LUPINE PUBLISHERS

Concepts of Dairy & Veterinary Sciences

DOI: 10.32474/CDVS.2022.05.000202

ISSN: 2637-4749

Mini Review

Significative Inactivation of SARS-CoV-2 3CL- Protease by *Camellia sinensis* Galloylated theaflavins: Processing conditions to maximize TF-3,3'-DG content

Lisete Paiva[1,2], Catarina Rato[2,3] and José Baptista [1,2],*

[1]*Department of Science Physics, Chemistry and Engineering (DCFQE) and Institute of Agricultural and Environmental Research and Technology (IITAA), University of Azores, Portugal*

[2]*Faculty of Science and Technology, University of Azores, Delgada, São Miguel, Portugal*

[3]*Faculty of Medicine, University of Coimbra, Portugal*

***Corresponding author:** José Baptista, Department of Science Physics, Chemistry and Engineering (DCFQE) and Institute of Agricultural and Environmental Research and Technology (IITAA), University of Azores, 9500-321 Ponta Delgada, São Miguel, Portugal

Received: July 04,2022 **Published:** July 08, 2022

Abstract

Since the last decade of the 19th century, the *Camellia sinensis* tea plant is commercially produced in one unique place in Europe, the volcanic São Miguel Island of the Azores Archipelago (Portugal) [1,2]. The molecular constituents of *C. sinensis*, in particular the galloylated theaflavins, mainly theaflavin-3,3'-di-O-gallate (TF-3,3'-DG), have been reported to inhibit SARS-CoV-2 3-chymotrypsin-like protease (3CLpro), a crucial enzyme required for the cleavage of its polyproteins (1a and 1ab) to produce vital individual functional proteins for viral cell replication [3]. According to Ohgitani [4] the virus treated with galloylated theaflavins, particularly TF-3,3'-DG, at 100 μM showed less than 1/10.000 infectivity compared to untreated virus. The TF-3,3'-DG content increased 50.91% with increased fermentation time from 1 to 3 hrs at room temperature (RT) and increased 41.26% at 35 °C of fermentation temperature. Furthermore, TF-3,3'-DG increased 29.40% with increasing temperature from RT to 70 °C in short extraction time (1:30 hrs) and oppositely decreased 18.44% with increasing temperature from RT to 70 °C in long extraction time (16:00 hrs). Taking all of the *in vitro* reported studies by several research teams and the *in vivo* results comparing the COVID-19 infections (mortality per million of population) in high tea consumption (4.28%) as compared with low tea consumption countries [5], the aim of this study was to investigate the steps of tea manufacturing conditions which lead to maximum TF-3,3'-DG content and, given its potential impact as an inhibitor of the 3CLpro enzyme, to create a novel antiviral Azorean black tea.

Results

The Table 1 clearly shows a low percentage (average of 95.7% lower) of infections by SARS-CoV-2 (COVID-19 mortality per million population) in countries with a higher consumption of *C. sinensis* tea as compared to countries with low tea consumption. According to Storozhuk [6], *C. sinensis* tea constituents could reduce overall risks related to COVID-19. This implies that tea theaflavins may be effective in the prevention/treatment of COVID-19 or the amelioration of its severity. It is well known that theaflavins contents in different tea samples changed according to different plucking seasons and are affected by many factors, such as plant variety, origin of tea production, agronomic management, climate, as well as by the tea processing and storage conditions. The Table 2 shows the variability of the TF-3,3'-DG content in different processing conditions. The TF-3,3'-DG content increased 50.91% with increased fermentation time from 1 to 3 hrs at room temperature (RT) and increased 41.26% at 35 °C of fermentation temperature. Furthermore, TF-3,3'-DG increased from 3.98 to 5.15 mg/g DW with increasing temperature from RT to 70 °C in short extraction time (1:30 hrs) and oppositely decreased from 5.64 to 4.60 mg/g DW with increasing temperature from RT to 70 °C in long extraction time (16:00 hrs). On the other hand, the value of TF-3,3'-DG change in different parts of the tea plant showed higher content (three times more) in bud plus 1st and 2nd leaves as compared to 3rd plus 4th leaves (data not shown).

Table 1: Countries with "low" and "high" (above 150 g per/capita) tea consumption relatively to the total COVID-19 mortality per millions of people and percentage of COVID-19 mortality per total infections in different countries.

Country	Population in millions	Total COVID-19 Cases[a]	Total COVID-19 mortality[a]	COVID-19 mortality (per million population)[a]	% COVID-19 mortality per total cases[b]
High tea consumption countries					
China	1439.32	2139792	14631	10.2	0.68
Kenya	47.56	334249	5655	118.9	1.69
Mali	20.25	31171	737	36.4	2.36
Japan	125.8	9364954	31309	248.9	0.33
Low tea consumption countries					
Italy	59.55	18610011	168425	2828	0.91
Spain	47.35	12818184	108111	2283	0.84
Portugal	10.31	5171236	24149	2342	0.47
France	67.39	31410383	150624	2235	0.48

[a]Data from Johns Hopkins Coronavirus Resource Center on July 2, 2022; [b] Higher ratio means low support by Health Care Institutions for COVID-19 patients.

Table 2: Theaflavins content from *Camellia sinensis* in different processing conditions (mg/g DW)[a] .

Theaflavins	Extraction				Oxidation/Fermentation			
	1h30		16h00		1h00		3h00	
	RT	70 °C	RT	70 °C	RT	35 °C	RT	35 °C
TF	3.48±0.08	4.61±0.13	4.93±0.16	4.01±0.04	4.02±0.09	2.75±0.07	5.98±0.05	3.70±0.01
TF-3-G	3.38±0.02	4.11±0.07	4.82±0.05	3.52±0.13	5.93±0.13	4.65±0.06	6.93±0.03	5.38±0.03
TF-3'-G	3.31±0.07	4.25±0.04	4.72±0.08	3.88±0.08	4.46±0.11	2.88±0.04	5.43±0.06	3.57±0.04
TF-3,3'-DG	3.98±0.04	5.15±0.03	5.64±0.13	4.60±0.11	3.28±0.08	2.06±0.06	4.95±0.06	2.91±0.01

[a]Values are mean ± SD (n = 3); DW, dry weight; RT, room temperature; TF, theaflavin; TF-3-G, theaflavin-3-gallate; TF-3'-G, theaflavin-3'-gallate; TF-3,3'-DG, theaflavin-3,3´-di-gallate.

Conclusion

The TF-3,3'-DG content in Azorean black tea was affected by the different processing conditions. Countries with higher *C. sinensis* tea consumption presented lower SARS-CoV-2 infectivity. The best extraction time for all theaflavins is 16 hrs at room temperature and for fermentation time the higher values was observed at 3 hrs at RT as compared with 35 °C. This study revealed the possibility to create a novel Azorean antiviral tea, investigating the black tea processing conditions, to maximize the TF-3,3'-DG content as an inhibitor of 3CLpro enzyme and, consequently, reducing the SARS-CoV-2 infectivity as already reported by several research teams.

References

1. Baptista JAB, Tavares JFP, Carvalho RCB (1999) Comparison of catechins and aromas among different green teas, using HPLC/SPME-GC. Food Res Int 31(10): 729–736.
2. Baptista J, Lima E, Paiva L, Castro AR (2014) Value of off-season fresh Camellia sinensis leaves. Antiradical activity, total phenolics content and catechin profiles. LWT–Food Sci Technol 59(2): 1152–1158.
3. Zhu Y, Xie D-Y (2020) Docking Characterization and in vitro Inhibitory Activity of Flavan-3-ols and Dimeric Proanthocyanidinis Against the Main Protease Activity of SARS-Cov-2. Frontiers Plant Sci 11: 601316.
4. Ohgitani E, Shin-Ya M, Ichitani M, Kobayashi M, Takihara T, et al. (2021) Significant inactivation of SARS-CoV-2 in vitro by a green tea catechin, a catechin-derivative, and black tea galloylated theaflavins. Molecules 26(12): 3572.
5. (2021) Data from Johns Hopkins Coronavirus Resource Center.
6. Storozhuk M (2020) COVID-19: Could green tea catechins reduce the risks? medRxiv.

DOI: 10.32474/CDVS.2022.05.000202