

1 Article

2 *Cistus incanus* L. as an innovative functional additive 3 to wheat bread

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22 **Abstract:** *Cistus incanus* L. (CI) has been proposed as an innovative functional supplement of food
23 products, and hence the present study aimed to evaluate the effect of the addition of dried CI on
24 the properties of bread. Bread was prepared from white wheat flour supplemented with the
25 addition of 1%, 2%, 3%, 4%, and 5% of ground CI. After the completion of baking process, various
26 characteristics of the obtained bread product, such as yield, volume, porosity, acidity, color, and
27 texture, were evaluated. In addition, total phenolic content (TPC), ABTS radical scavenging
28 activity, CHEL chelating power, and ability to quench OH· radicals were measured. The results
29 showed that the addition of CI to bread caused a reduction in the volume of bread, but texture of
30 the crumbs was acceptable. Acidity and moisture content of bread were found to be increased
31 following CI enrichment. Significant changes in the ash content and the color of bread crumbs were
32 also observed. Bread incorporated with CI was characterized by significantly higher TPC and much
33 higher antioxidant activity, as measured by ABTS, CHEL, and OH· radicals, compared to control
34 bread. Supplementation of bread with 3% CI produced a product with desirable characteristics
35 which was also favored by consumers.

36 **Keywords:** *Cistus incanus*; wheat; bread; baking; physical properties; antioxidants

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39 1. Introduction

40 Cistaceae is a small family of plants comprising shrubs that are commonly found in the
41 Mediterranean climate [1,2]. The plants of *Cistus* species are used in the preparation of folk
42 medicines, in the form of anti-diarrheal agents, to provide protection against various skin diseases,
43 and as anti-inflammatory agents [2,3]. Different species of *Cistus* are used as herbal infusions, food
44 additives, or nutraceutical products [4].

45 *Cistus incanus* L. (CI) is included in the class of medicinal plants because it exhibits
46 anti-inflammatory, anti-microbial, cytotoxic, and anti-ulcerogenic properties [5]. The anti-microbial
47 potential of different extracts was tested against *Escherichia coli* and *Staphylococcus aureus* [1]. Other
48 studies demonstrated that CI has the ability to reduce the risk of caries disease caused by
49 *Streptococcus mutans* [6]. The anti-aflatoxigenic efficacy of the CI plant against *Aspergillus parasiticus*
50 was also studied [7]. The tested extracts of CI [8] inhibited the growth of *A. parasiticus* and *Aspergillus*
51 *carbonarius*.

52 The water extract of CI tea was tested to determine the concentrations of flavonols, organic
53 acids, vitamin B, and alkaloids present in the extract [5]. Attaguile et al. [2] investigated the effects of
54 the aqueous extracts of CI and *Cistus monspeliensis* on DNA cleavage, and their free-radical
55 scavenging capacity was also analyzed. The impact of the extracts on lipid peroxidation in rat liver
56 microsomes was also evaluated [9]. These products are characterized by a high content of phenolic
57 substances and strong antioxidant activity [9,10]. Gori et al.'s study [3] aimed to characterize the
58 major polyphenolic compounds present in a crude ethanolic leaf extract of CI.

59 Phenolic compounds can undergo severe changes when exposed to various processing
60 techniques [9]. Therefore, standardizing the processing parameters is of utmost importance.
61 Domcheva et al. [11] showed that the technique and conditions adopted for extraction process have a
62 significant impact on the yield of polyphenols and flavonoids, as well as on the antioxidant capacity
63 of the final product. In other study [12], the influence of plant parts and particle size of CI on the
64 extractability of phenolic compounds was demonstrated. Riehle et al. [9] investigated the influence
65 of the brewing process, involved in the preparation of CI herbal infusions, on their phenolic
66 compound profile.

67 In recent years, there has been a global trend toward the use of natural substances present in the
68 food as a source of antioxidants and functional ingredients. In particular, natural antioxidants
69 present in food have received considerable interest because of their safety profile and potential
70 nutritional and therapeutic effects [13]. Additional ingredients are added during the bread-making
71 process to obtain fortified bread with higher nutritive value. Dried or fresh herbs can be added to the
72 basic recipe of bread to obtain an enriched product [13–16]. For example, Zhumaliyeva et al. [17]
73 developed a technology to prepare bakery products possessing anti-diabetic properties using a
74 mixture of herbal supplements (rose, Jerusalem artichoke, Stevia leaf, and celery root).

75 Singh et al. [18] attempted to develop a functional formulation of bread by incorporating
76 shatavari (*Asparagus racemosus* Willd.), an important medicinal plant found in India. In other study,
77 bread products were supplemented with bioactive components such as green tea powder, herbs,
78 and tomato paste. The bread thus obtained was called as prebiotic antioxidant bread (pre-aox-bread)
79 [19]. The effect of addition of oregano in formulation was examined in order to obtain herbal,
80 antioxidant-enriched bread [20].

81 Dietary products supplemented with CI plant are increasingly offered by food manufacturers.
82 Skąpska et al. proposed the addition of herbal CI extracts to drinks like cloudy Aronia juice [21]. In
83 another study [22], CI leaves were proposed as an additive to pasta.

84 Till date, no research has been done to assess the effect of the addition of dried CI on the
85 properties of bread. Therefore, the present study aimed to determine the effect of the addition of
86 dried and ground leaves of CI on the final quality of wheat bread and to determine the appropriate
87 concentration of this additive that is acceptable to the consumers.

88 2. Materials and Methods

89 2.1. Raw material

90 The raw materials used for the preparation of bread dough were white wheat bread flour
91 (Polskie Młyny, Warsaw, Poland) and dried leaves of CI (Malwa Tea, Lubiszyn, Poland). The dried
92 leaves of *Cistus* were ground into particles (measuring below 0.3 mm) in a laboratory hammer mill
93 (POLYMIX-Micro-Hammermill MFC). Various concentrations of dried herbs (1%, 2%, 3%, 4%, and
94 5%) were added to the flour to obtain fortified bread. Compressed yeast (Lesaffre, Poland) and salt
95 (Kłodawa, Poland) were purchased from the local market.

96

97 *2.2. Chemical composition of raw material*

98 Total protein content in the wheat flour was determined by Kjeldahl method [23] using FOSS
99 Kjeltex 8200. Wet gluten content and gluten index were evaluated by mechanical means [24]. The
100 falling number of flour was determined according to Hagberg-Perten method [25]. Total fat content
101 was measured using solvent extraction method in a Soxhlet's extractor [26]. The ash content in flour
102 and herbs was evaluated by incinerating the sample in a muffle furnace at 900°C for 60 min [27].
103 Water content was determined using drying method [28].

104

105 *2.3. Baking procedure of bread*

106 The bread dough was prepared using a straight dough method [29] and as described previously
107 by Romankiewicz et al. [30]. The basic dough formula consisted of wheat flour (100%), water, salt
108 (1.5% flour basis), and compressed yeast (3%). Different bread compositions were prepared using
109 varying concentrations of CI: 1, 2, 3, 4, and 5%. Water was added to the flour according to
110 farinograph water absorption measurements (Farinograph model 810114, Brabender, Germany),
111 and was equal to 60.0% for the control bread and 61.5%, 62.8%, 63.7%, 64.0%, and 65.4% for bread
112 with 1%, 2%, 3%, 4%, and 5% addition of herbs, respectively. All ingredients were mixed and
113 fermented at 30°C and 75% relative humidity for 60 min (with 1 min transfixion after 30 min). The
114 dough was mixed in a laboratory mixer (SP 800A, Sweden) and after fermentation was cut into 250 g
115 pieces and then placed in the molds. The dough was proofed again for 60 min. The bread was baked
116 (230°C, 30 min) in an electric oven (Sveba Dahlen, Sweden). After baking, loaves were cooled and
117 packed in polyethylene bags and stored for 24 h at 20°C.

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119 *2.4. Physical properties of bread*

120 After the completion of baking process, the bread yield and baking losses were calculated. In
121 addition, bread volume, crumb porosity [30], and bread acidity were estimated as previously
122 described [31]. The loaf volume converted to 100 g of bread was determined using a 3D scanner
123 (NextEngine, USA) and calculated using a computer program (MeshLab). The porosity of crumbs
124 was measured by kneading and determining the differences in the volumes between uncompressed
125 and compressed crumbs.

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127 *2.5. Color measurements*

128 The color parameters of CI herb and synthesized bread samples were recorded using the
129 formula CIE-L*a*b*, where L* indicates lightness, a* redness/greenness, and b* yellowness/blueness
130 [30, 32]. The instrumental measurement of the color of dried herbs and bread products was carried
131 out using the Minolta (CR-200, Japan) photocolormeter [30]. The total crumb color differences, ΔE ,
132 between bread crumbs enriched with CI and control bread sample were calculated using the
133 formula as described previously [30, 32].

134

135 *2.6. Textural properties of bread crumbs*

136 The textural properties of the bread were analyzed using texture analyzer type TA.XT2i (Stable
137 Microsystems, Surrey, UK). A cylindrical samples (20 mm diameter) were cut from loaves slices with
138 a thickness of 20 mm and were compressed using a capital equipped with a 25 mm plug until a 15
139 mm depth at a crosshead speed of 1 mm·s⁻¹. The samples were compressed twice (curves 1 and 2) to
140 give a two-bite TPA (Texture Profile Analysis) curve [33], from which textural parameters, such as
141 hardness, springiness, cohesiveness, and chewiness, were calculated [30].

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143 *2.7. Total phenolic content*

144 The TPC was determined by performing solvent extraction of bread samples. One gram of
145 dried bread powder was extracted with 5 ml mixture of methanol and water (1:1, v/v, 30 min). Then
146 the extracts were separated by decantation in a centrifuge (15 min). The residues were extracted
147 again with 5 ml of methanol, and the extracts were combined and stored (-20°C, darkness). TPC was

148 determined according to the Folin–Ciocalteu method [34]. For each sample, 0.1 ml of extract was
149 mixed with 0.1 ml of distilled water, 0.4 ml of Folin’s reagent (1:5 H₂O), and later 2 ml of 10% Na₂CO₃
150 was added. The TPC was expressed in mg as gallic acid equivalents after measuring the absorbance
151 of the mixtures in a spectrophotometer (720 nm).

152

153 2.8. Antioxidant activity

154 Antioxidant activity of CI and bread samples was evaluated by estimating the DPPH radical
155 scavenging activity [35], the ABTS radical scavenging activity [36], the chelating power CHEL [37],
156 and the ability to quench OH· radicals [38]. All anti-radical activities were expressed as EC₅₀—
157 extract concentration (mg_{d.w.}·mL⁻¹) that provided 50% of antioxidant activity based on a
158 dose-dependent mode of action. EC₅₀ value (mg·mL⁻¹) is the effective concentration at which the
159 absorbance was 0.5 for RED and was obtained by interpolation from linear regression analysis. The
160 lower EC₅₀ value indicates a higher antioxidant activity.

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162 2.9. Sensory evaluation of bread

163 After 24 h of baking process, sensory analysis of bread was performed under stable temperature
164 and light conditions by a team of 67 trained panelists at Warsaw University of Life Sciences,
165 Warsaw, Poland. Bread was cut into slices, and samples were scored using a nine-point hedonic
166 scale according to the appearance, taste, smell, color, texture, and overall acceptability of the final
167 product, where 1 represents “dislike extremely”, 5 indicates neither like nor dislike, and 9 represents
168 “like extremely” [39].

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170 2.10. Statistical analysis

171 All tests were performed in four replicates. Statistical analyses of the obtained results were
172 performed using Statistica software (Statsoft Inc.) at a significance level of $\alpha=0.05$. The data were
173 analyzed using analysis of variance (ANOVA), and the means were compared using the Tukey’s
174 range test.

175 3. Results and Discussion

176 3.1. Physical properties of bread fortified with *Cistus incanus*

177 Wheat flour used as a basic raw material for bread baking was characterized by a protein
178 content of 11.3±0.01%, a wet gluten content of 27.7±0.14%, a gluten index of 91±0.71, a falling number
179 of 293±1.41 s, a fat content of 1.8±0.09%, and an ash content of 0.71±0.007%. As demonstrated in an
180 earlier study [40] and according to practical recommendations and adopted standards, wheat flour
181 used for bread baking should have a minimum protein content of 11% and falling number must not
182 be below 220 s. The value of gluten index indicates that the flour used in our study is characterized
183 by good technological quality [41].

184 The addition of CI to bread caused noticeable changes in baking properties. Bread yield (Figure
185 1a) was found to be significantly increased when 3% concentration of herbal sample was added. The
186 bread yield depends on the amount of water absorbed by the flour during the baking process. The
187 herbal samples of CI used in this study were characterized by 9.1±0.48% water content and caused
188 significant changes in the water absorption capacity of the bread sample, which was evident by
189 farinograph studies which showed an increase in absorption from 60.0% to 65.4%. Other studies
190 reported that oregano addition to bread formulation increased the water absorption capacity and
191 dough development time, which was associated with a significant decrease in dough stability [20].
192 Baking loss (Figure 1b) in our studies increased significantly when 3%, 4%, and 5% concentrations of
193 CI were used in bread formulation. The volume of bread decreased (Figure 1c) and the porosity of
194 bread (Figure 1d) increased gradually with an increase in the proportion of these herbs from 1% to
195 5%. A significantly lower volume of bread was observed for the 2% concentration of CI, whereas a
196 significantly higher porosity was characterized for bread enriched with a 4% share of herbs. A

197 similar trend was observed in other studies, where specific volume was found to be decreased at
 198 higher concentrations of green tea powder [42]. With an increase in the level of green tea extract, no
 199 significant difference with regard to the cell diameter (porosity) was observed in the histogram [43].
 200 The addition of green tea extract and encapsulates to bread formulation did not show any change in
 201 terms of volume, which was almost similar to control [44].

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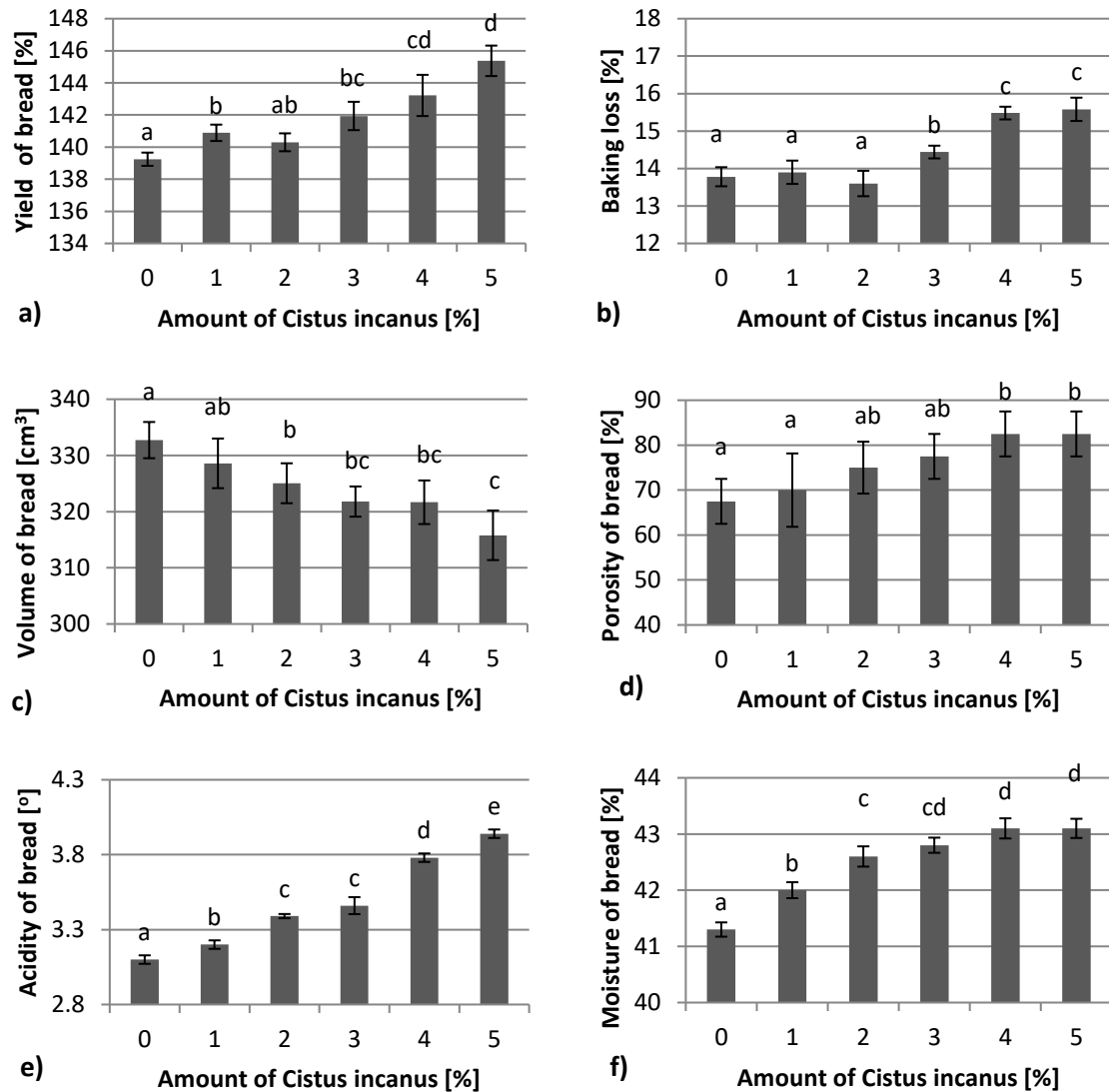


Figure 1. Baking properties of bread enriched with *Cistus incanus* L. additives.

Fortification of wheat bread with CI caused significant changes in acidity (Figure 1e) and moisture content of bread (Figure 1f). As mentioned earlier, higher moisture content could be attributed to higher water absorption capacity of dough. The acidity of bread could be increased by adding different kinds of additives. One study showed that acidity of bread can be altered by the addition of crushed safflower seeds [45] or dry powder of *Chamerion angustifolium* (L.) Holub leaves [31].

3.2. Color and texture of bread crumbs modified with CI

229 *Cistus incanus* L. used in this study was characterized by ash content of $5.0\pm 0.21\%$, and an
 230 addition of these herbs to bread resulted in significant changes in the ash content and color of the
 231 bread (Table 1). The differences in color parameters of breads are caused by the pigments present in
 232 CI. These results are consistent with previous studies, which reported that the lightness of crumb
 233 and crust decreased with increasing amounts of Moringa leaf powder, while the redness (a^*) value
 234 of crumbs increased with an elevation in the concentration of this additive [46].

235

236 Table 1. Ash content and color parameters of *Cistus incanus* L. (CI) and bread samples
 237 supplemented with this additive

Sample	Ash content [%]	L*	a*	b*	ΔE	
CI herbs	$5.01^c\pm 0.211$	$54.93^e\pm 0.75$	$0.68^b\pm 0.03$	$20.04^d\pm 0.44$	-	
Proportion of CI added	0%	$0.82^a\pm 0.018$	$82.44^a\pm 0.176$	$0.09^a\pm 0.003$	$10.35^a\pm 0.062$	-
	1%	$0.90^b\pm 0.037$	$75.71^b\pm 0.217$	$2.39^c\pm 0.038$	$10.65^a\pm 0.094$	7.20
	2%	$1.00^b\pm 0.041$	$72.89^c\pm 0.267$	$2.38^c\pm 0.065$	$12.67^b\pm 0.206$	9.99
	3%	$1.01^b\pm 0.086$	$72.64^c\pm 0.356$	$2.39^c\pm 0.037$	$12.69^b\pm 0.235$	10.12
	4%	$1.06^b\pm 0.027$	$70.90^d\pm 0.206$	$2.91^d\pm 0.063$	$14.45^c\pm 0.184$	12.21
	5%	$1.10^b\pm 0.064$	$69.98^d\pm 0.330$	$2.88^d\pm 0.065$	$14.59^c\pm 0.227$	13.09

238 Note: Means with different letter in the same column are significantly different ($\alpha=0.05$).

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240 The addition of CI caused small changes in the textural properties of bread (Table 2). There was
 241 no significant change in the hardness of bread with the supplementation of 1%, 2%, and 3% herbs
 242 when compared to the control bread. The higher proportion, in particular 5%, of this component
 243 contributed to an increase in the hardness of the final product. The bread formulated with a 4% share
 244 of these herbs was characterized by the least springiness. The cohesiveness of bread crumbs did not
 245 show a significant change with all concentrations of herbs compared to the control bread sample. A
 246 significant increase in chewiness was observed for bread fortified with a 5% CI. Similar results were
 247 obtained for bread incorporated with black tea extract, which showed little effect on textural
 248 properties [47]. The bread sample incorporated with green tea extract and encapsulates retained
 249 their quality in terms of crumb firmness which was almost similar to control [43]. The addition of
 250 shatavari (*A. racemosus* Willd.) to the investigated bread sample affected the textural properties by
 251 increasing its crumb hardness, adhesiveness, chewiness, and cohesiveness and by decreasing the
 252 springiness [18]. Crumb hardness, cell diameter, and chewiness characteristics of whole wheat pan
 253 bread increased at higher concentrations of green tea powder [41]. With an increase in the
 254 amount of green tea extract added, the brightness and sweetness of the bread decreased, whereas the
 255 hardness, stickiness, and astringency increased [42]. The hardness and chewiness of bread slightly
 256 decreased with the addition of 2.5% and 10% Moringa powder, whereas springiness was not found
 257 to be affected by this additive [46]. Many authors had previously established that textural attributes
 258 of bread crumbs are influenced by the nature and the amount of ingredients [48,49]. The changes
 259 related to bread crumb texture are probably a result of dilution of wheat gluten, change in the nature

260 of starch, and enrichment of fiber content due to addition of increased amounts of CI in our study
 261 [50].
 262

263 Table 2. Texture parameters of bread samples enriched with *Cistus incanus* L. (CI)

Sample	Proportion of CI	Hardness [N]	Springiness [-]	Cohesiveness	Chewiness
				[-]	[N]
	0%	12.9 ^a ±0.59	0.91 ^a ±0.031	0.61 ^a ±0.041	7.2 ^{ab} ±0.53
added	1%	12.7 ^a ±0.78	0.88 ^{ab} ±0.037	0.57 ^a ±0.038	6.4 ^b ±0.41
	2%	12.8 ^a ±0.68	0.89 ^a ±0.028	0.56 ^a ±0.031	6.5 ^b ±0.36
	3%	12.9 ^a ±0.94	0.88 ^{ab} ±0.045	0.55 ^a ±0.018	6.6 ^{ab} ±0.40
	4%	13.7 ^{ab} ±0.63	0.86 ^b ±0.019	0.56 ^a ±0.011	6.7 ^{ab} ±0.35
	5%	14.3 ^b ±0.46	0.89 ^a ±0.026	0.57 ^a ±0.026	7.4 ^a ±0.41

264 Note: Means with different letter in the same column are significantly different ($\alpha=0.05$).

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266 3.3. Total phenolic content and antioxidant activity of bread incorporated with *Cistus incanus* L.

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268 The bread sample incorporated with CI was characterized by significantly higher TPC already
 269 at 1% of the amount of this additive (Table 3). A steady increase in the concentration of CI caused
 270 gradual and significant changes in the content of phenolic acids, and a more than twofold increase
 271 was observed following the addition of 5% CI. Antioxidant activity measured by ABTS, CHEL, and
 272 OH[·] was also improved by the proposed additive. The ability to quench ABTS radical, measured by
 273 EC50 value of bread, was decreased by about two times with 1% CI. The least OH[·] radical
 274 scavenging activity and chelating power (CHEL) was observed for control bread, whereas these
 275 activities increased with increasing concentrations of CI in the formulated bread recipe. A few other
 276 studies confirmed the antioxidative potential of CI when included as a functional additive to pasta
 277 [22]. Antioxidative potential of bread was also improved by green tea powder, herbs, and tomato
 278 paste [19]. Bread supplemented with 1%, 2%, 3%, and 4% oregano samples showed high TPC and
 279 radical scavenging activity [20]. Black tea incorporation enhanced the antioxidant activity of Chinese
 280 steamed wheat bread [47]. Other results suggest that the addition of green tea powder at a
 281 concentration of 1.00 g/100 g of flour effectively increases the antioxidant activity of bread [41]. The
 282 TPC and antioxidant activity of bread extracts increased with the addition of Moringa leaf powder.
 283 The addition of Moringa powder already for 2.5% resulted in large increase in the content of TPC.
 284 With regard to ABTS scavenging capacity, high activity was observed for 5.0%, 7.5%, and 10% levels.
 285 Regarding OH[·] scavenging capacity, gluten-free breads fortified with 2.5–10% of Moringa leaf
 286 powder presented significantly higher activity comparing to control bread [46].

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291 Table 3. Total phenolic content and antioxidant activity of bread supplemented with *Cistus incanus* L.
 292 (CI)

Sample	[mg GAE·g ⁻¹ d.w. ⁻¹]		EC ₅₀ [mg d.w.·ml ⁻¹]		
	TPC	ABTS	CHEL	OH·	
Amount of CI added	0%	4.8 ^a ±0.06	156.9 ^a ±9.83	65.3 ^a ±0.36	39.9 ^a ±2.11
	1%	5.6 ^b ±0.06	77.5 ^b ±0.92	43.8 ^b ±2.35	35.0 ^b ±1.10
	2%	6.7 ^c ±0.10	48.6 ^{cd} ±1.25	40.7 ^{bc} ±0.58	26.9 ^c ±1.21
	3%	7.9 ^d ±0.25	38.3 ^{de} ±1.55	38.1 ^{cd} ±0.32	25.7 ^c ±0.83
	4%	8.3 ^e ±0.12	33.1 ^e ±1.15	36.7 ^d ±0.56	24.8 ^{cd} ±0.95
	5%	10.1 ^f ±0.06	24.8 ^e ±0.70	33.9 ^e ±0.83	22.5 ^d ±0.89

293 Notes: TPC—total phenolic content; ABTS—ability to quench ABTS radicals; CHEL- chelating power;
 294 OH·—ability to quench OH· radicals. Values in the same column not sharing same letters are significantly
 295 different ($\alpha=0.05$).

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297 3.4. Sensory evaluation of bread enriched with *Cistus incanus* L. herb

298 Sensory analysis (Table 4) showed that the addition of CI did not cause any significant change
 299 in the appearance of the bread loaves. According to the evaluators, highest scores for the appearance
 300 were obtained for breads with a 3% addition of dried herbs and the lowest scores for breads with a
 301 5% addition, but these differences were not significantly different from the scores observed for the
 302 control bread. In terms of taste and smell, statistically significant differences were noted for bread
 303 samples fortified with 4% and 5% CI. These bread products were characterized by the lowest value
 304 compared to the control sample. Regarding the texture of crumbs, there was no statistical difference
 305 when compared to control wheat bread and bread incorporated with 1%, 2%, 3%, and 4.0% CI. Bread
 306 crumbs with 5% of herbs were characterized by the lowest score which was significantly different
 307 from the control bread. The least scores for overall sensory attributes were obtained for bread with
 308 4% and 5% CI, and addition of CI up to 3% concentration resulted in a product that was rated as
 309 desirable, thus obtaining a score comparable to the control sample.

310

311 Table 4. Sensory analysis of wheat bread enriched with *Cistus incanus* L. (CI)

Amount of CI added	Sensory attributes				
	Appearance	Taste	Aroma	Texture	Overall acceptability
0%	6.3 ^a ±0.62	6.7 ^a ±0.76	6.8 ^a ±0.93	7.0 ^a ±0.87	6.7 ^a ±0.84
1%	6.4 ^a ±0.87	6.6 ^a ±0.79	6.9 ^a ±0.85	6.6 ^{ab} ±0.73	6.5 ^a ±0.73
2%	6.7 ^a ±0.91	6.1 ^{ab} ±0.59	6.5 ^{ab} ±0.74	6.4 ^{ab} ±0.81	6.5 ^a ±0.82
3%	6.8 ^a ±0.83	5.6 ^{ab} ±0.63	5.8 ^{ab} ±0.62	6.3 ^{ab} ±0.75	6.4 ^a ±0.81
4%	6.0 ^a ±0.59	5.1 ^b ±0.71	5.3 ^b ±0.63	6.0 ^{ab} ±0.79	5.2 ^b ±0.37
5%	5.5 ^a ±0.64	5.0 ^b ±0.65	5.2 ^b ±0.59	4.9 ^b ±0.56	5.1 ^b ±0.44

312 Note: Values followed by the same letter in the same column are not significantly different ($\alpha=0.05$).

313 Till date, no research has been conducted to determine the effect of dried CI leaves on bread
314 characteristics. We are the first to carry out such a study with CI leaves; moreover, similar findings
315 were reported for pasta supplemented with CI leaves [22]. However, the proportion of this additive
316 should not exceed 3%, as it results in a change of sensory characteristics. Wheat bread can be
317 supplemented with the same amount of Moldavian dragonhead leaves [51]. Other studies
318 conducted on bread enriched with different herbs have shown their effect on sensory characteristics.
319 These results suggested that oregano can be added up to 2% level to bread sample without causing
320 any major change in baking and sensory properties, and at the same time exhibiting a better shelf life
321 [20]. The optimum acceptable level for shatavari in wheat bread was found to be 3.5 % [18].
322 Deterioration of the organoleptic properties of bread products with herbs can be explained by the
323 presence of essential oils that cause a specific taste and smell [52].

324 5. Conclusions

325 The addition of CI to bread caused significant changes in the baking properties. Bread yield and
326 baking loss were found to be significantly increased when 3% herbs was added. The volume of
327 bread was reduced with the increasing concentration of proposed herb. Acidity and moisture
328 content of bread were increased when CI was used as an additive. Significant changes in ash content
329 and color of bread crumbs were also observed. Unlike other parameters, no significant changes with
330 regard to textural properties were noted, and only 5% addition of herbs caused significant
331 deterioration in the texture parameters.

332 The bread sample incorporated with CI was characterized by significantly higher TPC and
333 much higher antioxidant activity, as measured by ABTS, CHEL, and OH· radical scavenging
334 activities, when compared to control bread sample.

335 Sensory analysis showed that the highest scores for appearance were obtained for bread
336 products fortified with a 3% addition of dried herbs and the lowest scores for bread samples with a
337 5% addition. Supplementation of bread with up to 3% concentration of CI produced a desirable
338 product, which was comparable to the control sample and acceptable to the consumers.

339

340 Author Contributions:

341 Conceptualization, Grażyna Cacak-Pietrzak and Dariusz Dziki; Data curation, Grażyna Cacak-Pietrzak,
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347 Visualization, Renata Różyło; Writing – original draft, Renata Różyło; Writing – review & editing, Grażyna
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349

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