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Oxidation of Vegetable Fats and Methods of Their Analysis

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Abstract: The production and storage of vegetable oil is accompanied by oxidation and hydrolysis processes, leading to a reduction in nutritional value and safe use. Traditional physical and chemical indicators such as acid, iodine, peroxide values and other indicators, often used to determine the quality of oil do not fully reflect the biochemical processes. The study gives an overview of the indicator analysis methods such as Anisidine Value (AV) which characterizes the process of secondary oxidation of vegetable oil and fat. For the indicator analysis of the anisidine value, spectrometry, chromatographic analysis and nuclear magnetic resonance are used. The quality of the AV analysis is influenced by various factors, for example, the speed of analysis in the flow and the temperature in the laboratory room. The most complete description of the oxidation processes of vegetable oil and fat gives the TOTOX indicator which takes into account the amount of primary and secondary oxidation products.

Key words: Vegetable fat, anisidine value, methods of analysis, oxidation, indicators, description

INTRODUCTION

On Kazakhstani market, the main products of the fat and oil industry are represented by vegetable oil from sunflower, rape, soybean, safflower, corn. These oils are produced from local raw materials as well as from imported palm oil. In addition, the consumer and industrial market uses a wide range of different combinations of the above-mentioned products.

Since, vegetable oil, fat and their variations have a very wide application in the food and processing industries, the quality control of used fat and oil is very important not only as an indicative indicator of technological processes but also as one of the required measures of food safety and human health.

In the structure of human nutrition, oil and fat of animal and vegetable origin, along with proteins and carbohydrates have one of the most important meanings. These products are an indispensable link throughout the biochemical chain of metabolism. Underconsumption of fatty acids leads to the destruction of oxidation-reduction reactions which is accompanied by the replacement of essential fatty acids due to inclusions in the biochemical reactions of structural proteins and carbohydrates. As a result, this can lead to various systemic dysfunctions of the body and in cases of chronic deficiency, to pathologies, since, fat is a part of cell membranes and one of the metabolic regulators.

Vegetable oil is a source of essential substances necessary for the normal functioning of the human body. Vegetable oil is characterized by a high content of fat-soluble vitamins, sterols and other biologically active components as well as essential fatty acids (Dolgolyuk *et al*., 2014).

In addition to human nutrition, fatty acids play an important role in the food and processing industries. This is connected to the functional characteristics of oil and fat which are natural emulsifiers and are part of the recipes for fat and oil, confectionery, bakery, meat-canning and other industries as well as medicine and pharmaceuticals.

The production and storage of vegetable oil is accompanied by oxidation and hydrolysis processes, leading to a reduction in nutritional value and safe use. Traditional physical and chemical indicators such as acid, iodine, peroxide values and other indicators, often used to determine the quality of oil, do not fully reflect the biochemical processes. This is due to the fact that along with the primary oxidation products, determined by the peroxide value, there are carbonyl and other secondary compounds. It is the secondary compounds that determine the occurrence of the anisidine value which reflects their content and reveals the potential for safety and preservation of vegetable oil; it also allows detecting deviations in the technological processes of preparation and/or transportation and storage of finished products (Kowalski *et al*., 2004).

Oxidation is not one single reaction but a complex series of reactions. When oil oxidises it produces a series of breakdown products in stages, starting with primary oxidation products (peroxides, dienes, free fatty acids),

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then secondary products (carbonyls, aldehydes, trienes) and finally tertiary products. Oxidation progresses at different rates depending on factors such as temperature, light, availability of oxygen and the presence of moisture and metals (such as iron).

Polyunsaturated Fatty Acids (PFA) which are especially, rich in vegetable oil, are the main substrates for oil oxidation and their oxidative degradation products deteriorate the chemical, organoleptic and nutritional properties of oil (Belitz *et al*., 2004).

In addition when using oil during roasting, several volatile and non-volatile compounds are formed including aldehydes and ketones.

MATERIALS AND METHODS

For the analysis of oil and fat products, along with the acid and peroxide values which determine the products of primary oxidation, anisidine value characterizing products of secondary oxidation of vegetable oil and fat is used more frequently.

Anisidine value is the quantitative number of anidisine in fats and oils of animal and vegetable origin is determined by the content of mostly α and β unsaturated aldehydes.

The p-anisidine value is defined by convention as 100 times the optical density measured at 350 nm in a 1 cm cuvette of a solution containing 1.00 g of the oil in 100 mL of a mixture of solvent and reagent according to the method described. This method determines the amount of aldehydes (principally 2-alkenals and 2, 4-dienals) in animal and vegetable fats and oils by reaction in an acetic acid solution of the aldehydic compounds in an oil and the p-anisidine and then measuring the absorbance at 350 nm.

Simone Pucci from QCL (USA) determined that anisidine and p-Anisidine (4-methoxyaniline) is therefore used as a reagent to point out the secondary stage of the oxidation: it is one of the three possible isomers of the anisidine or methoxyaniline. The other two isomers are o-anisidine (2-methoxyaniline) and m-anisidine (3-methoxyaniline). p-anisidine value actually measures the secondary oxidation products like aldehydes, carbonyls, trienes, ketons. Together with a test like peroxide value it can really give a deep information about the state of an animal or vegetable oil and fat.

Lee *et al*. (2013) from the Singapore National University to determine the level of aldehydes in sunflower, palm and vegetable (for frying) oil used a test of p-anisidine and AlkalSafeTM. It is noted that during frying intense oxidation of lipids occurs with the formation of aldehydes in the form of secondary oxidation products. Both tests confirmed that sunflower oil contained the largest amount of aldehydes, followed by palm and vegetable oil. This confirmed that one of the factors that determined the level of aldehydes was the baseline level of unsaturated fatty acids (Lee *et al*., 2013).

The relevance of research in the fat and oil industry, especially in the field of vegetable oil oxidation is noted by South Korean scientists Jung-Mi Yun and Jeonghee Surh. These scientists studied ten types of vegetable fat in total 97 items, fatty acid compositions and products of lipid oxidation are analyzed. The purpose of the studies was to determine the degree of fatty acids composition influence in the oxidation processes of vegetable oil sold in South Korea, manufactured by an industrial and traditional method. The indicators of peroxide value, the basis of the fatty acid composition, ranged from 7.10-111.87. In the absence of induced oxidative stress, Malonic Dialdehyde (MDA), a product of lipids secondary oxidation was generated more in the oils with a higher peroxide value. The results of the studies suggest that fatty acid compositions can be a predictor of the stability of oxidation of vegetable oils in the early stage of oil oxidation but not for those that are in the later stage of oxidation (Tompkins and Perkins, 1999).

Katrina D. Placke and colleagues at the Department of Nutrition at the University of Applied Sciences in Hamburg note that, the most suitable indicators for controlling oil quality during frying are the acidity and p-anisidine while the quality of the incoming oil can be assessed by determining the acidity and peroxide. Therefore, it is necessary to determine experimentally the threshold values which represent the critical control points of the process. These values can effectively ensure the detection of values corresponding to the high quality requirements of the final product in the frying processes.

The studies conducted on soybean oil used to fry chips showed that the value of p-Anisidine (AnV) depended significantly on the concentration of aldehydes and polymers as well as other sensitive indicators (Labrinea *et al*., 2001).

Turkish researcher G. Yildirim revealed that the determination of the value of Anisidine (AV) is an empirical test for evaluating the oxidative rancidity of oils and fats. The AV test is particularly useful for oils with low Peroxide content (PV) and for assessing the quality of highly saturated oils. He estimates that the secondary oxidation products of unsaturated fatty acids are mainly conjugated dienals and 2-alkenals. Aldehydes are largely considered responsible for inactive fats and oils because of their low sensory thresholds.

When determining the anisidine value, there may be errors associated with the influence of external factors. For example, G. Gavrilov and his colleagues from the Ukrainian Research Institute of Oil and Fat, together with L. Peshuk and I. Radzinskaya from the National University of Food Technologies, revealed the influence of laboratory room temperature on the accuracy of AV measurements. Considering that the standard regulates the determination of the anisidine value at a temperature of 23±1°C, the researchers found the magnitude of AV depending on the measurement temperature (Wai *et al*., 2009). Depending on the interval and AV measurement temperature, the average deviation was 0.9 and 1.78°C for the laboratory room temperature of 15 and 30°C, respectively.

Eleni P. Labrinea with colleagues for optimization a Flow Injection (FI) spectrophotometric method for the determination of olive oil Anisidine Value (AV) is presented. A 40-l aliquot of 10% (w/v) olive oil samples in 2-propanol are injected in a p-anisidine in 2-propanol/acetic acid stream. The reagents are premixed in the FI system using a two-line manifold. Detection is achieved by monitoring absorbance continuously at 350 nm. The proposed FI method achieves a high analysis rate (110 samples/h), minimizes solvent consumption (2 mL 2-propanol and 0.4 mL acetic acid per analysis) and compares well with the manual spectrophotometric method (relative difference 0.5-6.8% for the analysis of 25 olive oil samples). Precision was found to be better than 1% relative standard deviation (RSD; n = 7). The linear range was 6-85 AV and is suitable for analysis of abused olive oils.

Similar research with using stream injection to determine anisidin in palm oil were conducted by a group of scientists led by Saad *et al*. (2007). Their work describes the methodology A Flow Injection Analysis (FIA) procedure for the determination of Anisidine Value (AV) in palm olein using a triiodide detector is described. Undiluted oil sample and chloramine-T reagent were added to a reaction chamber and reaction was accelerated by applying a short vortex action (typically for 30 sec). The optimized FIA procedure is linear over 1.0-23.0 AV. The method exhibits good repeatabililty (RSD of ±3.16% (n = 4) for the determination of 5.0 AV) and a sampling rate of 40 samples per hour was achieved. Good correlation (r^2 = 0.996 (n = 4)) between the proposed method and the manual American Oil Chemist's Society procedure was found when applied to the determination of twenty different types of palm olein samples.

Later the same group of scientists developed a flow injection analyzer for the determination of Total Oxidation value (TOTOX) in palm oleins was developed based on the sequential determination of Peroxide Value (PV) and Anisidine Value (AV) using a common triiodide potentiometric detector. The PV and AV parameters were sequentially determined by manipulating a switching valve. The mixing of the oil and reagents was effective by applying a short vortex (30 sec). The TOTOX value is obtained from the potentiometric readings of PV and AV (TOTOX = 2PV+AV). Using palm olein as standard, the method gives TOTOX value that is linear over 1.7-79.0 with a detection limit of 1.64. The sampling frequency is about 60 h^{-1}. Twenty aged and thermally stressed palm olein samples were analyzed and good correlations (r^2 = 0.9852) were found between the proposed FIA and the official methods.

Qualitative characteristics and oxidative stability for long-term storage of coconut oil were studied by Romanian scientists Mariana-Atena Poiana and Ioan Gogoasa Banat's. The main objective of this study is to investigate some quality characteristics based on which we can evaluate oxidative stability of coconut oil during 12 month of storage. The progress of lipid oxidation was assessed by measuring Peroxide Value (PV), p-Anisidine Value (AV) and Total Oxidation value (TOTOX). The low peroxide value (0.24-0.49 meq/kg oil) signifies a high oxidative stability while p-anisidine values were in the range 0.19-0.87. Fourier Transform Infrared (FTIR) spectroscopy was used to monitor the peak changes as effect of oxidation during storage. The prominent peak change observed during storage of coconut oil was at frequency 1742 cm^{-1} which corresponded to the ester carbonyl functional group of the triglycerides resulted from the hydroperoxide decompositions. These results suggest that coconut oil during 12 months on storage keeps its good chemical properties. The PV obtained in the present study showed that 0.24-0.49 meq/kg which was far below the maximum limits. PV increases from month to month but after 9 months the values decreased from 0.49 by 0.44 meq/kg.

The level of saturation and antioxidant value from the effects of temperature and spices during cooking on animal fats was studied by Natnael Tadesse with colleagues from the Department of Food Science and Nutrition from the Jiggyga University (Ethiopia).This study aimed to determine the effect of heat and spice treatment on the level of saturation and oxidative stability of both fresh and rancid animal butter. The spices used were ginger, garlic and korarima. Two different ratios, 80% animal butter: 20% spices and 90% animal butter: 10% spices were prepared from each butter treatment and various spices. The treatments were packaged in a transparent plastic jar. One group of the treatments stored at 25°C and the other group stored at 65°C to

accelerate the oxidation for 3 days while samples were taken for analysis at 0, 36 and 72 h of storage. Samples were examined for bromine value, Peroxide Value (PV), p-anisidine value and Free Fatty Acid (FFA) value. At 65°C spice treated butter exhibited lower values of FFA and PV when compared to untreated samples for both fresh and rancid butter but it showed higher bromine and p-anisidine value. Higher FFA, PV, p-anisidine and totox values indicated the poor quality contributed by the type of butter and storage deterioration.

The group led by Skiera *et al.* (2012) who used aldehydes as the markers in nuclear magnetic resonance, suggested an interesting alternative to the classical method of determining the anisidine value.

Aldehydes formed as secondary oxidation products during the autoxidation process of oils and fats, are analytical markers used to assess the lipid deterioration status. Generally, the level of aldehydes is expressed as the p-Anisidine Value (AV). This deterioration index is based on the reaction of the carbonyl group with p-anisidine leading to the formation of an intensively coloured Schiff base which is determined spectroscopically (UV/ViS). 1H NMR provides an alternative approach by enabling the quantification of individual aldehydes like n-alkanals, 2-alkenals or (E, E)-2, 4-alkadienals. This work illustrates that the AV can be modelled as a linear combination of the NMR integrals of aldehydes. A functional relationship was derived on the basis of calibration experiments. The suitability of the model is shown by comparing the NMR-determined AVs with the classical AVs of 79 commercially available edible oils of different oil types.

Schiff base (or azomethines) is a functional group named after Hugo Schiff. Contains a double bond of carbon nitrogen where nitrogen is linked to an aryl or alkyl group but not to hydrogen.

Kobylinski *et al.* (2016) evaluated the effect of Specific Oil Surface (SOS) during pan frying of rapeseed oil on its thermal stability and Antioxidant Capacity (AC) was evaluated. Rapeseed oils with different oil layer heights (OLH = 0.5, 1.0, 1.5, 2.0 and 2.5 cm) were heated on an electric frying pan coated with Teflon at 180±10°C (Kobylinski *et al.*, 2016). The changes of chemical parameters of oil samples such as peroxide value, p-anisidine value, TOTOX value, free fatty acids, total polar compounds and AC using the 2,2-diphenyl-1-picrylhydrazyl assay were determined. Irrespective of the applied methods, the highest changes in oil with OLH = 0.5 cm were observed. Heating in low OLH also led to the fastest time of TPC formation in rapeseed oil; the 0.5-cm layer reached 25% TPC in a relatively short time (71.5 min) compared to the highest OLH = 2.5 cm (t = 315.1 min). The SOS and the rate of change in the heated oils decreased with increasing OLH. Crucial effects of SOS on physicochemical oil changes were observed. The present study demonstrated the protective effect of increasing the OLH on the quality of the heated rapeseed oils.

Solati and Baharin (2015) investigated effect of supercritical CO_2 extracted *Nigella sativa* L. seed extract (NE) on frying performance of sunflower oil and Refined, Bleached and Deodorized (RBD) palm olein was investigated at concentrations of 1.2 and 1.0%, respectively. Two frying systems containing 0% *N. sativa* L. extract (Control) and 0.02% Butylated Hydroxytoluene (BHT) were used for comparison (Solati and Baharin, 2015). Physicochemical properties such as Fatty Acid Composition (FAC), Peroxide Value (PV), Anisidine Value (AV), Totox Value (TV), Total Polar Content (TPC), C18:2/C16:0 ratio and viscosity of frying oils were determined during 5 consecutive days of frying. Results have shown that *N. sativa* L. extract was able to improve the oxidative stability of both frying oils during the frying process compared to control. The stabilizing effect of antioxidants were in the order of BHT>NE. RBD palm olein was found to be more stable than sunflower oil based on the ratio of linoleic acid (C18:2) to palmitic acid (C16:0) and fatty acid composition.

RESULTS AND DISCUSSION

The determination by physical and chemical indicators such as acid, iodine, peroxide values and other indicators, often used to determine the quality of oil, do not fully reflect the biochemical processes during the storage. The oxidation is affected by many external factors (oxygen, temperature, ultraviolet radiation, salts and other metal compounds), it occurs in stages and with different dynamics. However, the internal factors affect the oxidation of vegetable oil which include the polyunsaturated fatty acids that are substrates for irreversible chemical transformations. The determination of secondary oxidation signs of vegetable oil is based on the use of spectrometric, chromatographic method and nuclear magnetic resonance.

For the analytical determination of the vegetable oil quality, the most significant indicator is the determination of p-Anisidine, the secondary signs of oxidation, since, they reveal compounds that can be carcinogen in human body. The influence of external factors in determining the anisidine value can also be in the form of errors caused by temperature deviations in the laboratories, from those stipulated in the standard.

In addition, the oxidation ability of oil during frying depends on the specific surface, i.e., the amount of used oil where the lowest layer was subjected to the maximal oxidation.

The use of flow injection of reagents affects the speed of the analysis and its purity. It is notable that fat of animal origin have a similar susceptibility to oxidative reactions. The researched topic of fat heat treatment influence with various spices revealed new aspects of frying processes biochemical reactions. According to the results of these researches, it is possible to make analogies with vegetable fat.

Thanks to the most complete analytical data the nuclear magnetic resonance has wide prospects of application at enterprises of the fat and oil industry where aldehydes are used as markers.

CONCLUSION

As most scientists in this industry note, the anisidine value reflects the dynamics and result of the anisidine and aldehydes interaction in the production and storage of vegetable oil and fat. However, the most complete picture of chemical processes in vegetable oil and fat in technological processes is reflected by the TOTOX-indicator that includes the values of both primary and secondary oxidation characteristics.

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