

**The incorporation of plant materials in “Serra da Estrela” improves its antioxidant activity without changing the fatty acids profile and visual appearance**

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**Abbreviations:**

DPPH: 2,2-diphenyl-1-picrylhydrazyl

EC<sub>50</sub>: Effective concentration 50

TBARS: Thiobarbituric acid reactive substances

MDA-TBA: Malondialdehyde-thiobarbituric acid

GAE: Gallic acid equivalents

GC: Gas chromatography

FID: Flame ionization detector

FAME: Fatty acids methyl esters

ANOVA: Analysis of variance

MUFA: Monounsaturated fatty acids

PUFA: Polyunsaturated fatty acids

SFA: Saturated fatty acids

## **Abstract**

“Serra da Estrela” cheese is a Portuguese delicacy, which has been produced for centuries from the milk of cattle pasturing in the protected Serra da Estrela natural park. Transforming this cheese into a functional food would be a huge benefit for the market and the consumer. Decocted extracts and dried chestnut flowers and lemon balm plants were incorporated into the cheese to functionalize it, granting antioxidant activity to this foodstuff. The functionalized cheeses showed higher antioxidant activity, especially lipid peroxidation inhibition. The incorporation of dried plants appeared to be more effective than using decoctions, but the influence of the plant species was less observable. Furthermore, the fatty acids profile of the cheeses was also determined through gas chromatography. C18:1 and C16:0 were the most abundant fatty acids; saturated fatty acids prevailed over the unsaturated ones. Between the control and the incorporated samples no significant differences were found. In addition, the external color was measured through a spectrophotometer for lightness, yellowness and redness. There were some differences recorded for each samples’ color. In general, the results indicated that the functionalization of this exquisite dairy product with natural plant extracts provided beneficial characteristics, both for consumers (healthier product) and producers (added-value products).

## Introduction

The worldwide food market represents one of the highest grossing money exchange networks. The investments on new foods and healthy food have gained interest in the last years, aligned with a new way of consumers to look upon what they eat [1-3]. This awareness has brought pressure on the food market to reduce the chemicals added to food, while demanding more information on the labels and fostering the use of natural products and extracts to either substitute synthetic extracts or to enhance features of the food. Many plants, mushrooms and algae, which have been consumed for centuries, are finding their way into other foodstuffs, providing beneficial effects towards the food (by conserving and altering it) and also to the consumer (which retains the bioactive molecules of the plants) [4].

In Portugal, and throughout the world, the dairy industry has a deep impact in the global economy and the cheese production is one of the most important. Within it, there is a vast number of different kinds of foodstuffs that have maintained the manufacturing procedure the same way for many years; but recently, innovations have been introduced, namely by incorporating plants or plant extracts to enhance flavor, reduce microbial load or alter the appearance of cheeses [5-10]. One of the most famous Portuguese cheeses is the “Serra da Estrela” which is made from raw ewe’s milk, mixed with salt and thistle. It is a soft cheese that has an estimated maturation of one month before consumption, although it can be transformed into a hard cheese if maturation stands for at least 6 months. There are some studies regarding this cheese, but to the authors best knowledge it is the first time that plants and their extracts are incorporated into it to provide beneficial effects towards the consumers’ health [11-15]. These effects are carried out through the antioxidant activity present in the plants, which is known to have

influence against oxidative stress, while preventing diseases like cancer, Alzheimer, diabetes, and other illnesses [16].

*Melissa officinalis* L., known commonly as lemon balm, has been thoroughly studied for its medicinal purposes and has proven to have extraordinary effects as anti-diarrheal, anti-ulcer, anti-viral, anti-bacterial, anti-fungal, anti-inflammatory and antioxidant effects, among others [17, 18] when consumed in infusions and decoctions. Regarding the antioxidant activity, [19] reported the highest values for commercial bags (lower than 1 mg/mL). Chestnut flowers are sub-products of the intense harvest of its nut, which represents in Portugal a revenue of 32 million euros [20]. The flowers have shown outstanding potential as antioxidants and antimicrobial agents, although only its consumption in infusions and decoctions is reported [21]. In terms of antioxidant activity, in various assayed antioxidant procedures, all results displayed an EC<sub>50</sub> (sample concentration responsible for 50% of antioxidant activity) value under 200 µg/mL for both infusions and decoctions [22]. These extractions are used in typical and ancestral claims [21] and although some molecules (and therefore bioactivity) can be lost with the heat process, others are activated or released in higher extension, resulting in a rich plant extract [23]. Accordingly, incorporating these ingredients in dairy products as cheese, therefore functionalizing them, is an advantage to the food industry, while also aiding farmers, by providing market opportunities for their byproducts.

## **Experimental**

### **Plant material**

*Castanea sativa* Mill. flowers belonging to *Judia* and *Longal* cultivars were collected in June 2013 in Oleiros, Bragança (north-eastern Portugal) (41°51'02'' N, 6°49'54''W). The samples were lyophilized (FreeZone 4.5, Labconco, Kansas, USA) and milled

down to a fine powder (841 microns). About 5 g of the powder was submitted to a decoction extraction in 1 litre of cold distilled water. After heating, it was left to boil for 5 min, and stood at room temperature for 5 additional minutes. After filtration through a Whatman N°4 filter paper, the obtained decoctions were frozen and lyophilized.

*Melissa officinalis* L. dried stems and leaves were provided in February 2014 by the company “Mais Ervas”, based in Trás-os-Montes, Portugal. Some samples were also submitted to the same extraction method as the chestnut flowers.

### **Cheese production**

The cheese was produced in a certified manufacturing plant (Queijos Casa Matias, Lda) based in Seia, on the mountain foot of Serra da Estrela. The milk, obtained from ewe's of the breed “Churra Mondegueira” arrived at the facility on the 7<sup>th</sup> of April and the cheese production took place on the same day. Upon arrival, the milk had a stable temperature of 5 °C and a pH of 6.88. After being transferred to a tank, artichoke thistle (*Cynara cardunculus* L.) was added to induce the milk clotting, along with salt. After the milk clotted, it was processed through an automated machine, which by pressing the cheese into a mold, removed the excess serum. A second pressing took place in horizontal pressing machines to remove almost all the serum. After pressing, the cheese was placed in maturing chambers with a controlled temperature ranging from 6 to 14 °C and a relative humidity between 85 and 95%. They were kept in these chambers for 35 days. During maturation, the cheese was washed with water every 15 days to remove possible exterior contamination.

### **Plants or extracts incorporation**

Five lots of 3 cheeses were produced under the same conditions: 1 lot represented the control, which was not incorporated with any plant or extract; 2 lots were reserved for incorporation of dried chestnut flowers and decoction extracts of these flowers; 2 lots were incorporated with lemon balm and its decoction extracts.

To determine the quantity of dried chestnut flowers extract to incorporate, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used. The  $EC_{50}$  value of the DPPH scavenging activity of pure chestnut flowers decoction has been previously reported by our research group [22]. This value, 99.47  $\mu\text{g/mL}$ , was then adjusted for the milk used for one cheese (0.250 litres), resulting in 248 mg of extract per cheese. The other lot was incorporated with dried flowers. The amount, 799 mg/cheese, was added based on the extraction yield of decoctions (31%). Therefore, 31% corresponded to the  $EC_{50}$  of the decoction (99.47  $\mu\text{g/mL}$ ), and 319.7  $\mu\text{g/mL}$  to a 100% yield. Considering the amount of milk used for each cheese, the final dried plant extract was obtained (799 mg/cheese).

The final two lots were also incorporated in the same manner, but with lemon balm. In this case, the  $EC_{50}$  value of the decoction extract was 60  $\mu\text{g/mL}$ , which corresponded to 380 mg/cheese. For the plant incorporation, by yielding 38.9% per decoction, each cheese was incorporated with 368 mg/cheese.

Just after the milk clotted and became semi-solid, it was processed through an automated process. The molds containing the cheese were let out of the machine and were manually placed in racks to initiate the pressing step. To incorporate the extracts in the cheeses, they were taken just before the pressing phase, and manually mashed. Finally they were thoroughly mixed together with the extracts and placed inside the molds to undergo pressing and further processing. All the subsequent steps of

maturation, refrigeration and washing were common to both the control samples and the incorporated ones.

### **Laboratorial preparation**

After one month of maturation, the cheeses were brought to the laboratory to be analysed. Initially they were peeled, cut into cubes, frozen and lyophilized. After lyophilisation they were submitted to various antioxidant assays to verify the bioactivity conferred by the plants and extracts.

### **Standards and reagents**

$\beta$ -carotene, ascorbic acid, iron chloride, and potassium ferricyanide were obtained from Alfa Aesar (Ward Hill, MA, USA). Folin-Ciocalteu's reagent, iron sulfate, phosphate buffer, sodium carbonate, thiobarbituric acid, trichloroacetic acid and Tween 80 were acquired from Fisher Scientific (Waltham, MA, USA). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA). All other materials and solutions were obtained from scientific retailers. All the water used in the methodology was treated with a purification system (TGI Pure Water Systems, Greenville, SC, USA).

### **Antioxidant assays**

The antioxidant activity assays were performed following a previously described methodology [24]. In order to determine the antioxidant activity, after lyophilisation, the cheeses were extracted with distilled water for 1 hour, filtered through a Whatman N°4 paper and further extracted for another hour. The resulting solution was evaporated under reduced pressure in a rotary evaporator and re-dissolved in water to obtain a



concentration of 200 mg/mL, of which successive dilutions were used. Reducing power was evaluated by the capacity to reduce  $\text{Fe}^{3+}$  into  $\text{Fe}^{2+}$ , measuring the absorbance at 690 nm in the microplate reader mentioned above. Inhibition of  $\beta$ -carotene bleaching was evaluated through the  $\beta$ -carotene/linoleate assay; the neutralization of linoleate free radicals avoids  $\beta$ -carotene bleaching, which is measured by the formula:  $(\beta\text{-carotene absorbance after 2 h of assay}/\text{initial absorbance}) \times 100$ . Lipid peroxidation inhibition in porcine (*Sus scrofa*) brain homogenates was evaluated by the decrease in thiobarbituric acid reactive substances (TBARS); the colour intensity of the malondialdehyde-thiobarbituric acid (MDA-TBA) was measured by its absorbance at 532 nm; the inhibition ratio (%) was calculated using the following formula:  $[(A - B)/A] \times 100\%$ , where  $A$  and  $B$  were the absorbance of the control and the sample solution, respectively. Trolox was used as positive control. The results of the antioxidant activity were expressed in  $\text{EC}_{50}$  value (sample concentration providing 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay). Total phenolics were determined by the Folin-Ciocalteu assay, measuring the absorbance at 765 nm. Gallic acid was used as a standard, and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

### **Fatty acids determination**

Fatty acids were determined by gas chromatography (GC) (DANI 1000, Contone, Switzerland) coupled to a split/splitless injector and a flame ionization detector (FID) [25]. The identification was carried out by comparing the relative retention times of the fatty acid methyl esters (FAME) to commercial standards. The quantification was

achieved through CSW 1.7 (DataApex 1.7, Prague, Czech Republic). The results were expressed in relative percentage of each fatty acid.

### **Colour measurements**

For the colour measurements, a Konica Minolta spectrophotometer (Konica Minolta, Chroma Meter CR-400, Tokyo, Japan) was used to determine the colour of the cheeses, with 6 readings on the top and bottom part. Illuminant C was used, with an 8 mm opening of the diaphragm. The CIE colour  $L^*$ ,  $a^*$  and  $b^*$  values were reported through the Spectra Magic Nx software (version CM-S100W 2.03.0006, Konica Minolta, Tokyo, Japan). The instrument was previously calibrated with standard white tiles.

### **Statistical analysis**

For the antioxidant assays and fatty acids analysis, and to have representative results, the lyophilized powder of two randomly chosen cheeses were joined for each case sample. All assays were carried out in triplicate and the data was expressed as means $\pm$ standard deviations, maintaining the decimal places allowed by the magnitude of standard deviation. The results for each parameter were compared through one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference *post hoc* test with  $\alpha = 0.05$ . All statistical analyses were carried out using the SPSS v.22.0 program (IBM Corp, USA).

## **Results and Discussion**

The choice of chestnut flowers (*Castanea sativa* Mill.) and lemon balm (*Melissa officinalis* L.) was based on previous studies of our research group, which proved their high antioxidant action *in vitro* [19, 23]. The very low EC<sub>50</sub> values justified their

inclusion into the cheeses to achieve a satisfactory functionalization, aiming that their high antioxidant activity would prove to have similar effects, although at a lower extent on the cheese, functionalizing this foodstuff that did not have any of these properties alone. This type of incorporation with plant extracts has been carried out for other foodstuffs, like snack crackers and pig patties, among other foodstuffs, with satisfactory results in terms of antioxidant and lipid peroxidation inhibition [26-30]. By providing functional properties to food through vegetables, their consumption is increased, translating into a higher intake of polyphenols and other antioxidant molecules that have proven healthy effects and which are found in vegetable tissues [31, 32].

Although solvents like methanol or ethanol could be used in the extracts preparation, this approach was not considered because these solvents are potentially harmful to humans. Therefore, the extracts for incorporation in cheese were prepared by decoction in water; plants were also directly incorporated in order to evaluate if it is worthy to prepare an extract or if the entire plant would be able to achieve the desired antioxidant properties in the “Serra da Estrela” cheese.

The EC<sub>50</sub> values for each antioxidant assay may be depicted from **Figure 1 A-D**. As a first remark it should be highlighted that the functionalized cheeses presented better antioxidant activity in all assays. In the reducing power assay, for the *M. officinalis* incorporation, the best results were obtained for the dried flower incorporations, while in the *C. sativa* incorporated cheeses, the decoction had slightly better activity. For the  $\beta$ -carotene bleaching inhibition, the incorporated cheeses with the *M. officinalis* decoction had the lowest EC<sub>50</sub> values, while for the *C. sativa* incorporations, once again a very slim difference favored the dried flowers. Regarding TBARS inhibition, for both incorporations, the dried flowers showed the best values. Finally, in terms of phenolics, the decoctions showed a higher quantity in both cases when compared to the dried

flowers incorporation. Overall, comparing the two types of functionalizing agents, dried plants tended to present the most effective antioxidant activity, except for *C. sativa* in the reducing power and *M. officinalis* in  $\beta$ -carotene bleaching inhibition, while the decoctions showed a higher amount of phenolics. These conclusions show that in terms of antioxidant activity, the decoctions may have lost some important molecules, probably due to the heating process to achieve the extraction (**Figure 1A-C**). On the other hand, the higher quantity of phenolics in the decoctions could be explained by the same process, with the heat aiding the extraction and therefore concentration of phenolics or reducing agents into the decocted extracts (**Figure 1D**). Nevertheless, none of the assayed plants showed unequivocal higher functionalizing power (as measured by the obtained antioxidant activity).

One interesting finding was the great increase in the lipid peroxidation inhibition capacity (as given by  $\beta$ -carotene bleaching inhibition and TBARS formation inhibition). This particular type of antioxidant activity might be much more useful to prevent the occurrence of undesired reactions in cheese, since this is a highly lipidic matrix.

The beneficial effects of functionalizing the cheese were also verified for the levels of phenolic compounds (**Figure 1D**), which were always higher in the functionalized formulations (except for cheeses added with lyophilized *M. officinalis*). The increased levels might be due to trigalloyl-HHDP-glucoside, pentagalloyl glucose and quercetin 3-*O*-glucoside, in chestnut flowers [23] and rosmarinic acid and luteolin-3'-*O*-glucuronide [18].

The antioxidant activity is usually correlated with the phenolics content of the matrices [16]. Still, in this particular case, after attempting different combinations between the assays and the phenolic content, the only correlation obtained was for the reducing power of the decoction of *C. sativa* flowers ( $R^2 = 0.0505$ ). The phenolic content of

chestnut flowers and lemon balm extracts has been reported previously by the authors, showing in both cases phenolic compounds with strong antioxidant activity [18, 22]. The lack of acceptable correlations in this case could be explained by the interferences and false positives that the methodology (Folin-Ciocalteu assay) provides, by reacting with all reducing species in the matrix [18]. Another surprising fact was the alleged presence of phenols in the control samples. Being the polyphenols a group of molecules belonging exclusively to plants, it would be unlikely that they were present in the control samples. Thus this false positive could be due to enzymes (glutathione peroxidase) [33], aminoacids (histidine, methionine, tryptophan, cysteine and tyrosine) [13] and organic acids (uric acid) [34] present in the milk, that, having reducing attributes, could have contributed to an apparent high quantity of phenols in this sample. To further understand the effect of the plant addition in the cheeses, the free fatty acids were detected through gas chromatography, coupled to a flame ionization detector. In all samples, 26 fatty acids were detected, and can be depicted on **Table 1** (containing only the fatty acids with a percentage over 2%). The most abundant fatty acids were saturated (SFA), followed by monounsaturated (MUFA) and finally, the least abundant were polyunsaturated fatty acids (PUFA). Individually, C18:1 (oleic acid) was the most abundant molecule, followed by C16:0 (palmitic acid). The detected fatty acids profile is in line with previous reports of these molecules for “Serra da Estrela” cheese [11], in which the most abundant fatty acids were the same in both manuscripts. To aid the understanding of results, an ANOVA was carried out, followed by a Tukey’s test to find statistical differences among the different incorporations. C4:0, C18:1, SFA and MUFA didn’t show any significant differences among the samples. Furthermore, the control samples did not show significant differences with the incorporated ones regarding all fatty acids with the exception of C4:0, C16:0 and C18:2. C6:0 was detected in a

statistically higher amount for the cheeses with *C. sativa* decocted flowers, and in a lower percentage for the samples with *C. sativa* dried flowers and decocted *M. officinalis*. Regarding C8:0, C10:0 and C12:0, the cheeses with decocted chestnut flowers showed the highest quantity, while there was no significant differences between the other samples, which showed the lowest values. In terms of C14:0, once again the highest values were recorded for the samples with chestnut decocted flowers, and the lowest values for this fatty acid were found in the cheeses with decocted lemon balm. For C16:0, the second most abundant fatty acid, the cheeses with dried chestnut flowers had the highest values and the lowest were recorded in the control samples. For C18:0 and C18:2, the samples with the least quantity of this fatty acid was the cheeses containing chestnut decocted flowers; the highest amount was observed in samples with dried chestnut flowers, although in C18:2, there were no significant differences between the cheeses with decocted chestnut flowers and lemon balm. These results underline a desired behavior of both plants in both formulations, by not altering in a significant way the fatty acids profile of this type of cheese.

Despite the successful inclusion of functionalizing agents, the final products must have an exterior appearance that allows their acceptability by the consumer. The products obtained with the decoctions of both plants were slightly darker than the control sample, while the inclusion of dried plants resulted in a spotted appearance, especially in the case of *M. officinalis* (**Figure 2**). Nonetheless, this change in the visual appearance is not likely to drive off the consumers, which might even look to this new feature and relate it with a healthier product (the presence of herbs is generally associated with desirable health effects) [35].

Besides evaluating the visual appearance empirically, the produced cheeses were evaluated by some technical parameters. The colour of the samples was measured with a

spectrophotometer using illuminant C at 2 degrees. The colour parameters CIE  $L^*$ ,  $a^*$ ,  $b^*$  values were measured and are shown on **Table 2**. The highest values reported for the  $L^*$  parameter belonged to the control sample, although it was not statistically different from the samples incorporated with *M. officinalis* decoctions. This parameter varies between black ( $L^* = 0$ ) and white ( $L^* = 100$ ), and all samples varied between 62 and 67, showing a very slight overall difference in lightness. The samples with the lowest value (darker samples) were those functionalized with chestnut flowers (**Figure 2**). The  $a^*$  value parameter measures the greenness-redness tendency, and all samples showed values close to 0, which indicated the absence of intense red or green colours, despite the incorporation of *M. officinalis* green dried plant. When positive, the  $b^*$  value indicates a yellow tone, while if negative, it indicates the presence of blue tones. All samples had positive and similar values, ranging from 22 to 24. Despite these similar values, for  $L^*$  all samples were significantly different between each other, apart from the cheese incorporated with lemon balm decoction which was not different from both the control sample and the chestnut flower decoction. The same happened with  $a^*$  values, in which the control sample was not statistically different from lemon balm, both for the dried plant and the decoction samples. Finally,  $b^*$  values also showed the same tendencies, with only one sample not being different from two others. In this case, the sample incorporated with dried chestnut flowers correlated with dried lemon balm and dried chestnut flowers. The reported differences in colour appearance could be an important factor in the marketing of cheeses, for the different colour could be pleasing to the potential consumer. In fact, to consumers, food appearance is favored when compared to flavor, being harder to sell a badly colored product than a badly tasting one. Studies regarding flavored yoghurts have shown that changing the colors of this

foodstuff increases intake, due to the fact that color catches the eye, directs attention and advertises that the food will be pleasant to eat [36].

## **Conclusions**

The functionalized cheeses showed higher overall antioxidant activity, when compared to the control samples. This improvement was especially noted for the lipid peroxidation inhibition ( $\beta$ -carotene bleaching inhibition and TBARS formation inhibition). The undoubted effects that these plants display towards health can be passed on to this dairy product, helping the dairy market to promote healthy foodstuffs. The external appearance can be something new, but in most cases the produced changes are not relevant. In fact, they might exert an appealing effect, due to the visible herbs (mainly in *M. officinalis*), with beneficial marketing effects. In general, no statistical differences were found in the fatty acids profile of control and incorporated samples.

Nevertheless, the search for other individual molecules as well as a complete nutritional profile should be carried out to see the alterations induced by these plants. At the same time, studies carried out during the maturation of the cheese are also interesting, allowing to understand the chemical (nutritional, antioxidant, toxicological) and physical (weight, colour, evaporation) transformations occurring during this period. Other concentrations, extraction methods and plants could also be employed to functionalize cheese. The introduction of innovation and development of new foodstuffs is always welcomed in the food industry, and moreover if these innovations bring bioactive properties to traditional foodstuffs.

## **Competing interests**

The authors declare no competing financial interests.



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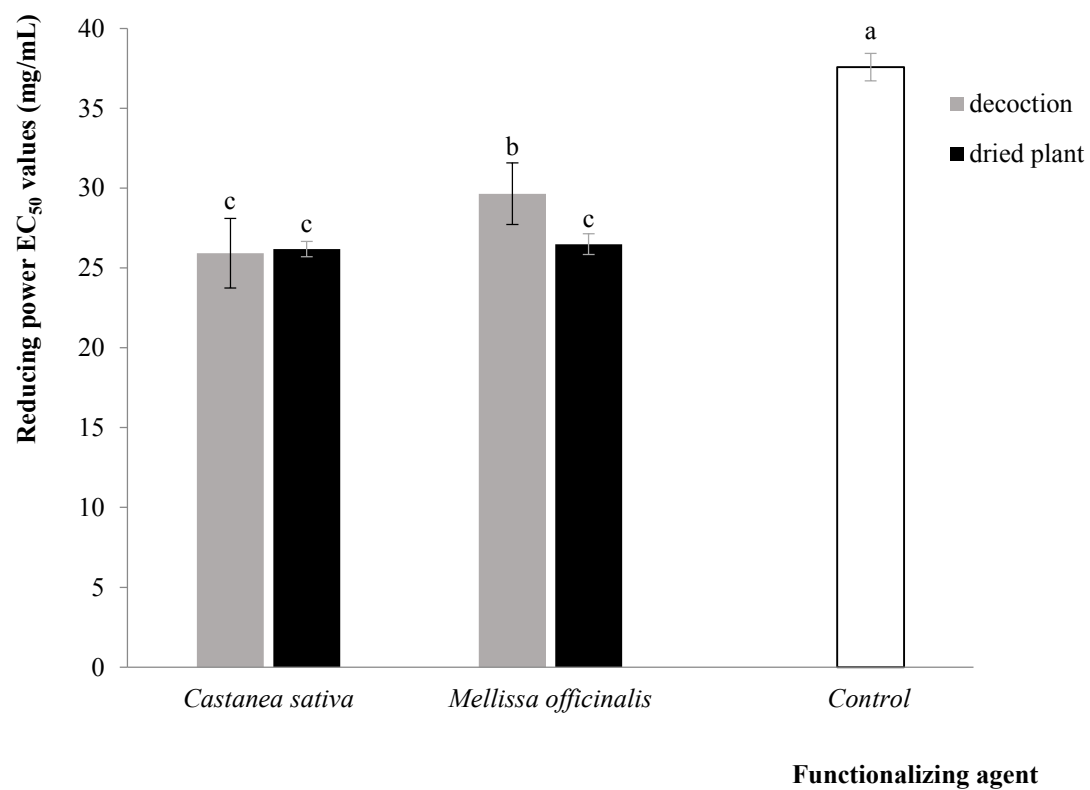
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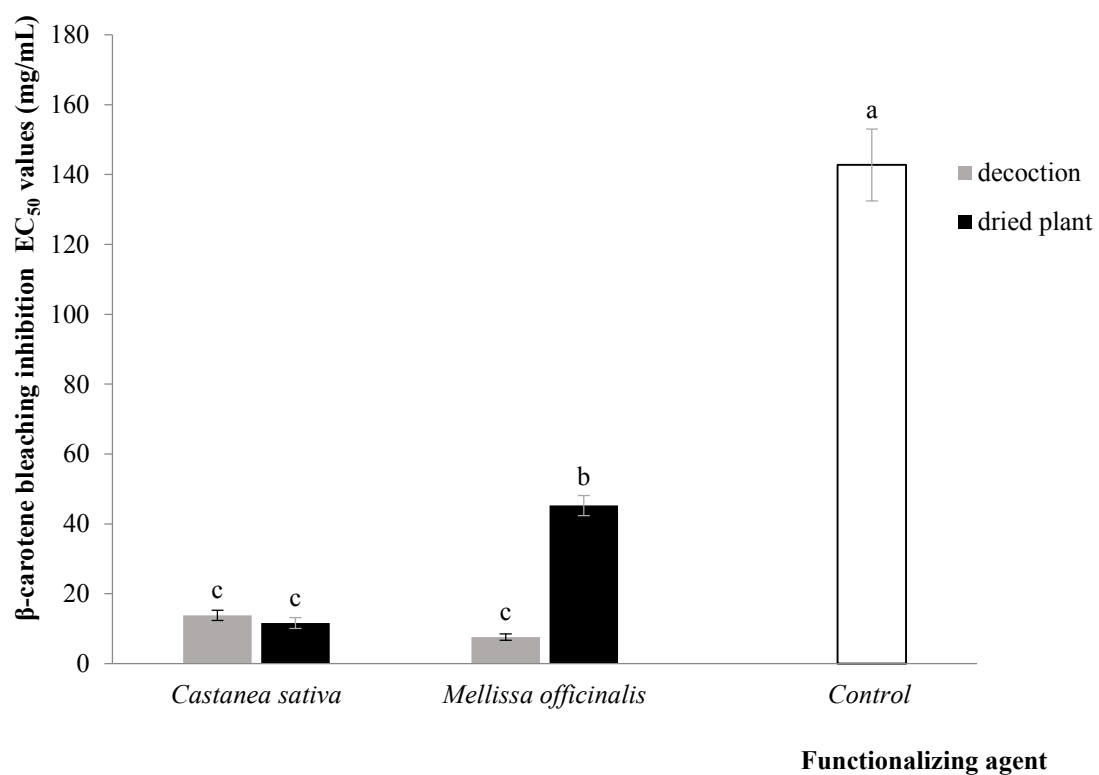
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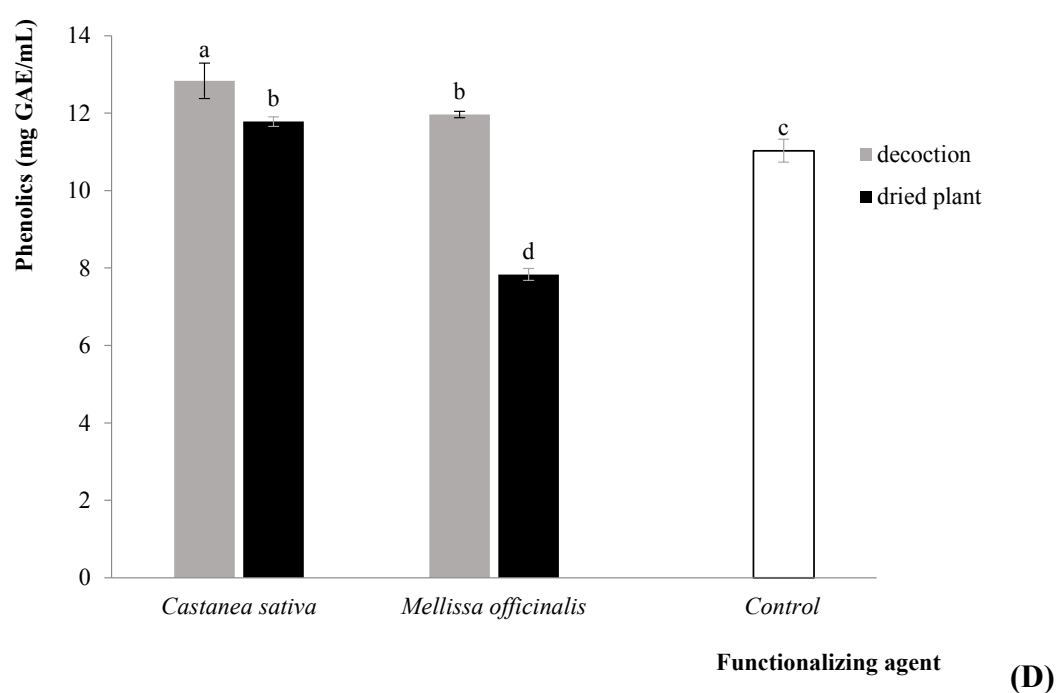
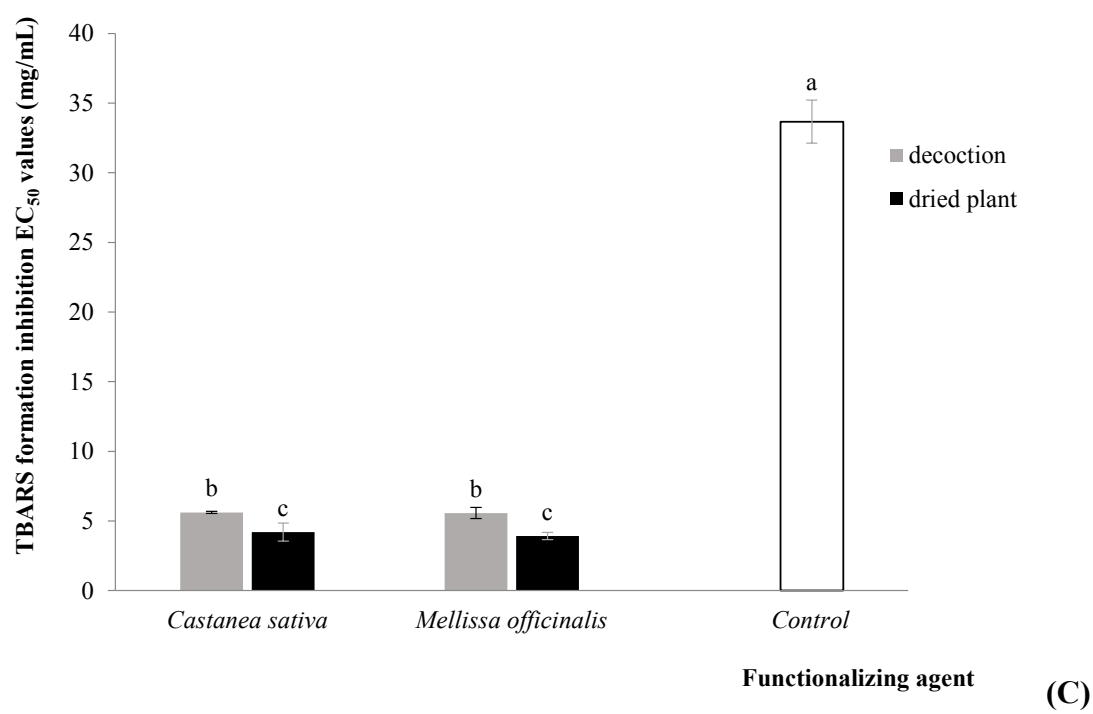
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(A)



(B)



**Figure 1.**  $EC_{50}$  values (mg/mL) for each antioxidant assay: (A) Reducing power; (B)  $\beta$ -carotene bleaching assay; (C) TBARS formation inhibition; (D) corresponding phenolic content (mg GAE/g extract). In each bar, different letters mean significant differences between samples ( $p < 0.05$ ,  $n = 10$ ).

**Table 1.** Most abundant fatty acids detected in the samples, represented in relative percentage. The results as represented as mean  $\pm$  SD (n = 9).

	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1n9	C18:2n6	SFA	MUFA	PUFA
Control	2.3 $\pm$ 0.2 <sup>a</sup>	2.6 $\pm$ 0.1 <sup>ab</sup>	2.4 $\pm$ 0.05 <sup>ab</sup>	6.9 $\pm$ 0.1 <sup>ab</sup>	4.3 $\pm$ 0.1 <sup>ab</sup>	10.6 $\pm$ 0.1 <sup>ab</sup>	23.3 $\pm$ 0.2 <sup>b</sup>	11.5 $\pm$ 0.3 <sup>ab</sup>	26.4 $\pm$ 0.2 <sup>a</sup>	2.60 $\pm$ 0.04 <sup>a</sup>	67.0 $\pm$ 0.1 <sup>a</sup>	25.5 $\pm$ 0.2 <sup>a</sup>	5.4 $\pm$ 0.1 <sup>ab</sup>
Dried chestnut flower	2.1 $\pm$ 0.1 <sup>a</sup>	2.3 $\pm$ 0.2 <sup>b</sup>	2.2 $\pm$ 0.13 <sup>b</sup>	6.4 $\pm$ 0.2 <sup>b</sup>	4.1 $\pm$ 0.1 <sup>b</sup>	10.3 $\pm$ 0.1 <sup>ab</sup>	24.1 $\pm$ 0.2 <sup>a</sup>	11.8 $\pm$ 0.3 <sup>a</sup>	26.9 $\pm$ 0.4 <sup>a</sup>	2.62 $\pm$ 0.05 <sup>a</sup>	66.5 $\pm$ 0.4 <sup>a</sup>	28.0 $\pm$ 0.4 <sup>a</sup>	5.4 $\pm$ 0.1 <sup>ab</sup>
Chestnut flower decoction	2.4 $\pm$ 0.1 <sup>a</sup>	2.7 $\pm$ 0.1 <sup>a</sup>	2.7 $\pm$ 0.01 <sup>a</sup>	7.4 $\pm$ 0.1 <sup>a</sup>	4.4 $\pm$ 0.1 <sup>a</sup>	10.7 $\pm$ 0.3 <sup>a</sup>	23.4 $\pm$ 0.1 <sup>b</sup>	10.6 $\pm$ 0.3 <sup>b</sup>	26.4 $\pm$ 0.1 <sup>a</sup>	2.38 $\pm$ 0.01 <sup>b</sup>	67.4 $\pm$ 0.1 <sup>a</sup>	27.6 $\pm$ 0.1 <sup>a</sup>	5.0 $\pm$ 0.2 <sup>b</sup>
Dried lemon balm	2.1 $\pm$ 0.1 <sup>a</sup>	2.4 $\pm$ 0.1 <sup>ab</sup>	2.3 $\pm$ 0.12 <sup>b</sup>	6.5 $\pm$ 0.4 <sup>b</sup>	4.1 $\pm$ 0.1 <sup>ab</sup>	10.6 $\pm$ 0.1 <sup>ab</sup>	23.8 $\pm$ 0.1 <sup>ab</sup>	10.7 $\pm$ 0.4 <sup>b</sup>	27.8 $\pm$ 0.5 <sup>a</sup>	2.59 $\pm$ 0.05 <sup>a</sup>	65.5 $\pm$ 0.5 <sup>a</sup>	30.0 $\pm$ 0.5 <sup>a</sup>	5.5 $\pm$ 0.2 <sup>a</sup>
Lemon balm decoction	2.0 $\pm$ 0.1 <sup>a</sup>	2.3 $\pm$ 0.1 <sup>b</sup>	2.21 $\pm$ 0.03 <sup>b</sup>	6.3 $\pm$ 0.1 <sup>b</sup>	4.0 $\pm$ 0.1 <sup>b</sup>	10.2 $\pm$ 0.1 <sup>b</sup>	23.8 $\pm$ 0.1 <sup>ab</sup>	11.5 $\pm$ 0.1 <sup>ab</sup>	28.1 $\pm$ 0.4 <sup>a</sup>	2.54 $\pm$ 0.02 <sup>ab</sup>	65.4 $\pm$ 0.3 <sup>a</sup>	29.2 $\pm$ 0.4 <sup>a</sup>	5.4 $\pm$ 0.1 <sup>ab</sup>

In each column, different letters mean significant differences between samples ( $p < 0.05$ ).



**Table 2.** Colour  $L^*$  (lightness),  $a^*$  (redness) and  $b^*$  (yellowness) of the cheese samples.

The results are presented as mean  $\pm$  SD (n = 12).

	$L^*$	$a^*$	$b^*$
Control	67 $\pm$ 2 <sup>a</sup>	-4 $\pm$ 1 <sup>cd</sup>	24 $\pm$ 1 <sup>a</sup>
Dried chestnut flower	62 $\pm$ 3 <sup>c</sup>	0 $\pm$ 1 <sup>a</sup>	22 $\pm$ 1 <sup>c</sup>
Chestnut flower decoction	65 $\pm$ 2 <sup>b</sup>	-2 $\pm$ 1 <sup>b</sup>	23 $\pm$ 1 <sup>bc</sup>
Dried lemon balm	63 $\pm$ 3 <sup>c</sup>	-4 $\pm$ 1 <sup>d</sup>	23 $\pm$ 1 <sup>b</sup>
Lemon balm decoction	65 $\pm$ 2 <sup>ab</sup>	-3 $\pm$ 1 <sup>c</sup>	24 $\pm$ 1 <sup>a</sup>

In each column, different letters mean significant differences between samples ( $p < 0.05$ ).



**Figure 2.** External appearance of the different cheeses after 1 month of maturation and before being submitted to the assays.