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ORIGINAL RESEARCH PAPER

EFFECTS OF SUPPLEMENTATION WITH ALGERIAN *NIGELLA SATIVA* BIOACTIVE COMPOUNDS ON QUALITY OF YOGURT

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Abstract

The characteristics of Algerian *Nigella sativa* (Ns) seeds were investigated after extraction with three aqueous ethanol concentrations (40, 60 and 80%) at three seed concentrations (2.5, 5 and 7.5%) referred as T, F and S, respectively. Aqueous ethanol extraction (40%) at 2.5 and 5% Ns seed concentration [T40% and F40%] had the highest total phenolic and total flavonoid contents. Higher seed (7.5%) and aqueous ethanol concentrations (60 and 80%) extracts [S60% and S80%] exhibited the maximum antioxidant activity. T80% and S40% extract exerted high antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, respectively. Four stirred yogurts formulated with 0, 0.5, 1 and 2% of S60% extract were evaluated for physicochemical and sensory properties; two of these formulated yogurts were further investigated for stability under refrigerated storage (4 °C for 21 days). Yogurt formulated with 0.5% of S60% extract exhibited the highest antioxidant activity, high titratable acidity, low pH and increasing syneresis during storage. However, the control yogurt had higher syneresis than the fortified yogurt (60 vs 46% at 21 days storage).

Keywords: *Nigella sativa*, bioactive compounds, optimization, antioxidant activity, antibacterial activity, functional food, enriched yogurt, sensory analysis

Introduction

In the last few decades, emphases on the role of foods have shifted from substances consumed merely to quell hunger or to provide needed nutrients for normal cellular function to substances that can potentially promote health and wellness and, particularly, reduce risk of disease. These foods are frequently referred to as

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nutraceuticals and/or functional foods with various reported bioactive functions (immunomodulators, antihypertensives, osteoprotectives, hypocholesterolemics, antioxidatives, and antimicrobials) (Aryee and Boye, 2014). Numerous studies have demonstrated beneficial impact of polyphenol rich diet against different oxidative stress induced maladies owing to their free radical scavenging perspective (Singh *et al.*, 2009; Hameed *et al*., 2019), and showed various biological effects, including anti-inflammatory, anti-carcinogenic activities, etc. The association between the antioxidative properties of food and health has recently been extensively investigated. Natural antioxidants are also in high demand for application as nutraceuticals/functional foods and biopharmaceuticals because of consumer preferences and their therapeutic effect (Aggarwal *et al.*, 2013).

Nigella sativa L. is one of the most popular plants around the world; its seeds are traditionally used in food as well as "medicine". Many studies reported the pharmacological properties of *Nigella sativa* seeds considered as antioxidant, anti-inflammatory, immunomodulatory, antitumor, antidiabetic agent and it plays a significant role in the cardiovascular and gastrointestinal systems (El-Dakhakhni *et al.*, 2000; Burits and Bucar, 2000; Gilani *et al.*, 2004; Salem, 2005). The seeds are small and black, and possess a scented odor, which is pungent and bitter in feel with a crunchy texture. The plant is an annual herbaceous, which belongs to the Ranunculaceae family. It is indigenous to the Mediterranean regions, but is also cultivated in Saudi Arabia, Africa, and Southwest Asia. The seeds are extensively sold in markets to be used as a condiment and native medicine. The seed oil is also considered among newer sources of edible oils (Cheikh-Rouhou *et al*., 2007).

Yogurt is easily digested milk product, has high nutritional value, and is a rich source of carbohydrates, protein, fat, vitamins, calcium, and phosphorus. Milk protein, fat, and lactose components undergo partial hydrolysis during fermentation (Sanchez-Segarra *et al*., 2000). It is an increasingly popular cultured dairy product in most countries due to its nutritional and potentially therapeutic characteristics. This is partly because of an increased consumer awareness regarding possible health benefits of yogurt ascribed to its nutraceutical, therapeutic and probiotic effects, such as improved digestion, enhanced immune system, anticarcinogenic activity and serum cholesterol reduction (Bertolami, 1999; Shah, 2001; Milo-Ohr, 2002; Aguirre-Mandujano *et al.*, 2009).

Food fortification/enrichment is one of the most cost-effective and the best approach to prevent malnutrition, especially in developing countries, and can be a valuable solution to develop functional foods (Nazari *et al.*, 2023). Previous studies confirm the use of dairy products, notably yogurt, as suitable food for enrichment/fortification with medicinal herbal extracts (Matter *et al*., 2016; Abdel-Hamid *et al*., 2020; Marand *et al*., 2020; Arab *et al*., 2020). To the best of our knowledge, very few studies has been conducted on the yogurt fortification with *Nigella sativa* seeds extract.

The objectives of this study were to optimize the extraction of bioactive compounds from *Nigella sativa* seeds and to further use the obtained extracts for formulating yogurt enriched in antioxidants. The sensory acceptability of the yogurt formulations

was tested, as well as the physicochemical parameters and antioxidant activity over storage under refrigeration condition (4 °C for 21 days).

Materials and methods

Materials

Our study was carried out on nigella seeds (*Nigella sativa* L.) grown in greenhouses in an agricultural region: Ain Oulmene, located at 33 km south of Sétif (the highlands) and at 335 km from Algiers. After cleaning and eliminating impurities and dust, the seeds were crushed by a mortar followed by mechanical grinding (Vevor 3000W, Grain Grinder Mill, China) and sieving (diameter ≤ 0.5mm). The obtained powder was stored in a hermetically sealed bottle away from light and humidity.

Strains of *Escherichia coli* and *Staphylococcus aureus* used for antibacterial activity were provided from the microbiology laboratory (Ecole Supérieure des Sciences de l'Aliment et des Industries Agroalimentaires [ESSAIA], Algiers).

Raw cow milk and the freeze-dried mixture of starter cultures (*Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarus* ssp. *thermophilus*) were provided from ONALAIT COLAITAL unit, Algiers.

Proximate composition

Proximate composition of Nigella seed's powder was determined including moisture, ash, fat and crude protein (nitrogen-to-protein conversion factor of 6.25) following the official AOAC methods 925.10, 923.03, 920.39, and 954.01 (AOAC, 1995). The percentage of carbohydrates was estimated by difference.

Extraction procedure

Extraction of phenolic compounds was carried out by maceration according to the method described by Oomah *et al.* (2011) using the mass of powder and the solvent concentration as parameters of optimization. An aliquot of Nigella powder (2, 4, 6 g) was mixed with 80 mL of aqueous ethanol (40, 60, 80%) by constant magnetic stirring for 2 h at room temperature. After centrifugation (5000g for 10 min), the recovered crude extracts were stored at -4°C in dark until analysis. The extracts obtained were coded as: T40%, T60%, T80%, F40%, F60%, F80%, S40%, S60%, S80%, where the different letters refer to the amount of samples used to prepare the extract: T two (2g), F to four (4g) and S to six (6g).

Determination of total phenolic content (TPC)

TPC was measured by the Folin method (Singleton and Rossi, 1965). Briefly, to 50µL of crude extract were added 250µL of Folin reagent and 3mL of distilled water. The absorbance was measured at 760nm after incubation in dark at room temperature; 750µL of sodium carbonate (Na_2CO_3) at 20% (w/v) and 950µL of distilled water were added. Gallic acid (0-0.1mg/mL) was used as standard. The results were expressed in mg gallic acid equivalent (GAE)/100g of powder.

Determination of total flavonoids content (TFC)

TFC was measured as described by Lamaison and Carnat (1990). Crude extract (2 mL) was added to 2 mL $AlCl_3$, $6H_2O$ at 2% (w/v); the mixture was homogenized,

incubated at room temperature for 15 min, and the absorbance measured at 430 nm. Quercetin (0-0.03 mg/mL) was used as standard and the results expressed in mg quercetin equivalent (QE)/100 g of powder.

DPPH radical-scavenging activity

Antioxidant activity was measured by free radical (DPPH) scavenging activity according to the method described by Brand-Williams *et al.* (1995). Briefly, 100 µL of the extract was added to 1 mL of DPPH solution (60 mM). After 30 min of incubation in the dark, the decrease in absorbance was determined at 517 nm. The % inhibition of DPPH was calculated according to equation 1.

$$\% \text{ inhibition} = (A_{control} - A_{sample})/A_{control} \times 100 \quad (1)$$

where: $A_{control}$ - absorbance of DPPH solution without extract; A_{sample} - absorbance of DPPH solution mixed with extract.

Antibacterial activity

Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method described by Bauer et *al.* (1966) with minor modifications (Rathan, 2000); to assess the presence of antibacterial activities of the extracts obtained from *Nigella sativa* seeds. Inoculum was prepared after collecting well-isolated bacterial colonies using a sterile platinum loop, which were then transferred into a sterile tube containing sterile nutrient broth (5mL), followed by incubation (37 ˚C for 24 h). At 1×10^8 CFU/mL (colony forming unit per mL), the optical transmission was measured at 640 nm. This was designated as the inoculum that was used for antibacterial activity in this work. A previously liquefied media appropriate for the test (Muller Hinton) was inoculated with the necessary quantity (0.1 mL) of the strains ($1x10^8$ CFU/mL); the suspension was added to the media at 40–50 °C and the inoculated media was poured immediately into dried Petri dishes to occupy 3 to 4 mm depth. The paper disc (N°.1Whatmann) was cut into small disc (6 mm diameter) and sterilized, then impregnated with Nigella extracts and placed on the surface of the media. The control disc was impregnated with the extraction solvent. The dishes were left standing at room temperature to allow Nigella extracts to diffuse into the media before the bacteria began to multiply. Subsequently, dishes were incubated for 24 h at 37 °C, prior to measuring the diameter of the inhibition zone.

Enriched yogurt formulation

The yogurts were formulated at ONALAIT COLAITAL unit, Algiers, according to the production diagram of a stirred natural yogurt. Briefly, raw cow milk was homogenized for 5 min, pasteurized (95 °C, 20 seconds), then cooled quickly to 45 °C. The milk was inoculated with the starter culture (3%) and the mixture stirred continuously then divided in 125 g plastic yogurt pots. The Nigella crude extract, with higher antioxidant activity S60% was first concentrated in a rotary evaporator at 45°C under reduced pressure, until reaching an extraction yield of 12.82%. This concentrated extract was then added at different concentrations (0%, 0.5%, 1% and 2%) to the yogurt. The mixture was fermented at 45 °C for 4h until the control

samples reached the pH of 4.5. The samples were gently stirred and stored at 4 °C until analysis. No additive that could influence the organoleptic and rheological characteristics was added to the yogurts. Four formulated yogurts were obtained: CY (control yogurt), EY1, EY2, and EY3 (yogurts enriched with 0.5%, 1%, and 2% extract, respectively).

Determination of pH, total dry extract (TDE) and titratable acidity (TA)

The pH, TDE and TA of the four formulated yogurts were measured after one day of storage. pH was measured at room temperature by electrode immersion with a pH meter (Starter 2100). TDE was measured using infrared desiccator (Sartorius MA35). TA was measured by mixing 1 mL of yogurt with 9 mL of distilled water and few drops of phenolphthalein (0.1% w/v), followed by titration with NaOH solution (0.1N) until the appearance and persistence of the pink color. The volume of NaOH solution required for titration was noted, and the TA was expressed in °Dornic according to equation 2.

$$TA\ (°D) = V \times 10 \quad (2)$$

where: V - Volume of NaOH (mL) required for titration; 10 - dilution factor.

Sensory analysis

A descriptive sensory analysis was performed for evaluating the sensory characteristics of the yogurts (in agreement with the evaluation protocol approved by the university ethics committee). The sensory analysis was carried out with the four formulated yogurts on the first day of storage, presented in pots and labeled with a code (1, 2, 3, and 4). The sensory analysis was based on a five-point 1 to 5 hedonic scales for some sensory parameters including smell (absent to very strong), color (not presentable to very presentable), texture (very grainy to very smooth), consistency (liquid to very firm), taste and acidity (absent to very strong). Thirty untrained panelists performed the sensory analysis, all non-smokers (students from ESSAIA, Algiers). Based on the results of the sensory analysis, we decided to use only CY and EY1 yogurts for the assessment of the evolution of physicochemical characteristics and antioxidant activity during storage (from day 1 to day 21).

Evolution of physicochemical characteristics during yogurt storage

Evolution of pH, TA and syneresis during storage was carried out by analyzing yogurt samples CY and EY1 at day 1 (D1), day 7 (D7), day 14 (D14) and day 21 (D21). The pH, and TA were measured as described above. Syneresis was determined using centrifugation method (Koegh and O'kennedy, 1998). Briefly, 20g of yogurt was centrifuged at 3000 g for 10 min at 4 °C. The clear supernatant was weighed, and syneresis was calculated according to equation 3.

$$\text{Syneresis}\ (\%) = (\text{weight of supernatant/weight of yogurt}) \times 100 \quad (3)$$

Evolution of antioxidant activity by DPPH inhibition assay during storage

Evolution of antioxidant activity during storage was carried out by analyzing yogurt samples (CY and EY1) at day 1 (D1), day 7 (D7), day 14 (D14) and day 21 and measured as described above.

Statistical Analysis

All determinations were performed in triplicate. The results are expressed as the mean ± standard deviation. The results were subjected to a multifactorial analysis (Factor 1: solvent concentration and Factor 2: mass of the sample) for the phenolic extracts, and (Factor 1: storage time, Factor 2: extract concentration) for the enriched yogurt, followed by a multiple comparison of the means (LSD test) using Statgraphics plus Centurion XVI software (Statpoint Technologies. Warrenton. USA). Differences are considered significant at $P<0.05$.

Results and discussion

Proximate composition

The proximate composition of *Nigella sativa* powder used in the experiment is provided in Table 1. The carbohydrates content was estimated at 40.86%, being the most dominant nutrient in Algerian black cumin seeds. This value is similar to the previously reported results on *Nigella sativa* seeds from Saudi (Al-Jassir, 1992), Turkey (Nergiz and Ötles, 1993), Tunisia and Iran (Cheikh-Rouhou *et al*., 2007) but lower than the Bengladeshi seeds (Mamun and Absar, 2018). Fat content was the second main nutrient in this variety (33.92%) which is similar to the fat value of Turkey and Bangladeshi seeds (Nergiz and Ötles, 1993; Mamun and Absar, 2018), but less than Saudi seeds (Al-Jassir, 1992). The crude protein content (15.05%) was lower than reported values (18.09 to 26.7%) (Al-Jassir, 1992; Nergiz and Ötles, 1993; Cheikh-Rouhou *et al.*, 2007; Mamun and Absar, 2018). However, the moisture and ash contents were very similar to the results available in the literature (moisture content of 4.6 – 6.4 % and ash content of 4.0 – 4.86%).

Table 1. Proximate composition of *Nigella sativa* powder.

Proximate composition	Moisture	Ash	Fat	Crude protein	Carbohydrates
Content (%)	6.16±0.33	4.10±0.08	33.92±0.24	15.05±0.51	40.86±0.22

Determination of total phenolic content (TPC)

The statistical analysis demonstrated a significant effect of the two factors studied for the optimization of extraction: the solvent concentration and the mass (*P1* and *P2*, respectively = 0.0000). Results summarized in Table 2 show that the significantly higher TPC content ($P<0.05$) was in favor of T40% crude extract (520.16 mg GAE/100g), while the S80% crude extract displays the lowest concentration (317.30 mg GAE/100g). Overall, for the same mass of Nigella powder, we note that the crude extracts T (40, 60, 80%) are significantly ($P=0.0000$) the richest in TPC, while the crude extracts S (40, 60, 80%) are the least concentrated in TPC. The concentrations obtained in this study (317.30-520.16 mg GAE/100g) are higher than those found in Saudi Nigella seeds extracts (Mechraoui *et al.*, 2018), where extraction was carried out using 70% methanol and 70% acetone and TPC

were estimated at 137.14 and 59.62 mg GAE/100g, respectively. The variations in TPC found among the seeds produced in different regions may be due to the geographical and climatic differences, cultivated regions, storage conditions and maturity stage. And also may be due to many experimental factors which influence the rate of extraction of the phenolic compounds; like the concentration and the type of solvent, because their polarity and concentration play an essential part in the extraction yield (Chirinos *et al*., 2007; Mamun and Absar, 2018; Tuong et *al.*, 2020).

Determination of total flavonoids content (TFC)

Results of TFC of different extracts are presented in Table 2. The statistical analysis shows a significant effect of the two factors studied for the optimization of extraction: the solvent concentration and the mass ($P1$= 0.0000 and $P2$= 0.0003). The F40% extract displays the highest content of TFC (57.76 mg QE/ 100g), while the S80% extract was found to be the least concentrated in TFC (19.28 mg QE/ 100g). According to our results, we found that for the same mass of Nigella powder, the TFC decreased significantly (P<0.05) by increasing the concentration of the extraction solvent. TFC obtained in our study (19.28 to 57.76 mg QE/ 100g) are similar to those found by Mechraoui *et al.* (2018) in Nigella seeds methanolic extracts (44.18 mg QE/ 100g) and acetonic extract (27.46 mg QE/ 100g), while the study carried out by Mamun and Absar (2018) showed incomparable values, where TFC was estimated at 3.78 mg QE/100g. The difference observed between the values obtained in the different extracts, can be justified by the composition of the soil where the different seeds were grown, the storage conditions, the extraction technique, the ratio: mass of the sample/volume of the solvent, the concentration and the polarity of the solvent which play an important role in the extraction yield (Bimakr *et al.*, 2011).

DPPH radical-scavenging activity

Phenolic compounds represent the majority of the antioxidant activity in plants, in which the antioxidant properties are mainly due to their redox potential, which allows them to act as reducing agents, hydrogen donors, metal chelators and singlet oxygen quenchers (Belhachat *et al.*, 2017). The antioxidant activity, determined by DPPH radical-scavenging activity of our different extracts is summarized in Table 2. The statistical analysis shows a significant effect of the two factors studied for the optimization of extraction: the solvent concentration and the mass ($P1$= 0.0000 and $P2$= 0.0000). The S60% exhibited the optimal inhibition property (32.43%), we noticed that for the same mass of Nigella powder, the antioxidant activity is significantly (P<0.05) the highest when the extraction solvent at 60% was used. In parallel, we observed that for same concentration of the solvent extract, the antioxidant activity is more considerable using 6 g powder. The antioxidant activity of S60% extract is slightly lower than those reported by Chauhan *et al.* (2018). The different intrinsic and extrinsic factors of the plant material influence the levels of antioxidants from one species to another and even between the different varieties of the same genus, this variation is probably related to the difference in the extraction methods, the nature and concentration of the solvents used. The affinity of the target compounds with the extraction solvent affects the scavenging of the DPPH radical

of the spices, as well as the methods of the assay, which does not give a complete quantitative composition of extracts (Bimakr *et al.*, 2011; Hameed *et al.*, 2019).

Table 2. Total phenolic compounds content (TPC), total flavonoids content (TFC) and antioxidant activity (AA) of *Nigella sativa* extracts.

Extracts	TPC (mg GAE/100g)	TFC (mg QE/ 100g)	AA (%)
T 40%	520.16±6.84 ^{f}	51.99±1.00 ^{e}	5.06± 0.41 ^{a}
T 60%	504.37±1.77 ^{e}	27.90± 2.13 ^{cd}	12.34± 1.87 ^{b}
T 80%	369.72±10.13 ^{bc}	26.54± 2.35 ^{c}	5.53± 0.24 ^{a}
F 40%	421.77±9.64 ^{d}	57.76± 1.02 ^{f}	14.22± 0.41 ^{c}
F 60%	413.97±12.59 ^{d}	26.39± 0.34 ^{c}	23.00± 2.87 ^{f}
F 80%	360.12±4.93 ^{b}	23.32± 1.03 ^{b}	19.16± 0.81 ^{e}
S 40%	368.92±0.00 ^{bc}	29.60± 0.07 ^{d}	16.22± 0.41 ^{d}
S 60%	375.68±3.89 ^{c}	23.97± 1.73 ^{b}	32.43± 2.45 ^{h}
S 80%	317.30±7.82 ^{a}	19.28± 1.25 ^{a}	28.17± 1.90 ^{g}
	P value		
Factor 1: concentration	0.0000	0.0000	0.0000
Factor 2: mass	0.0000	0.0003	0.0000

Means on the same column with different letters are significantly different ($P< 0.05$)

Antibacterial activity

The appearance of inhibition zones around impregnated discs with most of different extracts reflects the presence of antibacterial activity (Table 3). All extracts had anti *E. coli* activity manifested by different inhibition diameter; an extreme sensivity of *E.coli* was noticed toward T60%, T80%, F60%, F80% and S60% (D = 20, 34, 24, 26 and 20 mm, respectively). In contrast, the S80 displayed the weakest anti *E.coli* activity (D= 9mm). This result is similar to that reported previously (Zuridah et *al.*, 2008) for the Nigella extracts from Malaysia. *Staphylococcus aureus* showed resistance (D= 0 mm) to T60%, T80% and F40% extracts. However, this bacteria is extremely sensitive to S40% and S60% extracts (D of 20 and 30 mm, respectively). These results are comparable with the results of Özmen *et al.* (2007). This difference in sensitivity may be due to the concentration of polyphenols in the extracts, the nature of the molecules present in our extracts and the nature of the outer wall of the studied bacteria (Sharif *et al.*, 2009).

Determination of pH, total dry extract (TDE) and titratable acidity (TA)

Yogurt pH, TDE and TA were significantly (P= 0.0000) affected by *Nigella sativa* extracts addition, all tested parameters increasing with the increase of the extract concentration in yogurts (Table 4). The EY3 samples exhibited the highest pH, TDE and TA values. Our results are similar to those reported for the yogurts enriched with cantaloup or sesame (Kermiche *et al.*, 2018; Arab *et al.*, 2020).

Table 3. Antibacterial activity of *Nigella sativa* extracts.

Strain	Extract	Inhibition diameter (mm)	Reading
***Escherichia coli* (Gram -)**	T40%	15	VS
	T60%	20	ES
	T80%	34	ES
	F40%	19	VS
	F60%	24	ES
	F80%	26	ES
	S40%	14	S
	S60%	20	ES
	S80%	9	S
***Staphylococcus aureus* (Gram +)**	T40%	12	S
	T60%	0	R
	T80%	0	R
	F40%	0	R
	F60%	10	S
	F80%	11	S
	S40%	20	ES
	S60%	30	ES
	S80%	10	S

R: resistant (D ≤ 8), S: sensitive (8 < D ≤14), VS: very sensitive (15< D ≤19), ES: extremely sensitive (D ≥20), D: diameter.

Table 4. pH, total dry extract (TDE) and titratable acidity (TA) of the yogurt samples supplemented with 0% (CY), 0.5% (EY1), 1% (EY2) and 2% *Nigella sativa* extract (EY3).

Yogurt samples	pH	TDE (%)	TA (°D)
CY	4.55± 0.01 ^{a}	20.90± 0.25 ^{b}	55.00± 0.00 ^{a}
EY1	4.61± 0.01 ^{b}	21.47± 0.20 ^{c}	57.00± 0.57 ^{b}
EY2	4.64± 0.00 ^{c}	21.61± 0.04 ^{a}	58.00± 0.00 ^{c}
EY3	4.80± 0.01 ^{d}	21.71± 0.01 ^{a}	65.00± 0.28 ^{d}
***P* value**	0.0000	0.0000	0.0000

Means on the same column with different letters are significantly different (P< 0.05).

Sensory analysis

The collected scores of the sensory evaluation after 1 day of refrigerated storage performed in all yogurt samples are reported in Figure 1 as averages of scores given by the panel members. The enriched yogurts were appreciated by the sensory panel. The *Nigella sativa* extract has a very strong odor, therefore, EY1 was rated as the best for its smell and its slightly beige color. In terms of taste and consistency, the panel considered that EY1 was more appreciated than other enriched yogurts. Concerning texture, all enriched yogurts were highly appreciated than control yogurt (CY). EY2 and EY3 had a less acceptable sensation of acidity than the other yogurts;

this may be due to the concentration of the added extract. Overall, since the intensity of the taste varies with the concentration of Nigella extract, and since *Nigella sativa* seeds has a very marked taste, the best rated yogurt is the one with the lowest concentration (EY1). Similar results were observed in yogurt supplemented with germinated nigella (Nazari *et al.*, 2023).

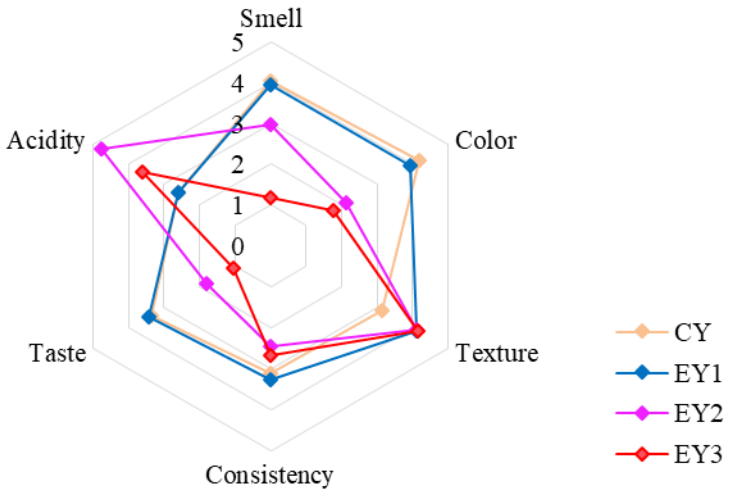

Figure 1. Sensory profile of the yogurt samples supplemented with 0% (CY), 0.5% (EY1), 1% (EY2) and 2% (EY3) *Nigella sativa* extract.

Evolution of physicochemical characteristics during storage

The pH, TA and syneresis values of yogurts during 21 days of storage at 4 °C are shown in Figure 2 (a, b, and c, respectively). Yogurt fortification and storage time significantly ($P< 0.05$) affected the physicochemical characteristics of the samples.

The pH values markedly decreased throughout the post acidification period, from 4.55 to 4.2 in case of the CY samples, and from 4.61 to 4.14 for EY1 (Figure 2 a).

Similar trends were observed in yogurts supplemented with sesame, with omija and with germinated black cumin seeds (Arab *et al.*, 2020; Cho *et al.*, 2020; Nazari *et al.*, 2023). pH is an important factor in the processing of fermented milks, as provides information about the shelf life of yogurts. Its decrease during storage is mainly due to lactic acid bacteria, which continue to transform lactose into lactic acid (Abdalla and Ahmed, 2010).

TA measurement is an indicator of bacterial metabolic activity in fermented dairy product. TA increased for the two yogurts (Figure 2 b). The initial TA values of CY and EY1 were 55°D and 57°D, respectively. The acidity of yogurt continued to evolve during storage until reaching values of 81°D for CY and 86°D for EY1, this development is due to the degradation of lactose into lactic acid (Hassan and Amjad, 2010). Similarly, El-Batawy *et al.* (2014), incorporated mango and pomegranate

peels in yogurt, and reported gradual TA increase during the storage period. EY1 is marked by a slight difference in TA compared to CY; this may be due to the bioactive extract of *Nigella sativa* incorporated in the yogurt.

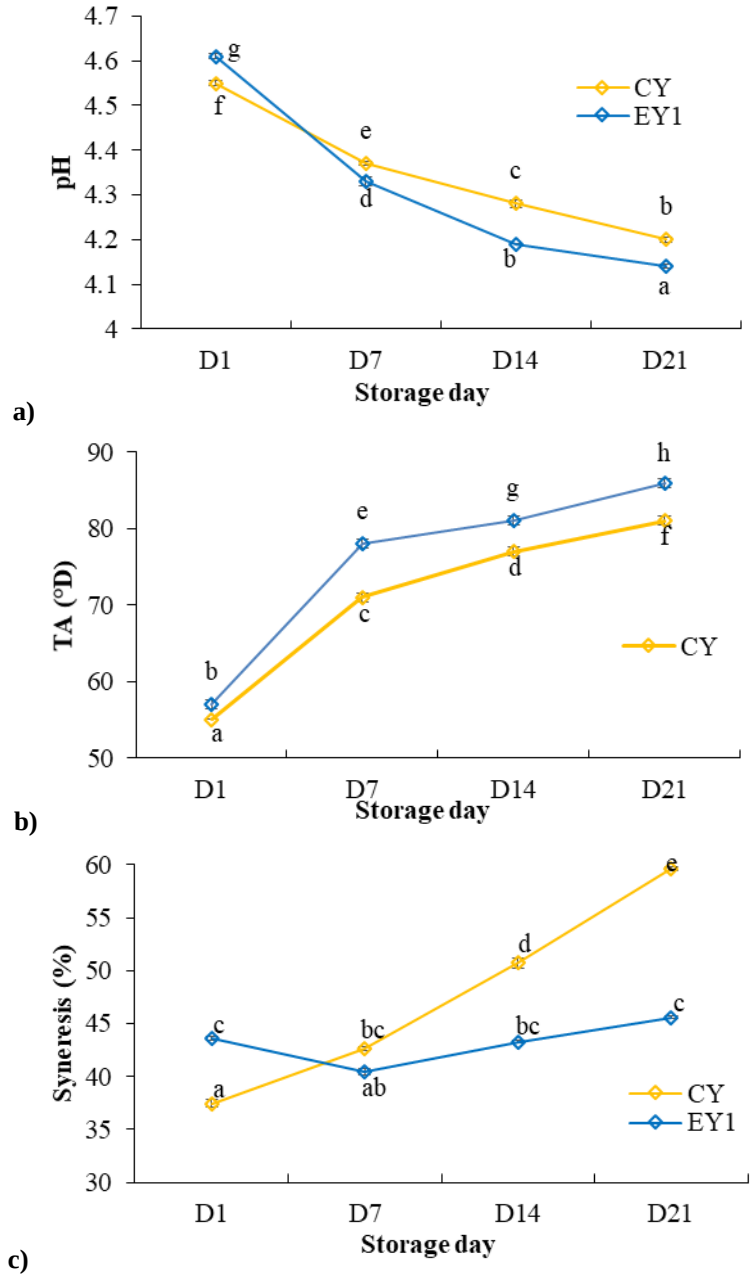

Figure 2. Evolution of pH (a), titratable acidity (b) and syneresis (c) during storage of the control (CY) and yogurt supplemented with 0.5% *Nigella sativa* extract (EY1). For a yogurt sample, means with different letters are significantly different ($P< 0.05$).

Syneresis is a physical phenomenon that occurs during yogurt storage; it is the separation of the whey from the yogurt due to the aggregation of the casein particles during storage (Tseng and Zhao, 2013). Syneresis increased for both samples during the storage period (Figure 2c). At the end of the storage period, the CY exhibited significantly higher syneresis of 59.65 % (P<0.05) compared to EY1 (syneresis of 45.58 %) (Figure 2 c). The pH decrease during storage can be the cause of the syneresis phenomenon; it also affects the structure of the gel, casein network and whey protein (Delikanli and Ozcan, 2014). The enrichment of yogurt with bioactive extract of *Nigella sativa* significantly reduced the spontaneous release of whey. This reduction can be linked to the abundance of polyphenols in Nigella, since polyphenols bind to the protein molecules from the yogurt matrix, and form complexes that limit syneresis (Siebert *et al*., 1996). These results are in agreement with previous reports on yogurts supplemented with fruits (Matter *et al*., 2016), or enriched with grape skin flour (Marchiani *et a*l., 2016).

Evolution of antioxidant activity by DPPH inhibition assay during storage

Fortification and storage time significantly (P< 0.05) affected the AA of the yogurt samples (Table 5). The enriched yogurt samples (EY1) exhibited three times higher DPPH radical scavenging activity compared to CY (54.33 and 16.63%, respectively). The AA decreased during the storage period eight times faster for CY until reaching a lower value (3.35%). A small reduction of thee AA was noticed for EY1 (28.73%) on day 21. This means that the addition of *Nigella sativa* extract to the yogurt, significantly, increased the inhibitory effect against the DPPH radical compared to the control yogurt. This confirms the antioxidant activity of the *Nigella sativa* extract. These results are in agreement with those reported by Georgakouli *et al*. (2016) and Cho *et al*. (2020), where the increase in the AA of the enriched yogurts was directly related to the phenol content. The decrease in AA of yogurts during storage may be related to the milk-polyphenol interaction, which can lead to reduced antioxidant capacity. Proline-rich casein can lead to precipitation of phenolic compounds and reduce antioxidant potential (Yuksel *et al*., 2010).

Table 5. Evolution of antioxidant activity (%) of the control (CY) and yogurt sample supplemented with 0.5% *Nigella sativa* extract (EY1), over storage for 21 days at 4°C.

Yogurt sample	**Storage time, days**			
	1	**7**	**14**	**21**
CY	16.63±0.49^{d}	8.90±0.24^{c}	6.94±0.56^{b}	3.35±0.49^{a}
EY1	54.33±0.76^{h}	51.02±0.54^{g}	42.06±0.52^{f}	28.73±0.82^{e}
	***P* value**			
Factor 1: storage time	0.0000			
Factor 2: concentration	0.0000			

Means on the same line with different letters are significantly different (P< 0.05).

Conclusions

Our study aimed at characterizing and optimizing the extraction of polyphenols from *Nigella sativa* seeds powder, and then developing functional food with health

benefits. Variability in the extraction rate of polyphenols and flavonoids was observed between the different crude extracts, and revealed an appreciable antiradical activity; the antimicrobial activity of some of these Nigella extracts proved to be satisfactorily effective against *Escherichia coli* and *Staphylococcus aureus*. The development of a dietary yogurt enriched with bioactive compounds from the *Nigella sativa* extract required a series of tests at the COLAITAL ONALAIT unit, Algiers. Microbiological analyzes of formulated yogurts revealed good compliance with standards and remarkable hygienic quality. Physicochemical results obtained from the present study during 21 days of storage at 4°C, indicated that the addition of *Nigella sativa* extract to yogurt could provide better product stability, viability of microorganisms and extend its shelf life by preserving its sensorial properties. Bioactives content and antioxidant activity of yogurts, increased with *Nigella sativa* extract addition. This implies that addition of Nigella extract in the yogurt formulation can be recommended; it improves the overall yogurt quality without altering its physicochemical and organoleptic properties.

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