valorization of *Calamintha nepeta* (L.) Savi. as an alternative source to synthetic antioxidants and motivate application of OD strategy in food industry to obtain functional end products to promote public health through their biological properties against ailments related to oxidant stress and to fulfill the developing consumers interest for natural-based foods.

CONCLUSION

The present study researched, for the first time, on the possibility to valorize herbal extracts in food fortification using OD strategy. The data obtained demonstrated that *Calamintha nepeta* (L.) Savi. is a rich source of phenolics compounds with great antioxidant properties that can found application in the creation of functional foods. The enrichment process introduced totality of phenolics constituents of the plant extract in the solid food model; the gain rate depends on the concentration of phenolics in osmotic solution.

The findings of this work reveal the possibility to explore MPs in food fortification, using OD process, to improve public health by introducing desirable bioactive molecules in diverse diets and to increase their daily intake. However, further investigations are needed to invest this new approach on real food and to optimize the operating conditions of OD for profiting maximally from the bioactive mixture of medicinal herbal extracts. Additionally, enrichment of real food products should be accompanied with sensorial characterization to examine their acceptability by consumers.

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Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: A.M., Design: A.M., A.N., Processing: S.F., A.N., Analysis or Interpretation: A.M., S.F., A.N., Literature Search: A.M., S.F., Writing: A.M.

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