

Characterization of ready-to-eat tahini-oat bars with added value from *Prunus avium* L.

Maria Dimitrova-Dimova^{1*}, Dasha Mihaylova², Aneta Popova¹, Pavlina Doykina¹ and Bogdan Goranov³

¹University of food technologies, Department of Biochemistry and nutrition, 4002 Plovdiv, Bulgaria

²University of food technologies, Department of Biotechnology, 4002 Plovdiv, Bulgaria

³University of food technologies, Department of Microbiology, 4002 Plovdiv, Bulgaria

Abstract. There is an increasing interest in foods with added nutritional value. This study presents the prospect of creating tahini-oat bars as an accessible snack option with added value from *Prunus avium* L. dried fruits. Four new formulations, containing different amounts of tahini, oats, bee honey, cherries, pumpkin flour, and “In shape mix”, were developed in order to cater the changing demands of consumers. Physical characteristics, texture analysis, water activity, microbial load, antioxidant potential and sensory profile aided in the evaluation of the newly developed products. The newly presented products are such of enhanced functional characteristics. Their colour spectra were significantly different from the control sample. The texture attributes also revealed a difference from the control sample in terms of increased hardness and cohesiveness. The moisture content ranged from 2.90±0.78 % (control sample) to 10.92±1.86 % (Formulation 3). The measured water activity was the lowest in the control sample and the highest in Formulation 1 (0.788±0.006). The study revealed a potential in the development of ready-to-eat healthy snacks.

1 Introduction

Nutrition is essential to the human body. However, it can be equally beneficial or harmful if the correct nutrients have not need introduced in the daily diet. A distinctive negative trend in today’s society is the lack of regular exercise and the consumption of processed foods with refined sugar, trans fats, high levels of salt [1]. A consequence of the abovementioned is the manifestation of non-communicable diseases like cardiovascular problems, endocrine disorders, among others.

It has been suggested that a consumption of various unprocessed foods like fruit, vegetables, nuts, herbs, and others may positively influence the health status of the individual [2]. This is mainly due to the presence of bioactive components in their composition. Polyphenols along with vitamins, minerals, and certain carbohydrates are reported to maintain a balance in the human body and to alter several unfavourable health conditions [3,4]. *Prunus* spp. is a genus that is spread worldwide and its fruits are often widely cherished. The cherry fruit is not only delicious but also contains health beneficial components. For example, cherry fruits can have up to 300 mg of polyphenols per 100 g of fresh weight [5].

Each meal can be presented with different amount of not only calories but bioactive molecules as well. A continuous research topic is food intake as a possible microbiota supporter [6]. In this view, snacking throughout the day can play an important pole in the introduction of beneficial nutrients to the body. Low protein intake is currently identified as a common

obstacle to achieving a balanced nutrition. Fibers along with vitamins, minerals are also consumed in insufficient amounts [7]. Consequently, fruits, vegetables, and nuts are often suggested as healthy snack options that can add up their intake [8]. Researchers have identified the need for the development of new products that can be used as daily snacks [9]. Bars with pumpkin and seed flour [10], mango-oat-chia bars [11], and sweet potato, carrot and banana protein bars [12] are only few of the examples. The modern consumer is constantly becoming more aware of the influence food choice can have on the human body. The hectic everyday life presents another challenge in the view of read-to-eat or on-the-go options. That is why there is a rising need for healthy meal options with health beneficial ingredients.

The object of this research was to provide ground for the development and comparison of four formulations of ready-to-eat tahini-oat bars with added value from *Prunus avium* L. A control sample was used for reference and aided in their characterization in terms of physico-chemical characteristics (moisture and ash content, titratable acidity and pH, amount of total soluble solids, colour), texture analysis, water activity, microbial load, antioxidant potential and sensory profile.

2 Materials and methods

* Corresponding author: mcollege_plovdiv01@abv.bg

2.1 Materials

Fresh cherry samples from the “Kosara” variety were provided from the Fruit Growing Institute, Plovdiv, Bulgaria. The cherries were washed and placed on flat trays with holes in a fruit dryer (Sencor SFD 6600BK, Japan) at 35°C for 60 h. The dried fruit were then de-pitted and placed in a air-tight plastic container and stored in a refrigerator for further usage. Other ingredients were purchased from local stores. The “In shape” mix (Dragon superfoods) was purchased in a 200g packaging from the online store zelen.bg. The pumpkin seed tahini (“Balcho”, 350g), acacia bee honey (“Ekobiomed”, 400 g), dates without pits (“D-r Keskin” 350g), pumpkin seed flour (“D-r Keskin” 200g) were purchased at a local “Baharica” store, and the fine oats flakes (“Crownfield”, 500g) were purchased from a local “Lidl” store.

2.2 Preparation of tahini-oat bars

The tahini-oat bars were prepared in laboratory conditions at the University of food technologies. Table 1 provides information about the percentage distribution of the ingredients used to prepare the formulations.

Table 1. Distribution of ingredients (%) in tahini-oat bars formulations.

| Ingredients/ Formulation | Control | 1 | 2 | 3 | 4 |
|-----------------------------|---------|----|----|----|----|
| Tahini | 26 | 26 | 26 | 26 | 26 |
| Oats | 26 | 26 | 26 | 26 | 26 |
| Bee honey | 26 | - | 11 | - | 11 |
| Cherries | - | 37 | 26 | 37 | 26 |
| Dates | 11 | - | - | - | - |
| Pumpkin flour | 11 | 11 | 11 | - | - |
| “In shape” mix | - | - | - | 11 | 11 |

The dried cherries and dates were separately finely chopped using Silver Crest chopper SMZ 260 J4 (260 W) at the turbo boost button speed for approximately 30 s. After that all ingredients depending on the variation of the recipe were hand-mixed until a soft plastic mass. The mass was placed in a silicone tray to ensure unity of the shape and thickness of each tahini-oat bar. The tahini-oat bars (Figure 1) were stored in a refrigerator (for 24 h) until further analysis.



Fig 1. Tahini-oat bar formulation.

2.3 Nutritional data

The calculation method was used to determine the nutritional data. Supplier specifications for each of the ingredients (tahini, bee honey, pumpkin flour, dates, “in shape mix”) were used to calculate the nutritional value of the finished products per 100 g. Reference about the nutritional value of the cherry fruit is based on unpublished data of the “Kosara” variety.

2.4 Moisture and ash content

The moisture content (%) of the studied samples was measured using an infrared moisture analyser PMB 53 (Adam Equipment Inc., Oxford, UK). Ash content (%) was determined by burning in a muffle furnace according to AOAC Official Method 935.52 [13].

2.5 Titratable acidity (TTA) and pH

The titratable acidity (TA) was measured by titration with 0.1n NaOH. Results are expressed as citric acid equivalents.

The pH was determined using a Jenway 550 Benchtop handheld pH meter (Cambridgeshire, UK) with the electrode standardized to pH 4.0; 7.0 and 10.00 (Product No GE17-6000-88) buffers (Sigma-Aldrich, Darmstadt, Germany).

2.6 Total soluble solids (TSS)

TSS (%) were evaluated using a digital handheld refractometer (Opti Brix 54, Bellingham + Stanley, Kent, UK).

2.7 Water activity (a_w)

The water activity was assessed using a LabSwift-aw, Novasina AG, Lachen, Bassersdorf, Switzerland.

2.8 Vitamin C content

The vitamin C content (mg %) was determined using the titration method as described by Petkova et al. [14].

2.9 Evaluation of colour

A PCE-CSM 2 (PCE-CSM instruments, Meschede, Deutschland) with a measuring aperture of 8 mm was utilized to examine the colour parameters (L^* , a, b, c, h) of each tahini-oat bar formulation. The total colour difference (ΔE) was calculated according to the CIE76 colour difference equation using values for L^* , a^* and b^* corresponding to the CIELAB colour measurements.

2.10 Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (ABTS, DPPH, FRAP, CUPRAC)

The extraction procedure followed the description of Mihaylova et al. [15]. The TPC was analysed following a modified method of Kujala et al. [16]. The TPC was

expressed as mg gallic acid equivalents (GAE) per g dw. The total flavonoid content was evaluated according to the method described by Kivrak et al. [17]. Results were expressed as μg QE/g dw, and quercetin (QE) was used as a standard. The antioxidant potential was tested using four methods: DPPH, determined by a slightly modified method of Brand-Williams et al. [18]; ABTS, estimated according to Re et al. [19]; FRAP, according to the procedure of Benzie and Strain [20], and CUPRAC according to the procedure of Apak et al. [21]. Trolox was used as a standard, and the results were expressed as TEAC values (μM TE/g dw).

2.11 Microbiological count

The tahini-oat bars were tested using the spread-plate method to determine yeasts and molds (YM) using potato dextrose agar. Potato dextrose agar plates were incubated at 30°C and counted after 72 h. The aerobic mesophilic microorganisms (AMM) count was evaluated according to ISO 4833-1:2013 [22] using plate count agar as a culture medium. The results are expressed as colony-forming units (CFUs)/g.

2.12 Texture analysis

The texture profile analysis was executed using a CT3 texture analyser (Brookfield, Stable Micro Systems, USA) in TPA mode. The experiments were conducted under ambient conditions using fixture – TA39, trigger

– 5.0g, deformation – 13 mm, speed – 0.5 mm/s. The parameters hardness, cohesiveness, springiness, adhesiveness, gumminess and chewiness were presented.

2.13 Sensory evaluation

A quantitative descriptive assay [23] was conducted with 12 trained panellists to evaluate parameters as follows: appearance (n=5); colour (n=3); taste (n=7); aroma (n=6); texture (n=3); aftertaste (n=2). The samples were coded, served in clean white plastic plates at room temperature in individual booths with adequate fluorescent lights. The sensory evaluations were collected on 9-point Hedonic scale with a maximum score of 9.

2.14 Statistical analysis

MS Excel software was used for data analysis. All assays were performed in triplicates. Results were presented as mean \pm SD. Additional statistical analyses of the data were presented using one-way ANOVA and a Tukey-Kramer post hoc test ($\alpha = 0.05$), as described by Assaad et al. [24].

3 Results and discussion

The physico-chemical characteristics of the tahini-oat bar formulation are presented in Table 2.

Table 2. Physico-chemical parameters in tahini-oat bars formulations.

| Parameter/ Formulation | Control | 1 | 2 | 3 | 4 |
|------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Moisture content, % | 2.90 \pm 0.78 ^b | 8.16 \pm 1.72 ^a | 9.11 \pm 0.42 ^a | 10.92 \pm 1.86 ^a | 8.31 \pm 0.01 ^a |
| Ash content, % | 2.92 \pm 0.46 ^a | 3.00 \pm 0.69 ^a | 2.99 \pm 0.94 ^a | 3.04 \pm 0.55 ^a | 3.46 \pm 0.40 ^a |
| Titrateable acidity | 0.02 | 0.07 | 0.06 | 0.10 | 0.11 |
| pH | 7.07 | 6.79 | 6.70 | 6.65 | 6.69 |
| Water activity | 0.622 \pm 0.006 ^d | 0.788 \pm 0.006 ^a | 0.749 \pm 0.004 ^b | 0.762 \pm 0.005 ^b | 0.718 \pm 0.005 ^c |
| Total soluble solids | 0.45 \pm 0.07 ^c | 0.40 \pm 0.28 ^c | 3.95 \pm 0.63 ^b | 5.70 \pm 0.14 ^a | 3.90 \pm 0.00 ^b |
| Vit. C content, mg% | 0.29 \pm 0.02 ^c | 0.65 \pm 0.03 ^a | 0.19 \pm 0.00 ^d | 0.33 \pm 0.01 ^c | 0.44 \pm 0.02 ^b |

Different letters in the same row indicate statistically significant differences ($p < 0.05$), according to ANOVA and the Tukey test.

The moisture content was the highest in Formulation 3 of the tahini-oat bars and the lowest in the control sample. This hinted that the inclusion of conventionally dried cherries increased the moisture content between 2.5 and 3 times. The inclusion of bee honey in the formulations did not lead to a trend concerning the moisture content. Other authors reported a moisture content from 16.02 to 18.48 % in sweet potato and mango bars [25]. Additionally, a moisture content from 68.78 to 74.51% was documented for apple-banana-cashew bars [26]. The water activity was the lowest in the control sample and the highest in

Formulation 1. The presence of dried cherries in the recipe provided more free water compared to the dried dates in the control sample. Mango-based fruit bars [27] had higher water activity than all the current new formulations.

The established ash content varied from 2.92 \pm 0.46 in the control sample to 3.46 \pm 0.40 in Formulation 4. The ash content of the control sample was comparable to other available research [28]. The presence of “In shape mix” (Dragon Superfoods) in the recipe increased the ash content. The titrateable acidity was the highest in the formulation where the “In shape mix” (Dragon Superfoods) was included in the recipe. The dried

cherries increased the titratable acidity and decreased the pH which could be attributed to their distinct acidic taste. The total soluble solids content was the highest in Formulation 3 but the value was lower than the data cited in other research [26]. The Vitamin C content was relatively low in all formulations including the control sample, which suggested that none of the ingredients contributed significantly to its content.

Table 3 is a visual representation of the nutritional data of the tahini-oat bar formulations.

Table 3. Nutritional data of tahini-oat bars formulations.

| Parameter/ Formulation | Control | 1 | 2 | 3 | 4 |
|------------------------|---------|-------|-------|-------|-------|
| Energy, kcal | 400 | 340 | 325 | 356 | 307 |
| Proteins, g | 17.56 | 17.61 | 14.34 | 17.49 | 14.23 |
| Carbohydrates, g | 50.51 | 36.72 | 37.57 | 41.23 | 42.07 |
| Sugars, g | 30.75 | 4.28 | 4.67 | 11.99 | 3.58 |
| Fibre, g | 5.05 | 4.16 | 7.95 | 4.44 | 8.23 |

Table 4. CIE lab colour spectra of tahini-oat bars formulations.

| Parameter/ Formulation | Control | 1 | 2 | 3 | 4 |
|------------------------|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| L* | 44.06±0.74 ^a | 24.61±3.61 ^b | 27.93±2.89 ^b | 26.34±3.24 ^b | 24.81±1.96 ^b |
| a | 11.41±0.08 ^a | 5.19±0.47 ^c | 8.45±1.48 ^b | 3.77±0.61 ^c | 3.62±0.26 ^c |
| b | 27.91±0.96 ^a | 4.82±2.27 ^b | 9.67±2.14 ^b | 7.49±3.54 ^b | 6.22±1.39 ^b |
| c | 30.15±0.90 ^a | 7.18±1.86 ^b | 12.85±2.55 ^b | 8.43±3.46 ^b | 7.22±1.27 ^b |
| h | 67.74±0.59 ^a | 40.71±11.33 ^c | 48.64±2.68 ^{bc} | 61.49±5.87 ^{ab} | 59.23±5.34 ^{ab} |

Different letters in the same row indicate statistically significant differences ($p < 0.05$), according to ANOVA and the Tukey test.

The colour of food is strongly dependent on the specific ingredients and the treatment it receives. That is why a comparison between the currently studied and other ready-to-eat bars with different ingredients was not suitable. The newly developed formulations were similar and differ from the control sample. The control sample had higher L* values which made it lighter than the others. Between the four formulations there were very little differences in the colour. However, it could be distinguished as different as seen by the calculated ΔE . The only formulations that were more difficult to

| | | | | | |
|-------------------|-------|-------|-------|-------|-------|
| Fat, g | 13.97 | 14.18 | 13.02 | 14.11 | 12.95 |
| Saturated fats, g | 2.16 | 2.15 | 2.2 | 2.15 | 2.2 |

The presented new formulations had less energy values compared to the control samples. There was a noticeable difference in the carbohydrate content and the sugars on a 100 g basis. The fibre content did not change drastically with recipe alteration. Following Regulation (EU) 1924/2006 [29] it can be stated that the new formulations are sources of fibre, and formulations 2 and 4 are high fibre. Additionally, they contained naturally occurring sugars, and are with no added sugar.

The protein content the new formulations and the control sample cannot account for a big percentage of the daily needed protein of the healthy individual. However, formulations 2 and 4 were more similar to the control and each other, than formulations 1 and 3.

Table 4 shows the CIE lab colour spectra of the studied formulations. Colour is very important in the food industry as it gives initial information to the consumer about attributes like expected taste, possible freshness among others.

differentiate and needed a closer observation were Formulations 3 and 4 with a ΔE 1.99.

All tahini-oat formulations had a tendency for the green and blue colour. They could be described as unsaturated based on their chroma (colourfulness). The established hue values showed a red-yellow tendency.

Figure 2 presents the total phenolic content, total flavonoid content, and the respected antioxidant activity tested with four complementing methods i.e. DPPH, ABTS, FRAP, and CUPRAC).

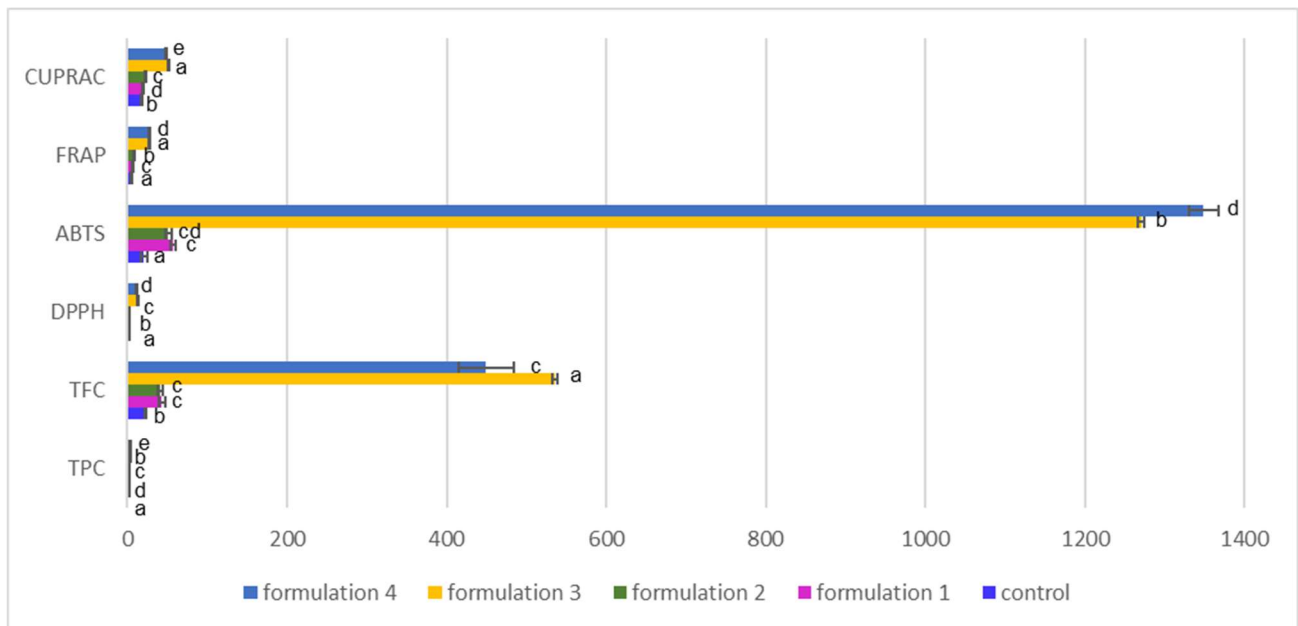


Fig 2. TPC (mgGAE/g), TFC (µgQE/g), and antioxidant activity of tahini-oat bar formulations measured on dry mass basis by ABTS, DPPH, FRAP and CUPRAC (µM/g) assays. Different letters in the same assay indicate statistically significant differences ($p < 0.05$), according to ANOVA and the Tukey test.

The highest values of antioxidant potential were measured in the ABTS assay ($1348.67 \pm 18.61 \mu\text{M/g dw}$). Similar trend was observed in muffins with added peach powder [30]. The antioxidant activity can positively influence the shelf life of food stuff as indicated by do Nascimento et al. [31].

It can be suggested that the phenolic compounds present in the tahini-oat bar formulations were mainly represented by flavonoids based on the total flavonoid content, having the highest value in Formulation 3 ($535.70 \pm 29.31 \mu\text{gQE/g dw}$). Formulations 3 and 4 had the highest values in all assays and similar to each other results. This was most likely due to the ingredients in those formulations. It can be suspected that not only the dried cherries but also the “In shape mix” was responsible. Correspondingly, Formulations 1 and 2 had similar to each other values. The control sample on the other had, had the lowest values which confirmed that the addition of dried cherries increased the biological activity of the tahini-oat bar formulations.

Microbial contamination is important to food safety and basic human health security [32]. The recorded microbial count (Table 5) showed that all Formulations are acceptable for immediate consumption. However, some papers reported no microbial contamination in snack bars, like a presented sunroot snack bar [33].

Table 5. Yeasts and molds (YM) and aerobic mesophilic microorganisms (AMM) count (CFUs/g) in tahini-oat bars formulations.

| Parameter/ Formulation | AMM, CFU/g | YM, CFU/g |
|---------------------------|-------------------|-------------------|
| Control | 2.4×10^4 | 3.3×10^2 |
| 1 | 2.1×10^4 | 3.5×10^2 |
| 2 | 1.1×10^4 | 3.4×10^2 |
| 3 | 3.0×10^4 | 1.2×10^2 |
| 4 | 1.8×10^4 | 5.0×10^1 |

The results showed that the introduction of dried cherries positively influenced the microbial status of the formulations. Contrarywise, the inclusion of a powdered functional mix did not follow a specific trend. In future, more efforts have to be paid in improving the microbial quality of the proposed formulations. Some simple changes might include a preparation on different surfaces with different utensils [34].

Since texture is an important multi-parameter property that is essential to food stuff [35], Table 6 presents the data of the hardness, cohesiveness, springiness, adhesiveness, gumminess, and chewiness of the studied tahini-oat bar formulations.

Table 6. Texture profile analysis of tahini-oat bars formulations.

| Parameter/ Formulation | Control | 1 | 2 | 3 | 4 |
|---------------------------|---------------------|--------------------------|-------------------------|------------------------|----------------------|
| Hardness 1, g | 90.17 ± 24.26^b | 315.17 ± 52.70^a | 250.33 ± 70.49^a | 361.5 ± 52.69^a | 367.67 ± 79.15^a |
| Hardness 2, g | 45.00 ± 1.80^b | 141.33 ± 122.57^{ab} | 162.33 ± 41.17^{ab} | 237.5 ± 83.07^{ab} | 246.50 ± 56.14^a |

| | | | | | |
|------------------|---------------------------|----------------------------|----------------------------|-----------------------------|----------------------------|
| Cohesiveness | 0.11±0.04 ^a | 0.09±0.02 ^a | 0.15±0.05 ^a | 0.16±0.08 ^a | 0.16±0.02 ^a |
| Springiness, mm | 9.30±0.98 ^a | 9.3±1.05 ^a | 9.7±0.36 ^a | 11.3±0.88 ^a | 10.93±0.64 ^a |
| Adhesiveness, mJ | 0.62±0.45 ^a | 0.71±0.02 ^a | 0.71±0.05 ^a | 1.09±0.32 ^a | 1.01±0.38 ^a |
| Gumminess, g | 10.34±7.41 ^b | 22.92±0.68 ^{ab} | 35.34±4.10 ^{ab} | 56.44±28.48 ^a | 58.39±11.99 ^a |
| Chewiness | 100.78±82.79 ^c | 214.78±40.41 ^{bc} | 342.99±44.66 ^{bc} | 441.88±143.04 ^{ab} | 640.79±151.84 ^a |

Different letters in the same row indicate statistically significant differences ($p < 0.05$), according to ANOVA and the Tukey test.

The newly developed tahini-oat bars appeared with an increased hardness compared to the control sample. All new formulations had an increased adhesiveness, gumminess, and chewiness. The established data was comparable to the reported by other authors about plant-based snack bars [36]. Additionally, the results showed similarity to those published by Munshi et al. [37] regarding nutrient bars.

The presence of more sugars is usually associated with an increased hardness [38]. This did not comply with the current results. Another possible explanation for the increased hardness of the new tahini-oat formulation is the migration of water and the formation of bonds between sugars and proteins [39].

Compared to each other, the formulations showed that the addition of dried cherries improved their cohesiveness which is associated with less crumbliness [40]. The cohesiveness reflects the internal bonds' strength, and the amount of force needed to deform them before rupture [41] as well as their binding ability [42]. Springiness manifests as elasticity of the sample meaning that increased springiness requires more force in order to chew the food and form bolus prior to swallowing due to their increased chewiness [44].

The sensory profile of the tahini oat bars was determined by evaluation of different parameters as follows: appearance (Figure 3), color (Figure 4), taste (Figure 5), aroma (Figure 6), texture (Figure 7), aftertaste (Figure 8).

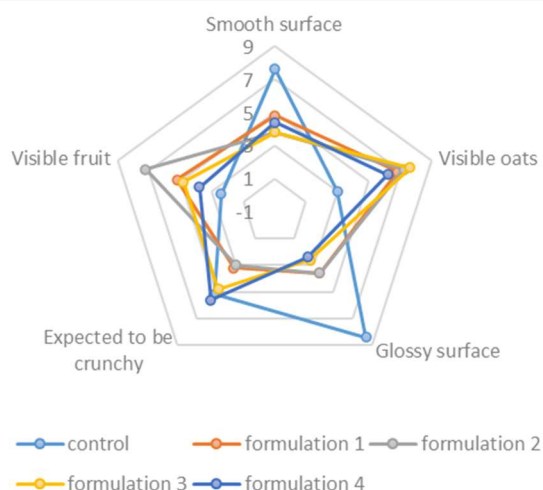


Fig 3. Appearance attributes of tahini-oat bar formulations based on sensory evaluation.

The new formulations differed from the control sample in terms of glossiness. They appeared more matte than the control. They had visible oats and fruit but the surface of the new formulations was not as smooth as the control sample. In addition, Formulation 3 was expected to be crunchier than Formulations 1, 2, and 4.

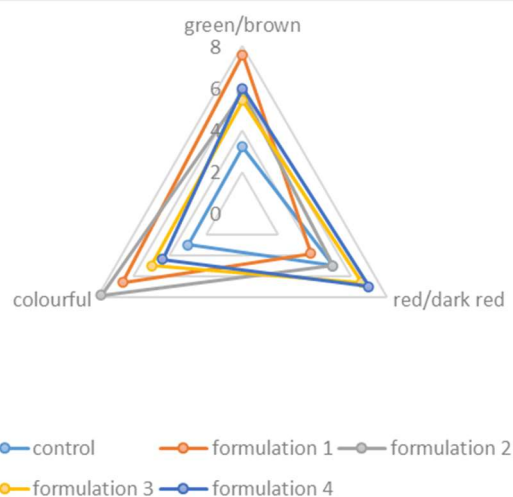


Fig 4. Color attributes of tahini-oat bar formulations based on sensory evaluation.

The control sample was different in color compared to the newly developed formulations. Formulation 2 appeared the most colorful compared to the others. Formulation 1 was browner, and Formulation 4 appeared red to the panelists. Color is a very important attribute when it comes to food. It is associated with an expected taste, as well as freshness, among other quality attributes [45].

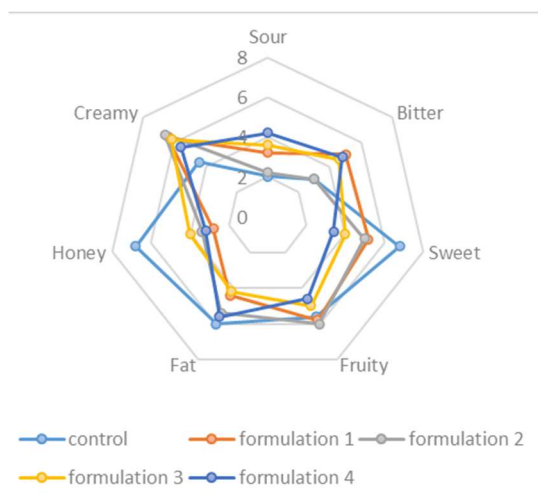


Fig 5. Taste attributes of tahini-oat bar formulations based on sensory evaluation.

The evaluation of taste revealed that the honey-like and sweet tastes in the control sample were less perceptible in the newly developed formulation. The creamy taste was more distinct in the new formulations. The fruity-like taste was comparable to the control sample. However, Formulation 4 was described as less fruity and more sour.

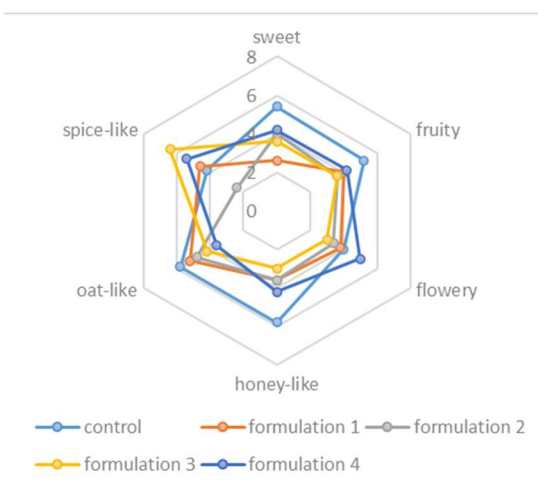


Fig 6. Aroma attributes of tahini-oat bar formulations based on sensory evaluation.

The human individual characterizes smell by its strength and character (smell-like) [46]. The aroma evaluation showed that the control sample was dominated by the honey-sweet-fruit scent. The new formulations were perceived as less sweet, fruity, and flowery. They were also described as spice-like, especially Formulation 3. The presence of the “In shape mix” which is a compilation of dried plant, incl. spices might have led to those results. The oat-like aroma was the most perceptible in the control sample and the least noticeable in Formulation 4.

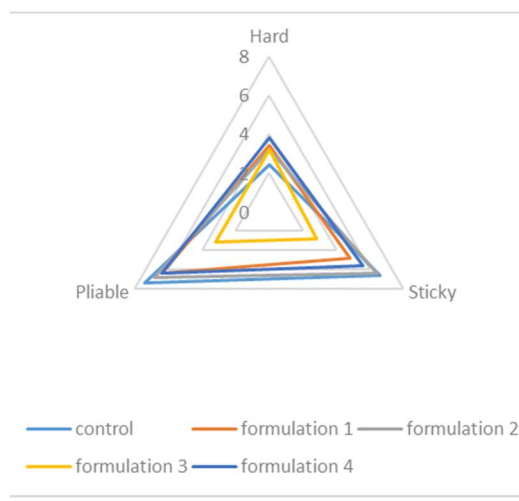


Fig 7. Texture attributes of tahini-oat bar formulations based on sensory evaluation.

There was a resemblance between the new formulations and the control sample as they appeared sticky and pliable. Formulation 3 was the one that was different and referred to as less sticky and crumblier.

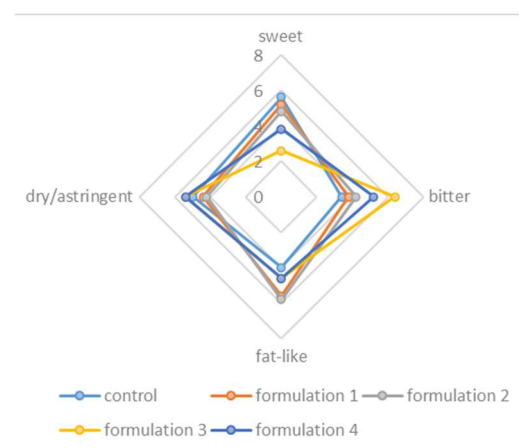


Fig 8. Aftertaste attributes of tahini-oat bar formulations based on sensory evaluation.

Aftertaste is important as it characterizes the intensity and presence of sensations in the mouth after a product is swollen [47]. The aftertaste is also a dynamic sensation [48]. The aftertaste of Formulations 1 and 2 was more similar to the control sample than of Formulations 3 and 4. The tahini-oat bars left a sweet and fat-like aftertaste. Some dryness was also perceived. Formulation 3 had a distinct bitter aftertaste.

4 Conclusions

This study presented the opportunity for the development of ready-to-eat tahini-oat bars with added value from *Prunus avium* L. The newly developed formulations can be a quick snack throughout the day or an enrichment to one’s daily healthy meal plan due to the presence of phenolic compounds, fibres, along with the lack of added sugar. It can be suggested that flavonoids mostly represent the phenolic compounds in

the tahini-oat formulations. All new formulations showed better adhesiveness compared to the control sample. Their hardness, gumminess, and chewiness were also greater than the control.

The current results can be used as a milestone for new product development as well as a good reference for comparison.

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The data presented in this study are available on request from the corresponding author.

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