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Effect of replacing olive oil with oil blends on physicochemical and sensory properties of taralli

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CHEMICAL-PHYSICAL AND SENSORY EVALUATION OF ITALIAN CEREAL-BASED SALTED SNACKS MADE WITH DIFFERENT OILS

Abstract

 Edible fats and oils is usually taken into consideration to produce bakery products for technological and economic reasons, but the nutritional and sensory quality of the final product must be considered. Four different lipid mixtures (high oleic sunflower oil, 87.5% extra virgin olive oil + 12.5 sunflower 28 oil, 75% extravirgin olive oil + 25 sunflower oil and 87.5% extra virgin olive oil + 12.5 rice oil) were used in the taralli production as an extravirgin olive oil alternative. The lipid fraction, rheological and sensory characteristics and oxidative evaluation of final products were studied. The use of the blend made of 75% extravirgin olive oil and 25% rice oil resulted in the best compromise for the content of sterols, tocols, and antioxidants, resulting in a combination able to give an induction period to the accelerated oxidation test that was comparable to that of the reference control containing only extravirgin olive oil.

 Keywords: salted snack, lipid mixtures, lipid characterization, rheological characteristics, oxidative evaluation.

> Institute of Food Science and Technology

1. Introduction

 Baked foods serve as one of the main staple food sources for consumers, and lipids are one of the principal classes of molecules present in this kind of food. Lipids play an important role in most baked products, influencing the nutritional and physical characteristics of the final product. In fact, lipids in baking contribute to air incorporation, heat transfer, tenderness, moisture, mouthfeel, lubricity, flavor, structure and shelf life (Manley, 2011). For these reasons, a wide choice of edible fats and oils is usually taken into consideration to produce the various kinds of bakery products. The lipid choice is often driven by technological and economic reasons, but it is important to also consider the nutritional and sensory quality of the final product.

 One of the simplest ways to improve the nutritional, organoleptic and technological properties is blending oils with different characteristics. Through the blends obtained by mixing oils already used for foods, it is possible to achieve structured oils with an improved composition (e.g., higher content of oleic acid, reduced content of saturated fatty acids, excellent ω-3/ω-6 ratio, greater content of minor lipid bioactive compounds), meeting the interest of modern consumers who are increasingly looking for natural and little manipulated foods.

 Taralli are common Italian salted snack foods that are usually formulated with wheat flour, oil, water, salt and white wine. The lipid fraction is traditionally represented by olive oil or extra virgin olive oil (EVOO), making up approximately 20% of the product formulation to ensure adequate crispiness and consistency. EVOO is well recognized as a health-promoting ingredient due to the abundant presence of polyunsaturated fatty acids, phytosterols, tocopherols and phenolic compounds (Farinetti et al. 2017; Salas-Salvadó et al. 2018). However, other vegetable oils or new blends thereof could be used to improve the shelf life and nutritional quality of the products in which they are included. Caponio and collaborators (Caponio et al. 2009) investigated taralli prepared with palm oil and found that the kneading phase caused an increase in the primary and secondary oxidation compounds, accompanied by a decrease in the unsaturated fatty acids. To maintain a high content of unsaturated fatty acids, other oils can be used, such as sunflower oil or high-oleic sunflower oil (HOSO), the latter presenting

 a percentage of oleic acid of approximately 80%, which makes HOSO similar to olive oil due to its acidic composition and represents a good characteristic for the cardiovascular system, reducing LDL cholesterol in blood (Allmann-Farinelli et al. 2005). Rice oil is characterized by important antioxidant 67 properties thanks to the high level of tocopherols and oryzanols, with γ -oryzanol in particular (Rodriguez-Estrada et al. 2017) and a pattern of fatty acids made of palmitic acid (12-18%), oleic acid (40-50%) and linoleic acid (29-42%). Coconut oil (CO) shows a significant content of medium- chain fatty acids such as lauric (45-53%), myristic (16-21%), palmitic (7-10%), stearic (2-4%), oleic 71 (5-10%) and linoleic (1-2%) acids that are easily digestible from the human body (DebMandal $\&$ Mandal 2010; Rodriguez-Estrada et al. 2017). These medium-chain fatty acids also have antiviral, anti-inflammatory, antibacterial and anti-obesity effects (Gopala Krishna et al. 2010).

 All these factors considered, the aim of this study was to formulate innovative blends of oils as alternatives to those already used for taralli production. In addition to the lipid fraction characterization, rheological, sensory and oxidative evaluations of the taralli samples were carried out.

2. Materials and Methods

2.1. Materials

 Wheat flour type 0, extravirgin olive oil (EVOO), high oleic sunflower oil (HOSO), sunflower oil (SO), rice oil (RO), coconut oil (CO), white wine and sodium chloride for the preparation of taralli were purchased at a local supermarket. All solvents and chemicals used were of analytical grade and 84 were purchased from Sigma-Aldrich (St. Louis, MO; USA).

2.2. Samples

 The taralli samples were formulated with the following ingredients: 59% wheat flour 0 type, 24% white wine, 16% fat and 1% salt. In particular, five different types of lipid fractions were used, as shown in Table 1.

 The ingredients were weighed and mixed with a professional kneader equipped with a hook mixing tool until the dough reached a suitable consistency (approximately 10 minutes). Successively, the dough was left relaxing for 20 minutes and then shaped manually in small rings (typical taralli shape). The cooking phase was performed in two stages: 1) boiling in water until the product rose to the surface and 2) approximately 35 minutes in a rotational oven at 180 °C. After cooling, taralli were stored in closed bags, leaving the same head space for each sample, and stored at room temperature.

2.3. Lipid extraction

 According to the AOAC Official Method, the lipid fraction of ground taralli (10 g) was extracted with *n*-hexane by using a Soxhlet apparatus (Behr Labor-Technik, Fischer Scientific Italia, Milano). Each extraction was performed twice $(n=2)$.

2.4. Fatty acid analysis

The fatty acid composition of taralli and relative lipid matrices was determined as fatty acid methyl esters (FAMEs) by capillary gas chromatography analysis after alkaline treatment according to Marzocchi et al. (2018). FAME composition was measured in 2 replicates for each lipid extract $(n=4)$.

2.5. Tocols analysis

 For the tocol determination, approximately 0.05 g of fat was dissolved in 0.5 mL of *n-*hexane. The solutions were filtered through a 0.45 µm nylon filter. The tocols were determined by HPLC (Agilent 1200 series, Palo Alto, CA, USA) equipped with a fluorimeter detector (Agilent, Palo Alto, CA, USA) according to Ben Lajnef et al. (2017). Analysis was achieved in 2 replicates for each extract $(n=4)$.

2.6. Sterols analysis

 To determine the phytosterol content, 0.5 mL of dihydrocholesterol (c =2 mg/mL) was added to 250 mg of oil, and saponification was conducted at room temperature according to Sander et al. 1989.

 Before injection, samples were silylated (Sweeley et al. 1963), and sterol separation was performed by GC/MS (GCMS-QP2010 Plus, Shimadzu, Tokyo, Japan) under the same chromatographic conditions reported by Cardenia et al. (2012). Phytosterol identification was achieved by comparing peak mass spectra with peaks of a standard mixture and by comparing them to the GC/MS data reported in the literature (Pelillo et al. 2003). Analysis was conducted in 2 replicates for each lipid extract $(n=4)$. 10 119 15 121

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2.7. Oxidative stability with OXITEST®

Ten grams of ground taralli were placed in the appropriate oxidation reactors in the $OXITEST[®]$ instrument (Velp Scientific, Usmate Velate, MB, Italy) at 90 °C and 6 bar of oxygen pressure, as reported by Riciputi & Caboni (2017). The analysis was repeated twice for each replicate (*n*=2). 24 125 26 126

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2.8. Texture analysis

 The fracturability hardness and consistency of taralli were determined with a texture analyzer TA-XT2i (Stable MicroSystem) using a cell load of 25 kg and a P/2 probe for the penetration test. The test settings were as follows: prespeed 2,0 mm/s; test speed 2,0 mm/s; postspeed 2,0 mm/s; distance 50,0%; trigger value 0.010 kg. The test allowed us to determine the sample height (mm), the fracturability, as force for the first rupture (g) , registered on the sample after penetration, the hardness as the maximum force (g) and the consistency measured as the area of the penetration graph (g x s). 136 Each result was expressed as the mean of at least 10 repetitions \pm standard deviation. 38 131 40 132 42 133 134 47 135

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2.9. Sensory analysis

The descriptive sensory aspects of taralli were evaluated by a panel of ten trained assessors recruited from the staff of the Department of Agricultural, Environmental and Food Sciences of the University of Molise for their experience and familiarity with the product. The different samples were randomly 56 139

 aroma and flavor (overall aroma, wine aroma, overall flavor, cereal flavor) and 5 for the tactile/textural sensations (crispiness as the crushing at first bite, consistency as resistance to chewing, friability, fat perception on the palate and palatability, i.e., the ease of swallowing the product after chewing). The panelists rated the intensity of each attribute using a grading scale from 1 to 9, where 1 indicated the absence of sensations and 9 the maximum intensity of sensations. Scores for two replicates and averages were calculated. Furthermore, the assessors were asked to give an additional to express levels of satisfaction/appreciation for the following attributes: appearance, shape, overall aroma, overall flavor, crispiness, and palatability.

2.10. Statistical analysis

 The relative standard deviation was obtained for all data collected. One-way analysis of variance (ANOVA) was evaluated using Statistica 8 software (2006, StatSoft, Tulsa, OK, USA). *p* Values lower than 0.05 were considered statistically significant using Tukey's honest significant difference 156 (HSD) test. All chemical analyses were carried out in 2 replicates for each extract ($n = 4$ for each sample).

Three-way ANOVA was used to evaluate the output of the sensory analysis, and the results were expressed according to Fisher's least significant difference (LSD) test.

- **3. Results and Discussion**
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3.1. Determination of fatty acids

As shown in Table 2, a total of 14 fatty acids were identified and quantified in oil, oil blends and in the respective taralli. Monounsaturated fatty acids (MUFA) represented the principal class of FA in all samples, with a content in a range of 72-83% for the raw lipidic matrices and 68-81% for taralli. Saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) were detected in the range of 8- 16% and 7-15%, respectively. In general, PUFA showed an increase in all the taralli samples 54 164

 compared to their respective lipidic matrices, mainly reported by Tctrl, TC and TD. The TA, TB and TC samples also reported an increased SFA concentration with respect to the corresponding lipid fraction. The same samples presented significant decreases (approximately 3-6%) in MUFA content, while Tctrl and TD did not show significant differences in their lipidic matrices. These changes could be linked both to the presence of fatty acids in the taralli of wheat flour and to the influence of the process, particularly the cooking step.

The individual fatty acid profile of taralli totally reflected the profile of the lipidic matrices used in their formulation. In fact, lauric acid (C12:0) was registered only in the HOSO:CO (4.4%) lipid blend and in its corresponding final taralli, TD (4.3%). Oleic acid (C18:1*cis9*) was the most abundant fatty acid in all the raw and final samples; in particular, HOSO and TA taralli made with 100% HOSO as the lipid ingredient showed the highest contents of C18:1*cis9* (82.5% and 80.5%, respectively). Palmitic acid (C16:0) and linoleic acid (C18:2*n6*) were the most abundant SFA and PUFA, respectively, found in the samples. In particular, palmitic acid registered the highest values in EVOO (13.4%) and EVOO:RO (12.4%) blend and in the taralli where these lipidic matrices were used, thus Tctrl (12.1%) and TC (13.6%), respectively, whereas EVOO:SO blend showed the highest percentage of linoleic acid (13.5%) as it was for its corresponding taralli, TB (14.3%). The fatty acid profiles of these vegetable oils were consistent with the results already found in the literature (Boskou et al. 2006; Chowdhury et al. 2007; Ichihara et al. 2021). 17 174 22 176 24 177 26 178 31 180 33 181 38 183 40 184 42 185

- 22 194 24 195 26 196 31 198 33 199 38 201 52 207
	- **3.2. Determination of tocols**

 Tocols are important for their health role as potent anticancer, antioxidant, immune stimulatory, anti- inflammatory, and nephroprotective agents (Belo et al. 2017). As reported in Table 3, six tocols were identified and quantified in all raw lipidic and taralli samples in this order of elution: α -tocopherol, α-tocotrienol, β-tocopherol, γ-tocopherol, β-tocotrienol and δ-tocotrienol. α-Tocopherol was the principal tocol in all samples, with a content ranging from 18 to 35 mg/100 g of fat. Among raw lipidic matrices, the EVOO:SO blend reported the highest value of α-tocopherol (35 mg/100 g of fat), followed by HOSO:CO and EVOO:RO samples with similar contents (~30 mg/100 g of fat) and HOSO and EVOO with concentrations of 27.7 and 27.4 mg/100 g of fat, respectively. 10 189 15 191 17 192

 β-Tocotrienol was found only in all final formulated taralli and not in the raw materials because it is a typical tocol of grain flour, as reported in the literature (Panfili et al. 2003; Engelsen & Hansen, 2009). Sample TC, formulated with EVOO:RO, showed the highest (p<0.05) concentration of total tocols (43 mg/100 g of fat), reflecting the raw material trend, where the EVOO:RO blend registered the highest concentration of tocols $(50.3 \text{ mg}/100 \text{ g} \text{ of } \text{fat})$ among lipids. In fact, these taralli and this 200 lipidic fraction reported the highest content of α -tocotrienol and γ -tocopherol compared to the other samples.

TB and TD, formulated with EVOO:SO and HOSO:CO, followed by TC in the total tocols content, showing a concentration of 29.5 and 28.6 mg/100 g of fat. Last, samples Tctrl and TA showed the significantly ($p<0.05$) lowest concentration of tocols, with values of 26.1 and 24 mg/100 g of fat, respectively. In general, the results showed a decrease in the total tocolic content from the lipid raw 206 materials to the final products, and in particular, α -tocopherol showed a decrease of approximately 40% due to its thermolability at heating temperatures (Kiczorowska et al., 2019). 40 202 45 204 47 205

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3.3. Determination of sterols

 Sterols represent a group of important health compounds and potent antioxidants of vegetable oils, and it is well known that their consumption can significantly lower the levels of serum LDL cholesterol (Moreau, 2015). In accordance with the reported literature on plant phytosterols, a total of 8 sterols were identified and quantified in the raw lipid matrices and in the final taralli samples (Table 4). Similar to the tocopherols for the sterol component, the lipid mixture EVOO:RO and its corresponding taralli TC represent the richest samples $(472.3 \text{ mg}/100 \text{ g of fat})$, reporting the highest 215 (p<0.05) content of the principal compounds β-sitosterol (241.3 mg/100 g of fat), stigmasterol (30 mg/100 g of fat) and avenasterol (19.6 mg/100 g of fat), as already reported in the literature (Moreau, 2015; Liu et al., 2020). The following sample with a high sterol content was TA with a concentration of 308 mg/100 g of fat, showing the highest ($p<0.05$) content of avenastanol (52.5 mg/100 g of fat) compared to other taralli samples. TB (EVOO:SO) and TD (HOSO:CO) showed phytosterol concentrations of 259.5 and 279.2 mg/100 g of fat, respectively, whereas sample Tctrl, formulated with 100% EVOO, reported the lowest ($p<0.05$) phytosterol content (225.3 mg/100 g of fat) among 222 all the taralli samples. In addition, this sample was also the only one without stigmasterol and Δ^7 avenasterol in its sterol profile.

Unlike tocols, taralli samples reported a slightly higher concentration of phytosterols than their corresponding lipidic fractions used in formulation. This increase could be due to the contribution of the other ingredients, first of all wheat flour and to the hydrolysis of sterols from steryl esters or γ orizanol during baking (Mandak & Nyström, 2013). As widely reported in the literature, wheat is a good source of sterols and is mainly characterized by sitosterol, campesterol and the corresponding saturated forms of stanol and stigmasterol (Rajhi et al. 2020; Loskutov & Khlestkina, 2021)

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3.4. Oxidative stability of taralli

 The oxidative stability of taralli was tested to evaluate the impact of the different lipidic fractions used in the formulation on the oxidative quality of the final product. The results obtained with OXITEST® (Table 5) are expressed as the *induction period* (IP) in hours (h), which is the time required to obtain a complete oxidation cycle of the samples. Tctrl and TC, formulated with EVOO and EVOO:RO, respectively, presented the highest (p <0.05) IP values equal to 38.7 and 40.4 h, respectively. In addition to the presence of tocols and sterols, the highest oxidative stability of these 239 two samples could also be due to the elevated content of phenolic compounds in EVOO and γ oryzanol in rice oil, which is a compound with well-known antioxidant properties (Massarolo et al. 2017; Jung et al., 2017). The other samples, TA, TB and TD, reported significantly lower $(p<0.05)$ IP values than Tctrl and TC and without significant differences among them (23.0, 20.7 and 23.5 h, respectively). For these taralli samples, the high percentage of MUFAs and PUFAs (Table 1) could influence their oxidative stability more than their total antioxidant content. Considering the literature (Marzocchi & Caboni, 2018), these results can be considered satisfactory; in fact, in this study, taralli formulated with sunflower oil and the consequent addition of a synthesized antioxidant compound reported IP values of 6-23 hours.

3.5. Texture and sensory analysis

Taralli samples were tested for their texture characteristics through the penetration test. The heights of the products were very similar, with values varying between 11.68 (\pm 0.89) and 12.02 (\pm 0.26) mm. Figure 1 shows the response to the test in terms of fracturability and hardness. No differences were detected among samples for the above parameters. The high variability of samples was confirmed by the high values of SD, which was mainly attributable to the hand-made process of taralli production. Sensory data were analyzed through 3-way ANOVA that evidenced the significant differences for taralli relative to the different attributes. The results of the statistical analysis, expressed as the output of Fisher's least significant difference (LSD) test, are shown in Table 6.

 As reported in Table 6, the overall aroma was perceived differently for Tctrl and TA compared to TB, TC and TD, as was the aroma of wine. The overall flavor was perceived to be more intense in Tctrl and TD compared to the other samples, the cereal flavor was perceived to be stronger in TD taralli, while the crispiness was lower compared to the other samples. Consistency was perceived to be lower in the TC sample, while it was higher in Tctrl. Higher scores for friability and palatability were assigned to TA taralli, and the perception of fat on the palate was stronger for taralli made with fat blends (TB, TC and TD).

 The results of the appreciation test for the different taralli samples are reported in Figure 2 in the form of a spider plot representation. As seen from the figure, Tctrl, TB and TD were rated similarly for overall appearance and shape, while TA and TC scored lower on the same attributes (TC had a lower score than TA). The aromas of Tctrl and TB received the highest scores, followed by those of TA, TD and TC. The latter still has the lowest score for both aroma and flavor. The flavor score of Tctrl and TA was higher than that of TC but lower than that of TD and TB. The product with the highest crispiness score was TB, and the others had similar scores. Despite the higher intensity of palatability, the TA sample was the least valued for this attribute, while TD was the most valued. Finally, Tctrl, TA, TB and TD were assigned a similar overall rating for acceptability, and TC received a slightly lower rating. As a whole, for several parameters, such as appearance, shape and palatability, the TB $(87.5\%$ EVOO and 12.5% SO) and TD $(87.5\%$ HOSO and 12.5% CO) samples were accepted as Tctrl, and considering the overall acceptability, TB and TD scored higher than Tctrl. Consequently, for most of the parameters, TC (75% EVOO and 25% RO) and TA (100% HOSO) were more distant from Tctrl.

 Considering the texture attributes measured instrumentally (hardness) and through sensory analysis, it emerges that, in the first case, samples did not present significant differences, while some differences among samples were perceived for sensory consistency. This discrepancy was in agreement with what was observed by Barbieri and colleagues (Barbieri et al. 2018), who attributed the low correlation between the two methods, both to the sample heterogeneity and to the different

 way to carry out the evaluation, since during the sensory test, samples are subjected to different moisture and temperature conditions compared to those of the penetration test.

 The present study has demonstrated that the use of fat blends in taralli formulation can improve the nutritional and qualitative profile of the final products without significantly affecting the textural characteristics measured through objective instrumental tools, whereas many sensorial attributes were perceived as different on the different products. The use of the fat blend made of 75% extravirgin olive oil and 25% rice oil resulted in the best compromise for the content of sterols, tocols, and antioxidants, resulting in a combination able to give an induction period to the accelerated oxidation test that was comparable to that of the reference control containing only extravirgin olive oil. On the other hand, taralli made with the unusual combination of 87.5% high oleic sunflower oil and 12.5% coconut oil, despite the lower induction time, presented fatty acid composition (in terms of SFA, MUFA and PUFA), tocol and sterol content very close to that of the control and were also better accepted than the latter. 15 289 17 290 22 292 24 293 26 294 31 296 33 297

Acknowledgments 40 300

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Author contributions: $\frac{31}{52}$ 305

 Conceptualization: F.P., S.M., F.C., M.C.M., M.F.C.; Data curation: F.P., S.M., C.R., F.C., M.C.M.; Formal analysis: F.P., S.M., C.R., F.C., M.C.M.; Funding acquisition: M.C.M., E.M., M.F.C.; Investigation: F.P., S.M., C.R., F.C., M.C.M.; Methodology: F.P., S.M., C.R., F.C., M.C.M; 54 306 56 307

Figure 1. Fracturability (A) and hardness (B) of taralli samples measured through penetration test. Different superscript letters indicate significant difference (Tuckey HSD *p* < 0.05).

Figure 2. Spider plot illustrating the appreciation of the products through mean score assigned by the assessors to different descriptors.

Samples	Composition of fat fraction
Tetrl	100% EVOO (Extra-Virgin Olive Oil)
TA	100% HOSO (High Oleic Sunflower Oil)
TB	87.5% EVOO + 12.5% SO (Sunflower Oil)
TC	75% EVOO + 25% RO (Rice Oil)
TD	87.5% HOSO + 12.5% CO (Coconut Oil)

Table 1. Fat fraction composition and sample coding of Taralli samples.

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Table 4. Sterol composition and content (mg /100 g of fat) of raw lipidic matrices and taralli.

Table 5. IP (Induction Period) values recorder for the different samples

Different letters in the same row show significant different mean values (Tukey HSD $p < 0.05$).

Table 6. Sensory data on taralli samples.

Different letters in the same row indicate significant difference among samples (Fisher LSD $p <$ 0.05).