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The development of value-added yogurt based on pumpkin peel powder as a bioactive powder

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ABSTRACT

This study aimed to explore the utilization of pumpkin by-products as a bioactive powder in the development of value-added yogurt. Pumpkin peel resulting from food processing contains antioxidant components like phenolics, flavonoids, and carotenoids, compounds characterized by high bioactivity. This study investigated the potential of incorporating pumpkin peel powder (PPP) into yogurt to enhance its nutritional value and sensory attributes.

Results indicated that the incorporation of PPP into yogurt resulted in improvements in nutritional composition, particularly in terms of β-carotene and bioactive compounds. Additionally, the addition of PPP positively influenced the textural properties of the yogurt. The sensory evaluation revealed that the incorporation of pumpkin peel had no negative impact on the overall acceptability of the yogurt, with some samples (YPP2) even exhibiting preferred sensory characteristics compared to the control. The utilization of PPP as a bioactive powder in yogurt presents a promising strategy for reducing food waste and creating innovative, value-added dairy products. The development of such products can not only contribute to sustainable food production but also provide consumers with more diverse food choices with enhanced characteristics.

1. Introduction

A circular economic model could be used to conduct agro-food by-product re-valorization in order to reduce created wastes and save the environment. Recent studies have focused on valuing agri-food wastes as a sustainable source of natural pigments while taking the "circular economy" model into consideration. Food waste, particularly from the processing of fruits and vegetables, offers a variety of natural pigments (secondary metabolites), such as carotenoids (yellow to orange), anthocyanins (blue to purple), chlorophylls (green), and betalains (dark red to pink), as well as natural additives to enhance the quality of food products [1].

Milk and dairy products are one of the potential resource categories for generating value added food items since they are rich in several essential nutrients. Milk and dairy products are nutrient-dense foods that provide energy, high-quality protein, as well as a variety of necessary micronutrients in an easily absorbed form, particularly calcium, magnesium, potassium, zinc, and phosphorus. One of the most well-known fermented milk products produced worldwide is yogurt. The most common fermented dairy food is yogurt, which has a high concentration of probiotics and nearly all of the necessary nutrients, easily digestible proteins. Because of its high nutritional value and the positive benefits on health that come from the presence of living bacteria like *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*, it is of significant economic importance throughout the world [2]. It can be easily digested even by people who are lactose intolerant because it has a low lactose content. Protein, unsaturated fatty acids, calcium, phosphorus, magnesium, zinc, and vitamin B are all abundant in yogurt [3].

Yogurts with different additions have, however, been produced more

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frequently in recent years in an effort to boost consumption and provide alternatives. Due to the growing demand from customers for taste and nutrient intake, adding fruits or vegetables to yogurt is currently a new trend in the modern food industry. Yogurt is often fortified with ingredients to enhance flavor, including a variety of fruits, some vegetables, coffee, oats, hazelnuts, almonds, and chocolate. Yogurts with fruit added have become more popular recently as a result of the growing popularity of fruit additions. However, yogurt is an excellent food matrix for adding probiotic microorganisms to and a significant source of calcium and protein [4].

Curcurbita pepo, C. maxima, and C. moschata are the three economically significant species of pumpkin that are grown around the world. Pumpkins are members of the *Cucurbitaceae* family. It offers a beneficial supply of carotenoids (β-carotene), protein, pectin, dietary fiber, certain vitamins (A, B1, B2 and C), minerals (K, P, Mg, Fe and Se), and other nutrients [5]. Pumpkin is frequently utilized in the manufacturing of specialty sweets, it is also processed in many different nations into jams, marmalades, pumpkin dessert, puree, and ready-to-eat dried snacks. Pumpkin can be used as a value added and coloring element in flour combinations, pasta, soups, and sauces. It can also be processed into flour (in its powder form). The primary portion of pumpkins intended for processing and eating is the pulp. In the form of peel or seeds, about 18–21% of the fruit is wasted. Large amounts of peel and other byproducts produced by the food sector are used as fertilizer or animal feed [6]. Numerous phytochemicals and antioxidants found in these byproducts could be utilized as food additives, bioactive molecules, or nutritional supplements. The bioactive compounds found in pumpkin byproducts (total phenolic, total flavonoid, tocopherols total carotenoid, mineral, and dietary fiber) have a variety of biological properties including antioxidant, antimicrobial, antihypertensive, and immunomodulatory activities [7,8].

According to studies, pumpkin peels, the main by-product of pumpkin processing (accounting for 2.6–16%), are used as an ingredient in the development of new value-added foods enriched in fiber [9] and have shown to have a high level of antioxidant activity [6,9].

Byproducts, like peel, offer useful qualities and can be employed as food ingredients or nutritional supplements. Regarding the carotene content determined from the pumpkin peel extract, many studies have been carried out, given the fact that pumpkin is an excellent source of carotenoids specially flesh and peel of pumpkin [7]. Vitamin A, which is mostly found in carotenoids, is important for human health since it supports the eyesight, immunological system, reproductive system, growth, and development. Vitamin A insufficiency is a leading cause of infant death and blindness [10]. Vegetables naturally include bioactive substances such as carotenoids, phenolic compounds, fiber, vitamins, and minerals. These goods can be made more valuable and perform better nutritionally thanks to the concentration or addition of bioactive substances. In order to create new food ingredients with useful qualities and a high concentration of carotenoids, there is growing industrial interest in the extraction of carotenoids from pumpkins. As a result, the extraction of carotenoids from pumpkin peels and other by-products that have been denied may be a good option to enhance the nutritional qualities while boosting the market value of these materials [7].

The present research focused on the extraction of the carotenoids from pumpkin peel powder (PPP) and obtain value-added yogurt. The use of pumpkin peel powder in the composition of yogurt can improve the product's nutritious value and, therefore the quality of life. Therefore, the objective of this study was to extract the carotenoids from pumpkin peel powder and analyzed the extract for total carotenoids content, phenolic compounds and antioxidant activity. In the end, the study aimed to obtain a value-added yogurt by the incorporation of the pumpkin peel powder. Investigations were also carried out on the impact of pumpkin peel powder (PPP) supplementation on the phytochemical composition, sensory characteristics, color and textural properties of yogurt.

2. Materials and methods

2.1. Materials

In total, 20 mature pumpkin fruits (*Cucurbita maxima*) with an average of 4.9 ± 0.5 kg were gathered from the Iasi local market. The specimen was brought to the "Ion Ionescu de la Brad" University of Life Sciences' Department of Plant Science, Faculty of Agriculture, for identification. The Rediu Iași Research Station, which is a part of the University of Life Sciences, provided the cow's milk (125 L). The farm is inhabited by a number of 55 Fleckvieh/Simmental cattle.

2.2. Pumpkin peels powder preparation

Pumpkin fruits (*Cucurbita maxima* with *Golden Nugget* variety) at full maturity were bought in November 2022 from a local supermarket in Iasi County, Romania. The fruits were processed right away after being sorted. When they got to the laboratory, the fruits were cleaned with distilled water, and the peels, flesh, and seeds of pumpkins were carefully separated and chopped into small pieces of 1 mm thickness, using a knife. The peels were cleaned with ultrapure water, dried with paper towels, and then freeze-dried for 48 h at 42 °C under a pressure of 0.10 mBar to reach 98% dry weight using CHRIST Alpha 1–4 LD plus equipment (Germany). Additionally, the freeze-dried peels were pulverized using MC 12 machinery (Stephan, Germany) into a fine powder (60 μm) and kept at room temperature in the dark in glass jars until analysis. The final powder was subjected to sterilization with a UV lamp for decontamination.

2.3. Extraction of phytochemicals from pumpkin peels powder (PPP)

With a few minor adjustments, the ultrasound-assisted extraction technique reported by Lima et al. [11] was used to extract the phytochemicals from pumpkin peel powder. In brief, 1.0 g of pumpkin peel powder was combined with 10 mL of n-hexane/acetone solvent mixture (3:1, v/v) or 70 % ethanol (only for total polyphenols and flavonoids extraction) and subjected to ultrasound treatment for 40 min at 40 °C and a frequency of 40 kHz by Smart MRC LLC, Holon, Israel. After recovering the resulting crude extract, it was then centrifuged for 15 min at 6500 rpm and 10 °C. The supernatant was collected after separation and the residue was extracted repeatedly by using 10 mL of n-hexane/acetone (3:1, v/v) until it became colorless. Moreover, the supernatant was collected and concentrated under reduced pressure at 40 °C using AVC 2–18 system from Christ (Osterode am Harz, Germany). The concentrated extracts were then analyzed by solubilization in the extraction solvent to calculate the amount of lycopene, β-carotene, total carotenoids, total flavonoids, and total polyphenols in pumpkin peel powder (PPP).

2.4. The quantification of carotenoids, phenolic compounds and evaluation of antioxidant potential of pumpkin peels powder (PPP)

2.4.1. Total carotenoid, β-carotene, and lycopene contents

Spectrophotometric analysis was performed to measure and determine the total carotenoids, β-carotene, and lycopene concentrations of extract as described by Nistor et al. [12] with slight modifications. In brief, 0.2 mL of the extract was dissolved in the extraction solvent mixture, then introduced in the UV quartz cuvette and a Libra S22 UV-VIS spectrophotometer was used to measure the absorbance at $\lambda =$ 450 nm for total carotenoids, $\lambda = 470$ nm for β-carotene, and $\lambda = 503$ nm for lycopene (Biochrom, Cambridge, UK). The results were reported as mg/g of dry weight (DW). Their concentrations were calculated using the following Equation:

$$\text{Contents (mg/g DW)} = (\text{A x Mw x Df})/(\text{m x L x Ma})$$

A—Absorbance of the sample;
Mw —molecular weight;
Df—sample dilution rate;
m—Mass/weight of concentrated extract;
L—length of the optical path of the cuvette (1 cm);
Ma—molar absorptivity, which is 2500 L mol^{-1} cm^{-1} for carotenoids, 2590 L mol^{-1} cm^{-1} for β-carotene, and 3450 L mol^{-1} cm^{-1} for lycopene.

2.4.2. Total flavonoid content

Using the technique described by Horincar et al. [13], the total flavonoid content of samples of pumpkin peel powder was assessed using the aluminum chloride method. In short, 0.25 mL of extract solution was combined with 0.075 mL of NaNO2 5% solution in 2 mL of distilled water. After 5 min of rest, 0.15 mL of AlCl3 10% solution were added in the mixture and then was given another 6 min to rest. After that, 0.5 mL of a 1 M NaOH solution was added, and the mixture's absorbance at 510 nm was immediately measured utilizing a Libra S22 UV-VIS spectrophotometer. A calibration curve for catechin as a standard was created, and the total flavonoid concentration was calculated using the calibration curve's linear regression equation ($R^2 = 0.9968$). The results were expressed as milligrams catechin equivalents per gram of dry weight (mg CE/g DW).

2.4.3. Total polyphenolic content

According to the method outlined by Horincar et al. [13], the total soluble phenolics present in PPP extract were measured using the Folin-Ciocalteu assay. Briefly, 1.0 mL of the Folin Ciocalteau solution and 0.2 mL of the PPP extract were put into tubes containing 15.8 mL of distilled water. 3 mL of Na_2CO_3 20% was added to the mixture after 10 min. The resulting combination was kept at room temperature and in the dark for 60 min before the absorbance at 765 nm was measured in comparison to a control (pure ethanol). To determine the amount of TPC in the sample, the extract's absorbance was compared to a standard curve for Gallic acid. The results were expressed as milligrams of Gallic acid equivalents per gram of dry weight (mg GAE/g DW).

2.4.4. Antioxidant activity (DPPH)

The antioxidant activity was assessed using the DPPH method, and the results were expressed as μmol of Trolox equivalents per gram of dry weight (μmol TE/g DW) [13]. A calibration curve utilizing Trolox as standard was used. In a nutshell, the blank's absorbance was determined at 515 nm using a 3.9 mL DPPH solution 0.1 M in methanol (A0). After adding 0.1 mL of PPP extract to the reaction mixture of 3.9 mL of 0.1 M DPPH solution, the mixture was allowed to sit at room temperature in the dark for 1 h and 30 min before the absorbance at 515 nm was measured (Af). The inhibition percentage was calculated as follows:

$$\% \text{ Inhibition} = (A0\text{- } Af)/A0 \text{ x } 100.$$

2.4.5. Color evaluation of pumpkin peel powder (PPP)

The MINOLTA Chroma Meter model CR-410 (Konica Minolta, Osaka, Japan) with a CIE Lab scale was used to measure the color characteristics of the powder. By placing the probe into the powder, the procedure allowed for reading the Chroma parameters. A* (red (>0) to green (<0) color, b* (yellow (>0) to blue (<0) color, and L* (black: L* = 0 and white: L* = 100) were used to express the results of the color measurements. Following equipment calibration against a white plate, the CIELAB color parameters were collected in triplicate. Hue angle, (Hue angle = arctan (b*/a*) for quadrant I (+a*, +b*), which describes the color of the powders (0° or 360° = red color, 90° = yellow color, 180° = green color, and 270° = blue color), and Chroma (Chroma = $\sqrt{(a^*)^2 + (b^*)^2}$)) which describes the purity or saturation of color, were also determined [14].

2.4.6. HPLC investigation of the carotenoids from the extract

The separation of carotenoids from the extract obtained from pumpkin peels was carried out in a concentration gradient using acetonitrile 90% (*v/v*) (solvent A) and ethyl acetate 100% (solvent B) as described by other authors [15]. The identification of carotenoids in the analyzed samples was carried out at a wavelength of 450 nm with the help of a MWD detector (Multiwavelength Detector) connected to an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, USA). Individual calibration curves were used for the quantitative analysis of the identified compounds. We were able to determine carotenoids characteristics by comparing their retention times and absorption spectra to those of readily available actual carotenoids and published data, respectively.

2.5. Raw milk collecting, sampling and analysis

A volume of 125 L of milk were taken out of the farm's storage tank. A truck with a thermo-regulated refrigeration tank delivered milk to the dairy processing Centre (at the University of Life Sciences). The milk in the delivery tank was maintained at a temperature of 5 °C. Each sample weighed 300 mL, and it was taken from the tank in sterile containers. It was transported to the laboratory in a separate box with ice packs, and it was refrigerated at 4 °C for 24 h. Prior to analysis, an average sample from each of the four original samples was created for each group. Milk was then completely homogenized and added to the analytical laboratory investigations (10 replications per trait/method under analysis).

The physicochemical parameters of milk samples (moisture content, solid non-fat content, fat content, protein content, ash content, lactose and pH) were determined in according with methods of AOAC.

2.6. Yoghurt manufacturing

The technological process of yogurt preparation with Pumpkin Peel Powder begins with the reception stage of the raw material and ingredients (cow's milk, lactic cultures, PPP) and auxiliary materials (Fig. 1). The following is the procedure for storing raw materials and auxiliary materials: milk is kept in isothermal tanks with temperature monitoring (0–4 °C); yogurt lactic cultures are kept at −18 °C with temperature and humidity monitoring; the bioactive powder (PPP 2 and 4%); auxiliary and packaging materials are kept in well-ventilated spaces at room temperature, free from foreign odors, and with natural light. The temperature and relative humidity of the air are also monitored to ensure product compliance. The milk undergoes the pasteurization process after filtration (10 min at a temperature of 90 °C). Subsequently, the milk was cooled while the temperature was monitored, which was maintained at 42 °C. The inoculation of milk with lactic starter cultures (*Lactobacillus delbruckii* subsp. *Bulgaricus and Streptococcus thermophilus*, YF-L812 commercial product, Chr. HANSEN, Denmark) was the next step in the technological process after they had previously been dosed and prepared (starting from the standard 50 U culture per 250 L of milk, according to the manufacturer's guidelines). Milk was divided into three parts (35 L for each part). The first part, without the addition of PPP in the composition was considered to be the control batch (YC - control batch). In the second part of milk, 2% PPP was added (YPP2 – yogurt with 2% PPP) and in the last part 4% PPP was added (YPP4 – yogurt with 4% PPP) (Fig. 1). The yogurt was dosed into PET glasses with a capacity of 120 mL, followed by their thermo-welding at 227 °C. The quantity and number of glasses were recorded. The product was incubated in plastic glasses at 43 °C for 360 min using a thermostatic camera (IT 40 thermostatic chamber, produced by Electronic April S.R.L. Romania) until a hard clot formed and the pH reached a range of 4.3–4.5, followed by gentle cooling (Fig. 1). The yogurts were stored at 5 ± 1 °C and the five replicates of the stirred yogurt were analyzed after 1, 7 and 14 days of manufacturing.

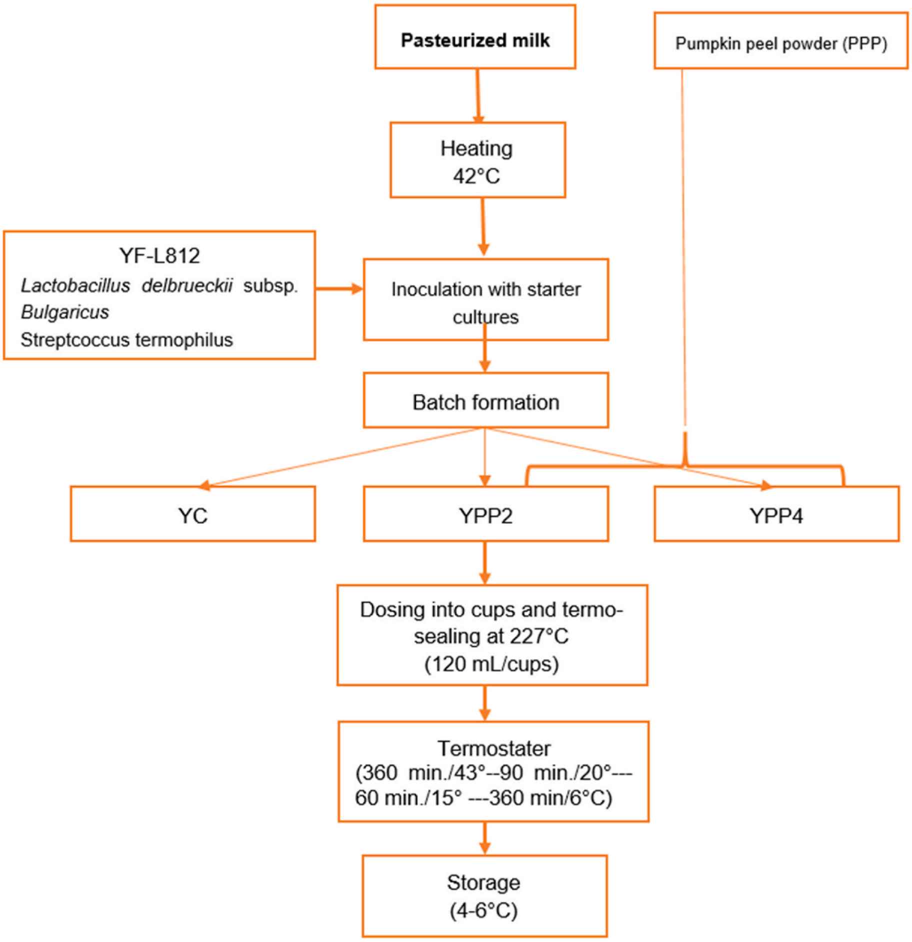

Fig. 1. Value added yogurt prototype processing flow diagram.

2.7. *Characterization of phytochemicals, physicochemical, syneresis and antioxidant activity of yogurts supplemented with PPP*

Moisture, total solids, total protein, fat, ash and pH of samples were determined in accordance with methods of AOAC. The approach described by Mbaeyi-Nwaoha et al. [16] was used to determine the crude fiber. It was identified as the portion that was still present after being digested with regular sulfuric acid and sodium hydroxide. In a nutshell, 2.0 g of the material was hydrolyzed in 299 mL of 1.25% sulfuric acid before being heated for 30 min. The mixture was vacuum-filtered, the residue rinsed three times with hot distilled water, heated for another 30 min with 200 mL of 1.25% sodium hydroxide, and then vacuum-filtered once more. The digested sample was rinsed three times with hot distilled water after being first neutralized with hydrochloric acid. The leftover material was placed in a crucible, dried for 2 h at 100 °C in an oven, and then cooled in a desiccator before being weighed. The sample in the crucible was burned at 500 °C for 5 h to completely burn out all carbonaceous material. The ash-containing crucible was then dried in the desiccator, cooled, and weighed.

$$\%\ \text{crude fiber} = (\text{loss in weight (g)after ignition})/(\text{weight of the original sample (g)}) \times 100$$

Total energy value was determined by Atwater method as described by Ezeonu et al. [17]. This method involves multiplying % carbohydrate content by 4%, protein content by 4% and fat content by 9%. Kilocalories per kilogram (Kcal/100 g) were used to measure the energy.

$$\text{Energy value} = (\%\text{CP x } 4) + (\%\text{CFT x } 9) + (\%\text{CHO x } 4)$$

Where: %CP – percentage crude protein; %CFT – percentage crude fat; % CF = percentage crude fiber; % CHO = percentage carbohydrate.

Igbabul et al. [18] provided the mathematical formula used to calculate carbohydrates.

$$\text{CHO} = 100 - \%\ (\text{ash} + \text{protein} + \text{fat} + \text{crude fiber} + \text{moisture})$$

The proportion of free whey is employed to quantify the degree of syneresis, which refers to the natural release of the aqueous component of yogurts caused by gel contraction. The measurement was conducted using the approach proposed by Wijesinghe et al. [19]. Concisely, 10 g of each yogurt sample were individually placed on a sheet of filter paper and left to rest on the top of a funnel. The remaining yogurt was weighed after undergoing vacuum drainage for 10 min, and the syneresis was calculated using the following equation:

$$\text{Free whey } (\%) = \frac{mass\ of\ initial\ sample(g) - mass\ of\ sample\ after\ filtration(g) \times 100}{weight\ of\ initial\ sample\ (g)}$$

Using the methods outlined in Sections 2.3.1-2.3.4, the total carotenoids, total polyphenolic contents, total flavonoid contents and antioxidant activity of yogurts enhanced with PPP were evaluated.

2.8. Color evaluation of yogurts supplemented with PPP

The MINOLTA Chroma Meter model CR-410 (Konica Minolta, Osaka, Japan) with a CIE Lab scale was used to measure the color characteristics of the yogurt. The method is similar to that described in section 2.3.5.

2.9. Texture analysis of yogurts supplemented with PPP

Using a texturometer with a digital dynamometer of 25 N, the Mark 10 ES M 300 texturometer (Mark-10 Inc., USA) was used to analyze the texture of yogurt samples (resolution 0.005 N). Three yogurt samples of each kind were subjected to analysis while the probe was moved through the yogurt mass twice through in a non-stationary manner. This test procedure results in the texture curve profile [20]. Regarding the principle of the method, it consists in determining the texture by exerting compressive stress on the coagulum using a Brookfield TA4/1000 cylindrical probe (h = 20 mm, D = 38.1 mm, Brookfield AMETEK Inc., USA). The force was continuously recorded throughout the experiment. As a working approach, the cylindrical probe applies various compression forces over the duration of the experiment depending on how hard the clot is. The information was gathered by acquiring the texture profile, from which a number of textural characteristics, including cohesiveness, elasticity, hardness, gumminess, consistency, resiliency, adhesiveness, and coagulum breaking force, could be calculated. To ascertain the clot's durability, a fresh compression cycle is carried out by rein-traducing the probe into the bulk of the clot after it has been removed from it. Ten repetitions were used to get the desired experimental results.

2.10. Rheological characterization of yogurts supplemented with PPP

The rheological properties of the yoghurt samples supplemented with different amounts of PPP were measured using the AR2000ex rheometer (TA Instruments, Ltd, New Castle, DE, USA), fitted with a plate-cone geometry (diameter of 20 mm and angle of 2°), and a closing gap of 1 mm. Prior to rheological measurements all samples were gently stirred, using a spatula. The samples equilibrated at 5 °C were subjected to dynamic strain sweep and steady shear rate sweep tests.

The dynamic strain sweep tests were carried out at constant frequency of 1 Hz, while gradually increasing the applied strain from 0.1 to 100%, such as to identify the linear viscoelastic range (LVR) specific to each yoghurt sample. The values of the main dynamic rheological parameters, namely storage modulus (G'), loss modulus (G''), the complex modulus (G^*) and the loss factor ($\tan \delta$) were registered within the LVR, at 0.80% deformation. The flow behavior of the yoghurts was investigated by gradually increasing the shear rate (γ) from 0.1 to 100 s^{-1}, while measuring the shear stress (σ, Pa) and apparent viscosity (η, Pa·s) over a steady rate sweep test. The Ostwald de Waele rheological model was used to fit the σ vs. γ experimental results:

$$\sigma = K \cdot \gamma^n$$

where K is the consistency coefficient (Pa·s^n), while n is the flow behavior index.

The rheological measurements were performed in duplicate.

2.11. Sensorial analysis of yogurts supplemented with PPP

Several organoleptically attributes of the different yogurt formulas was carried out. Thirty students and 18 specialists (a total of 48 tasters) from the Food Technologies department of the University of Life Sciences in Iasi evaluated the samples. The following criteria were evaluated: flavor (30 points), color (20 points), consistency (20 points), tongue feel (20 points), acidity (10 points), and overall acceptability (100 points) [21]. The average of the mean values of the aforementioned qualities and their standard error were obtained after statistical analysis of the results.

2.12. Statistical analysis

The statistical analysis was performed using the SPSS program (ver. 19), which has a multi-function utility with regard to the experimental design. Multiple comparisons were performed using LSD in accordance with Steel et al. [22].

3. Results and discussions

3.1. The PPP's phytochemical characterization and color evaluation

The phytochemical content and antioxidant activity of the PPP extract were determined and the results are exhibit in Table 1.

The ultrasound-assisted method applied in the present study allowed us to obtain a bioactive-enriched extract, containing total carotenoids of 15.107 ± 0.070 mg/100 g DW, total flavonoids of 2.698 ± 0.019 mg CE/g DW, with a total polyphenolic content of 4.616 ± 0.043 mg GAE/g DW. Regarding the β-carotene content, the average value was 12.513 ± 0.032 mg/100 g DW and for Lycopene a 3.001 ± 0.048 mg/100 g DW value was obtained. The extract showed a DPPH radical scavenging capacity of 15.349 ± 0.118 μmol TE/g DW, with 89.855 ± 1.021 % inhibition of DPPH radical. The obtained results are in agreement with the data reported in other studies.

Therefore, our results in terms of total carotenoids are higher than those reported by Jang et al. [23] who determined total carotenoid content and total flavonoid content in peel pumpkin powder from freeze-dried pumpkin powder and values were 2.75 mg/100 g and 81.5 mg CE/100 g powder. Lima et al. [24] indicate that the total carotenoids in the ethanolic extract are 771.5 ± 3.7 μg/g and for β-carotene the average value was 527.0 ± 6.0 μg/g. The β-Carotene contents in the peels of three species of pumpkins *Cucurbitaceae pepo*, *Cucurbitaceae moschata*, and *Cucurbitaceae maxima* were 3.948 ± 0.024, 6.830 ± 0.202, and 12.319 ± 3.061 mg/100 g, respectively [25]. Carvalho et al. [26] when quantifying carotenoids in pumpkin (*Cucurbita moshata*), found 234.2 μg/g to 405.0 μg/g of total carotenoid content lower than our study and total *E*-β-carotene content ranged from 142.0 μg/g to 244.2 μg/g. Hussain et al. [27] reported lower content of total carotenoids (23.7 ± 0.19 mg/100 g powder), β-carotene (4.60 ± 0.05 mg/100 g powder), total phenolics (93.40 ± 0.69 mg GAE/100 g powder) and total flavonoids (45.0 ± 0.59 mg CE/100 g powder) in pumpkin peels as compared with our findings.

There may be a number of reasons for these variations in total carotenoid concentration between studies, including genotype, harvesting method, postharvest storage, meteorological circumstances, and various pumpkin cultivars [28].

Table 1
Phytochemical content and color parameters of the PPP.

Parameters	Sample PPP
Total Carotenoids (mg/100g DW)	15.107 ± 0.070
β-caroten (mg/100g DW)	12.513 ± 0.032
Lycopen (mg/100g DW)	3.001 ± 0.048
Total flavonoids (mg CE/g DW)	2.698 ± 0.019
Total polyphenols (mg GAE/g DW)	4.616 ± 0.043
DPPH (μmol TE/g DW)	15.349 ± 0.118
Inhibition (DPPH) %	89.855 ± 1.021
L*	78.33 ± 0.02
a*	0.57 ± 0.02
b*	27.89 ± 0.04
Hue angle	1.55 ± 0.01
Chroma	27.90 ± 0.03

Plants' well-known active substances, phenolics and flavonoids, have a healing effect on humans. Using three different extraction solvent types, Jarungjitaree and Naradisorn [29] investigated the total phenolic contents of pumpkin peel. Significantly, various values of the total phenolic contents were obtained utilizing various solvent types and methanol extraction concentrations (92.25 mg GAE/100 g DW in 95% methanol peel extract, 73.44 mg GAE/100 g DW in 95% ethanol peel extract, and 57.41 mg GAE/100 g DW in 95% acetone peel extract).

According to data from Saavedra et al. [6], in the best operational conditions assessed, pumpkin peels had a higher phenolic content than seeds, with values of 11 and 6.1 mg GAE/g DW, respectively.

In other studies, conducted resulted that total flavonoid contents in 70% methanolic extracts of pumpkin peel were 13.81 ± 0.23 mg quercetin equivalent/100 g and in 70% ethanolic extract of pumpkin peel were 14.62 ± 0.29 mg quercetin equivalent/100 g this being larger compared to the one identified in the pulp [30].

The DPPH radical method is one way to gauge an antioxidant's capacity to scavenge free radicals. In this experiment, the antioxidants in the extracts counteract the violet DPPH radical, which results in the solution being discolored. Abdullahi and Santhose [31] compared the level of DPPH radicals' inhibition for different parts of pumpkin: peel, seeds and flesh adjacent to the seeds, the so called "brain". They showed that the highest antioxidant activity was observed in the flesh peel (74.05% of inhibition). The brain (66.00%) and seeds (56.90%) showed slightly lower ability to inhibit DPPH radicals. Nyam et al. [9] confirmed that pumpkin peel (69.38% inhibition) shows higher antioxidant activity than the seeds (36.97%).

Different solvents used to extract the same plant material produce different amounts of phenolic compounds even when the extraction temperature and time are the same. This is likely because of the solvent's polarity and the composition of the matrix being extracted, as some nonvolatile compounds are also extracted when the water content in other polar solvents is raised [32].

Table 1 also shows the color measurement (L*, a*, b* and parameters Chroma and hue angle) of the pumpkin powder (PPP). In terms of the color characteristics, could be noticed that the PPP had luminosity (L*) parameter closer to the value corresponding to white, a* parameter closer to green, and b* parameter closer to yellow. The parameter of L* (78.33 ± 0.02) found in the powder made from the peel was high, indicating the presence of β-carotene [33]. As can be observed, the high b* value suggests that the sample predominate color is yellowness, while the a* value indicates that the reddish color is quite low. The values stated by Staichok et al. [34] for pumpkin peel flour with 66.95 ± 3.91 for L*, 23.09 ± 2.75 for a* and 1.39 ± 0.22 for a*, lower than those discovered in this study (except for a*), were also comparable to the values observed for the instrumental parameters of color for the PPP, presented in Table 1. Couto [35] evaluated pequi peel flour and discovered a value for b* parameter of 28.86, slightly higher than the value discovered for the PPP (27.89). Chroma exhibited the same pattern as the parameter b*, indicating that the yellow color was the most expressive in determining the powder color. The hue angle was placed in the first quadrant of the color solid and indicate the yellowness of the sample. All data were placed in the first quadrant (+a*, +b*) based on the results for the values of a* and b*, which indicated a tendency to yellow and green, which is characteristic of carotenoid pigments.

3.2. HPLC investigation of carotenoids from the PPP extract

An HPLC technique-based chromatographic examination was carried

Table 2

Quantitative evaluation of carotenoid compounds in the extract obtained from the pumpkin peel.

Peak no.	Retention time, minutes	Bioactive compound	Area, mUA × s/g DW	Concentration, µg/g DW
1	1.25	Zeaxanthin	1854.85 ± 14.36	1106.95 ± 47.66
5	5.75	Lutein	58375.21 ± 35.05	ND
6	9.20	β-cryptoxanthin	1840.39 ± 13.60	312.39 ± 23.57
10	12.029	Lycopene	20648.08 ± 134.46	ND
11	13.77	α-carotene	144144.22 ± 1988.08	28884.19 ± 35.92
14	14.888	α-cryptoxanthin	8338.28 ± 14.54	ND
15	15.347	β-carotene	855.19 ± 7.35	2079.18 ± 5.89

ND-not detected, DW-dry weight.

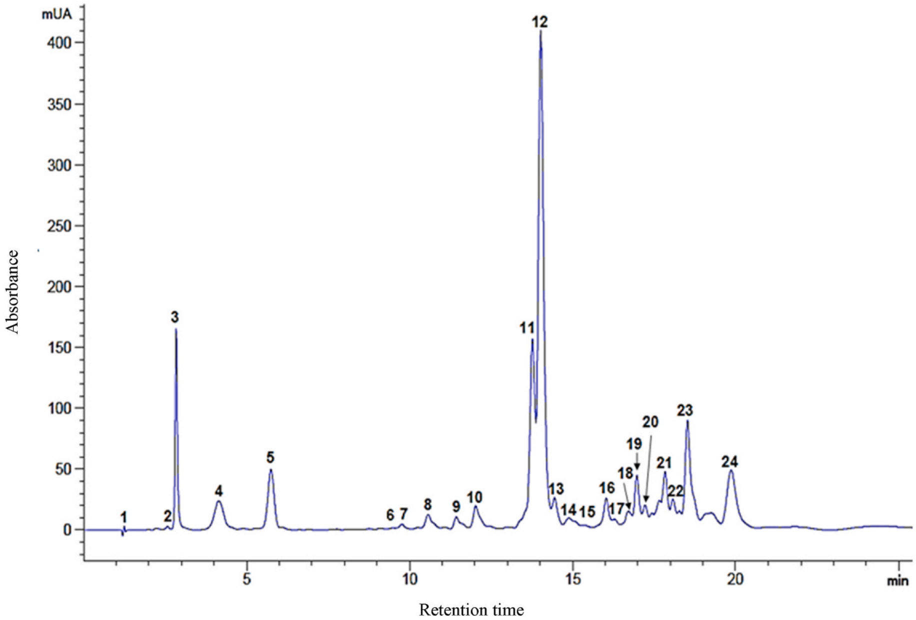

Fig. 2. Chromatographic profile of carotenoids (450 nm) from the pumpkin peel extract. Peaks: 1 – zeaxanthin, 5- lutein; 6 – β-cryptoxanthin, 10 – lycopene; 11 – α-carotene, 14 - α-cryptoxanthin; 15 - β-carotene, 2–4, 7, 8, 12, 13, 16–24 – unidentified compounds.

out in order to characterize the pumpkin carotenoids profile. The typical HPLC chromatogram of carotenoids compounds from the pumpkin peel extract is given in Fig. 2.

α-Carotene was the main carotenoid tentatively identified in pumpkin peel extracted by ultrasound assisted extraction (Table 2). The other carotenoids tentatively identified, such as zeaxanthin, lutein, β-cryptoxanthin, lycopene, α-cryptoxanthin and β-carotene were also presented but in small amounts.

The acquired results were not in fully accordance with literature data. Therefore, Sathiya Mala and Anjali [36] used HPLC to assess the β-carotene content of pumpkin peel and pulp powder. After 8 min, the β-carotene is eluted and was detected using standard β-carotene. According to the study the pulp waste of pumpkin (142.38 mg/100 g fresh matter) was found to be a rich source of β-carotene than peel (11.89mg/100 g). Carotenoid content of *Cucurbita maxima* peel of different varieties was reported by Mirjam et al. [37], by HPLC, and the total carotenoid content of the pumpkin peel depends on the variety with 12 mg/kg for „Butternut” up to 1751 mg/kg for „Rouge”. Carotenoids were most abundant in the peel and pulp of the *C. maxima* L. var. “Rouge” (mean 1751 mg/kg dry weight and 683 mg/kg dry weight, respectively). Also in the peel of „Baby bear” (mean 1070 mg/kg) and „Hokkaido” (mean 1048 mg/kg) high carotenoid concentrations were found. In the pumpkin peel, β-carotene was the main carotenoid in „Baby Bear” (403 mg/kg) „Muscat”(61 mg/kg) and „Butternut” (8 mg/kg). α-carotene was also identified in the study of Mirjam et al. [37], as a second carotenoid in the peel of „Baby Bear” (40 mg/kg), and „Butternut” (4 mg/kg). Other carotenoids were also identified in the peel such as zeaxanthin „Rouge” (72 mg/kg), β-crypthoxanthin „Rouge” (289 mg/kg). α-crypthoxanthin „Rouge“ (542 mg/kg). Kim et al. [25] determined carotenoids concentrations (mg/kg raw weight) in three major varieties of pumpkin peel (*Cucurbitaceae pepo, C. moschata, and C. maxima*) using HPLC and β-carotene was reported to be 39.48 ± 0.24 for *Cucurbitaceae pepo*, 68.30 ± 2.02 for *C. moschata*, and 123.19 ± 30.61 for *C. maxima*. β-cryptoxanthin and α-tocopherols was also identified but in small concentrations. Growing conditions, the total number of sunny days, and the composition of the soil are potential causes of the variance of the results.

3.3. Chemical composition of raw cow’s milk

Results of chemical composition of cow’s milk samples which are the raw material for the yogurt are in Table 3.

Regarding the water content, it had an average value of 87.12 ± 0.094% and that of TS was 12.89 ± 0.094%. Water is the medium in which all other components of milk (total solids) are dissolved or suspend. For the fat content, the average value was 4.21 ± 0.05%, which led to an average of 8.67 ± 0.10% for the SNF content. The protein level recorded an average value of 3.33 ± 0.027%. For the lactose content, an average value of 0.5 ± 0.02% was obtained and the ash content recorded a level of 0.51 ± 0.02% with variation limits between 0.41% and 0.56%. The results obtained by the proximate analysis revealed that all milk samples fulfilled the World Health Organization and other national and international standards.

To produce dairy products of exceptional quality, raw milk of top quality is necessary. Once raw milk has a flaw, it cannot be fixed during processing, and flaws frequently worsen. As a result, raw milk must be produced and handled from farm to processing facility in a manner that doesn’t compromise its quality or, by extension, the quality of the final product. Our results for raw milk are similar to those obtained by others. Similar results were also obtained by Tesfaye and Gebre [38]. Data regarding the pH value indicated values between 6.50 and 6.60, the average value being 6.54 ± 0.012. These results were relatively similar to those reported by Mahboba and Ibtisam [39] and also by Hajirostamloo and Mahastie [40].

Table 3
Chemical composition (%) of raw cow’s milk samples (n = 10).

Parameters	Mean
Water (%)	87.12 ± 0.094
Total Solids (%) (TS)	12.89 ± 0.094
Fat (%)	4.21 ± 0.05
Solid-non fat (SNF) (%)	8.67 ± 0.10
Protein (%)	3.33 ± 0.027
Lactose (%)	0.5 ± 0.02
Ash (%)	0.51 ± 0.02
pH	6.54 ± 0.012

3.4. Chemical composition and storage stability of yogurts supplemented with PPP

The chemical composition of the prepared yogurts is described in

Table 4
Chemical composition and storage stability of added-value yogurt (n = 4).

Component (%)	Product	Storage period (day)			Mean
		1	7	14	
Moisture	**YC**	86.78 ± 0.12^{xA}	86.86 ± 0.12^{xA}	86.93 ± 0.14^{xA}	86.86 ± 0.13^{x}
	YPP2	83.52 ± 0.07^{yA}	83.40 ± 0.07^{yA}	83.15 ± 0.07^{yBA}	83.35 ± 0.17^{y}
	YPP4	80.51 ± 0.41^{zA}	80.42 ± 0.41^{zA}	80.34 ± 0.41^{zA}	80.42 ± 0.38^{z}
TS	**YC**	13.25 ± 0.12^{zA}	13.14 ± 0.12^{zA}	13.05 ± 0.12^{zA}	13.15 ± 0.14^{z}
	YPP2	16.49 ± 0.07^{yB}	16.61 ± 0.07^{yB}	16.86 ± 0.07^{yAB}	16.65 ± 0.17^{y}
	YPP4	19.50 ± 0.41^{xA}	19.58 ± 0.41^{xA}	19.67 ± 0.41^{xA}	19.58 ± 0.38^{x}
Fat	**YC**	3.81 ± 0.01^{yA}	3.83 ± 0.03^{xA}	3.81 ± 0.01^{xA}	3.82 ± 0.02^{y}
	YPP2	4.02 ± 0.02^{xA}	3.88 ± 0.09^{xB}	3.83 ± 0.01^{xCB}	3.91 ± 0.10^{x}
	YPP4	3.85 ± 0.04^{yA}	3.84 ± 0.02^{xA}	3.84 ± 0.02^{xA}	3.84 ± 0.03^{y}
Total protein	**YC**	3.70 ± 0.08^{zA}	3.61 ± 0.08^{zA}	3.77 ± 0.05^{zyA}	3.70 ± 0.09^{z}
	YPP2	4.17 ± 0.01^{yA}	4.02 ± 0.01^{yA}	3.91 ± 0.01^{yA}	4.04 ± 0.11^{y}
	YPP4	4.53 ± 0.45^{xyA}	4.42 ± 0.45^{xA}	4.33 ± 0.45^{xA}	4.42 ± 0.41^{x}
Ash	**YC**	0.75 ± 0.03^{yzA}	0.69 ± 0.03^{yzA}	0.60 ± 0.03^{zBA}	0.68 ± 0.07^{z}
	YPP2	0.80 ± 0.06^{yA}	0.79 ± 0.05^{yA}	0.82 ± 0.05^{yA}	0.80 ± 0.05^{y}
	YPP4	1.12 ± 0.14^{xA}	1.12 ± 0.14^{xA}	1.13 ± 0.11^{xA}	1.13 ± 0.12^{x}
Crude fibre	**YC**	0.00 ± 0.00^{zA}	0.00 ± 0.00^{zA}	0.00 ± 0.00^{zA}	0.00 ± 0.00^{z}
	YPP2	1.49 ± 0.03^{yA}	1.49 ± 0.03^{yA}	1.48 ± 0.02^{yA}	1.49 ± 0.02^{y}
	YPP4	2.08 ± 0.06^{xA}	2.08 ± 0.06^{xA}	2.08 ± 0.06^{xA}	2.08 ± 0.05^{x}
Energy value (kcal 100 g^{-1} FW)	**YC**	68.94 ± 0.47^{zA}	68.96 ± 0.58^{zA}	68.94 ± 0.60^{zA}	68.94 ± 0.50^{z}
	YPP2	76.84 ± 0.38^{yA}	76.66 ± 0.59^{yA}	77.37 ± 0.28^{yA}	76.96 ± 0.50^{y}
	YPP4	84.42 ± 2.05^{xA}	84.73 ± 2.08^{xA}	85.00 ± 1.73^{xA}	84.72 ± 1.79^{x}
pH	**YC**	4.65 ± 0.10^{xA}	4.62 ± 0.02^{xA}	4.51 ± 0.01^{xB}	4.60 ± 0.09^{x}
	YPP2	4.51 ± 0.02^{yA}	4.39 ± 0.01^{yB}	4.24 ± 0.02^{yC}	4.38 ± 0.12^{y}
	YPP4	4.46 ± 0.01^{yA}	4.34 ± 0.01^{zB}	4.20 ± 0.01^{yC}	4.33 ± 0.11^{y}
Syneresis (%)	**YC**	19.62 ± 0.19^{xA}	20.71 ± 0.31^{xB}	22.23 ± 0.22^{xC}	20.85 ± 0.76^{x}
	YPP2	11.75 ± 0.31^{yA}	16.38 ± 0.23^{yB}	18.38 ± 0.23^{yC}	15.32 ± 0.15^{y}
	YPP4	9.14 ± 0.40^{zA}	11.08 ± 0.38^{zB}	12.54 ± 0.22^{zC}	10.92 ± 0.99^{z}

Table 4. The analysis of the obtained media indicates differences between YC and the yogurt with the addition of powder obtained from pumpkin peel (YPP2 and YPP4) for almost all the analyzed parameters. The only parameter that was not influenced by the addition of PPP used by us was fat.

FW –fresh weight, x,y&z: There is no significant difference ($P > 0.05$) between any two means, within the same column, that has the same superscript lower letter;/A,B&C: There is no significant difference ($P > 0.05$) between any two means, within the same row, that has the same superscript capital letter.

YC recorded a higher water content compared to that identified in YPP2 and YPP4, the differences being significant ($p < 0.05$). During the 14 days of storage, differences between batches ($p < 0.05$) were reported only in the case of YPP2. The same significant differences were noted in the case of the TS level where the average value in day 1 for YC was 13.20 ± 0.12% compared to 16.49 ± 0.07% obtained for YPP2 and 19.50 ± 0.41% value obtained for YPP4.

Following the obtained results, it can be observed that the addition of PPP did not influence the fat content of the obtained products nor the storage time ($p > 0.05$). On the other hand, the data regarding the protein level indicate a significant difference ($p < 0.05$) in the case of the products YPP2 (4.17 ± 0.01%) and YPP4 (4.53 ± 0.45 %.) compared to the value obtained for YC (3.70 ± 0.08 %), this being influenced by the PPP content added to each product. During storage, no changes were revealed in terms of the protein level ($p > 0.05$). The ash content of the yogurts recorded the highest level in the case of YPP4 (1.12 ± 0.05%) and the lowest in the case of YC (0.68 ± 0.07%), the differences being also significant ($p < 0.05$). During storage, differences were established only in the case of the YC product, which on the 14th day of storage registered an average value of 0.60 ± 0.03%, the difference being significant ($p < 0.05$) compared to the average obtained on the first day and to the control carried out after 7 days.

The data regarding the fiber content indicate differences ($p < 0.05$) between all three analyzed batches generated in the first phase by the fact that at YC they were not identified but also between YPP2 where the average on the first day was 1.49 ± 0.03% and YPP4 (2.08 ± 0.06%) ($p < 0.05$). During storage no differences were reported ($p > 0.05$). As expected, the differences in the energy value at the first control were significant ($p < 0.05$), the average value obtained for YC being 68.94 ± 0.47 kcal 100 g^{-1} FW, for YPP2 of 76.84 ± 0.38 kcal 100 g^{-1} FW. During the 14 days of storage, no differences were reported ($p > 0.05$) (Table 4).

Practically, the results of proximate chemical composition concluded that there are differences generated by the powder content added to the yogurt processing. There have been no studies on the quality of yogurt enriched with pumpkin peel powder, but Staichok et al. [34] developed a bread with partial addition of flour obtained from pumpkin peel, and then they demonstrated a significant difference ($p < 0.05$) between the formulations regarding protein, ash, carbohydrates, and caloric value. Mishra and Sharma [41] produced and standardized a biscuit made from pumpkin peel and examined the closest component of the finished product. On a 9-point hedonic scale of sensory evaluation, the panelists gave the highest ratings to the biscuits made with 20% pumpkin peel flour. The mean value of the proximate analysis, which included calculations of moisture, protein, fat, fiber, carbohydrate, and energy, was moisture (6.59%), fat (21.75 g/100 g), protein (0.08 g/100 g), carbohydrate (69.40 g/100 g), fiber (0.16 g/100 g), ash (1.91 g/100 g), and energy (473.71 kcal/100 g). As a result, the biscuit is quite nutrient-rich and suitable for both pregnant women and those fighting malnutrition. However, there are articles that discuss products created from pumpkin seeds and pulp. Energy, protein, iron, calcium, carotene concentration, and textural qualities are the main changes in the physicochemical properties of bakery, dairy, beverages, and snacks (hardness, chewiness, snapping force and viscosity) [42].

Table 4 also shows the evolution of pH during the storage period. The addition of PPP generated a significant ($p < 0.05$) decrease in pH in the samples that had the addition of PPP. Therefore, the pH value at the first control at YPP2 was 4.51 ± 0.02 and at YPP4 4.46 ± 0.01. As can be seen in Table 4, differences were only between YC which had a value of 4.65 ± 0.10 and YPP2 and YPP4 ($p < 0.05$). Therefore, yogurt with the addition of PPP recorded lower values compared to YC both at the first control and during storage the cause may be generated by the acidity of the pumpkin pulp [43,44]. During the storage period, the pH value decreased in all samples as a result of the growth of LAB [45].

Syneresis is an important index of yogurt quality. The addition of PPP and storage time had a significant ($p < 0.05$) impact on the syneresis of yogurt. Yogurt's syneresis was lowered by adding PPP in comparison to the control; the highest and the lowest values were seen in control on the 14th day and the sample contained 4% PPP on the first day respectively. The decrease in syneresis on days zero can be attributed to the increase in dry matter content. The syneresis revealed a simultaneous increase in all yogurts throughout storage. In their study, Mahdian and Tehrani [46] demonstrated a notable reduction in yogurt syneresis as the total solid content increased. During the period of storage from day 14 to day 21, there was an increase in syneresis, which refers to the ability of the samples to retain water. According to Dönmez et al. [47], a decrease in syneresis by PPP increases the amount of water trapped within the gel network, which lowers yogurt's serum release. Concerning the increase in syneresis during storage, Ghadge et al. [48] discovered that the acidity of yogurt increased as a result of the increased syneresis during storage.

3.5. Phytochemical content and color evaluation of yogurts supplemented with PPP

Table 5 shows phytochemical content of added-value yogurts and stability during 14 days of storage. The contents of bioactive compounds of the PPP-enriched yogurt samples fortified by 2% and 4% PPP were assessed by determining the total polyphenolic compounds, total flavonoids and total carotenoids as well as antioxidant activity applying DPPH method.

The highest total polyphenolic compounds content (256.56 mg GAE/g DW) was found in the yogurt fortified with 4% PPP (YPP4) and the lowest content (203.93 mg GAE/g DW) was found in YPP2 yogurt. Control yoghurt (YC) exhibited 170.05 mg GAE/g DW at the first control carried out (day 1) reaching after 14 days of storage a value of 161.02 mg GAE/g DW. Total flavonoids data showed values of 1.48 mg CE/100 g for YPP2 and 1.73 mg CE/100 g for YPP4. In the case of the yogurt from the control group (YC), total flavonoids were not detected. As expected in YC, no carotenoids were identified. In the case of YPP2, the value identified at the first control was 0.50 ± 0.01 mg/100 g reaching the control on day 14 at a level of 0.41 ± 0.01 mg/100 g. In the case of YPP4, the level of carotenoids was higher compared to that identified in YPP2 ($p < 0.05$), namely 1.10 ± 0.01 mg/100 g reaching the control on day 14 at a level of 0.83 ± 0.11 mg/100 g. YPP2 and YPP4 had higher antioxidant activity than YC. The DPPH radical scavenging activity of YC was 17.17 ± 0.33 μmol TE/100 g DW, and DPPH radical scavenging activity of yogurt increased in pumpkin-yoghurt significantly ($p < 0.05$) not only in mean score but also during storage period. The phytochemical content of pumpkin and microbial metabolic activity may release some bound bioactive components, explaining the enhanced antioxidant activity in PPP-enriched yoghurt. Nevertheless, the findings show that the incorporation of pumpkin powder improved the yogurt's antioxidant properties, allowing the development of enhanced foods with a high antioxidant potential that may have health benefits while consumed. Nguyen and Hwang [49] had previously discussed findings of a similar nature. Therefore, the total polyphenol and total flavonoid contents of yogurt supplemented with aronia juice (1%, 2%, and 3%), increased proportionally with increasing amounts of aronia juice. Yogurt containing aronia juice had significantly higher antioxidant activity than the control and increased proportionately as aronia juice concentration increased.

Konrade et al. [50] describes a study on the application of pumpkin

Table 5
Phytochemical profile of plain and added-value yogurts and stability during 14 days of storage.

Parameters	Product	Storage period (day)			Mean
		1	7	14	
Total polyphenolic compounds (mg GAE/100 g DW)	**YC**	170.05 ± 1.32^{yA}	163.00 ± 1.32^{yB}	161.02 ± 1.32^{yB}	164.69 ± 4.22^{y}
	YPP2	203.93 ± 3.27^{xC}	222.67 ± 3.27^{xB}	230.56 ± 3.27^{xA}	219.05 ± 12.03^{x}
	YPP4	205.02 ± 3.27^{xC}	221.59 ± 3.27^{xB}	229.84 ± 3.27^{xA}	218.81 ± 11.17^{yx}
Total flavonoids (mg CE/100 g DW)	**YC**	0.00 ± 0.00^{zA}	0.00 ± 0.00^{zA}	0.00 ± 0.00^{zA}	0.00 ± 0.00^{z}
	YPP2	1.48 ± 0.03^{yC}	1.65 ± 0.03^{yB}	1.81 ± 0.03^{yA}	1.65 ± 0.14^{y}
	YPP4	1.73 ± 0.03^{xC}	1.92 ± 0.03^{xB}	1.99 ± 0.03^{xA}	1.88 ± 0.12^{x}
Total Carotenoids (mg/100 g DW)	**YC**	0.00 ± 0.00^{zA}	0.00 ± 0.00^{zA}	0.00 ± 0.00^{zA}	0.00 ± 0.00^{z}
	YPP2	0.50 ± 0.01^{yA}	0.44 ± 0.01^{yB}	0.41 ± 0.01^{yB}	0.45 ± 0.04^{y}
	YPP4	1.10 ± 0.01^{xA}	0.99 ± 0.04^{xB}	0.83 ± 0.11^{xC}	0.97 ± 0.13^{x}
DPPH (μmol TE/100 g)	**YC**	17.17 ± 0.33^{zA}	14.09 ± 0.33^{zB}	13.07 ± 0.33^{zC}	14.77 ± 1.84^{z}
	YPP2	39.12 ± 0.77^{yC}	40.22 ± 0.77^{yB}	41.34 ± 0.77^{yA}	40.23 ± 1.18^{y}
	YPP4	40.13 ± 0.77^{xC}	$42.43 \pm 0\ .77^{xB}$	44.33 ± 0.77^{xA}	42.30 ± 1.93^{x}
L*	**YC**	92.38 ± 0.44^{xB}	93.44 ± 0.44^{xA}	93.45 ± 0.44^{xAB}	93.09 ± 0.66^{x}
	YPP2	73.72 ± 0.72^{yA}	74.24 ± 0.72^{yA}	74.47 ± 0.72^{yA}	74.15 ± 0.73^{y}
	YPP4	71.69 ± 0.72^{zA}	72.02 ± 0.72^{zA}	72.04 ± 0.72^{zA}	71.92 ± 0.67^{z}
a*	**YC**	-2.14 ± 0.04^{zA}	-2.18 ± 0.04^{zA}	-2.40 ± 0.04^{zB}	-2.24 ± 0.13^{z}
	YPP2	0.27 ± 0.05^{yB}	0.36 ± 0.07^{yA}	0.27 ± 0.07^{yBA}	0.30 ± 0.07^{y}
	YPP4	0.43 ± 0.07^{xB}	0.77 ± 0.07^{xA}	0.75 ± 0.07^{xBA}	0.65 ± 0.18^{x}
b*	**YC**	10.88 ± 0.11^{zA}	11.05 ± 0.11^{zA}	11.03 ± 0.11^{zA}	10.99 ± 0.13^{z}
	YPP2	38.21 ± 0.12^{yB}	38.52 ± 0.12^{yA}	38.00 ± 0.12^{yC}	38.24 ± 0.25^{y}
	YPP4	40.62 ± 0.12^{xC}	41.02 ± 0.12^{xB}	41.21 ± 0.12^{xA}	40.95 ± 0.28^{x}
Chroma	**YC**	10.88 ± 0.11^{zA}	11.05 ± 0.11^{zA}	11.03 ± 0.11^{zA}	10.99 ± 0.13^{z}
	YPP2	38.21 ± 0.12^{yB}	38.52 ± 0.12^{yA}	38.00 ± 0.12^{yC}	38.24 ± 0.25^{y}
	YPP4	40.62 ± 0.12^{xC}	41.03 ± 0.12^{xB}	41.21 ± 0.12^{xA}	40.95 ± 0.28^{x}
Intensity	**YC**	-1.38 ± 0.01^{yB}	-1.38 ± 0.01^{zB}	-1.36 ± 0.01 zA	-1.37 ± 0.01^{z}
	YPP2	1.56 ± 0.01^{xA}	1.56 ± 0.01^{xA}	1.56 ± 0.01^{xA}	1.56 ± 0.01^{x}
	YPP4	1.56 ± 0.01^{xA}	1.55 ± 0.01^{yB}	1.55 ± 0.01^{yB}	1.55 ± 0.01^{y}

$^{x,y\&z}$: There is no significant difference ($P > 0.05$) between any two means, within the same column, that has the same superscript lower letter;/
$^{A,B\&C:}$ There is no significant difference ($P > 0.05$) between any two means, within the same row, that has the same superscript capital letter;/-: not detected.

(*Cucurbita pepo. L.*) peels to increase nutritional content in extruded crispbread products. The addition of pumpkin peels to the wheat flour dough serves as a measure of the final product's enhanced nutrients in addition to producing a soft and crispy crispbread. Initially, the number of total carotenoids found in dried, powdered pumpkin wastes was high. Carotenoids content in the samples enriched with pumpkin increased significantly from 0.77 ± 0.01 mg/100 g to 6.51 ± 0.02 mg/100 g after 20% of pumpkin peels were added to the ingredients.

Product quality affects consumer acceptability and preference. Consumers typically notice color as the initial sensory attribute, and color has the power to alter other impressions including flavor and aroma. Pumpkin peel is a beneficial source of β-carotene, so adding it to yogurt samples had a significant impact on the color characteristics (Fig. 3) [51].

Color properties as (L*, a*, b*, chroma and intensity) of stirred pumpkin-yoghurt are shown in Table 5. The L* values, which indicate the brightness, registered an average value of 73.72 ± 0.72 for YPP2 and 71.69 ± 0.72 for YPP4 on the first control day. Higher values were obtained for YC, 92.38 ± 0.44 compared to the samples that had the addition of PPP. As predicted, the addition of PPP resulted in a reduction in the whiteness of yogurts, which became more pronounced with an increase in PPP level, with a significant difference between the three samples ($p < 0.05$). The values of L* did not change significantly while being stored.

On the other hand, a* and b* values were higher in YPP2 and YPP4 yogurt. The a* values, which indicate redness, ranged between −2.14 and 0.43 in all yogurt samples in the first control. The b* values, which indicate the yellow color, varied from 38.21 in YPP2 to 40.62 in YPP4 while in YC the value of b* was 10.88. A decrease in the values obtained for a* and b* in the yogurt that had the addition of PPP was noticed at the control performed on the 14th day of the control. This can be the result of storage-related oxidation of β-carotene or any other carotenoids. Zhou et al. [52] reported that the increase of L* value in high hydrostatic pressure-applied pumpkin during storage was related with the occurrence of nonenzymatic browning reactions that also took place together with oxidation and isomerization of β-carotene.

As calculated from the L*, a* and b* results, the averages of chroma (C) and color intensity were also significantly different ($p < 0.05$) with the difference being caused by the addition of PPP as well as the concentration used. However, a pronounced yellowish color was determined visually in yogurts with PPP, compared to control. This yellow and bright color in the samples originates from carotenoids and lutein, respectively. Ayar and Gürlin [53] pointed out that the pumpkin had the distinct yellow color and b* value (13.25) in pumpkin-added yogurt was higher than the control sample.

3.6. *Texture analysis of yogurts supplemented with PPP*

Yogurt's textural characteristics are a key factor in determining its quality. The structural elements, various parameters, such as composition, and manufacturing procedures all have an impact on the textural characteristics of coagulated dairy products. The structural arrangement of proteins and the microstructure of the protein network affect the rheological and textural characteristics of fermented dairy products. In Table 6, an analysis of the TPA parameters (cohesiveness, hardness, springiness, adhesiveness, and gumminess) for the yogurt with PPP powder is shown. The most typical texture evaluation criterion for yogurt is firmness or hardness, which is defined as the amount of force necessary for ensuring a particular deformation [54].

The investigated parameters of the textural analysis were significantly increased ($p < 0.05$) with the higher concentration of PPP add-on, except springiness and adhesiveness. After up to 14 days of storage, the samples with the addition of PPP resulted in a significant increase in cohesiveness, springiness, and gumminess. Yogurt's structure and texture are primarily produced during the milk fermentation process, although they are also influenced by the addition of hydrocolloids. It is widely known that the inclusion of various hydrocolloids improves the textural characteristics of food products [55].

The hardness values grew as the amount of PPP increased from 2 to 4%. The maximum hardness was measured in YPP4 sample followed by

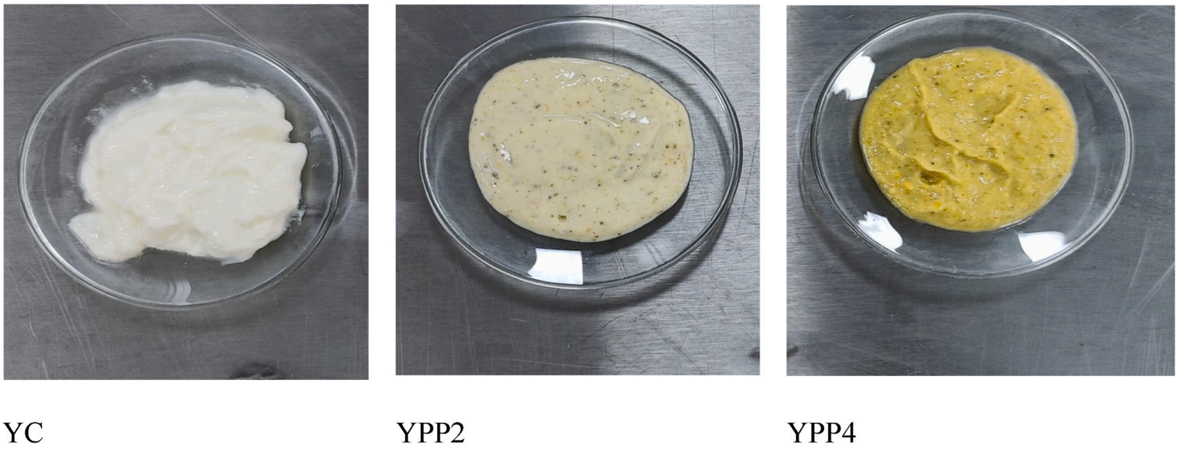
YC YPP2 YPP4

Fig. 3. Images of the yogurt without PPP, control (YC); yogurt with 2% PPP (YPP2); yogurt with 4% PPP (YPP4).

Table 6
Texture of yogurt samples enhanced after 1,7 and 14 days storage at 4 °C ± 1 °C.

Component	Product	Storage period (day)		
		1	7	14
Cohesiveness	**YC**	0.29 ± 0.01^{yB}	0.30 ± 0.01^{yB}	0.32 ± 0.01^{zAA}
	YPP2	0.35 ± 0.01^{xC}	0.37 ± 0.01^{xB}	0.38 ± 0.01^{yAB}
	YPP4	0.35 ± 0.01^{xC}	0.37 ± 0.01^{xB}	0.39 ± 0.01^{xA}
Springiness	**YC**	0.55 ± 0.03^{xA}	0.56 ± 0.03^{xA}	0.57 ± 0.03^{xA}
	YPP2	0.32 ± 0.02^{yC}	0.35 ± 0.02^{yB}	0.38 ± 0.02^{yA}
	YPP4	0.27 ± 0.02^{zC}	0.30 ± 0.02^{zB}	0.33 ± 0.02^{zA}
Hardness, N	**YC**	5.75 ± 0.03^{zA}	5.76 ± 0.03^{zA}	5.77 ± 0.03^{zA}
	YPP2	8.00 ± 0.01^{yA}	8.01 ± 0.01^{yA}	8.02 ± 0.01^{yA}
	YPP4	12.14 ± 0.02^{xA}	12.15 ± 0.02^{xA}	12.16 ± 0.02^{xA}
Gumminess, N	**YC**	1.65 ± 0.01^{zB}	1.66 ± 0.01^{zB}	1.67 ± 0.01^{zAB}
	YPP2	2.82 ± 0.01^{yB}	2.83 ± 0.01^{yB}	$2.84 \pm 0.01y^{AB}$
	YPP4	4.23 ± 0.01^{xC}	4.25 ± 0.01^{xB}	4.26 ± 0.01^{xAB}
Adhesiveness, mJ	**YC**	-2.55 ± 0.01^{xA}	-2.57 ± 0.01^{xA}	-2.58 ± 0.01^{xA}
	YPP2	-7.15 ± 0.04^{yA}	-7.17 ± 0.04^{yA}	-7.19 ± 0.04^{yA}
	YPP4	-12.29 ± 0.06^{zA}	-12.31 ± 0.07^{zA}	-12.33 ± 0.08^{zA}

x,y&z: There is no significant difference ($P > 0.05$) between any two means, within the same column, that has the same superscript lower letter;/A,B&C: There is no significant difference ($P > 0.05$) between any two means, within the same row, that has the same superscript capital letter.

YPP2. The springiness of the yogurt decreased with the increase in the PPP levels. The internal strength of yogurt is also indicated by its cohesiveness, which is a crucial textural factor in determining the quality of the yogurt [56]. It has been showed that the addition of pumpkin powder to yogurt contributed to the increase of cohesiveness compare with the control. The tendency for yogurt samples supplemented with PPP to become more cohesive may be caused by the strength of the structure provided by pumpkin powder.

Another important parameter for the examination of yogurt's textural characteristics is its gumminess [56]. Similar to hardness, the gumminess values of the yogurt samples increased as PPP concentration was increased, going from 2.82 ± 0.01 N (2% YPP2) to 4.23 ± 0.01 N (4% YPP4). The adhesiveness of the yogurts with PPP exhibited a tendency toward decrease. According to some estimates, the value of yogurt stickiness rises inversely with consumer acceptance [57].

The pumpkin powder addition into the yogurt increased the gel characteristic of the value-added product so transforms the weak gel profile of the yogurt into semisolid food and a higher hardness was observed for all yogurt samples. Numerous dietary fibers, including oat, orange, carrot, and β-glucan, have been added to yogurt to increase viscosity, avoid syneresis, and enhance textural properties [58,59]. The texture of yogurts that contain by-products such as carrot cell wall particles (1% and 2%) [58], blueberry pomace (4.5%) [60], and passion fruit peel powder (0.5% and 1.0%) [61], has also been observed to be enhanced.

3.7. *Rheological properties of yogurts supplemented with PPP*

The impact of PPP addition on the rheological behavior of the yoghurt samples was determined by carrying out dynamic and steady shear sweep tests. Analyzing the results of the dynamic sweep test, presented in Fig. 4 and Table 7, one can observe the significant increase of the G' with PPP addition. These results suggest the enhanced solid-like behavior of the yoghurt samples with increasing amounts of PPP. No important differences were found between YC and YPP2 samples in terms of G'' (21.16 ± 1.41 and 26.82 ± 0.33), but raising the PPP addition to 4% resulted in significant increase of the G'' to 93.48 ± 4.42 Pa. The viscous and elastic components contribute equally to defining the complex modulus, G^*, which is a direct measure of the sample's stiffness or resistance to deformation. As expected, the composition of the yoghurt was found to significantly influence the G^* values ($p < 0.05$), which increased from 85.80 ± 2.56 Pa to 350.30 ± 0.71 Pa, with the increase of the PPP addition from 0 to 4%.

The PPP addition to the yoghurts resulted in no significant differences ($p > 0.05$) between tan δ values (Table 7), which provide additional information on the extent of the viscoelastic behavior of the samples. The measured tan δ values ranged between 0.25 and 0.28, suggesting the prevalence of the elastic behavior [62].

All investigated samples exhibited shear-thinning behavior, characterized by apparent viscosity decrease over the entire domain of tested shear rates (Fig. 5). This behavior is the result of the yoghurt gel network gradual destruction, appearing as a consequence of alteration of the intermolecular associations, mainly the protein-protein interactions [82]. When investigating the rheological behavior of the concentrated yoghurt, Mohameed et al. [83] indicated that the weak electrostatic and hydrophobic interactions involved in consolidating the gel network of the yoghurt are easily disrupted while shearing the samples. No important differences in terms of apparent viscosity measured at high shear rates were found between control and yoghurt samples supplement with 2% PPP (Table 7). Anyway, a significant increase of the apparent viscosity measured at shear rate of 100 s^{-1} ($p < 0.05$) was noticed in case of the YPP4 sample supplemented with even higher PPP amounts, most probably as the result of the total solids increase. A

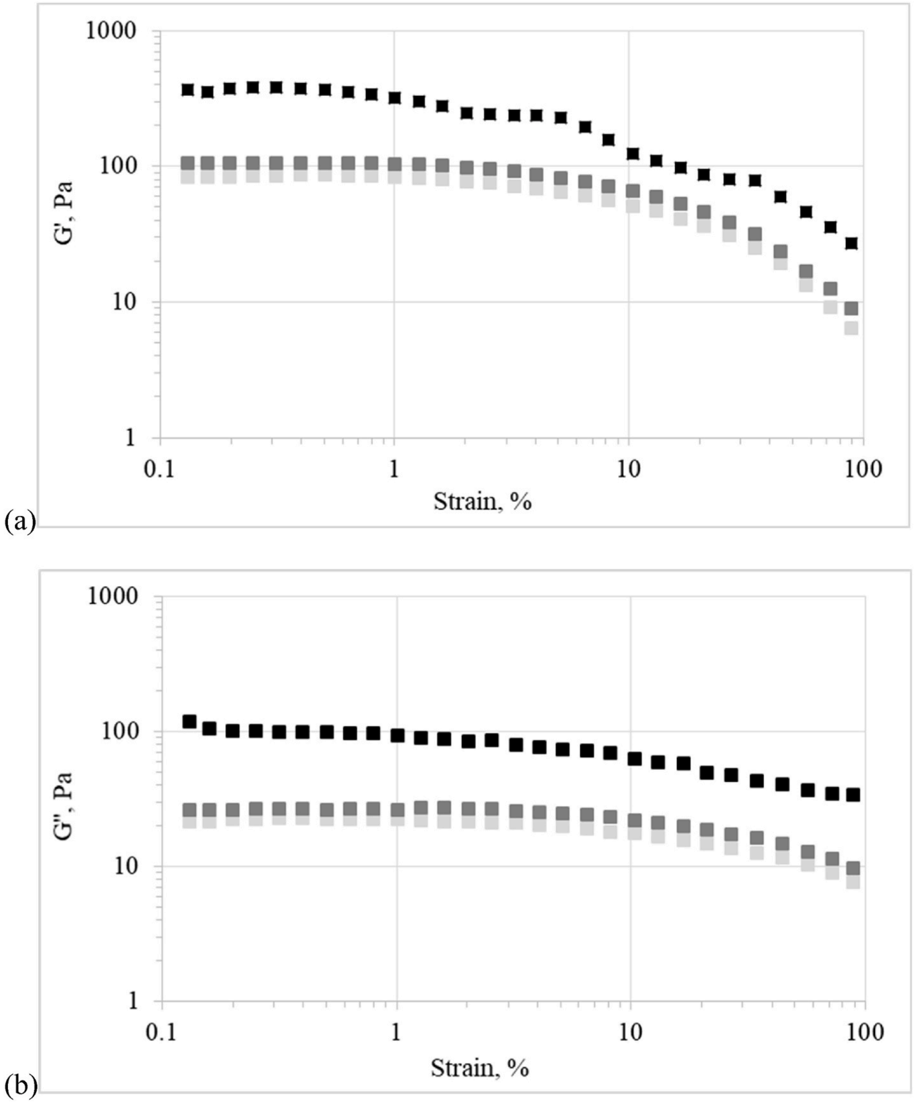

Fig. 4. Rheological behavior of the yoghurt samples measured under dynamic strain sweep test. Evolution of the (a) storage modulus (G′) and (b) loss modulus (G″). YC, YPP2 and YPP4 samples are represented with light grey, dark grey and black, respectively.

Table 7
Rheological parameters of the yoghurt samples measured while running dynamic strain sweep and steady shear rate sweep tests.

Rheological parameter	Yogurt sample		
	YC	YPP2	YPP4
Strain sweep test			
G’, Pa	83.15 ± 2.28^{c}	109.15 ± 5.30^{b}	337.65 ± 0.49^{a}
G″, Pa	21.16 ± 1.41^{b}	26.82 ± 0.33^{b}	93.48 ± 4.42^{a}
G*, Pa	85.80 ± 2.56^{c}	112.40 ± 5.23^{b}	350.30 ± 0.71^{a}
tan δ	0.25 ± 0.01^{a}	0.25 ± 0.01^{a}	0.28 ± 0.01^{a}
Steady rate sweep test			
K, Pa·s^{n}	6.38 ± 0.05^{b}	6.26 ± 0.72^{b}	21.93 ± 2.23^{a}
N	0.41 ± 0.02^{a,b}	0.46 ± 0.02^{a}	0.33 ± 0.01^{b}
η at 100 s^{-1}	0.40 ± 0.06^{b}	0.48 ± 0.03^{b}	0.90 ± 0.06^{a}

There is no significant difference ($P > 0.05$) between any two mean values, within the same row, that share the same superscript letter.

similar trend was observed in case of the consistency index values, estimated upon applying the Ostwald de Waele relationship to fit the σ vs. γ results. The significantly higher value ($p < 0.05$) of the consistency index obtained in case of YPP4 sample might be the result of the more densely aggregated proteins network, entrapping high amounts of low molecular weight compounds arising from PPP [63]. Our results agree with the observations of Mohameed et al. [64], who reported the increase of the consistency index with the solids concentration and yoghurt viscosity. Regardless of the PPP addition, the yoghurt sample exhibited non-Newtonian thing behavior, as confirmed by the $n < 1$ (Table 7). In agreement with our results, Mohameed et al. [64] found subunitary n values decreasing with the increase of the solids concentration in the yoghurt samples.

3.8. *Sensorial analysis of yogurts supplemented with PPP*

Table 8 displays the sensory assessments of plain and PPP-enriched yogurts. The addition of pumpkin peel powder to yogurt had a statistically significant impact on sensory indices such as flavor, color, consistency, mouth feel, and overall acceptance, even though the panelists did not observe a significant variation ($p > 0.05$) in acidity. The yogurt fortified with 2% PPP (YPP2) had the highest flavor score (26.01 ± 1.96), while the yogurt fortified with 4% PPP (YPP4) received the lowest (23.21 ± 1.64).

When compared to the first day of storage, the control yogurt (YC) displayed a noticeably higher score on day 7 ($p < 0.05$) when compared to the first day of storage and furthermore displayed a modest intensity

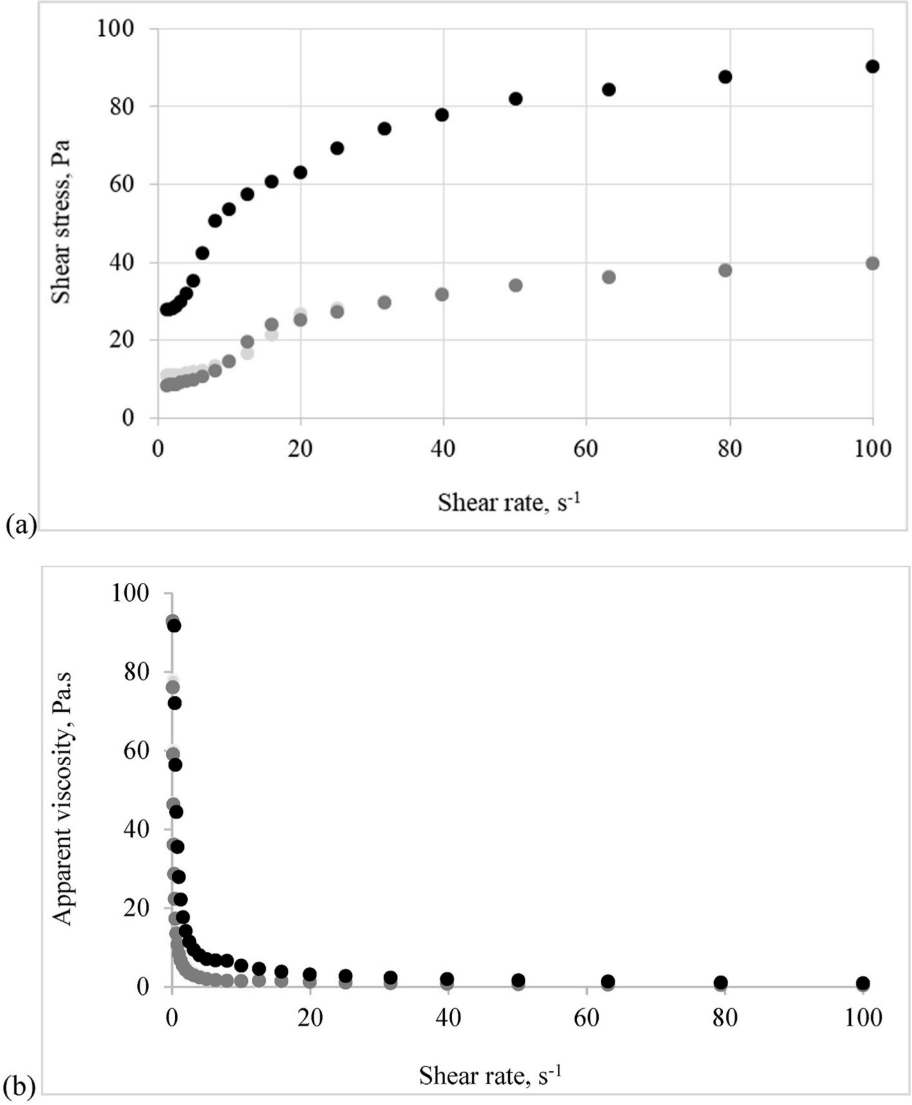

Fig. 5. Evolution of (a) shear stress and (b) apparent viscosity of the yoghurt samples measured while running steady shear rate sweep tests. YC, YPP2 and YPP4 samples are represented with light grey, dark grey and black, respectively.

loss ($p > 0.05$) when compared to the beginning of the shelf-life period, the same pattern is observed significantly and at the formulation yogurts.

Plain yogurt's color score was the lowest, while pumpkin yogurts had the highest significance. The mean color score for plain yogurt had the lowest value ($p < 0.05$), while the highest score ($p < 0.05$) was for pumpkin yogurt formulation treatments. The formulation of 4% PPP (YPP4) on days 7 and 14 of storage, with a slight reduction noticed on day 14, had the most overt impact ($p < 0.05$) on the panelists' evaluation. The better rating for attractiveness and desirability was given to the 2% PPP (YPP2) formulation. Carotenoids from pumpkin give a yellow hue that may not be to panelists' tastes. Yogurt also benefits from this color. Literature [43,61] has demonstrated that adding fruits and vegetables to yogurt improves its flavor and color.

Mouthfeel of pumpkin-yoghurt was significantly different ($p < 0.05$) as pumpkin was added when compared to the control formulation. Scores of 2% (YPP2) yogurt formulation showed the highest score which differed significantly from the control and YPP4. The opposite conclusion was reached by Nguyen and Hwang [58] who established that adding 3% aronia juice to plain yogurt had no statistically significant effect on flavor, mouthfeel, thickness, or overall acceptance.

For overall acceptability, YPP2 showed the highest score ($p < 0.05$) followed by YPP4, while control recorded the lowest score ($p < 0.05$). It is obviously shown that panelists were favored to the prepared pumpkin yogurt in 2% proportion.

4. Conclusions

The current study aimed to assess the possibility to incorporate pumpkin peel powder from pumpkin by-products, mainly peel, into yoghurts and its effect on phytochemical composition, sensory characteristics, color and textural properties of yogurt, while assessing their potential to replace synthetic colorants and antioxidants. The characterization of the pumpkin by-products extract proved that pumpkin peel extract exhibited higher carotenoids content. The results also highlighted that the pumpkin peel extract is an important source of bioactive compounds (phenolics) with high antioxidant activity.

Results of this work display that pumpkin peel powder could be a promising alternative for the food industry to provide carotenoids-enriched yoghurts. As a result, the current study demonstrates that incorporating pumpkin peel powder into yogurt considerably increases its physicochemical, phytochemical, texture, color, sensory qualities, and antioxidant activity. The yellowish color of enriched yogurts was improved by adding pumpkin peel powder.

Table 8
Average scores of attributes assessed by sensorial analysis of plain and value-added yogurts during 14 days of storage.

Item	Product	Storage period (day)			Mean
		1	7	14	
Flavor	**YC**	23.06 ± 0.93^{yB}	25.22 ± 1.84^{yA}	23.78 ± 0.75^{yB}	24.02 ± 1.53^{y}
	YPP2	24.22 ± 0.91^{xC}	28.33 ± 0.79^{xA}	25.48 ± 1.05^{xB}	26.01 ± 1.96^{x}
	YPP4	21.46 ± 0.99^{zC}	24.70 ± 1.18^{xyA}	23.47 ± 0.77^{xyB}	23.21 ± 1.66^{z}
Color	**YC**	17.01 ± 1.26^{zB}	17.73 ± 1.21^{zB}	18.10 ± 1.07^{yAB}	17.61 ± 1.2^{z}
	YPP2	18.35 ± 1.09^{yA}	18.83 ± 1.06^{yA}	18.69 ± 1.16^{yA}	18.63 ± 1.10^{y}
	YPP4	19.53 ± 1.45^{xB}	20.91 ± 1.01^{xA}	19.95 ± 1.09^{xB}	20.13 ± 1.31^{x}
Consistency	**YC**	16.28 ± 1.25^{zB}	17.49 ± 1.17^{yA}	17.40 ± 0.90^{yBA}	17.05 ± 1.23^{z}
	YPP2	19.08 ± 1.06^{xA}	18.35 ± 0.55^{xB}	18.42 ± 0.93^{xAB}	18.62 ± 0.92^{x}
	YPP4	17.99 ± 1.22^{yA}	17.54 ± 0.95^{yA}	18.21 ± 0.84^{xA}	17.91 ± 1.04^{y}
Mouth feel	**YC**	12.25 ± 0.76^{yB}	13.29 ± 1.16^{yA}	12.55 ± 0.76^{yBA}	12.70 ± 0.99^{y}
	YPP2	13.98 ± 1.31^{xB}	15.58 ± 0.91^{xA}	14.64 ± 1.21^{xB}	14.73 ± 1.31^{x}
	YPP4	11.20 ± 1.14^{zA}	11.34 ± 1.12^{zA}	10.59 ± 1.02^{zAB}	11.04 ± 1.12^{z}
Acidity	**YC**	9.72 ± 0.94^{xA}	9.47 ± 0.53^{xA}	9.06 ± 0.81^{xBA}	9.42 ± 0.81^{x}
	YPP2	8.52 ± 0.77^{yA}	8.66 ± 0.86^{yA}	8.88 ± 0.63^{xA}	8.69 ± 0.76^{y}
	YPP4	8.84 ± 0.95^{yA}	9.05 ± 0.75^{xyA}	8.03 ± 0.84^{yB}	8.64 ± 0.94^{y}
Overall acceptability	**YC**	83.64 ± 0.95^{zC}	88.84 ± 0.77^{yA}	85.25 ± 1.09^{zB}	85.91 ± 2.38^{z}
	YPP2	89.09 ± 1.02^{xC}	93.61 ± 1.12^{xA}	92.25 ± 1.29^{xB}	91.65 ± 2.22^{x}
	YPP4	$86.81 \pm 1.05_{yC}$	87.93 ± 0.56^{zB}	89.57 ± 0.64^{yA}	88.10 ± 1.38^{y}

x,y&z: There is no significant difference ($P > 0.05$) between any two means, within the same column, that has the same superscript lower letter;/A,B&C: There is no significant difference ($P > 0.05$) between any two means, within the same row, that has the same superscript capital letter;/-: not detected.

In addition, according to the sensory evaluations, the enriched yoghurts with added powder may be potentially acceptable to consumers due to their generally acceptable quality characteristics. Thus, adding pumpkin powder to yoghurts shows improvement of sensory, textural, certain nutritional characteristics compared with the control sample, that provide consumers real benefits.

In order to develop value-added products, this study emphasized the possibilities of using pumpkin peel powder derived from agro-industrial by-products in food like yogurts as a bioactive powder and a replacement for synthetic preservatives.

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CRediT authorship contribution statement

Roxana Nicoleta Gavril (Rațu): Writing – original draft, Validation, Funding acquisition, Data curation, Conceptualization. **Petru Marian Cârlescu:** Methodology, Investigation, Formal analysis, Data curation. **Ionut Dumitru Veleșcu:** Visualization, Validation, Software, Investigation, Data curation. **Vlad Nicolae Arsenoaia:** Project administration, Methodology, Funding acquisition, Conceptualization. **Florina Stoica:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Nicoleta Stănciuc:** Visualization, Validation, Supervision, Methodology, Data curation. **Iuliana Aprodu:** Writing – original draft, Validation, Software, Methodology, Data curation. **Oana Emilia Constantin:** Writing – review & editing, Software, Resources, Methodology, Investigation. **Gabriela Râpeanu:** Writing – review & editing, Validation, Supervision, Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data availability

Data will be made available on request.

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