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QUALITY INDICES OF CHEESE OXIDATION DURING STORAGE

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ABSTRACT

The objective of the work was to identify the quality indices of lipid oxidation of Grana Padano cheese and to optimize the methods for their determination. Cheese was stored at 4°C and at 20°C for 60 days. Free fatty acids (FFA) content, peroxide value (PV) and volatile organic compounds (VOC) were studied during storage. No difference for FFA values in cheese during the storage was found, while PV increased after 60 days at 20°C. The concentration of VOC increased during the storage. Results showed that VOC were the best quality indices to discriminate samples during storage.

Keywords: cheese, lipid oxidation, quality indices, shelf-life, storage, volatile compounds

1. INTRODUCTION

Lipid oxidation is one of the processes responsible for the reduction in food shelf-life since it leads to off-flavour, off-odour and has been linked to oxidation reactions that cause product discoloration and loss of nutrients (DALSGAARD *et al*., 2010).
Non-enzymatic browning and lipid oxidation are the main alteration process of grana Padano cheese. The main effects of alteration process are: change of organoleptic value due to rancid flavors production as well as production of reactive oxygen species implicated in inflammation and cardiovascular diseases (KRISTENSEN *et al*., 2001; FEDELE and BERGAMO, 2001). Storage conditions affect the oxidative stability of cheese, and both modified atmosphere and light exposure have an impact on the formation of oxidation products.
So, the objective of this work was to identify both the critical quality indices of lipid oxidation and lipolysis able to describe the kinetics of alteration and the analytical methods for their determination during the storage of cheese at 4°C and 20°C.

2. MATERIAL AND METHODS

2.1. Sampling cheese

Grana Padano cheese (10 months-aged) has been used as a case study. The cheese was packed in air with a barrier film and stored at 4°C and at 20°C for 60 days. After 20, 40, and 60 days the following quality indices of the lipid oxidation and lipolysis were studied: free fatty acid content, peroxides value and volatile organic compounds.

2.2. Extraction of fat by cheese

The fat content was gravimetrically determined by using with some modifications the method described in D.M. 1986 based on the Schimith-Bondzynski-Ratzla traditional method of extraction of lipids. Ten grams of grated cheese were hydrolyzed using 10 mL of 37% hydrochloric acid and 7 mL of 95% (v/v) ethanol. The cheese suspension was homogenized with an ultrasound for 10 min and it was incubated for 30 min at 50°C in thermic bath. After cooling, the fatty matter was extracted using 10 mL of ethyl ether-petroleum ether (1:1) solution and the suspension was centrifugated for 10 min at 6000 rpm. The organic phase was collected and the extraction protocol was repeated 3 times.
Three organic extracts were pooled, dried over anhydrous sodium sulfate, filtered with a paper filter, evaporated under reduced pressure in a rotary evaporator and weighed.

2.3. Peroxide value

To evaluate the number of peroxide value (PV), a titration method was used, followed by the procedure of SENGUL *et al*., (2014) with some modification. Briefly, 500 mg of fat was weighed after dissolving in acetic acid/chloroform (3:2 v/v) solution, then stored in the dark for 5 min after the addition of saturated potassium iodide. Finally, 7.5 mL deionized water and 1% starch solution was added to mixture and titrated with 0.001 N $Na_2S_2O_3$ solution. PV was expressed as meqO_2/kg fat.

2.4. Free fatty acids

To quantify free fatty acids (FFA) a titration method was used, followed by the procedure of KONIECKO (1979) with some modifications. Briefly, 500 mg of fat was weighted and 5 ml of ethanol/ethyl-ether (1:2) were added. NaOH 0.01N was used to neutralize sample acidity and phenolphthalein was used as indicator. Results are expressed as % of oleic acid.

2.5. Volatile organic compounds analysis

The extraction and analysis of volatile organic compounds (VOC) was performed using SPME-GC/MS, according to LEE *et al*. (2013) and MANZO *et al*. (2019) with modifications. The solid-phase microextraction (SPME) device equipped with a 50/30-μm thickness divinyl-benzene/carboxen/polydimethylsiloxane fiber coated with 2-cm length stationary phase was used. Then, 1.5 g of frozen grated cheese was transferred into a 10 mL vial added with 3 mL of deionized water and 15 μL of 2-methyl-3-heptanone as internal standard (408 mg/L). Samples were homogenized and heated on a heating magnetic stirrer. Then the SPME device was hermetically put in the vial containing the samples and left for 1 hour at 50°C. The SPME was introduced directly into the GC injector where the thermal desorption of the analytes was performed at 250 °C for 10 min. A GC system 6890N equipped with a mass detector 5973 was used. The VOC were separated on a 30 m × 0.250 mm capillary column coated with a 0.25 μm film of 5% diphenyl l95% dimethylpolysiloxane. Splitless injection was used for the samples. The column oven temperature was held to 40°C for 2 min and increased from 40°C to 160°C at 6°C/min and from 160 to 210°C at 10°C/min, which was held for 10 min. The injection and ion source temperatures were 250 and 230 °C, respectively. Helium was used as carrier gas at a flow rate of 1 mL/min. The ionizing electron energy was 70 eV and the mass range scanned was 40-450 amu in full-scan acquisition mode. The compounds were identified using the NIST Atomic Spectra Database version 1.6 and verified by the retention indices. The VOC were calculated by the internal standard method and were expressed as mg/kg of cheese.

2.6. Statistical analysis

Results were expressed as means ± standard deviation (SD). Statistical analysis was performed with the statistical package SPSS for Windows (version 20, SPSS Inc. Chicago, IL, USA). The difference between means was tested using Student's t-test. Statistical significance was set at level of $p<0.05$.

3. RESULTS AND DISCUSSION

Table 1 shows the results about the PV and FFA during the storage at different temperatures.
Results showed that no significant difference for FFA values in cheese stored at different time was found. The PV was increased after 60 days at 20°C, while no difference was found with the storage at 4°C.
Fig. 1 shows the results about the VOC concentration during the storage at different temperatures.
In terms of the VOC, an increase of ketones and alcohols during the storage at both the temperatures was found, while the concentration of aldehydes increased significantly only after 60 days at 20°C (Fig. 1).

Table 1. PV and FFA during the storage at 20°C and 4°C. Different letters indicate significant difference ($p<0.05$) during the time at same temperature, while the asterisk indicates significant difference ($p<0.05$) between the different storage temperatures.

T (°C)		Time (days)			
		0	20	40	60
4	PV (meq O2/Kg)	8.5±0.4a	9.1±0.2a*	9.1±0.01a*	8.6±0.01a
20	PV (meq O_2/Kg)	8.5±0.4b	7.5±0.3b*	7.6±0.2b*	9.1±0.4a
4	FFA (% oleic acid)	2.6±0.1a	3.1±0.02a	2.9±0.01a	2.8±0.01a
20	FFA (% oleic acid)	2.6±0.1a	2.9±0.01a	3.1±0.01a	2.9±0.01a

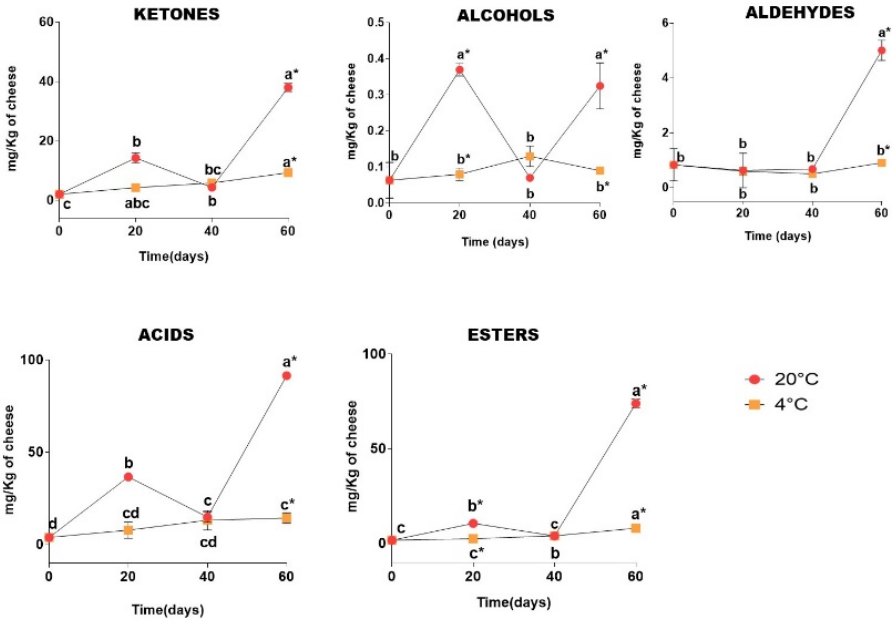

Figure 1. VOC concentration during the storage. Different letters on the lines indicate significant difference ($p<0.05$) during the time at same temperature, while the asterisk indicates significant difference ($p<0.05$) between the different storage temperatures.

Furthermore, the concentration of acids increased during the storage at both temperatures, but the highest concentration of acids was found after 60 days at 20°C (Fig. 1). Finally, the concentration of esters increased after storage at 20°C with the highest concentration after 60 days, while at 4°C, an increase of esters after 60 days was found (Fig. 1).
The results showed that the VOC were the best quality indices to discriminate samples during storage.

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