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Effect of Black Cumin Cake Extract, Octyl Caffeate, and Active Packaging on Antioxidant Properties of Egg-Free Mayonnaise during Storage

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Abstract: The egg-less mayonnaise (CM) based on aquafaba and refined rapeseed oil was prepared and enriched with black cumin cake extract (BCCE) at two concentrations of 0.1% (BCCM0.1%) and 3% (BCCM3%) and 0.1% of octyl caffeate (OCM). The obtained mayonnaises were placed in glass jars without and with active film incorporating BCCE at the jar bottom (AFM). The influence of antioxidants on oxidative stability and antioxidant capacity (AC) of the prepared mayonnaises was estimated by the shelf-life test. Peroxide value (PV), anisidine value (AnV), total oxidation (TOTOX) value, acid value (AV), and amounts of conjugated dienes (CD) and conjugated trienes (CT) were used to assess the extent of mayonnaise deterioration during storage of up to 4 weeks in a refrigerator. The synthesized octyl caffeate (OC) and natural antioxidants from BCCE added to mayonnaises directly and released from active film enhanced the AC of the studied mayonnaise samples determined using the QUick, Easy, New, CHEap, and Reproducible-2,2-diphenyl-1-picrylhydrazyl (QUENCHER-DPPH), and QUENCHER-2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (QUENCHER-ABTS) methods. All enriched mayonnaises had higher antioxidant potential than the AC of the control sample without antioxidants (CM) and commercial plant-based mayonnaise (CPBM). Therefore, the proposed antioxidants and active film can be used in the food industry to suppress lipid oxidation.

Keywords: mayonnaise; black cumin cake extract; octyl caffeate; active packaging; antioxidant capacity; shelf-life test



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1. Introduction

A plant-based diet has been a growing trend in recent years. This causes an increased production of food without any animal products. One of them can be vegan mayonnaise, which is a healthy alternative to traditional mayonnaise containing 65–85% of fat [1]. Due to high oil content, oil-in-water emulsions such as mayonnaises are vulnerable to the oxidation processes of the unsaturated fats in the oil phase. These undesirable reactions lead to the formation of unpleasant flavor, rancid odor, degradation of color or texture, and the generation of potentially toxic compounds [2,3]. Recently, a wide variety of natural and synthetic antioxidants have been used to reduce the rancidity of emulsions. It is well known that plant materials are natural sources of different antioxidants, such as phenolic acids and their derivatives, that can inhibit the oxidative degradation of lipids. For this reason, various essential oils, plant extracts, and spices such as *Carum copticum* essential oil [4], rosemary essential oil, *Ferulago angulata* extract [2], *Rosa canina* fruit extract [5], and ginger [6] were successfully incorporated into mayonnaise samples to enhance their oxidative stability. Furthermore, by-products of the food industries, such as beetroot peel [7], pistachio green hull [8], grape seed extract [9], and purple corn husk extract [10] demonstrated high antioxidant potential; thus, they were used as antioxidant agents in

food emulsions to reduce their rancidity. This application of by-products is promissory to reduce industrial waste, assure safety against rancidity and extend shelf-life, i.e., the period during which oil-based food products maintain satisfactory quality.

Lately, to retard or inhibit the oxidative process and the prolongation of emulsions' shelf life, the addition of synthetic additives named phenolipids has been widely used. Interestingly, phenolipids as phenolic compounds modified with aliphatic chains using lipophilization have health benefits due to phenolic motif and amphiphilic and antioxidant properties [11]. The choice of antioxidants is based on their effectiveness in multiphase systems and depends on many factors, such as molecular structure, the reactivity of antioxidants, their physical location, and the presence of other compounds. Among them, the partitioning of the antioxidant into the different phases is the most important factor [12]. It is noteworthy that lipid oxidation in emulsion is initiated at the interface between oil and water phases. Therefore, the antioxidant should be placed at the surface of oil droplets in an oil-in-water emulsion. There are two hypotheses describing the efficiency of antioxidants in emulsion: the cut-off effect and the polar paradox theory. According to the second theory, non-polar antioxidants tend to be located in the interfacial region, whereas polar antioxidants have a tendency to accumulate in the water phase. Therefore, lipophilic/amphiphilic antioxidants should be desired to protect mayonnaise from oxidation [12,13].

Unfortunately, phenolic compounds, which mainly occur in plants, are very water-soluble antioxidants. Nevertheless, the esterification of phenolic acids with various alkyl alcohols was widely used to enhance the hydrophobicity of the synthesized phenolipids. However, alcohols with different carbon chain lengths affected the degree of lipophilization, which was associated with the antioxidant properties of phenolipids. This phenomenon is called the cut-off effect, and it is an extension of the polar paradox hypothesis [12,13]. Previous research demonstrated that phenolic acid alkyl esters added to oil-in-water emulsions increased their oxidative stability and antioxidant potential [11,14,15]. The addition of different caffeic acid esters to oil-in-water emulsions indicated the enhancement of their antioxidant activity up to octyl caffeate incorporation, whereas further elongation of the alkyl carbon chain caused a decrease in the antioxidant properties of the emulsions [11,16].

On the other hand, there is a growing demand among certain consumer groups for food products without synthetic additives. Consequently, active packaging could be a promising alternative for effectively preventing the deterioration of fat-based products [17]. In fact, films incorporating active substances have the ability to slow down or inhibit lipid oxidation by regulating the release of antioxidants into the food product [18]. For instance, high-density polyethylene active package containing rosemary extract powder effectively delayed the oxidation of sunflower oil [18]. Polyethylene terephthalate bottles enriched with titanium dioxide and zinc oxide nanoparticles efficiently acted as antimicrobial packaging for mayonnaise sauce [19]. Furthermore, Flórez et al. [20] reported that the oxidation of butter wrapped in chitosan film sachets enriched with *Santalum album* essential oil was delayed compared to butter stored without film or with chitosan film without the active additive. Thus, it can be assumed that both natural and synthetic packaging materials reinforced with active substances significantly extend the shelf life of high-fat food products.

Recently, black cumin oil has been used to increase the oxidative stability of mayonnaise [21], but no data have been found regarding the application of black cumin cake extract (BCCE) as a direct additive or as an active component of the packaging material of mayonnaise to inhibit its oxidation processes. Moreover, the scientific literature has presented little information on studies to determine the shelf life of new vegan mayonnaises with functional additives. However, the evaluation of the shelf life of these products is crucial in the fat industry because it ensures the quality and safety with which the oil-based product will be distributed.

Thus, the main objective of this research was to estimate the changes in the antioxidant capacity (AC) and oxidation stability of egg-free mayonnaises with natural antioxidants present in BCCE, synthesized octyl caffeate (OC), and contacted with active film incorporating BCCE during storage for up to 4 weeks. The antioxidant effect of BCCE and OC on the

antioxidant properties, oxidative status, and shelf life of aquafaba-based mayonnaise was investigated. The AC of prepared mayonnaise samples was compared with the antioxidant potential of commercial plant-based mayonnaise (CPBM). The modified QUick, Easy, New, CHEap, and Reproducible-2,2-diphenyl-1-picrylhydrazyl (QUENCHER-DPPH) and QUick, Easy, New, CHEap, and Reproducible-2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (QUENCHER-ABTS) procedures were applied for AC evaluation. However, the amounts of primary and secondary oxidation products and free fatty acids in all studied mayonnaise samples were analyzed using the official methods. Moreover, principal component analysis (PCA) was applied for grouping the studied mayonnaise samples, their characterization, and detecting differences.

2. Materials and Methods

2.1. Chemicals and Materials

All chemicals used in the study were analytical or HPLC grade. 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, TE), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), caffeic acid were supplied from Merck, Warszawa, Poland. Chloroform, ethanol, methanol, octanol, n-heptane, and n-hexane were purchased from Chempur, Piekary Śląskie, Poland. All solutions were prepared with redistilled water.

The ingredients for mayonnaise preparation, namely canned chickpeas (*Cicer arietinum* L.), mustard, vinegar, salt, sugar, and nutritional yeast, were sourced from a local market. Refined rapeseed oil and egg-less mayonnaise (CPBM) were delivered by two different manufacturers shortly after production. The shelf life of unopened CPBM was assessed at 6 months from the production day and, after opening the jar, this emulsion should not be stored for longer than 2 weeks at 4 °C. These recommendations were determined based on durability tests conducted by the CPBM producer. Black cumin cake (BCC) was kindly donated by a local vegetable oil factory.

2.2. Preparation of Black Cumin Cake Extracts

To achieve black cumin cake extract (BCCE), approximately 3.0 g of ground BCC was combined with 20 mL of solvent (1:1 mixture of ethanol and water) in a round-bottomed flask and agitated at room temperature for 30 min. The extraction process was replicated three times. Subsequently, the residual black cumin flour was separated from the liquid phase using centrifugation (MPW-54 centrifuge, MPW MED. INSTRUMENTS, Warszawa, Poland) at 4500 rpm for 10 min. Once the extracts were pooled, they were filtered to remove any remaining impurities and then stored in a refrigerator prior to analysis.

2.3. Preparation of Active Film

In this study, the preparation of active film was carried out by adapting a modified procedure from a previous investigation [22]. To begin, separate solutions of gelatin (GEL) and polyvinyl alcohol (PVA) were prepared at concentrations of 5% *w/v* each, by dissolving them in distilled water. The GEL solution was heated at 60 °C, while the PVA solution was heated at 80 °C, both under continuous stirring for 20 min for GEL and 2 h for PVA. Once the solutions were ready, they were combined in a 5:3 volume ratio. Subsequently, glycerol (3% *v/v*), BCCE (12% *v/v*), and distilled water (5% *v/v*) were incorporated into the mixture, which was then heated at 60 °C and constantly stirred for 30 min to ensure homogeneity. The film-forming solution was poured into plastic Petri dishes and air-dried at room temperature for 72 h. Following the drying period, the films were carefully removed from the casting surface.

2.4. Octyl Caffeate Synthesis

The synthesis procedure of OC was described in our previous work [23]. In brief, 1-octanol was dried over 3 Å molecular sieves before starting the experiment. In turn, caffeic acid (6 mmol) with an excess 1-octanol (66 mmol) and catalyst (10 mol/L H₂SO₄) was

transferred in a 50 mL two-neck round bottom flask equipped with a thermometer, a Teflon-coated magnetic stirring bar, and a reflux condenser. Afterwards, activated molecular sieves were put into a round bottom flask to remove any traces of water, and the esterification was carried out at 100 °C for 2 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate, washed with brine and water, dried over magnesium sulfate, and filtered. In the next step, ethyl acetate was evaporated under a vacuum on a rotary evaporator. The synthesized OC (Figure 1) was purified by crystallization from n-heptane.

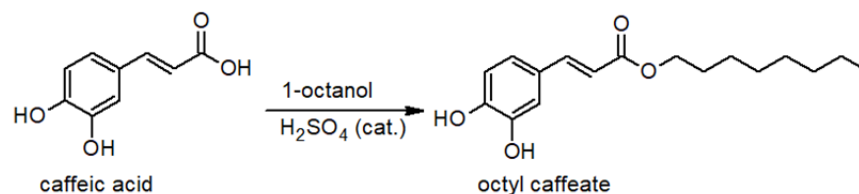


Figure 1. Synthesis of octyl caffeate (OC).

^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were collected to confirm the purity of OC at 700 and 170 MHz, respectively, on a Bruker Avance III 700 MHz spectrometer (Bruker Corporation, Karlsruhe, Germany) at 25 °C. Ester was dissolved in CDCl_3 -containing tetramethylsilane (TMS) as an internal standard. Chemical shifts were recorded in δ values in parts per million (ppm), and the coupling constant (J) was reported in Hertz (Hz).

Moreover, attenuated total reflectance–Fourier transform infrared (ATR–FTIR) spectra of OC were recorded by using a Bruker VERTEX 70v FTIR spectrometer (Bruker Optics, Ettlingen, Germany) equipped with a diamond ATR cell. The synthesized OC was directly placed and pressed on the crystal surface of the ATR device and analyzed from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} .

The purified OC (octyl (E)-3-(3,4-dihydroxyphenyl)-2-propenoate) was a white solid compound with the following characteristic: ^1H NMR (CDCl_3): δ 0.90 (t, $J = 7.2\text{ Hz}$, 3H), 1.24–1.37 (m, 9H), 1.37–1.43 (m, 2H), 1.67–1.74 (m, 2H), 4.20 (t, $J = 6.8\text{ Hz}$, 2H), 5.59 (br. s., 2H), 6.28 (d, $J = 15.9\text{ Hz}$, 1H), 6.88 (d, $J = 8.2\text{ Hz}$, 1H), 7.03 (dd, $J = 8.2, 1.9\text{ Hz}$, 1H), 7.10 (d, $J = 1.9\text{ Hz}$, 1H), 7.58 (d, $J = 15.9\text{ Hz}$, 1H). ^{13}C NMR (CDCl_3): δ 14.06, 22.64, 25.98, 28.74, 29.19, 29.24, 31.79, 64.75, 114.40, 115.57, 116.12, 122.44, 127.88, 143.66, 144.39, 145.98, 167.56. IR: ν 3488, 3313, 2955, 2917, 2870, 2849, 2467, 1680, 1624, 1604, 1531, 1473, 1440, 1309, 1280, 1239, 1177, 1153, 1107, 1031, 972, 813 cm^{-1} .

2.5. Preparation of Mayonnaises

Aquafaba from a commercial chickpea jar was separated by draining the chickpeas and reserving the liquid phase. A representative aquafaba sample was chosen for mayonnaise preparation.

Mayonnaise was prepared based on a previously established formula with some modifications [24]. The formulation contained the following ingredients in a weight ratio (w/w): refined rapeseed oil (74%), aquafaba (24%), mustard, vinegar, salt, sugar, and nutritional yeast (summary 2%). The prepared emulsion was divided into five equal portions: 1—control sample was packed into a glass jar (CM), 2—sample with 0.1% of BCCE packed into a glass jar (BCCM0.1%), 3—sample with 3% of BCCE packed into a glass jar (BCCM3%), 4—sample with 0.1% of OC packed into a glass jar (OCM), 5—sample packed in a glass jar with active film on its bottom (AFM). The mayonnaises were stored in a refrigerator at 4 °C and analyzed weekly for 4 weeks. Additionally, vegan mayonnaise containing 35% fat was supplied directly after production by one of the top-selling brand in the Polish market (CPBM). The appearance of the investigated samples is shown in Figure 2.



Figure 2. The appearance of the prepared egg-less mayonnaise samples: commercial plant-based mayonnaise (CPBM), control aquafaba-based mayonnaise (CM), aquafaba-based mayonnaise with 0.1% of black cumin cake extract (BCCM0.1%), aquafaba-based mayonnaise with 3% of black cumin cake extract (BCCM3%), aquafaba-based mayonnaise with 0.1% of octyl caffeate (OCM), and aquafaba-based mayonnaise with active film incorporating black cumin cake extract (AFM).

2.6. Determination of Antioxidant Capacity

The AC was determined using the QUENCHER-DPPH and QUENCHER-ABTS assays described in our previous publication [24].

2.6.1. QUENCHER-DPPH Assay

In brief, 0.0150–0.0400 g of each mayonnaise sample was placed into centrifuge tubes. Afterward, 6 mL of DPPH solution (60.8 $\mu\text{mol/L}$) was added, and the mixtures were vortexed for 5 min. After keeping the samples in darkness for 15 min and centrifugation at $3120 \times g$ for 3 min, their absorbance was read at 517 nm against a reagent blank (DPPH solution, $c = 60.8 \mu\text{mol/L}$) using a Hitachi U-2900 spectrophotometer in a 1-cm quartz cell.

2.6.2. QUENCHER-ABTS Assay

To determine AC using the QUENCHER-ABTS assay, 0.0120–0.0300 g of mayonnaises was placed into six pre-weighed dry clean centrifuge tubes and 6 mL of the working ABTS^{•+} reagent (diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm) was added to each sample. After vortexing for 5 min and centrifuging at $3120 \times g$ for 3 min, the absorbance was recorded at 734 nm against a reagent blank (2.5 mL of ABTS^{•+} solution).

2.7. Determination of Oxidative Status

For oxidative status analysis, the oil phase of each mayonnaise was separated based on the procedure used by Alizadeh et al. [2]. In brief, mayonnaises were frozen at -18°C for 12 h and then thawed and centrifuged for 5 min. Each oil phase, separated from the emulsion residue, was stored in a closed glass flask in the refrigerator at 4°C until further analysis.

The primary and secondary oxidation products in the oil phase of mayonnaise samples were determined and expressed as peroxide value (PV) and anisidine value (AnV), respectively.

The PV of each oil phase was assessed using the official procedure ISO 3960:2017 [25], whereas the AnV was carried out spectrophotometrically in accordance with the ISO 6885:2016 method [26].

The overall oxidation state of the oil phase was calculated as a TOTOX value based on the PV and AnV using the formula: $(\text{TOTOX} = 2 \text{ PV} + \text{AnV})$.

The conjugated dienes (CD) and conjugated trienes (CT) values were measured according to [27] as the absorbance of 1% solution of each oil from oil phase in hexane at 233 and 268 nm, respectively. Hexane was used as the blank sample.

The determination of acid value (AV) evaluating the amount of free fatty acids liberated by hydrolysis was performed according to the ISO 660:2020 [28].

2.8. Statistical Analysis

The obtained results of antioxidant properties and oxidative status were presented as means (c) $\pm$ standard deviation (SD). All experiments were performed in triplicate on the same day to verify the precision of the applied analytical methods. The results were tested statistically by one-way analysis of variance (ANOVA) and Duncan post hoc test to evaluate significant differences ($p < 0.05$). Principal component analysis (PCA) was employed to study the clustering and differentiation of mayonnaise samples without and with antioxidants incorporated in various forms and stored for 4 weeks based on AC, PV, AnV, TOTOX, CD, CT, and AV results. The data analyzed by PCA were depicted as a biplot, which combined score and loading plots. Statistica 8.0 (StatSoft, Tulsa, OK, USA) software was applied for the statistical data analysis.

3. Results and Discussion

3.1. Antioxidant Properties of Mayonnaises

The changes in AC of the studied mayonnaise samples without and with antioxidants added directly and to active packaging material during 4 weeks of storage at refrigeration temperature were determined by direct measurement using QUENCHER-DPPH and QUENCHER-ABTS assays, and the results are listed in Table 1.

Table 1. Changes in antioxidant capacity of mayonnaise samples before and after antioxidant supplementation during 4-week cold storage.

Week	Type of Mayonnaise					
	CPBM	CM	BCCM0.1%	BCCM3%	OCM	AFM
QUENCHER-DPPH $\pm$ SD ($\mu\text{mol TE}/100\text{ g}$)						
0	557 $\pm$ 5 a,b,A	850 $\pm$ 25 b,B	890 $\pm$ 25 c,B	978 $\pm$ 21 b,c,C	1846 $\pm$ 50 a,D	841 $\pm$ 21 a,B
1	534 $\pm$ 4 a,A	884 $\pm$ 41 b,B	914 $\pm$ 41 c,B	1014 $\pm$ 48 c,C	2185 $\pm$ 92 b,D	844 $\pm$ 36 a,B
2	578 $\pm$ 15 b,A	845 $\pm$ 34 b,B,C	829 $\pm$ 32 b,B	1006 $\pm$ 38 b,c,D	2435 $\pm$ 101 c,E	924 $\pm$ 35 b,C,D
3	692 $\pm$ 27 c,A	735 $\pm$ 33 a,A	776 $\pm$ 19 a,b,A	948 $\pm$ 26 b,B	2708 $\pm$ 133 d,C	1031 $\pm$ 50 c,B
4	704 $\pm$ 17 c,A	679 $\pm$ 30 a,A	727 $\pm$ 34 a,A	845 $\pm$ 28 a,B	2887 $\pm$ 139 d,D	1164 $\pm$ 38 d,C
QUENCHER-ABTS $\pm$ SD ($\mu\text{mol TE}/100\text{ g}$)						
0	2070 $\pm$ 94 c,A	2068 $\pm$ 39 b,A	2126 $\pm$ 40 b,B	2326 $\pm$ 52 b,C	2644 $\pm$ 41 a,D	2065 $\pm$ 38 a,b,A
1	1872 $\pm$ 84 b,A	2079 $\pm$ 57 b,B	2286 $\pm$ 61 b,C	2302 $\pm$ 46 b,C	2895 $\pm$ 149 b,D	2075 $\pm$ 34 a,b,B
2	1821 $\pm$ 57 b,A	1990 $\pm$ 54 b,B	2064 $\pm$ 95 a,B,C	2311 $\pm$ 71 b,D	3794 $\pm$ 80 c,E	2175 $\pm$ 43 b,C
3	1301 $\pm$ 50 a,A	1988 $\pm$ 68 b,B	1990 $\pm$ 41 a,B	2334 $\pm$ 96 b,D	3886 $\pm$ 131 c,d,E	2138 $\pm$ 59 b,C
4	1232 $\pm$ 42 a,A	1735 $\pm$ 81 a,B	1974 $\pm$ 64 a,C	2139 $\pm$ 97 a,C	4128 $\pm$ 198 d,D	1989 $\pm$ 95 a,C

$n = 3$; SD—standard deviation; different lowercase letters within the same column indicate significant differences between the AC of the studied mayonnaise samples during the shelf life (one-way ANOVA and Duncan test, $p < 0.05$); different capital letters within the same row indicate significant differences between the AC of the studied mayonnaise samples among antioxidant agent (one-way ANOVA and Duncan test, $p < 0.05$). Abbreviations: CPBM—commercial plant-based mayonnaise; CM—control aquafaba-based mayonnaise; BCCM0.1%—aquafaba-based mayonnaise with 0.1% of black cumin cake extract; BCCM3%—aquafaba-based mayonnaise with 3% of black cumin cake extract; OCM—aquafaba-based mayonnaise with octyl caffeate; AFM—aquafaba-based mayonnaise with active film incorporating black cumin cake extract.

QUENCHER-DPPH and QUENCHER-ABTS seem to be the most popular and willingly used tests due to their measurement simplicity and direct use without sample preparation, short experimental time, and the employment of the inexpensive spectrophotometer. However, the QUENCHER-DPPH method is specific for potential lipophilic antioxidant testing because the DPPH radical dissolves only in organic media, whereas the QUENCHER-ABTS is applicable to lipophilic and hydrophilic substances [29].

Regardless of the analytical assay performed, the commercially available CPBM sample revealed the lowest AC during a 4-week storage period at refrigeration temperature

in an opened jar (Table 1). In addition, a decrease in QUENCHER-ABTS results after each week of storage and QUENCHER-DPPH values after 1-week storage of CPBM were observed. The loss of antioxidants during CPBM storage is probably related to their decomposition and oxidation, which causes a decrease in the quality of commercially available mayonnaise. Higher QUENCHER-DPPH values after a more extended time indicate that cold storage promoted the significant increase of compounds in CPBM with the scavenging ability of DPPH radical. The polyphenol derivatives present naturally in mayonnaise were probably hydrolyzed to a free state, or new DPPH scavengers were produced during the storage period. Moreover, lipophilic antioxidants such as carotenoids commonly used in CPBM formulation were released from the oil phase during a longer storage time and became more effective in scavenging DPPH radical as days of storage passed. On the other hand, the oxidation processes of some flavonoids present in CPBM could generate metabolite mixtures that retained the scavenging properties of unoxidized flavonoids or had higher antioxidant activities, causing an increase in DPPH results [30]. A similar effect of DPPH increase for water-in-oil emulsions of *Pulicaria jaubertii* extracts without and with ultrasonic treatment during storage for 28 days was observed by Al-Maqtari et al. [31]. The emulsions treated with ultrasound released lower antioxidants with DPPH scavenging ability during the storage period, compared to the untreated emulsion.

It can be noted that the supplementation of CM with BCCE and OC caused a significant increase in AC after 1 week of storage, while insignificant differences for mean QUENCHER-DPPH and QUENCHER-ABTS values were observed between CM stored in a glass jar without and with an active film containing BCCE at the same storage time (Duncan test, Table 1). On the other hand, the addition of antioxidants to the proposed CM caused a significant increase in the antioxidant properties of the enriched mayonnaise samples. Enriched mayonnaises before and after each week of storage had higher values of QUENCHER-DPPH (727–2887 $\mu\text{mol TE}/100\text{ g}$) and QUENCHER-ABTS (1974–4128 $\mu\text{mol TE}/100\text{ g}$) than control and commercial samples (QUENCHER-DPPH = 534–884 $\mu\text{mol TE}/100\text{ g}$ and QUENCHER-ABTS = 1232–2079 $\mu\text{mol TE}/100\text{ g}$).

Interestingly, during the 4-week storage period, antioxidant properties of the CM contacted with active film (AFM) increased with storage time (except QUENCHER-ABTS value in the last week). This can be explained by the fact that the natural antioxidants present in BCCE added to GEL-PVA material were effectively released and distributed in mayonnaise. Moreover, the fortification of CM with the synthesized and purified OC caused an approximately 1.6-fold increase in the AC of OCM determined by both analytical methods at the end of the 28 days of storage (Table 1). This confirms that fortification with amphiphilic antioxidant (OC) significantly enhanced the concentration of lipophilic antioxidants in OCM, which can be detected by QUENCHER-DPPH and QUENCHER-ABTS assays. Additionally, the AC of studied OCM samples significantly increased with the extension of storage time (Duncan test, Table 1). This suggests that when natural and synthetic compounds with antioxidant properties were combined, different interactions occurred, having various effects that may be synergistic, antagonistic, or additive, and improved the antioxidant properties of mayonnaise.

On the contrary, the initial AC of CM before and after fortification of BCCE at 0.1 and 3% decreased during 4 weeks of storage at 4 °C in a refrigerator, indicating degradation of antioxidants naturally present and added to prepared mayonnaise samples (Table 1). However, natural antioxidants in BCCE had an important role in inhibiting these destructive reactions because of the significantly lower reduction of QUENCHER-DPPH (20% and 14–18% before and after supplementation, respectively) and QUENCHER-ABTS (16% and 7–8%, before and after supplementation, respectively) values observed during the same period. Moreover, the antioxidative effect of BCCE increased with increasing its concentration in the BCCM0.1% and BCCM3%, respectively (Table 1). This suggests that BCCE contained compounds which were responsible for free radical capture. Moreover, there were synergistic effects and relationships between the bioactive ingredients of CM and

concentrations of antioxidant compounds in BCCE extract, and their antioxidant potential depended on their structure.

For comparison, the obtained QUENCHER-DPPH (534–704 $\mu\text{mol TE}/100\text{ g}$) and QUENCHER-ABTS (1232–2070 $\mu\text{mol TE}/100\text{ g}$) results for CPBM were similar to those reported previously for vegan mayonnaise (QUENCHER-DPPH = 589 $\mu\text{mol TE}/100\text{ g}$ and QUENCHER-ABTS = 1371 $\mu\text{mol TE}/100\text{ g}$) [24]. The addition of beetroot peel powder to mayonnaise also increased the antioxidant activity (DPPH = 21.15–52.09 mM TE/100 g) of samples during storage compared to the control sample (DPPH = 1.60–1.81 mM TE/100 g) [7]. Moreover, the ABTS of W/O emulsions with *Pulicaria jaubertii* extract treated with different ultrasound powers (100–600 W) increased continuously from 2.93 to 12.88 mg TE/mL during storage conditions up to 28 days [31]. The prolongation of storage time also caused an increase in ABTS results (463–613 $\mu\text{mol TE}/100\text{ g}$ and 590–752 $\mu\text{mol TE}/100\text{ g}$ at 1st and 45th day of storage, respectively) of vegan mayonnaises enriched with hydroxytyrosol extracted from olive mill wastewater [32]. In contrast, after 45 days of storage, these samples exhibited a lower ability to scavenge DPPH radical (74–100 $\mu\text{mol TE}/100\text{ g}$) than the sample stored 1 day (78–134 $\mu\text{mol TE}/100\text{ g}$) [32].

3.2. Oxidative Status of Mayonnaises

The oxidative parameters of the proposed mayonnaise formulations were evaluated to predict the finished products' shelf life and ensure their functionality and safety during storage conditions. Results related to the oxidative status of the oil phase of each mayonnaise sample stored in the refrigerator for 4 weeks are presented in Table 2.

PV measures the amount of primary oxidation products—peroxides and hydroperoxides. It can be noted that the PV results of all prepared emulsions and commercially available mayonnaise significantly increased during the shelf life test (Duncan test, Table 2). The highest PV values (2.67–4.00 meq O_2/kg of oil) were observed for CPBM during storage conditions up to 28 days. This could be attributed to the absence of additional substances, such as antioxidants, in the mayonnaise sample, which would prevent the formation of peroxides and hydroperoxides in the oil phase. However, the prepared CM aquafaba-based mayonnaise samples with the addition of BCCE were characterized by the lowest PV values (1.10 meq O_2/kg of oil and 1.26 meq O_2/kg of oil for BCCM3% and BCCM0.1%, respectively) after 4 weeks of storage. This indicates that antioxidants present in BCCE successfully delayed the primary oxidation processes, mainly generating hydroperoxides in the oil phase.

Unexpectedly, the supplementation of CM with synthesized antioxidant OC caused a 1.5-fold increase in PV after 1 week of storage (Table 2). At the end of the storage, the amount of hydroperoxides in OCM (PV = 2.02 meq O_2/kg of oil) insignificantly differed from PV for non-supplemented CM (2.00 meq O_2/kg of oil). On the other hand, antioxidants present in active film incorporating BCCE gradually released to CM and markedly decreased hydroperoxide formation in the AFM sample after 28 days of storage in the refrigerator compared to the CM sample (Duncan test, Table 2).

A similar increase in PV (1.55–2.00 meq O_2/kg of oil) for mayonnaise samples fortified with different ginger concentrations (0–1.25%) after 4 weeks of storage was reported by Kishk et al. [6]. However, after 20 weeks of storage, a sample without ginger powder had the highest PV (53.0 meq O_2/kg of oil) with a significant difference compared to all fortified mayonnaise samples (PV = 8.9–36.8 meq O_2/kg of oil). Moreover, Alizadeh et al. [2] found that levels of primary oxidation products in mayonnaises with different antioxidant additives, such as tocopherol, rosemary essential oil, *Ferulago angulata* extract, and tertiary butylhydroquinone (TBHQ) were lower (PV = 1.5–2.75 meq O_2/kg of oil) after 1 month of storage than the PV of control sample (3.25 meq O_2/kg of oil).

Table 2. Changes in oxidative parameters of mayonnaise samples before and after antioxidant supplementation during 4-week cold storage.

Week	Type of Mayonnaise					
	CPBM	CM	BCCM0.1%	BCCM3%	OCM	AFM
PV (meq O ₂ /kg of oil)						
0	2.67 ± 0.05 ^{a,C}	0.50 ± 0.02 ^{a,A}	0.51 ± 0.03 ^{a,A}	0.50 ± 0.03 ^{a,A}	0.63 ± 0.03 ^{a,B}	0.50 ± 0.03 ^{a,A}
1	3.47 ± 0.03 ^{b,D}	1.01 ± 0.04 ^{b,C}	0.55 ± 0.01 ^{b,B}	0.50 ± 0.01 ^{a,A}	0.97 ± 0.01 ^{b,C}	1.00 ± 0.01 ^{b,C}
2	3.50 ± 0.01 ^{b,F}	1.24 ± 0.01 ^{c,E}	0.60 ± 0.01 ^{c,A}	0.80 ± 0.01 ^{b,B}	1.05 ± 0.05 ^{c,C}	1.09 ± 0.01 ^{c,D}
3	3.99 ± 0.02 ^{c,E}	1.48 ± 0.02 ^{d,C}	0.81 ± 0.02 ^{d,A}	1.00 ± 0.01 ^{c,B}	2.00 ± 0.01 ^{d,D}	1.49 ± 0.03 ^{d,C}
4	4.00 ± 0.01 ^{c,E}	2.00 ± 0.01 ^{e,D}	1.26 ± 0.02 ^{e,B}	1.10 ± 0.02 ^{d,A}	2.02 ± 0.02 ^{d,D}	1.75 ± 0.01 ^{e,C}
AnV						
0	2.19 ± 0.11 ^{a,B}	1.15 ± 0.06 ^{a,A}	1.13 ± 0.04 ^{a,A}	1.17 ± 0.07 ^{a,A}	1.19 ± 0.05 ^{a,A}	1.15 ± 0.03 ^{a,A}
1	2.39 ± 0.08 ^{a,b,D}	1.17 ± 0.07 ^{a,A,B}	1.07 ± 0.07 ^{a,A}	1.39 ± 0.07 ^{b,C}	1.26 ± 0.06 ^{a,B}	1.19 ± 0.05 ^{a,b,B}
2	2.21 ± 0.10 ^{a,C}	1.20 ± 0.05 ^{a,b,A}	1.17 ± 0.06 ^{a,A}	1.37 ± 0.06 ^{b,B}	4.43 ± 0.14 ^{b,D}	1.30 ± 0.07 ^{b,A,B}
3	2.21 ± 0.11 ^{a,C}	1.29 ± 0.03 ^{b,A}	1.38 ± 0.04 ^{b,B}	1.44 ± 0.07 ^{b,B}	4.78 ± 0.08 ^{c,D}	1.48 ± 0.06 ^{c,B}
4	2.53 ± 0.12 ^{b,C}	1.52 ± 0.07 ^{c,A}	1.57 ± 0.07 ^{c,A}	1.54 ± 0.07 ^{c,A}	4.70 ± 0.23 ^{c,D}	2.00 ± 0.10 ^{d,B}
TOTOX						
0	7.53	2.15	2.15	2.17	2.45	2.15
1	9.33	3.19	2.17	2.39	3.20	3.19
2	9.21	3.68	2.38	2.97	6.53	3.48
3	10.19	4.25	3.00	3.44	8.78	4.46
4	10.53	5.52	4.09	3.74	8.74	5.50
CD						
0	2.30 ± 0.07 ^{b,B}	2.20 ± 0.01 ^{c,A}	2.19 ± 0.01 ^{a,A}	2.20 ± 0.01 ^{a,A}	2.23 ± 0.02 ^{b,A}	2.20 ± 0.01 ^{b,A}
1	2.07 ± 0.02 ^{a,A}	2.03 ± 0.03 ^{b,A}	2.33 ± 0.09 ^{a,b,D}	2.32 ± 0.07 ^{b,c,C}	2.25 ± 0.02 ^{b,A,B}	2.19 ± 0.03 ^{b,B}
2	2.41 ± 0.05 ^{c,A}	2.38 ± 0.02 ^{d,A}	2.43 ± 0.10 ^{b,c,A}	2.47 ± 0.02 ^{d,e,A,B}	2.63 ± 0.05 ^{c,C}	2.57 ± 0.05 ^{d,B}
3	2.50 ± 0.05 ^{d,C,D}	2.42 ± 0.03 ^{d,C}	2.56 ± 0.08 ^{c,D}	2.54 ± 0.03 ^{e,C,D}	2.00 ± 0.04 ^{a,A}	2.28 ± 0.04 ^{c,B}
4	2.91 ± 0.02 ^{e,C}	1.98 ± 0.03 ^{a,A}	2.44 ± 0.06 ^{b,c,B}	2.39 ± 0.09 ^{c,d,B}	1.95 ± 0.02 ^{a,A}	1.95 ± 0.04 ^{a,A}
CT						
0	1.96 ± 0.09 ^{a,D}	0.53 ± 0.01 ^{c,B}	0.56 ± 0.02 ^{b,C}	0.51 ± 0.01 ^{b,A}	0.55 ± 0.01 ^{a,C}	0.53 ± 0.01 ^{c,B}
1	2.16 ± 0.05 ^{b,D}	0.55 ± 0.01 ^{d,B}	0.45 ± 0.01 ^{a,A}	0.46 ± 0.01 ^{a,A}	0.71 ± 0.01 ^{e,C}	0.58 ± 0.01 ^{e,B}
2	2.28 ± 0.04 ^{c,E}	0.50 ± 0.01 ^{b,B}	0.45 ± 0.01 ^{a,A}	0.46 ± 0.01 ^{a,A,B}	0.66 ± 0.02 ^{d,D}	0.54 ± 0.01 ^{d,C}
3	2.41 ± 0.03 ^{d,E}	0.49 ± 0.01 ^{a,B}	0.45 ± 0.02 ^{a,A}	0.46 ± 0.01 ^{a,A,B}	0.64 ± 0.01 ^{c,D}	0.51 ± 0.01 ^{b,C}
4	2.61 ± 0.01 ^{e,C}	0.48 ± 0.01 ^{a,A,B}	0.46 ± 0.02 ^{a,A}	0.48 ± 0.02 ^{a,A,B}	0.58 ± 0.02 ^{b,B}	0.48 ± 0.01 ^{a,A,B}
AV (mg KOH/g of oil)						
0	1.20 ± 0.01 ^{a,C}	0.08 ± 0.00 ^{a,A}	0.07 ± 0.00 ^{a,A}	0.09 ± 0.00 ^{a,A}	0.13 ± 0.01 ^{a,B}	0.08 ± 0.00 ^{a,A}
1	1.33 ± 0.01 ^{b,F}	0.12 ± 0.01 ^{c,D}	0.08 ± 0.00 ^{b,A}	0.09 ± 0.00 ^{a,B}	0.17 ± 0.00 ^{b,E}	0.11 ± 0.00 ^{b,C}
2	1.34 ± 0.02 ^{b,D}	0.12 ± 0.00 ^{c,B}	0.10 ± 0.00 ^{c,A,B}	0.09 ± 0.00 ^{a,A}	0.17 ± 0.01 ^{b,C}	0.11 ± 0.00 ^{b,A,B}
3	1.36 ± 0.01 ^{b,E}	0.11 ± 0.00 ^{b,C}	0.10 ± 0.00 ^{c,B}	0.09 ± 0.00 ^{a,A}	0.23 ± 0.01 ^{c,D}	0.11 ± 0.00 ^{b,C}
4	1.38 ± 0.05 ^{b,C}	0.12 ± 0.00 ^{c,A}	0.10 ± 0.00 ^{c,A}	0.11 ± 0.01 ^{b,A}	0.23 ± 0.00 ^{c,B}	0.11 ± 0.00 ^{b,A}

$n = 3$; SD—standard deviation; different lowercase letters within the same column indicate significant differences between the oxidative status of the studied mayonnaise samples during the shelf life (one-way ANOVA and Duncan test, $p < 0.05$); different capital letters within the same row indicate significant differences between the oxidative status of the studied mayonnaise samples among antioxidant agent (one-way ANOVA and Duncan test, $p < 0.05$). Abbreviations: CPBM—commercial plant-based mayonnaise; CM—control aquafaba-based mayonnaise; BCCM0.1%—aquafaba-based mayonnaise with 0.1% of black cumin cake extract; BCCM3%—aquafaba-based mayonnaise with 3% of black cumin cake extract; OCM—aquafaba-based mayonnaise with octyl caffeate; AFM—aquafaba-based mayonnaise with active film incorporating black cumin cake extract.

Nevertheless, unstable primary oxidation products transform into secondary oxidation products such as alkanes, alcohols, or aldehydes. The content of non-volatile components, primarily aldehydes generated during hydroperoxide decomposition, was measured and

expressed as AnV. As presented in Table 2, AnV results of all studied mayonnaises in the absence and presence of natural and synthetic antioxidants increased during storage time. The lowest amounts of secondary oxidation products after 4 weeks of storage were observed in CM, BCCM3%, and BCCM0.1% (AnV = 1.52, 1.54, and 1.57, respectively). Thus, only aquafaba as the main ingredient of the proposed mayonnaise can affect the hydroperoxide decomposition to form carbonyls and other compounds, in particular, aldehydes.

The obtained results match with those reported by other authors who found that the AnV results of mayonnaise enriched with apple peel extract (0–1.25%) decreased with increasing concentrations of this extract (AnV = 7.65, 4.71, 4.11, 3.51, and 3.42 for samples without and with 0.5, 0.75, 1.0, and 1.25% of extract after 60 days of storage, respectively) [33]. Alizadeh et al. [2] also observed an increasing trend in AnV changes of mayonnaises before and after the addition of tocopherol, rosemary essential oil, *Ferulago angulata* extract, and TBHQ during storage at ambient temperature for 6 months. Although at the end of storage, all fortified samples had lower AnV results (10.26–12.23) compared to the control sample (AnV = 18.32). Similarly, fortification of mayonnaises with various spices (oregano, rosemary, black pepper, and ginger), vitamin C, tocopherol, and butylated hydroxytoluene (BHT) affected their AnV values differently [34]. The increase in AnV (3.00) for the vitamin C-added mayonnaise was the lowest after 6 weeks of storage at 37 °C, while AnV (7.12) for mayonnaise with ginger was approximately 1.5 times higher than AnV (4.70) for a control sample.

Unfortunately, the contact of CM with the active film did not effectively inhibit the formation of secondary oxidation products. In fact, after 4 weeks of storage, the AnV (2.00) of AFM increased by approximately 30% compared with the AnV (1.52) of the control CM sample. This fact could be associated with gradually releasing antioxidants from packaging material to mayonnaise. Bioactive compounds from the active film were probably released too slowly to prevent the decomposition of primary oxidation products into secondary oxidation products.

Among studied mayonnaises, the highest AnV (4.43–4.70) data were observed for OCM samples between 2 and 4 weeks of storage (Table 2). Because OCM samples had the highest antioxidant potential (Table 1), the highest AnV results for OCM can be explained by interferences of the added OC on the AnV measurements determined by the official method.

Furthermore, the TOTOX indexes at the end of the 28 days of storage indicated that the addition of BCCE directly to mayonnaise samples inhibited oxidation reactions compared to the CM sample, without active additives (TOTOX = 5.52, 4.09, and 3.74 for CM, BCCM0.1%, and BCCM3%, respectively). It is noteworthy that the CPBM sample characterized by the highest TOTOX values (7.53–10.53) exhibited the lowest AC (QUENCHER-DPPH = 534–704 µmol TE/100 g, QUENCHER-ABTS = 1232–2070 µmol TE/100 g). Therefore, it could be assumed that the antioxidant content in this sample was insufficient to retard the generation of oxidation products.

Additionally, conjugated polyenes are also suitable parameters for the assessment of oxidative deterioration of emulsions. CD mainly form with the hydroperoxides generation as early indicators of oil phase oxidation. At the end of storage, the highest CD concentration was observed for CPBM (2.91), while OCM, AFM, and CM samples had significantly lower CD contents (1.95–1.98) (Duncan test, Table 2). The CD amounts in emulsions stabilized by BCCE increased remarkably to 2.39 and 2.44 for BCCM3% and BCCM0.1%, respectively, revealing the occurrence of primary oxidation during the storage period.

Moreover, the fresh and stored CPBM had the highest CT results (1.96–2.61), which corresponded to the highest TOTOX (7.53–10.53) of this sample. Similar to the AnV results, the OC addition as an amphiphilic antioxidant caused an increase in CT concentration in the OCM sample at each step of storage (CT = 0.55–0.71) compared with CM (CT = 0.48–0.55).

The obtained results of CD (1.95–2.91) and CT (0.45–2.61) for all investigated samples were lower than the results of CD (3.90–7.50) and CT (2.17–2.65) for mayonnaises enriched with different amounts (5–20%) of black cumin oil and stored in an incubator at 20 °C for

28 days [21]. Moreover, CD amounts in mayonnaises with 0.10 and 0.15% of grape seed extract did not change during storage at 20 °C for up to 8 weeks [9]. However, progress in CD generation in mayonnaise samples without and with the lowest amount of this extract (0.05%) was found. In contrast, CD levels (2.9–3.0) in mayonnaises without and with 0.03% of rosemary extract did not differ significantly after storage for 10 months [35]. Thus, rosemary extract did not exhibit protective effects towards the primary changes of oxidation in mayonnaise.

The formation of free fatty acids determined as AV due to the hydrolysis of triacylglycerols and hydroperoxide decomposition in the oil phase of emulsions during storage time is one of the most critical indicators of their deterioration. Consequently, AV results of the studied mayonnaise samples insignificantly increased with increasing storage duration, irrespective of the antioxidant type used (Duncan test, Table 2). However, BCCE added directly to mayonnaise and active film inhibited the generation of free fatty acids the most effectively. For this reason, at the end of the shelf life test, the AV of BCCM0.1%, BCCM0.3%, AFM, and CM did not differ significantly. However, the addition of OC to mayonnaise consistently increased its acidity from 0.13 to 0.23 mg KOH/g after 28 days of storage.

Similar tendencies were observed in our previous work after fortification of rapeseed oil with phenolipids (AV = 0.029–0.085 mg NaOH/g and 0.077–0.503 mg NaOH/g, for control rapeseed oil and rapeseed oil with phenolipids, respectively) [36]. It can be explained by the fact that OC, which contains the -OH group, can react with alkali and increase acidity expressed as AV. Nevertheless, the AV (0.23 mg KOH/g) of OCM was 6 times lower in comparison with the AV of CPBM (1.38 mg KOH/g) after 4 weeks of storage.

Interestingly, the studied mayonnaise samples had lower AV results (0.07–1.38 mg KOH/g) than the AV (0.85–1.44 mg KOH/g) of mayonnaises with beetroot peels powder in different concentrations (1.5–7%) [7] and the AV (0.1–2.9 mg KOH/g) of mayonnaise samples enriched with different ginger concentrations and were stored for 4 weeks [6].

The proposed formula of aquafaba-based mayonnaise enriched with BCCE and OC and stored contacting with active film incorporating BCCE was characterized by lower oxidative parameters after 4 weeks of storage compared to the oxidative status of CPBM. At the end of storage, the TOTOX values of CM, BCCM0.1%, BCCM3%, and AFM (5.52, 4.09, 3.74, and 5.50, respectively) were lower than the TOTOX of CPBM at the beginning of shelf-life (7.53). Only the AnV of OCM from 2 to 4 weeks of storage was higher than AnV of CPBM (4.43–4.78 and 2.21–2.53, for OCM and CPBM, respectively). These results encourage the use of aquafaba-based formulation and active packaging to extend the shelf-life of egg-free mayonnaises from 2 to 4 weeks.

3.3. Principal Component Analysis

Principal component analysis (PCA) was performed on the multidimensional variables of six various mayonnaises for visualization of the underlying structure in experimental data and relationships between data and samples. Among the eight components, PC1 and PC2 revealed eigenvalues > 1 (4.03 and 2.79), while the remaining principal components (PC3, PC4, PC5, PC6, PC7, and PC8) had eigenvalues < 1 (0.86, 0.20, 0.098, 0.025, 0.0023, and 0.000001, respectively); thus, they have not been discussed further. The first two components accounted for 85.21% of the total variance, of which the first component explained 50.34% of the total variance in the data set and the second component contributed 34.87%. For this reason, the first two PCs were plotted as a biplot on the X- and Y-axes to detect the association between variables as well as similarities or differences among mayonnaise samples (Figure 3).

PC1 correlated highly negatively with PV, TOTOX, CT, and AV with loading of −0.972, −0.903, −0.968, and −0.971, respectively. PC2 also negatively reflected a QUENCHER-DPPH, QUENCHER-ABTS, and AnV loading of −0.932, −0.918, and −0.879, whereas PC2 was positively contributed by CD (0.356).

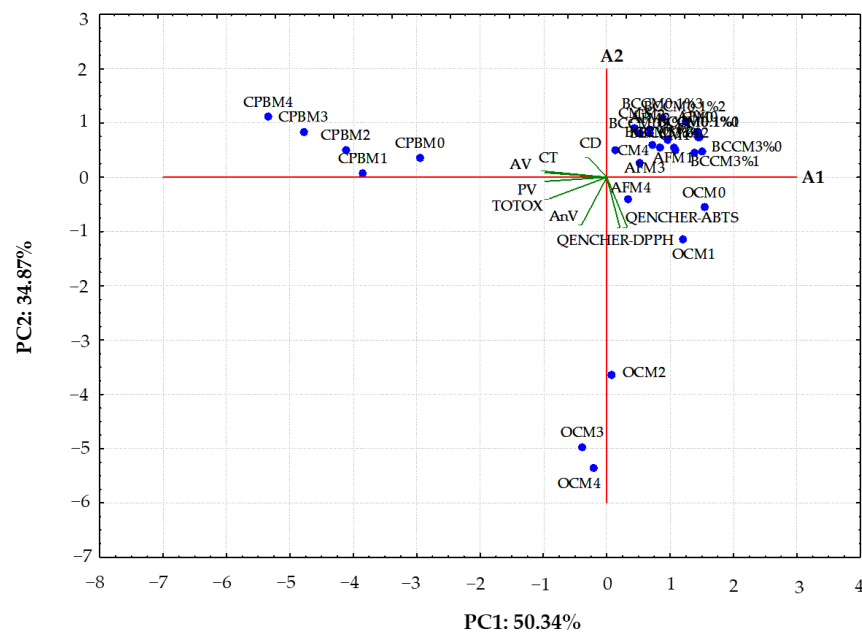


Figure 3. Biplot of the first two principal components (PC1 and PC2) of six various mayonnaise samples (CPBM, CM, BCCM0.1%, BCCM3%, OCM, and AFM) at 0, 1, 2, 3, and 4 weeks of storage.

According to the biplot in Figure 3, all CPBM samples with low antioxidant properties and high free fatty acid content, as well as mayonnaise after the fortification of synthesized OC and stored in the refrigerator at 4 °C for up to 21 and 28 days having high amounts of primary and secondary oxidation products, were located on the left side of the biplot. However, the proposed CM samples without and with BCCE at 0.1 and 3% concentrations and contacting with active film incorporating BCCE during the storage period were situated at the right in the diagram and had an excellent oxidative status and high antioxidant potential. Furthermore, mayonnaises after supplementation of synthesized OC with the highest ability to scavenge DPPH and ABTS radicals and the highest content of secondary oxidation products were located under the A1 axis.

It can be noted that the investigated mayonnaise samples fell into four distinct groups, respectively (Figure 3). The location of commercially available mayonnaise samples during the 4-week storage period in the top left-hand quadrant of Figure 3 can be explained by their high results of CT and AV (Table 2), which were co-located in this region of the PC space. On the contrary, CM samples without and with natural antioxidants present in BCCE had low oxidative parameters, so they were situated diametrically opposite commercial CPBM. The supplementation of OC and storage for a long time caused the longest distance of OCM2, OCM3, and OCM4 samples from other mayonnaises due to their highest antioxidant potential determined by QUENCHER-DPPH and QUENCHER-ABTS methods and amounts of secondary oxidation products. Nevertheless, OCM0, OCM1, and AFM4 with moderate antioxidant properties created an evidently distinct cluster.

The biplot revealed that the grouping of the 30 mayonnaise samples depended on their ingredients, bioactive compounds added directly to samples and packaging material, as well as storage time, which affected levels of antioxidants and oxidation products.

4. Conclusions

In this study, novel aquafaba-based egg-free mayonnaises (CM) enriched with bioactive compounds extracted from by-product (BCC) and synthesized phenolipid (OC) were prepared. The proposed mayonnaise samples without and with antioxidants and stored in contact with active film incorporating BCCE had higher antioxidant properties than commercially available plant-based mayonnaise (CPBM). Antioxidant potential increased with increasing the concentration of BCCE in the mayonnaise samples. Moreover, BCCE

inhibited the formation of free fatty acids and primary and secondary oxidation products in mayonnaise samples (BCCM0.1% and BCCM3%), improving their total oxidative status. Interestingly, the presence of OC or an active film in a jar and the extension of storage time positively affected the antioxidant properties of OCM and AFM. Although the OCM revealed the highest AC, the OC presence had low activity in preventing oil phase oxidation under storage conditions. However, the use of active film slightly slowed down the oxidation and hydrolysis reactions in the AFM sample during the storage period.

In conclusion, the addition of BCCE-enriched active film and BCCE added directly to mayonnaise samples had a beneficial effect on oxidative status and prolonged the shelf life of aquafaba-based egg-free mayonnaise. Thus, natural antioxidants extracted from fat industry waste can be used as a potential alternative to synthetic antioxidants in food emulsions to extend their shelf life.

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