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PESTO SAUCE TYPE PRODUCTS: INFLUENCE OF BEESWAX AND STORAGE CONDITIONS ON RHEOLOGY AND COLLOIDAL STABILITY

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ABSTRACT

One of the major problems for pesto sauce type products is the tendency of oil to cream, causing their alteration and decreasing consumer's acceptability. In this study, colloidal and oxidative stability of pesto samples with (0.5-1.2%) of beeswax was evaluated during storage at 4, 20 and 40°C. The rheological behavior and color analysis was evaluated at the same temperatures. Sensory evaluation by using a hedonic test was also perfomed. Increasing the samples beeswax content increased the pesto colloidal stability. The samples with 0.8-1.2% have the highest stability and overall performed well on sensory analysis. The peroxide values of samples increased significantly ($p<0.05$) with storage time and temperature, as well as the p-anisidine values. During the storage at 40°C, peroxide values of samples without wax and with 1,2% wax increased from 11.75 to 33.81 and 32.72 meq O_2/kg, respectively. In the same condition, p-Anisidine values increased from 4.51 to 10.71 and 9.94, respectively. For these same samples stored 793 h, peroxide values increased from 13.33 to 33.81 and to 32.72 meq O_2/kg, when the temperature increased from 4 to 40°C. The same observation was made for p-anisidine values, which increased from 8.40 to 10.71 and 9.94. As expected, the pesto samples showed the best oxidative stability at low temperature. It was shown that beeswax content, temperature and time of storage are important parameters for controlling pesto rheology, colloidal and oxidative stability. In fact, the pseudo plasticity of samples increases with increasing beeswax added and with decreasing the temperature. The colloidal and oxidative stability of samples decreased as the storage time and temperature increased.

Keywords: Pesto sauce, Storage test, Stability, Beeswax

INTRODUCTION

Pesto is a typical Italian basil-based pasta sauce **(Mitić-culafić *et al.*, 2014; Zunin *et al.*, 2009; Masino *et al.*, 2008)** which has recently met with the favor of the international market particularly in North America **(Zunin *et al.*, 2009; Fabiano *et al.*, 2000)**. The pesto sauce is the second most popular pasta sauce after tomato sauce **(Masino *et al.*, 2008)**. It is traditionally prepared by carefully mixing of basil, cheese, extra-virgin olive oil, pine nuts and/or walnuts and garlic **(Mitić-culafić *et al.*, 2014; Salvadeo *et al.*, 2007)**. It is a kind of semi-solid suspension.

This study is focused on pesto sauce based on salad rocket type *Eruca sativa*, a product that even though highly appreciated by occasional consumers, is not commonly known **(Fabiano *et al.*, 2000)**. Salad rocket has been grown in the Mediterranean area since Roman times and are being presently extensively cultivated in various places for commercial purposes **(Pasini *et al.*, 2012)**. In recent years, these crops have gained greater importance as vegetables and culinary herbs, especially among Middle Eastern and European populations **(Pasini *et al.*, 2012)**. Previous data have shown as rocket species are rich in glucosinolates **(Pasini *et al.*, 2012)**. The characteristic spicy flavor and the anti-carcinogenic properties may be related to the presence of these components and their associated hydrolytic products, in particular isothiocyanates **(Pasini *et al.*, 2012)**. The main quality problems for the pesto industry are first, the short shelf-life **(Fabiano *et al.*, 2000)** of this product and second, the tendency of the oil to separate at the top of containers. The challenge is to control the system stability and avoid its destabilization during storage, for instance when environmental conditions, such as temperature, change **(Drelich *et al.*, 2010)**. Unfortunately, limited information regarding essays of stabilization of pesto is available. The use of an oil structuring agent may be a solution to increase viscosity and physical stability of this kind of products. Beeswax is just an example of these oil structuring agents **(Doan *et al.*, 2015)**. The use of beeswax as suspension stabilizer may be a viable global alternative to other products more expensive. Beeswax (E 901), was permitted as a glazing agent on confectionery (excluding chocolate), for the surface treatment only of certain fruits (fresh citrus fruits, melons, apples, pears, peaches and pineapples), in food supplements and as a carrier for colors **(EFSA, 2007)**. Beeswax is a complex mixture of saturated and unsaturated linear and complex monoesters, hydrocarbons, free fatty acids, free fatty alcohols, and other minor substances produced by the worker honeybee **(Aichholz *and* Lorbeer, 1999)**. The biochemical and toxicological studies of beeswax showed the absence of toxicity of their main components. The available information concerning the potential allergenicity of beeswax was very limited **(WHO, 2006a)**. The European Food Safety Authority **(EFSA, 2007; WHO, 2003)** established an Acceptable Daily Intake of beeswaxes between 10 and 20 mg/kg body weight, according to total content of hydrocarbons with chain lengths (≥ C25). The aims of this study were (i) the physicochemical and rheological characterization of pesto sauce based of rocket salad (ii) to investigate its colloidal and oxidative stabilization by beeswax addition in function of temperature and time of storage.

MATERIALS AND METHODS

Preparation of pesto sauce

The prepared pesto sauce samples were based on fresh rocket leaves (*Eruca vesicaria* subsp *sativa*), purchased to supermarket and washed with water. The product contains (rocket salad 33 %, sunflower oil 32 %, virgin olive oil 17.1 %, cheese (Pecorine romano 0.8% and Grana Padanno 5 %), cashew nuts 4%, potato flake 3.9 %, garlic 0.4 %, ascorbic acid (Sigma-Aldrich, Germany) 0.2 %, and pinion 1%, NaCl (Sigma-Aldrich, Germany) 2.6 % and beeswax on different

percentages. All ingredients were mixed by a household blender during two minutes with maximum speed. The natural wax (yellow beeswax, Kahlwax 8109) was dispersed in olive and sunflower oils and mixed at 70°C under agitation (300 rpm) for 10 min. The clear oil dispersions were subsequently cooled to room temperature and mixed with the other ingredients (like mentioned above). A control sample and five different pesto samples were prepared with different concentrations of beeswax (0.5; 0.6; 0.8; 1; 1.2%) (w/w of pesto sauce). The products were packed in glass jars of 150 g and pasteurized at 90°C for 10 min and cooled to 13°C in an ice bath.

Rheological Measurements
Flow Behavior

Viscosimetry measurements of pesto sauces and oils mix (continuous phase) at 4, 20 and 40°C were performed by a Modular compact rheometer Anton Paar MCR 302 (Anton Paar Instruments, Austria) controlled stress rheometer equipped with a water temperature control unit. The measuring geometry employed was a cup for concentric cylinder CC24 (26 mm diameter and 50 mm height) and bob (24.005 mm diameter and 24.975mm height) system. The samples, vigorously sheared for 1 min in order to suppress the previous rheological history, were equilibrated for 3 min before the experiment. The shear stress and apparent viscosity were recorded as a function of the shear rate. Viscosimetry measurements were carried out in triplicate. The experimental data obtained for each sample were fitted to the Ostwald or power law model:

$(\sigma = K\gamma^n)$

where σ is the shear stress, K is the consistence coefficient and n flow behavior index

Vertical Scan Macroscopic Analysis

A Vertical Scan Macroscopic Analyzer Turbiscan Classic MA 2000 instrument (Formulaction Inc, USA), was used for following of the physical stability of pesto sauces. After preparation, all samples were vigorously mixed and 7 ml of each sample were placed into flat bottomed cylindrical glass tubes (140 mm height, 16 mm diameter). The measure of the transmission and backscattering profiles was made according to the height (70 mm). Three individual tubes from each pesto sauce were stored at 4, 20 and 40 °C, and measurements were done at randomly chosen time intervals during 33 days of storage (at 0, 72, 117, 167, 287, 314, 407, 479, 626 and 794 hours). For each sample, the percent colloidal stability **(Wu, 2001)** was determined using the equation below:

$$\mathbf{CS}\ (\%) = \left(\frac{\mathbf{H_t} - \mathbf{H_0}}{\mathbf{H_t}}\right) \times \mathbf{100}$$

where H_0 is the height of oil phase determined at each measurements from transmission Turbiscan profile, and H_t is the total height of pesto sauce in the test tubes assessed from the initial backscattering Turbiscan profile.

Fat Extraction

For all samples, oil was extracted according to a modified method described by **Folch *et al.* (1957)** described by **Muresan *et al.* (2015)**. The extracted oil was brought to dryness by flushing with nitrogen and was used for the analysis of the peroxide, p-anisidine indexes. For oxidative stability the measurements were done at randomly chosen time intervals during 33 days of storage (at 0, 72, 117, 167, 287, 314, 479 and 794 hours).

Peroxide Value (PV)

The primary oxidation products were measured using the peroxide value, according to a modified method of the AOCS Official Method Cd 8-53 described by **Crowe *and* White (2001)**. The titration was done by a sodium thiosulfate solution 0.02 N (Titrisol-Merck, Darmstadt, Germany). Glacial acetic acid and potassium iodide were from Merck (Darmstadt, Germany), while the chloroform used was from VWR (Leuven, Belgium). Peroxide values were determined in triplicate. PVs were expressed in 11meq O_2/kg oil and calculated using formula below:

$$PV = \frac{(S-B)\times N\times 1000}{m}$$

where *S* is the volume of titrant (ml) of sample; *B* is the volume of titrant (ml) of blank; *N* is the normality of sodium thiosulfate solution; *m* is masse (g) of the test portion.

p-Anisidine Value (pAV)

Secondary oxidation products were assessed by measuring the p-anisidine value, which was determined according to the AOCS Official Method Cd 18-90 **(AOCS, 1997)**. The spectrophotometric reading was done by a Shimadzu UV-2401PC. Isooctane used was for spectroscopy from Sigma-Aldrich (Bornem, Belgium). The p-anisidine analytical reagent and glacial acetic acid were from Merck (Darmstadt, Germany). p-Anisidine values were determined in triplicate. *pAV*s were without unit and calculated using formula below:

$$pAV = \frac{25\times(1.2\ As - Ab)}{m}$$

where *As* is Absorbance of the fat solutions after reaction with p-Anisidine reagent; *Ab* is the absorbance of the fat solution; *m* is masse (g) of the test portion.

Color measurements

Surface color measurements of pesto sauces were performed using a colorimeter (ColorFlex EZ, HunterLab, Verginia, USA) with the Windows-based computer program (EasyMatch QC Software (EZMQC-CFLX)). The instrument was calibrated using the standard white file. Color measurements were made on the surface of each paste in triplicate on three randomly selected locations. Color measurements were made at room temperature (≈25 °C) without dilution. The pesto samples color is expressed as chromatic ordinates a*, b* and L*. The parameters chroma (C*) and Hue (h°) were calculated using equations below:

$$C^* = (a^{*2} + b^{*2})^{1/2}$$

$$h^\circ = \arctan(\frac{b^*}{a^*})$$

Sensory evaluation

In this study, the sensory analysis was performed by a hedonic test by ranking of appreciation of tested product. This test measures consumer pleasure and / or satisfaction experienced at the sight or consumption / use of a product. Six pesto samples were freshly prepared for the hedonic test by 60 untrained subjects. The tasters choose, for each sample, the category that corresponds to their level of appreciation using a 7-point Hedonic scale (1 -Extremely dislike; 7-Extremely like). The description of the tested product through the five senses (Sight, hearing, smell, taste and touch) will define terms called descriptors. This will provide a sensory profile of the product studied. Sensory descriptors of the samples were appearance (visual appeal on sight), color, aroma (olfactory feeling on inhaling the head space volatiles), taste (response of taste bud on masticating), aftertaste (the last taste in the mouth after masticating) and texture at the spoon (force to fill the spoon), overall acceptability and ranking **(Kaur *et al.*, 2011; Ranganna, 1986).**

Statistical analysis

An IBM SPSS Statistics, version 23, software was used for ANOVA, and Tukey's test to detect significant differences among all pesto samples stored for 33 days at different temperatures for different parameters tested. Additionally, correlations between parameters were calculated by person test. A Microsoft Excel 2007 was used for determination of means (± standard deviations) and construction of viscosity and flow curves.

RESULTS AND DISCUSSION

Rheological Properties of pesto sauce and oil (continuous phase)

Time-independent rheological properties of different samples were evaluated at 4±1°C, 20±1°C and 40±1°C. A preliminary study revealed that for pesto and oil samples, the cone-plate geometry CP (diameter 50 mm and a=1°) was not suitable due to the wall slip. Consequently, the concentric cylinder CC24 was used in order to characterize the flow properties of different samples studied.
For values of shear rates higher than ~0.658 s^{-1}, a fracture was detected and confirmed also by the sharp change in shear stress after this value when plotting shear stress in function of shear rate **(Figure 1)**.

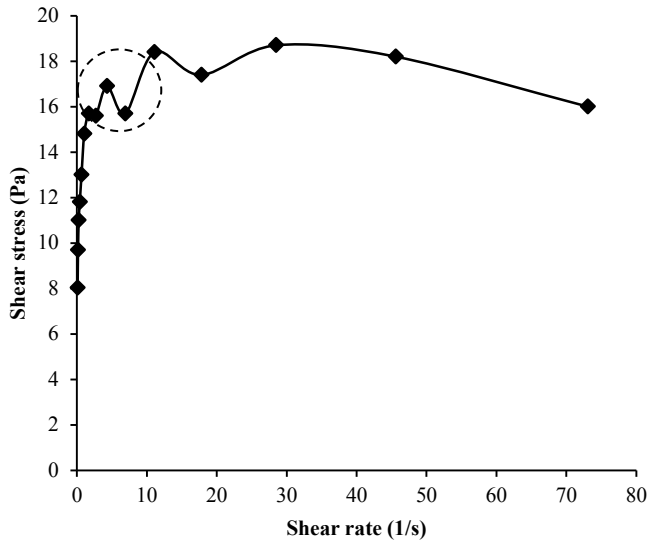


Figure 1 Flow curve of pesto sample with 0.5% of wax at 40°C. Circle in dashed line shows evidencing product fracture after 0.658 s^{-1}

Similar issues were observed for samples continuous oil phase, for shear rates higher than ∼11.1 s^{-1}. Thus, considering the above-mentioned product fracture issues, it was decided to use intervals of shear rate from 0.1 to 0.658 s^{-1} and from 0.1 to 11.1 s^{-1} for pesto and oil (continuous phase) samples, respectively. It was similar to the interval shear rates from 0.1 to 20 s^{-1} experienced in flow behavior of oleogels reported by **Doan *et al.* (2015)**.

The rheological analysis showed that for all studied temperatures and wax percentages, the shear stress– shear strain relationship was nonlinear, indicating that pesto and continuous oil phase behave as a non-Newtonian fluid, except for the oil (continuous phase) without wax which has a flow index (n=1) indicating a Newtonian behavior **(Table 1)**. For all temperatures and all samples studied, the apparent viscosity (η) decreased with increasing shear rate; it indicates that all samples behave as pseudo plastic materials **(Muresan *et al.*, 2014; Paredes *et al.*, 1988)** irrespective of temperature and percentage of wax. As an example, the pesto viscosity curves obtained at 40°C are given in **Figure 2**.

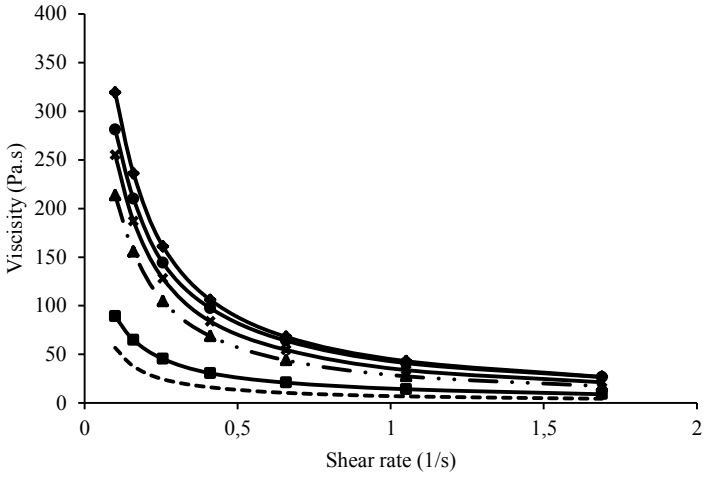


Figure 2 Viscosity curves of pesto samples at 40°C. (---) sample without wax; (■) sample with 0.5% of wax; (▲) sample with 0.6% of wax; (×) sample with 0.8%; (♦) sample with 1% of wax; (●) sample with 1.2% of wax .

The consistency coefficient (K) and flow behavior index (n) of samples obtained by fitting the experimental shear stress–shear rate data to the power law model as a function of beeswax percentage and temperature levels are given in **Table 1**.

Table 1 Rheological characteristics of different pesto sauce and oil phase continuous samples

PESTO	**Power low model**								
	K(Pa.s^n)			**n(-)**			***R*^{2}**		
Sample code	4°C	20°C	40°C	4°C	20°C	40°C	4°C	20°C	40°C
0%	49.60±0.00 ^{Da}	27.72±0.01^{Db}	7.36±0.11 ^{Fc}	0.28 ±0.00 ^{Ba}	0.21±0.00 ^{Ab}	0.11±0.01 ^{Dc}	0.99	0.99	0.99
0.50%	58.31±0.42^{Ca}	45.03 ±1.7 ^{Cb}	15.45±0.7 ^{Ec}	0.36±0.05 ^{Aa}	0.28±0.06 ^{Abc}	0.23±0.02 ^{Ac}	0.999	0.99	0.99
0.60%	68.78±0.01^{Aa}	56.00 ±1.9 ^{Bb}	35.33±0.02 ^{Dc}	0.29 ±0.00 ^{Ba}	0.22 ±0.00 ^{Ab}	0.17 ±0.00 ^{Cc}	0.99	0.99	0.95
0.80%	68.13±0.01^{Ba}	66.10 ±1.3 ^{Ab}	39.55±0.05 ^{Cc}	0.29 ±0.00 ^{Ba}	0.26 ±0.01 ^{Ab}	0.16 ±0.00 ^{Cc}	0.97	0.97	0.95
1%	68.75±0.01^{Aa}	53.47±0.78^{Bb}	52.12±1.99 ^{Ab}	0.20 ±0.00 ^{Ca}	0.21 ±0.00 ^{Aa}	0.16±0.01 ^{Cb}	0.95	0.96	0.95
1.20%	49.45±0.12^{Da}	41.71 ±2.11^{Cb}	45.94±1.77 ^{Ba}	0.23±0.01 ^{BCa}	0.24 ±0.00 ^{Aa}	0.20 ±0.00 ^{Bb}	0.96	0.96	0.97
OIL (continuos phase)	Power low model								
	K(Pa.sn)			n(-)			R^2		
Sample code	4°C	20°C	40°C	4°C	20°C	40°C	4°C	20°C	40°C
0%	0.11±0.001 ^{Fa}	0.06±0.00 ^{Fb}	0.03±0.00 ^{Dc}	1.01±0.01 ^{Aa}	1.01 ±0.00 ^{Aa}	1.00 ±0.00 ^{Aa}	1.000	1.000	1.000
0.50%	1.37±0.00 ^{Ea}	0.13±0.00 ^{Eb}	0.09±0.01 ^{Cc}	0.57±0.00 ^{Bc}	0.94 ±0.00 ^{Bb}	0.84 ±0.00 ^{Ba}	0.996	0.999	0.997
0.60%	2.01±0.01 ^{Da}	0.22±0.00 ^{Db}	0.12 ±0.01 ^{Cc}	0.52±0.01 ^{Cc}	0.87 ±0.00 ^{Ca}	0.78±0.01 ^{Cb}	0.997	0.995	1.000
0.80%	3.25±0.02 ^{Ca}	0.61±0.00 ^{Cb}	0.20 ±0.02 ^{Ac}	0.50±0.00 ^{Dc}	0.77 ±0.00 ^{Db}	0.58±0.01 ^{Ea}	0.996	0.992	0.994
1%	5.90±0.01 ^{Aa}	1.20±0.00 ^{Bb}	0.17 ±0.00 ^{Bc}	0.37±0.02 ^{Ea}	0.62 ±0 ^{Ea}	0.61 ±0.00 ^{Eb}	0.992	0.976	0.994
1.20%	5.79±0.01 ^{Ba}	1.52 ±0 ^{Ab}	0.19 ±0 ^{ABc}	0.49^{Dc}±0	0.62 ^{Eb}±0	0.67^{Da}±0.01	0.987	0.959	0.997

Mean (n = 3) ± SD; Means of consistence coefficient K and flow index n, with different lower-case superscripts (a-c) in each row or with different capital superscripts (A-F) in each column, are significantly different at α = 0.05 (one-way ANOVA and Tukey's test. a or A—the highest content); (1-1.2%) indicate samples with concentration of wax from 0 to 1.2 %

The coefficients of determination (R^2) obtained were close to unity (between 0.95 and 0.99) which confirm that power law model was adequate for describing the flow behavior of the pesto and continuous oil phase samples within the wax ratios studied. Because it only contains two parameters that can describe shear rate–shear stress data, the power law model has been used extensively to characterize fluid foods **(Muresan *et al.*, 2014; Steffe, 1996; Rao, 2007).**

For analyzed pesto samples, the value of consistency coefficient, K, ranged from 7.36 to 68.78 Pa.s^n and the flow behavior index, n, ranged from 0.11 to 0.36. Similar results were reported by **Doan et al. (2015)** for oleogels with 5% of candelila and bees waxs. Increasing the wax percentage induced a significant increase of consistency coefficient, K as revealed by the correlation coefficient r= +0.452 (p<0.01). At each temperature, the lowest K value was obtained for pesto control sample (without beeswax). This can be explained by the absence of the oleogelator, so the particles are more efficiently aligned in the flow direction as there are no wax crystals, consequenty phases separation and oil releasing on the top may occur. The shear-thinning behavior of pesto was confirmed by the fact that flow behavior indexes (n) obtained were less than unity. The smaller n values, the greater the departure from Newtonian behavior **(Muresan *et al.*, 2014)**. The shear-thinning behavior of pesto sauces was in agreement with the findings of other researchers about the rheological properties of salad dressing, sesame and sunflower tahini products **(Muresan *et al.*, 2014; Paredes *et al.*, 1988).**

The values of consistency coefficient, K, for oil (continuous phase) samples, ranged from 0.03 to 5.9 Pa.s^n and the flow behavior index, n, ranged from 0.37 to 0.94 Pa.s^n except the control sample which has flow index (n=1) (Newtonian fluid). Results of consistency were lower than K values for oleogel with 5% of wax stored at 5°C reported by **Doan *et al.* (2015)**, which were in the range of 2.03 – 91.10 Pa.s^n. However, flow index behavior was close to the results of these

authors, ranging from 0.2 to 0.73. This difference could be due to the higher quantity of wax which they have used.

For all temperature, there was a positive (+0.478) and very significant correlation ($p<0.01$) between consistency coefficient, K and wax ratios added to oil samples. The correlation (-0.770) between flow index, n and the wax ratios added was highly significant ($p<0.01$). Thus, the increasing of wax added to oil samples induced an increasing of consistency coefficient, K and a decreasing of flow index, n. The lower flow index n and the higher consistency index K are indicative of higher pseudo plasticity, higher consistency and viscosity, which are characteristics of a more complex and dense network **(Doan *et al.*, 2015; Mezger, 2006)**. Subsequently, the pseudo plasticity increases with increasing of percentage of beeswax added.

Samples of pesto showed a significant correlation (-0.670; -0.631), between coefficients (K, n) and temperature, respectively. Concerning oil phase, the correlation between consistency coefficient K and the temperature was significant (-0.640). It was confirmed by **Altay *and* Ak (2005)**, who found that coefficients (K and n) decrease with increase in temperature for sesame paste samples. This phenomenon was observed, also by **Paredes *et al.* (1988)** for five kinds of salad dressing. Consequently, the pseudo plastic behavior of all samples tested was more important with the decreasing of temperature. All pesto samples contained the same ingredients, but their rheological properties were different. This was due to the differences in wax ratios added. The trapping of the oil by wax crystals depended on amount of added wax **(Doan *et al.*, 2015)**. However, this behavior was observed until a quantity of wax threshold was attained. At this threshold the consistency starts to decrease. In other hand, the threshold of percentage of wax added increases with increasing of temperature, which was confirmed by K values **(Table 1)**. Indeed, the decreasing of K starts from 0.8%, 1%, 1.2% at 4, 20 and 40°C, respectively. The wide range of temperatures used during pesto and oil samples analysis allowed shows that the flow behavior of pesto samples was strongly influenced by temperature as seen in **Figure 3**.

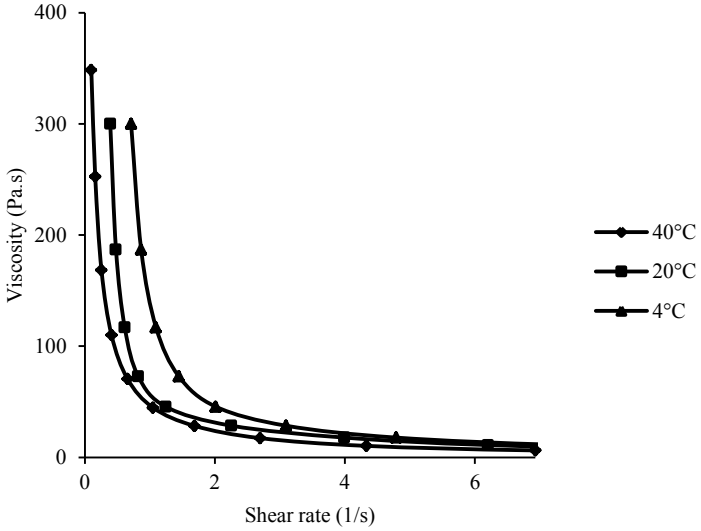


Figure 3 Effect of temperature on viscosity curves of pesto sample with 0.8 % of wax. (▲) sample stored at 4°C; (■) sample stored at 20° C; (♦) sample stored at 40°C.

A decrease in consistency coefficient was observed with the increasing temperature indicating a decrease in apparent viscosity at higher temperature. This is a trend observed with several food products **(Rao, 2007)**. Similar results were reported by **Wan *et al.* (2014)** for oleogel of sunflower oil developed by β-sitosterol and lecithin, by **Goupale *and* Rajkapoor (2011)** for oleogel of sesame oil and by **Yilmaz *and* ÖğüTcü (2014)** for oleogel of olive oil with beeswax.

The pesto samples with low percentage of wax added showed a higher decrease in viscosity than pesto samples with higher percentage of wax exhibiting lower decrease in viscosity and higher rate of resistance to flow **(Figure 2)**. Indeed, several studies have shown that the increasing of the amount of wax added in oleogels induiced a dense and crowded spatial distribution of the crystals which gives oleogels greater firmness and high rate of resistance to flow. This was confirmed by **ÖğüTcü *and* Yılmaz (2014)** and **Yilmaz *and* ÖğüTcü (2014)**, who have studied oleogels of olive oil with carnauba wax and beeswax respectively.

Colloidal stability (CS)

Tubes prepared **(Figure 4)** for periodical scanning, were filled with the pesto samples and stored at 4, 20 and 40 °C for 793 h. For instable samples, oil separation occurred at the top of the pesto vessels during storage. Vertical scan macroscopic analysis, with Turbiscan scanning profiles, was proved to be a suitable technique enabling destabilization phenomenon characterization. This technique gives a precise quantification of pesto samples' CS **(Muresan *et al.*, 2015)**.

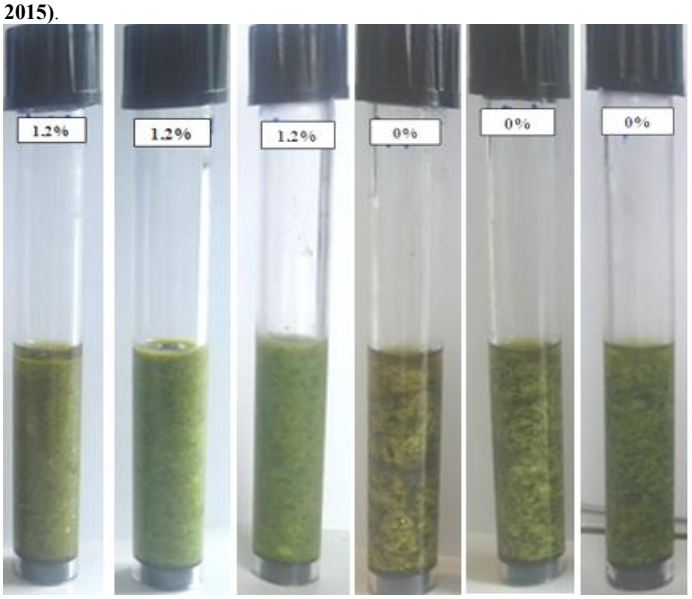


Figure 4 Photography showing turbiscan tubes filled with pesto samples 1.2% and 0% (repetition 1) at time 239 h, stored at: 40, 20 and 4 °C (from left to right side)

The variations in CS of different pesto samples stored at 4, 20 and 40 °C during 793 h are presented in **Table 2**.

Table 2 Colloidal stability of pesto sauces during 793 h of storage at 4, 20 and 40 °C

Storage temperature	Sample	Storage time												
		Colloïdal stability (%)												
Storage time (h)		2	72	76	117	141	167	239	287	314	407	479	626	793
4°C	0%	95.74±0.49 Ba	95.43 ±0.53 Bab	95.44±0.32 Cab	94.44±0.58 Babc	94.38±0.42 Babc	94.31±0.48 Babc	94.3±0.52 Babc	94.25± 0.32 Babc	94.19±0.86 Babc	94.13±0.8 Bbc	93.74±0.48 Bc	93.69±0.30 Bc	93.48 ±0.40 Cc
	0.50%	99.59±0.71 Aa	99.43 ±0.61 Aa	99.21 ±0.38 Ba	99.21 ±0.38 Aa	99.18 ±0.57 Aa	99.16 ±0.36 Aa	99.12 ±0.53 Aa	99.1 ±0.46 Aa	99.05 ±0.23 Aa	98.99 ±0.36 Aa	98.72 ±0.11 Aa	98.65 ±0.49 Aa	98.39 ±0.41 Ba
	0.60%	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	99.78 ±0.38 Aa	99.72 ±0.48 Aa	99.67 ±0.58 Aa	99.67 ±0.57 Aa	99.35 Aa ±0.74	99.29 ±0.73 Aa	98.96 ±0.90 Aa	98.96 ±0.90 Aa	98.96 ±0.93 Aa	98.35 ±0.76 Ba
	0.80%	100 ±0 Aa	100 ±0 Aa	100 Aa ±0	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	99.83 ±0.29 Aa	99.78 ±0.39 Aa	99.32 ±0.61 Aa	99.27 ±0.69 Aa	99.21 ±0.86 Aa	99.15 ±0.16 ABa
	1%	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	99.63 ±0.65 Aa	99.57±0.74 Aa	99.52±0.83 Aa	99.47±0.92 ABa
	1.20%	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa
20°C	0%	95.13 ±0.39 Ba	94.85±0.41 Cab	94.23 ±0.16 Cabc	94.18 ±0.78 Cabc	93.77 ±0.49 Dbcd	93.1 ±0.24 Ccde	92.99 ±0.26 Ccde	92.56 ±0.40 Cdef	92.19 ±0.60 Cefg	91.55 ±0.35 Cfgh	91.34 ±0.40 Cfgh	91.25 ±0.50 Cgh	90.69 ±0.26 Ch
	0.50%	100 ±0 Aa	96.84± 0.17 Bb	96.5± 0.17 Bbc	95.97 ±0.16 Bbc	95.74 ±0.05 Cc	94.71 ±0.03 Bd	94.67 ±0.34 Bd	94.49 ±0.34 Bd	94.10 ±0.16 Bde	93.59 ±0.16 Bef	93.53 ±0.31 Bef	93.18 ±0.56 Bf	92.84 ±0.56 Bf
	0.60%	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	96.28 ±0.20 Bb	95.60 ±0.46 Bbc	95.37 ±0.50 Bcd	94.86 ±0.13 Bde	94.52 ±0.13 Bef	94.30 ±0.30 Bef	93.84 ±0.14 Bfg	93.56 ±0.21 Bgh	93.30 ±0.14 Bgh	93.13 ±0.13 Bh
	0.80%	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	99.97±0.05 Aa	99.94 ±0.10 Aa	99.88 ±0.20 Aa	99.83 ±0.30 Aa	99.77 ±0.40 Aa	99.71 ±0.50 Aa
	1%	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	100 ±0 Aa	99.94	99.89	99.83	99.77

	1.20%	100 ±0 ^{Aa}	100 ±0 ^{Aa}	100 ±0 ^{Aa}	100 ±0 ^{Aa}	100 ±0 ^{Aa}	100 ±0 ^{Aa}	100 ±0 ^{Aa}	100 ±0 ^{Aa}	100 ±0 ^{Aa}	±0.10 ^{Aa} 100 ±0 ^{Aa}	±0.20 ^{Aa} 100 ±0 ^{Aa}	±0.30 ^{Aa} 100 ±0 ^{Aa}	±0.40 ^{Aa} 100 ±0 ^{Aa}
40°C	0%	96.54 ±0.27 ^{Ca}	93.01 ±0.42 ^{Cb}	92.53 ±0.08 ^{Cbc}	92.0 ±0.43 ^{Ccd}	91.97 ±0.23 ^{Ccd}	91.50 ±0.18 ^{Cde}	91.46 ±0.06 ^{Cdef}	91.39 ±0.20 ^{Cdef}	91.32 ±0.29 ^{Cdef}	90.80 ±0.12 ^{Cefg}	90.79 ±0.12 ^{Cfg}	90.58 ±0.08 ^{Cg}	90.47 ±0.26 ^{Cg}
	0.50%	98.58 ±0.10 ^{Ba}	93.96 ±0.37 ^{Bb}	93.00 ±0.72 ^{Ccd}	92.367 ±0.76 ^{Cde}	92.34 ±0.75 ^{Ccd}	91.95 ±0.01 ^{Ccde}	91.90 ±0.01 ^{Ccde}	91.76 ±0.05 ^{Cde}	91.70 ±0.01 ^{Cde}	91.61 ±0.01 ^{Cde}	91.71 ±0.29 ^{Cde}	91.13 ±0.01 ^{Ce}	91.13 ±0.01 ^{Ce}
	0.60%	99.83 ±0.30 ^{Aa}	99.77 ±0.40 ^{Aa}	96.51 ±0.68 ^{Bb}	96.35 ±0.77 ^{Bb}	96.34 ±0.79 ^{Bb}	95.16 ±0.20 ^{Bbc}	95.67 ±0.90 ^{Bbc}	95.67 ±0.90 ^{Bbc}	94.98 ±0.54 ^{Bbc}	94.98 ±0.55 ^{Bbc}	94.98 ±0.63 ^{Bbc}	94.64 ±0.62 ^{Bbc}	94.09 ±0.67 ^{Bc}
	0.80%	100 ±0 ^{Aa}	99.77 ±0.40 ^{Aa}	99.71 ±0.51 ^{Aa}	99.65 ±0.61 ^{Aa}	99.59 ±0.71 ^{Aa}	99.48 ±0.90 ^{Aa}	99.48 ±0.90 ^{Aa}	99.48 ±0.75 ^{Aa}	95.83 ±0.01 ^{Bb}	95.83 ±0.01 ^{Bb}	95.83 ±0.01 ^{Bb}	95.48 ±0.01 ^{Bb}	94.97 ±0.01 ^{Bb}
	1%	100 ±0 ^{Aa}	99.94 ±0.10 ^{Aa}	99.77 ±0.40 ^{Aa}	97.44 ±0.63 ^{Bb}	97.44 ±0.63 ^{Bb}	95.99 ±0.12 ^{Bc}	95.75 ±0.39 ^{Bc}	95.75 ±0.39 ^{Bc}	95.70 ±0.28 ^{Bc}	95.68 ±0.36 ^{Bc}	95.65 ±0.50 ^{Bc}	95.57 ±0.61 ^{Bc}	95.15 ±0.53 ^{Bc}
	1.20%	100 ±0 ^{Aa}	100 ±0 ^{Aa}	99.66 ±0.59 ^{Aab}	99.61 ±0.68 ^{Aab}	99.61 ±0.68 ^{Aab}	99.55 ±0.78 ^{Aab}	99.55 ±0.78 ^{Aab}	99.44 ±0.97 ^{Aab}	99.44 ±0.97 ^{Aab}	99.44 ±0.97 ^{Aab}	99.44 ±0.97 ^{Aab}	98.14 ±0.01 ^{Aab}	97.88 ±0.49 ^{Ab}

Mean (n = 3) ± SD; Means of colloidal stability, with different lower-case superscripts (a-h) in each row (according time storage), are significantly different (p< 0.05); Means of colloidal stability with capital superscripts (A-C), within columns for each temperature, are significantly different (p < 0.05); (one-way ANOVA and Tukey's test. a or A—the highest content); (1-1.2%) indicate samples with concentration of wax from 0 to 1.2 %

In this study, during the 793 h of storage at different temperatures, the increasing of beeswax added to pesto samples was accompanied by the decreasing of oil separation at the top of the tube. The stability and wax percentage added to pesto samples were correlated significantly (0.689, p<0.01). Thus, the increasing of wax ratios on pesto samples induced a significant increasing of the CS. Pesto stability depends directly on the stability of the oleogel. According to **Yılmaz and ÖğüTcü (2014)**, the increasing of the wax amount leads to the formation of a dense oleogel able to have a colloidal stability more important.

A three-way general linear model ANOVA for CS (%) revealed highly significant differences (p < 0.001) for beeswax ratios added, storage time and temperature, as well as for their first and second order interactions. Consequently, three-way interactions Sample × Temperature × Time were analyzed by several one-way ANOVA tests and compared by Tukey's method **(Table 2)**.

There was a significant correlation (p<0.01) between CS, storage time and temperature with correlation coefficients of -0.305, and -0.363, respectively. When the storage temperature increased, the CS of samples decreased **(Table 2)**, as reported by **Çiftçi *et al.* (2008)** and **Muresan *et al.* (2015)** for sesame and sunflower tahini.

This CS decreasing was due to the low viscosities of continuous phase at high temperatures, which facilitates the sedimentation of solids **(Muresan *et al.*, 2015)**. Indeed, the viscosity of continuous phase with 1.2 % of wax to shear rate of 0.1 s^{-1}, decreases from 15.25 Pa.s (at 4°C) to 0.388 Pa.s (at 40°C). Moreover, for the control, it decreases from 0.108 to 0,0327 Pa.s. Similar phenomenon was observed by **Goupale *and* Rajkapoor (2011)** and **Wan *et al.* (2014)** for sesame and sunflower oleogels, respectively. This may be explained by the loosening of oleogel under the effect of storage temperature and time, leading to oil separation. Indeed, **Yılmaz *and* ÖğüTcü (2014)** have showed that the temperature and time of storage affect the oleogel of olive oil with bees and sunflower waxs texture. However this loosening is less important for samples with a high amount of wax. Indeed, at the end of storage at 40°C CS values of samples without and with 1.2% of wax were 90.47 and 97.88%, respectively **(Table 2)**.

Overall, samples showed a decrease of CS with the increase of storage time **(Table 2)**. In function of storage time, CS was significantly different, except at 4°C and for pesto samples with 0.8%, 1% and 1.2% of beeswax at 20°C. Moreover, the duration required to observe a considerable drop of stability increases with increasing the wax amount added. Thus, the 72 h and 479 h periods were necessary to observe changes for the control sample and 1.2% wax, respectively.

After 793 h of storage at all temperature, pesto sample without beeswax showed the highest level of oil separation **(Table 2)**. From 72h of storage, CS values of pesto were significantly different (p<0.001) **(Table 2).** In the end of storage at 40°C samples of different percentage of beeswax added can be classified with respect to their oil separation level. Thus, control sample showed the highest level of oil separation, followed by 0.5; 0.6; 0.8; 1 and 1.2% **(Table 2)**. The high stability of samples with high ratio of wax may be explained by the fact that the continuous phase had a compact structure, conferring it a high viscosity, better pseudo plastic behavior and leading to a lower availability of oil prone to creaming in between particles. This was confirmed by the results of **Yılmaz *and* ÖğüTcü (2014)**, who have observed that the increasing of wax ratio added, for an oleogel of olive oil with beeswax, leads to obtain polarized light microphotographs, increasingly dense and a firmness, a work of shear and a stickiness increasingly important.

Oxidative stability

Our research was extended by assesing the oxidative stability of the studied pesto samples during a 793 h storage test .

Pesto samples

Peroxide values (PV) During Storage

PV is one of the best parameters for the evaluation of oxidation during storage **(Yilmaz *and* ÖğüTcü 2014)**. It is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation **(Muresan *et al.*, 2015)**. For pesto samples, the initial PV was measured after the production and pasteurization. Initially, peroxide value of all samples was 11.75±0.11meq O_2/kg oil.

Very significant differences were detected (p<0.01) between the PV of each sample stored for the same period at different temperatures **(Table 3)** especially from 72h to 793 h of storage, confirming the observation of oxidation increasing with storage temperature, made by **Lee et *al.* (2007)**. All samples stored during 793 h at 40 °C had a PV about three times higher than the ones stored at 4 °C, and about one time higher than ones stored at 20°C. The increasing of PV with temperature was explained by **List *et al.* (2005)** who mentioned that peroxides developed very slowly at 21.1 °C but increased markedly at 37.8 and 48.9 °C, probably because oxygen diffuses more rapidly into the reaction interface at higher temperatures **(Muresan *et al.*, 2015). Borompichaichartkul *et al.* (2009)** specified the 30 meq/kg limit according to Thai Industrial Standards. However, there are no specifications regarding the maximum allowable limit for the PV of pesto sauce. In this context, more biochemical and toxicological studies are needed. According to **Gotoh *et al.* (2006)** deteriorated fats and oils with PVs of at least 100 meq O_2/kg revealed neurotoxic effects in animal studies. In this study, none of pesto sauce samples was higher than the aforementioned limit during the storage at all temperatures.

A highly significant increasing was detected (p<0.001) for PVs of each sample during storage **(Table 3)**. This result was confirmed by **Lee *et al.* (2007)** who reported that as the oxidation time and temperature increased, PV of olive and sunflower oils increased. Similar results was reported by **Mihov *et al.* (2012)** for salad dressing and mayonnaise stored at 20°C during 20 days. Furthermore, **Iqbal *and* Bhanger (2007)** have observed similar results for sunflower oil containing garlic extract stored at high temperature during 15 days. During storage at 4°C, PVs of samples with different percentage of wax were not significantly different (p<0.05) **(Table 3),** indicating that the oxidation degree is not influenced by concentration of beeswax at low temperature. However, at 20 and 40 °C PVs were significantly different (p<0.05). This shows that the extent of oxidation decreases with the increases of beeswax concentration. Similar results was reported by **Paraskevopoulou *et al.* (2006)** for salad dressings stored at 23°C and stabilized with Arabic gum. That can be explained by the fact that, when the consistency of samples increases, the colloidal stability increases, and this leads to a lower creaming of the oil which was prone to oxidation in presence of oxygen **(Muresan *et al.*, 2015)**.

Pesto samples p-Anisidine Values (pAV) During Storage

The p-Anisidine values (pAV) is commonly used to follow the formation of aldehydic compounds in edible oils **(Tompkins *and* Perkins 1999)**. Changes in pAV of pesto samples during oxidation at 4, 20 and 40°C in the dark are shown in **Table 3**. The rate of pAV increased, with increasing of oxidation time and temperature as expected. A similar phenomenon was observed for sunflower oil, olive oil and avocado oil **(Muresan *et al.*, 2015; Lee *et al.*, 2007; Sun-Waterhouse *et al.*, 2011)**. Initially, pAV value was 4.51 **(Table 3)**. Similar result of initial pAV for sunflower and olive oils stored at 25 °C or 40 °C, was reported by **Lee et *al.* (2007)**. From 72h of storage, the pAVs of samples stored at different temperatures were very significantly different (p<0.01) **(Table 3)**, indicating that the pesto sauce oxidation increases with storage temperature. Similar results were reported by **Lee *et al.* (2007)** and **Muresan *et al.* (2015)** for sunflower, olive oils and sunflower tahini, respectively.

According to **Man *et al.* (1999)**, as a rule of thumb, the pAV should be less than 10 for good quality oil. Only pesto samples stored at 40°C slightly exceeded this threshold. Indeed, the highest level attained by pesto samples at 40°C is 10.71. Results are similar to ones of **Lee *et al.* (2007)** who reported that pAV of sunflower and olive oils, after 30 days of storage at 25 °C or 40°C, increased from (5.5 and 3.4) to (7.8 and 6.7) respectively.

A highly significant increasing of pAV values of each sample, were detected (p<0.001) during storage **(Table 3)**.

Table 3 Peroxide (PV) and p-Anisidine (pAv) values of pesto samples stored for 793 h at 4, 20 and 40 °C

	Storage temperature	Storage time (h)							
		0	72	117	167	287	314	479	793
Sample		Peroxyde values (meq O_2/kg oil)							
0%	4°C	11.75^{Ade}±0.11	12.16^{Cbcd}±0.06	12.07^{Cde}±0.06	12.52^{Cbc}±0.31	12.42^{Cbcd}±0.32	11.42^{Ce}±0.38	12.85^{Cab}±0.07	13.33^{Ca}±0.36
	20°C	11.75^{Af}±0.11	13.90^{Be}±0.1	17.18^{Bd}±0.31	17.88^{Bcd}±0.07	17.96^{Bcd}±0.06	18.56^{Bbc}±0.08	19.03^{Bab}±0.02	20.01^{Ba}±0.97
	40°C	11.75^{Ah}±0.11	14.94^{Ag}±0.05	20.50^{Af}±0.5	23.21^{Ae}±0.34	25.25^{Ad}±0.23	28.11^{Ac}±0.34	32.63^{Ab}±0.35	33.81^{Aa}±0.44
0.50%	4°C	11.75^{Acd}±0.11	12.09^{Cbc}±0.09	12.04^{Cbc}±0.06	12.42^{Cbc}±0.18	12.26^{Cbc}±0.37	11.32^{Cd}±0.46	12.71^{Cab}±0.19	13.24^{Ca}±0.26
	20°C	11.75^{Af}±0.11	13.87^{Be}±0.06	17.41^{Bd}±0.28	17.85^{Bcd}±0.08	17.91^{Bcd}±0.08	18.54^{Bbc}±0.05	19.01^{Bab}±0.02	19.73^{Ba}±0.64
	40°C	11.75^{Ah}±0.11	14.84^{Ag}±0.21	20.43^{Af}±0.51	23.15^{Ae}±0.22	25.22^{Ad}±0.28	28.04^{Ac}±0.31	32.58^{Ab}±0.37	33.73^{Aa}±0.40
0.60%	4°C	11.75^{Acd}±0.11	12.08^{Cbc}±0.09	12.03^{Cbc}±0.06	12.38^{Cbc}±0.17	12.26^{Cbc}±0.37	11.30^{Cd}±0.43	12.69^{Cab}±0.17	13.23^{Ca}±0.25
	20°C	11.75^{Af}±0.11	13.83^{Be}±0.12	17.40^{Bd}±0.30	17.81^{Bcd}±0.12	17.91^{Bcd}±0.09	18.53^{Bbc}±0.04	19.00^{Bab}±0.01	19.70^{Ba}±0.61
	40°C	11.75^{Ah}±0.11	14.82^{Ag}±0.19	20.37^{Af}±0.55	23.13^{Ae}±0.23	25.18^{Ad}±0.34	28.03^{Ac}±0.32	32.54^{Ab}±0.42	33.70^{Aa}±0.46
0.80%	4°C	11.75^{Acd}±0.11	11.98^{Ccd}±0.25	12.00^{Cbc}±0.1	12.35^{Cbc}±0.17	12.26^{Cbc}±0.37	11.27^{Cd}±0.38	12.69^{Cab}±0.17	13.20^{Ca}±0.3
	20°C	11.75^{Ag}±0.11	13.83^{Bf}±0.12	16.56^{Be}±0.03	17.53^{Bcd}±0.15	17.86^{Bcd}±0.15	18.36^{Bbc}±0.32	19.00^{Bab}±0.01	19.70^{Ba}±0.61
	40°C	11.75^{Ah}±0.11	14.72^{Ag}±0.37	19.84^{Af}±0.3	21.30^{Ae}±0.26	25.17^{Ad}±0.32	27.53^{Ac}±0.54	32.50^{Ab}±0.36	33.70^{Aa}±0.46
1%	4°C	11.75^{Acd}±0.11	11.74^{Bcd}±0.65	12.00^{Cbcd}±0.1	12.30^{Cabc}±0.1	12.26^{Cbc}±0.37	11.27^{Cd}±0.38	12.70^{Cab}±0.17	13.18^{Ca}±0.18
	20°C	11.75^{Ag}±0.11	13.60^{Af}±0.52	16.31^{Be}±0.45	17.30^{Bcd}±0.26	17.83^{Bcd}±0.12	18.37^{Bbc}±0.32	19.00^{Bab}±0.01	19.70^{Ba}±0.61
	40°C	11.75^{Ag}±0.11	14.52^{Af}±0.38	19.14^{Ae}±0.31	20.30^{Ad}±0.26	25.17^{Ac}±0.32	27.53^{Ab}±0.54	32.37^{Aa}±0.25	33.33^{Aa}±0.42
1.20%	4°C	11.75^{Acd}±0.11	11.73^{Ccd}±0.64	11.85^{Ccd}±0.25	12.03^{Cbc}±0.15	12.32^{Cabc}±0.33	11.14^{Cd}±0.06	12.71^{Cab}±0.19	13.13^{Ca}±0.14
	20°C	11.75^{Af}±0.11	13.20^{Be}±0.1	15.09^{Bd}±0.02	16.08^{Bb}±0.07	17.43^{Bb}±0.4	18.36^{Ba}±0.08	18.69^{Ba}±0.57	19.02^{Ba}±0.03
	40°C	11.75^{Ag}±0.11	14.20^{Af}±0.1	16.37^{Ae}±0.4	17.95^{Ae}±0.13	24.47^{Ac}±0.38	26.97^{Ab}±0.1	32.00^{Aa}±0.01	32.72^{Aa}±0.72
		p-Anisidine value							
0%	4°C	4.51^{Ae}±0.04	4.54^{Be}±0.12	4.70^{Bde}±0.10	5.10^{Cd}±0.1	5.68^{Bc}±0.19	5.93^{Cc}±0.21	6.46^{Cb}±0.04	8.40^{Ba}±0.31
	20°C	4.51^{Ac}±0.04	4.75^{Bc}±0.31	5.10^{Bc}±0.1	6.76^{Bb}±0.04	6.80^{ABb}±0.87	7.81^{Ba}±0.10	7.90^{Ba}±0.36	8.75^{Ba}±0.13
	40°C	4.51^{Ae}±0.04	5.81^{Ad}±0.25	7.41^{Ac}±0.46	7.62^{Ac}±0.33	8.00^{Ac}±0.36	8.79^{Ab}±0.10	9.22^{Ab}±0.08	10.71^{Aa}±0.20
0.50%	4°C	4.51^{Ae}±0.04	4.57^{Be}±0.15	4.70^{Bde}±0.1	5.13^{Cd}±0.12	5.68^{Bc}±0.19	5.94^{Cc}±0.21	6.45^{Cb}±0.04	8.41^{Ba}±0.30
	20°C	4.51^{Ad}±0.04	4.76^{Bd}±0.31	5.10^{Bd}±0.10	6.76^{Bc}±0.04	6.80^{ABbc}±0.87	7.80^{Bab}±0.09	7.90^{Ba}±0.36	8.73^{Ba}±0.15
	40°C	4.51^{Ae}±0.04	5.78^{Ad}±0.19	7.38^{Ac}±0.50	7.59^{Ac}±0.32	7.97^{Ac}±0.32	8.78^{Ab}±0.03	9.15^{Ab}±0.05	10.70^{Aa}±0.2
0.60%	4°C	4.51^{Ae}±0.04	4.50^{Be}±0.11	4.67^{Bde}±0.12	5.10^{Cd}±0.1	5.66^{Bc}±0.21	5.93^{Cc}±0.21	6.45^{Cb}±0.04	8.37^{Ba}±0.36
	20°C	4.51^{Ad}±0.04	4.74^{Bd} ±0.30	5.07^{Bd}±0.11	6.74^{Bc}±0.04	6.80^{ABbc}±0.87	7.79^{Bab}±0.08	7.88^{Ba}±0.34	8.71^{Ba}±0.18
	40°C	4.51^{Ae}±0.04	5.75^{Ad}±0.13	7.35^{Ac}±0.54	7.55^{Ac}±0.32	7.93^{Ac}±0.29	8.78^{Ab}±0.03	9.15^{Ab}±0.15	10.67^{Aa}±0.2
0.80%	4°C	4.51^{Ae}±0.04	4.47^{Be}±0.12	4.67^{Bde}±0.12	5.10^{Cd}±0.1	5.66^{Cc}±0.21	5.93^{Cc}±0.21	6.45^{Cb}±0.04	8.37^{Ba}±0.36
	20°C	4.51^{Ac}±0.04	4.73^{Bc}±0.29	5.07^{Bc}±0.11	6.74^{Bb}±0.04	6.75^{ABb}±0.83	7.79^{Ba}±0.08	7.85^{Ba}±0.4	8.71^{Ba}±0.18
	40°C	4.51^{Af}±0.04	5.75^{Ae}±0.13	7.35^{Ad}±0.54	7.51^{Ad}±0.15	7.93^{Acd}±0.29	8.61^{Abc}±0.27	9.12^{Ab}±0.10	10.64^{Aa}±0.15
1%	4°C	4.51^{Ae}±0.04	4.44^{Be}±0.16	4.67^{Bde}±0.12	5.10^{Bd}±0.10	5.66^{Bc}±0.21	5.93^{Cc}±0.21	6.45^{Cb}±0.04	8.37^{Ba}±0.36
	20°C	4.51^{Ac}±0.04	4.73^{Bc}±0.29	5.04^{Bc}±0.15	6.74^{Ab}±0.04	6.75^{ABb}±0.83	7.79^{Ba}±0.08	7.82^{Ba}±0.45	8.71^{Ba}±0.18
	40°C	4.51^{Af}±0.04	5.75^{Ae}±0.13	7.35^{Ad}±0.54	7.40^{Ad}±0.5	7.93^{Acd}±0.29	8.63^{Abc}±0.29	9.12^{Ab}±0.10	10.57^{Aa}±0.05
1.20%	4°C	4.51^{Ae}±0.04	4.37^{Bd}±0.26	4.63^{Bd}±0.15	4.97^{Bd}±0.06	5.59^{Bc}±0.3	5.93^{Cbc}±0.21	6.45^{Cb}±0.04	8.37^{Ca}±0.36
	20°C	4.51^{Ad}±0.04	4.72^{Bd}±0.29	5.00^{Bd}±0.01	6.71^{Ac}±0.07	6.75^{ABbc}±0.83	7.62^{Bbc}±0.29	7.80^{Bab}±0.48	8.71^{Ba}±0.18
	40°C	4.51^{Af}±0.04	5.58^{Ae}±0.16	7.28^{Ad}±0.57	7.32^{Ad}±0.55	7.90^{Acd}±0.26	8.54^{Abc}±0.38	9.02^{Aab}±0.13	9.94^{Aa}±0.14

Mean (n = 3) ± SD; Means of PV an pAV with different capital superscripts (A-C) within columns for each sample and with different lower-case superscripts (a-g) within rows are significantly different ($p < 0.05$); (one-way ANOVA and Tukey's test. a or A—the highest content); (1-1.2%) indicate samples with concentration of wax from 0 to 1.2 %

This was confirmed by Lee ***et al.* (2007)** for olive and sunflower oils stored at 25 and 40°C. Similar results was reported by **Kishk *and* Elsheshetawy (2013)** for mayonnaise stored at 20°C during 20 weeks.

Evolution of pH

For all studied samples, the initial pH was measured after the production and pasteurization, with initial values ranged from 4.9 to 5.33 **(Table 4)**. Similar results, were reported by **Hwang *and* Tamplin (2005)** for a mayonnaise stored at 4 °C which present an initial pH between (3.7- 5.1). The initial pH found, was also close to the pH threshold (4.6) of tomato purée in conserve, limited by Codex Alimentrius **(CODEX STAN 57-1981)**. In other hand, it is higher than the pH of tomato sauce ketchup indicated by **EAS (2010)** which must be between 3.3 and 4.2. The pH values **(Table 4)** decrease significantly ($p<0.05$) with the increasing of storage duration, at the same temperature. The lowest value of pH (3.49) has been found for the sample stored during 793 H at 40°C and the highest one (5.33) has been found initially for a sample stored at 4°C.

Table 4 pH values measured for pesto samples during 793 h of storage at 4 °C, 20°C and 40 °C

Samples	Temperature	Storage time (h)				
		0	72	287	407	793
0%	4°C	5.21±0.01^{Aa}	5.20±0.01^{Aa}	5.17±0.06^{Aa}	5.02±0.02^{Aab}	4.88±0.16^{Ab}
	20°C	4.90±0.18^{Ba}	4.85±0.11^{Bab}	4.80±0.01^{Bab}	4.69±0.08^{Bab}	4.63±0.01^{Bab}
	40°C	4.90±0.1^{Ba}	4.70±0.10^{Bb}	4.13±0.03^{Cc}	3.79±0.01^{Cd}	3.49±0.01^{Ce}
0.50%	4°C	5.20±0.01^{Aa}	5.19±0.01^{Aa}	5.19±0.21^{Aa}	4.97±0.02^{Aab}	4.92^{Ab}±0.02
	20°C	4.91±0.01^{Ba}	4.87±0.01^{Bb}	4.80±0.01^{Bc}	4.69±0.01^{Bd}	4.59^{Be}±0.01
	40°C	4.90±0.02^{Ba}	4.70±0.02^{Cb}	4.68±0.01^{Bb}	3.82±0.11^{Cc}	3.49^{Cd}±0.1
0.60%	4°C	5.19±0.01^{Aa}	5.18±0.01^{Aa}	5.21±0.03^{Aa}	5.03±0.02^{Ab}	4.98±0.02^{Ac}
	20°C	4.93±0.04^{Ba}	4.87±0.06^{Bab}	4.80±0.01^{Bb}	4.66±0.01^{Bc}	4.56±0.01^{Bd}
	40°C	4.92±0.02^{Ba}	4.72±0.02^{Cb}	4.71±0.02^{Cb}	3.80±0.01^{Cc}	3.50±0.01^{Cd}
0.80%	4°C	5.25±0.01^{Aa}	5.24±0.01^{Aa}	5.22±0.02^{Aa}	5.01±0.04^{Ab}	4.96±0.^{Ab}04
	20°C	4.97±0.01^{Ba}	4.93±0.01^{Bb}	4.80±0.02^{Bc}	4.78±0.01^{Bc}	4.68±0.01^{Bd}

	40°C	4.92±0.01 ^{Ca}	4.72 ±0.01 ^{Cb}	4.72 ±0.01 ^{Cb}	3.93 ±0.05 ^{Cc}	3.51 ±0.02 ^{Cd}
1%	4°C	5.24±0.01 ^{Aa}	5.23 ±0.01 ^{Aa}	5.23 ±0.06 ^{Aa}	5.11 ±0.02 ^{Ab}	5.06 ±0.02 ^{Ab}
	20°C	4.96±0.01 ^{Ba}	4.92 ±0.01 ^{Ba}	4.80 ±0.02 ^{Bab}	4.79 ±0.1 ^{ABab}	4.68 ±0.12 ^{Ab}
	40°C	4.94±0.01 ^{Ca}	4.74 ±0.01 ^{Ca}	4.73 ±0.01 ^{Bab}	4.38 ±0.38 ^{Bab}	4.08±0.38 ^{Bb}
1.20%	4°C	5.33±0.06 ^{Aa}	5.32 ±0.06 ^{Aa}	5.31 ±0.02 ^{Aa}	5.11 ±0.34 ^{Aa}	5.08 ±0.29 ^{Aa}
	20°C	5.02±0.12 ^{Ba}	4.94±0.05 ^{Bab}	4.81±0.02 ^{Bab}	4.79±0.01 ^{ABab}	4.69±0.18 ^{ABb}
	40°C	4.94±0.01 ^{Ba}	4.74 ±0.01 ^{Cb}	4.73 ±0.01 ^{Cb}	4.58 ±0.01 ^{Bc}	4.28 ±0.01 ^{Bd}

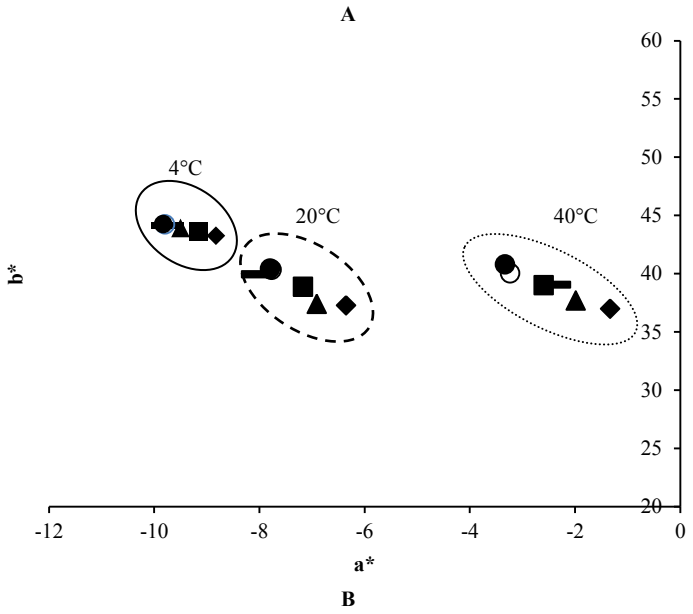
Mean (n = 3) ± SD; Means of pH values with different capital superscripts (A-C) within columns for each sample and with different lower-case (a-e) superscripts within rows are significantly different ($p < 0.05$); (one-way ANOVA and Tukey's test. a or A—the highest content); (1-1.2%) indicate samples with concentration of wax from 0 to 1.2 %

Samples stored at different temperatures were significantly different ($p < 0.05$). Indeed, at 793 h of storage when temperature increases from 4 to 40°C, pH values decrease from 4.88 to 3.49 and from 5.08 to 4.28 for samples with 0% and 1.2 % of wax, respectively. The decreasing of pH could be due to the development of microorganisms such as lactic bacteria, which grow at 37°C, and produce lactic acid **(Paulo *et al.*, 2012).**

From 287 h of storage at 40°C, a highly significant difference ($p < 0.001$) was detected between samples with different percentage of beeswax. The samples with 1% and 1.2% have the highest pH values at 40°C from 407 h of storage. In other words, the increasing of wax % could be responsible for the slow decreasing of pH values, the acidification and the alteration of pesto sauces studied.

Evolution of color parameters

The projection of the points yielded by pesto samples onto the (a*, b*) diagram, according to their percentage of beeswax added and temperature of storage, is shown in **Figure 5** and **Table 5**. The L*, a*, b* values of pesto samples were ranged (30.50 – 39.68), (-9.83 – -1.33) and (36.99 – 44.26), respectively. Similar results were reported by **Soliva *et al.* (2001)**, for avocado purée preserved by combined methods. They reported that at 4°C, L* a* and b* values were (40 – 80), ((-10) – 4) and (25 – 45) respectively. In other hand, **Lee *et al.* (2007)** have reported that L* value of sunflower and olive oils was 97.1 and 88.7 respectively. This result showed that pesto sauce is darker-colored than sunflower and olive oils due to the complexity of pesto ingredients, which influence its reflection power. In this study a* and b* values **(Figure 5 and Table 5)** were between sunflower and olive oil values reported by **Lee *et al.* (2007)**, which were (-2.7; 6.5) and (-13.2; 92.7), respectively. These results showed that studied pesto sauces have a greenish-yellowish tint. The green color of pesto sauce is mainly due to the presence of chlorophyll in olive oil and rocket leaves **(Masino *et al.*, 2008)**.



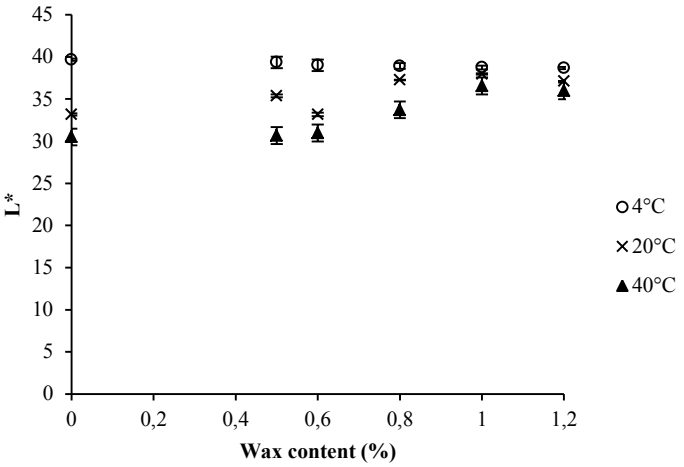


Figure 5 (A) Evolution of color coordinates a* and b*; **(B)** Evolution of L* of pesto sauces processed by different percentage of beeswax and temperature.
(♦) sample without wax; (■) sample with 0.5 % of wax; (▲) sample with 0.6 % of wax; ▬sample with 0.8 % of wax; (○) sample with 1 % of wax; (●) sample with 1.2 % of wax.

Highly significant differences were detected ($p<0.001$) between the samples stored at different temperatures for all color parameters (L*, a*, b* C* and h°) **(Figures 5A, 5B, 6 and Table 5)**.

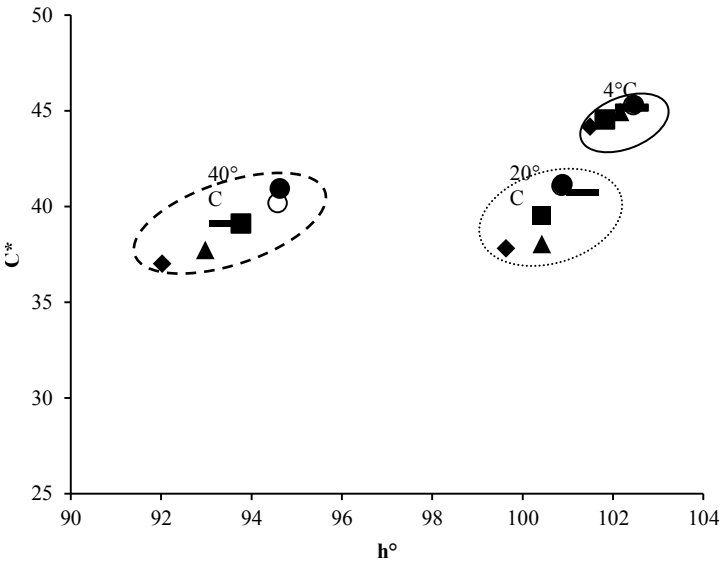


Figure 6 Evolution of chroma (C*) and hue (h°) of pesto sauces processed by different percentage of beeswax and temperature.
(♦) sample without wax; (■) sample with 0.5 % of wax; (▲) sample with 0.6 % of wax; (▬) sample with 0.8 % of wax; (○) sample with 1 % of wax; (●) sample with 1.2 % of wax

The L* values decrease very significantly ($p<0.01$) with the increasing of temperature **(Figure 5B)**. In other words, the lightness decreases with temperature increasing for all samples. Lightness and the green color of pesto were preserved at low temperature. Indeed, samples stored at 40°C presented the highest green component (a*) (negative values) and the lowest b* (positive values), and consequently the lowest greenish tint. This can be explained by the

fact that the increasing of temperature leads to a more important browning. These changes were observed by **López-Malo *et al.* (1998)** for avocado purée conserved at 5°C and 25°C with different pressures. They reported that, at 32 days of storage, L* value of avocado purée decreases from 60 to 53, at 5°C, 345 MPa and 25°C, 345 MPa, respectively. The results of a* and b* parameters was confirmed by those of chroma (C*) **(Figure 6)**, which represents the amount of color and is measured according to the distance from origin (point of the illuminant) of the coordinates **(Terrab *et al.*, 2004)**. Indeed, greater the temperature increases, greater the chroma C* decreases **(Figure 6)**.

Hue (h°) is the attribute according to which colors have been traditionally defined as reddish, greenish, etc. It is the attribute which allows us to distinguish a color with reference to a grey color with the same lightness, and it is related to the differences in absorbance at different wavelengths and is considered the qualitative attribute of color **(Terrab *et al.*, 2004)**. The hue results **(Figure 6)** shows that all samples were located in the second quadrant between 92.02 and 102,48. In other words, the samples are found in the green zone **(Figure 6)**. This was confirmed by **López-Malo *et al.* (1998)** who reported that avocado puree have a green color with hue angles in the range 101.4-102.8.

There is highly significant differences ($p<0.001$) between the samples with different percentage of beeswax stored at same temperature for all color parameters (L*, a*, b* C* and h°) at 20 and 40°C **(Figures 5A,5B and 6 and Table 5)**.

Table 5 Analysis of colors parameters (L, a*, b*, C* and h°)

Samples	Temperature	L*	a*	b*	C*	h°
0%	4°C	39.68±0.13 ^{Aa}	-8.83±0.11 ^{Ca}	43.26±0.12 ^{Aa}	44.15±0.14 ^{Aa}	101.49±0.11 ^{Aa}
	20°C	33.19±0.13 ^{Bd}	-6.35±0.06 ^{Ba}	37.28±0.56 ^{Bc}	37.82±0.56 ^{Bc}	99.63±0.05 ^{Bd}
	40°C	30.50±0.17 ^{Ce}	-1.33±0.04 ^{Aa}	36.99±0.66 ^{Bc}	37.02±0.65 ^{Bc}	92.02±0.09 ^{Ce}
0.5%	4°C	39.35±0.70 ^{Aa}	-9.16±0.68 ^{Ca}	43.59±0.68 ^{Aa}	44.55±0.81 ^{Aa}	101.82±0.68 ^{Aa}
	20°C	35.38±0.16 ^{Bc}	-7.17±0.03 ^{Bb}	38.87±0.06 ^{Bb}	39.53±0.06 ^{Bb}	100.4±0.04 ^{Bc}
	40°C	30.68 ±0.15 ^{Cde}	-2.60±0.06 ^{Ad}	39.02±0.09 ^{Bb}	39.11±0.09 ^{Bb}	93.76±0.07 ^{Cb}
0.6%	4°C	39.01±0.69 ^{Aa}	-9.50±0.61 ^{Ca}	43.93±0.68 ^{Aa}	44.94±0.80 ^{Aa}	102.16±0.59 ^{Aa}
	20°C	33.17±0.22 ^{Bd}	-6.91±0.15 ^{Bb}	37.41±0.07 ^{Bc}	38.05±0.07 ^{Bc}	100.42±0.23 ^{Bc}
	40°C	30.96±0.04 ^{Cd}	-1.99±0.02 ^{Ab}	37.67±0.16 ^{Bc}	37.73±0.16 ^{Bc}	92.97±0.02 ^{Cd}
0.8%	4°C	38.91±0.34 ^{Aa}	-9.74±0.23 ^{Ca}	44.10±0.1 ^{Aa}	45.16±0.08 ^{Aa}	102.42±0.3 ^{Aa}
	20°C	37.26±0.04 ^{Bd}	-8.03±0.05 ^{Bc}	39.96±0.06 ^{Ba}	40.76±0.06 ^{Ba}	101.32±0.07 ^{Ba}
	40°C	33.72±0.12 ^{Cc}	-2.37±0.06 ^{Ac}	39.06±0.13 ^{Cb}	39.13±0.13 ^{Cb}	93.43±0.07 ^{Cc}
1%	4°C	38.75±0.23 ^{Aa}	-9.79±0.14 ^{Ca}	44.23±0.08 ^{Aa}	45.30±0.11 ^{Aa}	102.44±0.15 ^{Aa}
	20°C	37.98±0.06 ^{Ba}	-7.77±0.03 ^{Bc}	40.35±0.13 ^{Ba}	41.09±0.13 ^{Ba}	100.86±0.05 ^{Bb}
	40°C	36.55±0.02 ^{Ca}	-3.24±0.02 ^{Ae}	40.05±0.09 ^{Ca}	40.18±0.08 ^{Ca}	94.58±0.03 ^{Ca}
1.2%	4°C	38.68±0.13 ^{Aa}	-9.83±0.11 ^{Ca}	44.26±0.12 ^{Aa}	45.34±0.14 ^{Aa}	102.48±0.11 ^{Aa}
	20°C	37.12±0.02 ^{Bb}	-7.80±0.17 ^{Bc}	40.43±0.04 ^{Ca}	41.17±0.05 ^{Ba}	100.88±0.24 ^{Bb}
	40°C	35.97±0.03 ^{Cb}	-3.33±0.03 ^{Ae}	40.81±0.04 ^{Ba}	40.94±0.04 ^{Ca}	94.63±0.05 ^{Ca}

Mean (n = 3) ± SD; Means of color parameters with different capital superscripts within columns for each sample and with different lower-case superscripts within rows are significantly different ($p< 0.05$); (one-way ANOVA and Tukey's test. a or A—the highest content); (1-1.2%) indicate samples with concentration of wax from 0 to 1.2 %

In other words, the increasing of temperature puts in evidence the difference lightness, color and chroma between samples with different percentage of wa These differences were in agreement with sensory test results.

At high storage temperature, L* values of samples with 0.8, 1 and 1.2 % of w was significantly ($p<0.001$) higher than the others samples. Indeed, t correlation between L* and percentage of wax was significant ($r=0.370$). Th showed that lightness increases significantly with increasing of wax percenta added.

The correlation between the concentration of wax and green component (a*) not significant. However, it is significant ($r=0.347$) for yellow component (b* This indicated that for a color component (a*) almost constant, the yello component (b*) (positive values) was high according to wax added increasin These results show clearly that the presence of beeswax affects the color studied pesto sauce. This behavior was confirmed by chroma (C*) results, whi presents a significant correlation ($r=0.330$) with the added beeswax percentag Thus, the amount of color increases with beeswax increasing. Consequently wi high percentage of beeswax, the lightness and the greenish-yellowish color pesto sauce is preserved irrespectively of temperature.

A correlation test between the colors parameters and p-Anisidine (pAV) a peroxide (PV) values was realised. From 72 h of storage, the L*, b, C* and parameters were negatively correlated ($r<-0.554$) with PV and pAV values, opposition with a* parameter ($r>+0.867$). The lightness and the green color samples decreases with oxidative stability values increasing. This was confirm by C* and h° values.

Sensory characteristics

In order to evaluate the normality of sensory scores, Kolmogorv-Smirnov and Shapiro-Wilk tests were realised. Indeed, all samples values were not normal ($p<0.05$) for all studied descriptors. The statistical analysis of samples homogeneity was done by the non parametric test (Kruskal-Wallis test with k samples). This analysis revealed that there was a significant difference ($p<0.05$) only for appearance, color, texture, overall acceptability and ranking between samples (Figure 7). The pesto sauce sample with 1.2% of wax have presented highest sensory scores for these descriptors, followed by samples with 1% and 0.8 % wax added. In other words, the sixty subjects have appreciate these samples more than the other ones.

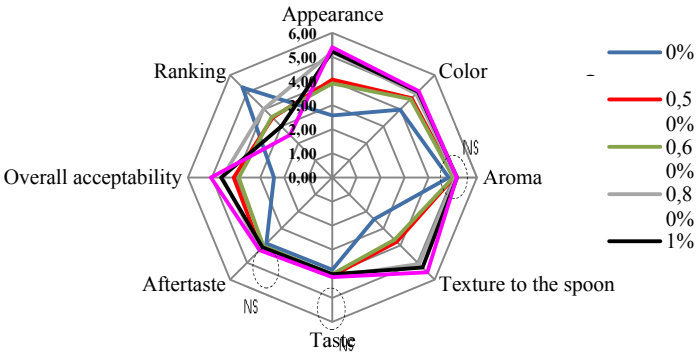


Figure 7 Sensory characteristics of pesto samples with or without wax
Different letters (a–b) for a same descriptor denote significant differences between samples ($p<0.05$); (one-way ANOVA and Tukey's test. a or A—the highest content); (1-1.2%) indicate samples with concentration of wax from 0 to 1.2 %

CONCLUSION

For all temperatures and all samples of pesto and oil (continuous phase), the apparent viscosity (η) decreased with increasing shear rate; it indicates that all samples showed pseudo plastic behavior **(Muresan *et al.*, 2014; Paredes *et al.*, 1988)**. This pseudo plasticity increases with increasing beeswax percentages and decreasing the temperature. In other hand, the colloidal stability of samples decreased as the storage time and temperature increased, reaching 94.97, 95.15 and 97.88 % for samples with 0.8%, 1% and 1.2% of wax, respectively, after 793h at 40 °C. The addition of wax allowed to obtain a more compact structure of wax crystals entrapping the liquid oil, thus preventing sedimentation and oil

creaming. PVs increases significantly according to time and temperature of storage. Indeed, samples stored during 793h at 40 °C had PVs about three times higher than the ones stored at 4 °C, and about one time higher than those stored at 20°C. The temperature increasing puts in evidence the fact that the extent of oxidation decreases with the increases of beeswax concentration. All color parameters L*, a*, b*, C* and h° of pesto samples was affected by storage temperature and percentage of beeswax. In other words, lightness and the green color of pesto can be preserved at low temperature and high beeswax concentration. The sensory test showed that consumer prefered the samples of pesto with 1.2 % of wax followed by 1% and 0.8%. Overall, it is strongly recommended to monitor the storage temperature and time of pesto, and to control the percentage of beeswax added in order to improve the pesto stability. Pesto sauce based on rocket can be a good alternative to traditional pesto based on basil and the use of beeswax as an oleogelator can be recommended for a greater stability of product and a greater consumer acceptability.

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