INFLUENCE OF WATER ACTIVITY ON WALNUTS (JUGLANS REGIA L.) MICROBIOLOGICAL AND OXIDATIVE QUALITY

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Abstract: The main objective of this study was to identify the storage environmental conditions where walnuts (*Juglans Regia L.*) would keep the lowest oxidation rate and the lowest level of microorganisms' development. Analyzing the obtained results, we concluded that to reduce the number of bacteria and molds, as well as peroxide value and moisture content from walnut kernel, with a view to preserve their quality, it is necessary that the water activity of storage rooms to fit in limits from 0.344 to 0.412.

Keywords: walnuts, storage, water activity, peroxide value,

Introduction

Walnut production is increasing globally. Walnuts have generated considerable interest in the last decade because the fatty acid profile found in walnut oil, in particular the presence of ω-3 and ω-6 PUFAs which are essential dietary fatty acids and to their favorable ratio in walnut oil (Amaral, 2003). Walnuts contain about 60% fat, of which about 80% is unsaturated. Oleic, Linolenic and linoleic acids account for the majority of the total unsaturated fatty acids in typical walnut. Although linoleic acid is essential for humans, it is susceptible to lipid oxidation (Nawar, 1996). In the presence of oxygen, light, moisture, and high temperatures, oxidation of fatty acids can occur (Nawar, 1996) and has been found to be a major source of off-flavors and decreased quality in walnuts (Ory et al., 1985). Walnut storage conditions have significant effects on overall quality. Water activity plays an important role in walnuts kernel oxidation. Walnut processors have reported shelf lives as short as one year due to oxidative rancidity. Control of water activity decreases oxidative rancidity in stored walnuts (Mate et al., 1996).

Unfortunately, during processing, walnut kernel being highly hygroscopic is susceptible to microbial deterioration and spoilage when not properly stored. One of the purposes of this study is therefore aimed at characterizing the interacting effect of water activity, moisture content and time on the storage stability of walnut at the prevailing environmental condition and identifying the various organisms involved.

Materials and methods

Materials

Performing research were used qualitative walnuts collected in 2015 year which correspond to the GOST 16833-71 demands.

Methods

Sample Preparation and Storage

In order to create different storage conditions, triplicate samples of walnuts were stored in glass desiccators containing solution of H_2SO_4 with concentration range: from 0% - 60% to create different storage environments with different water activities. The samples were maintained in desiccators during 16 weeks.

Lipid Extraction and Peroxide Value Determinations

Lipid was extracted, in duplicate, from the walnuts using a laboratory press. The Official American Oil Chemists' Society [AOCS] method was used to measure peroxide values and results reported as milliequivalents peroxide/kg oil (AOCS Cd 8-53).

Moisture determination

The moisture of the stored walnuts was determined, in triplicate, using the AOAC

Official Method 925.40 (moisture in nuts and nut products).

Microbiological analysis

Considering the design of the experimental set up of 0, 2, 4, 6, 8, 10, 12, 14 and 16 weeks at different relative humidity of storage, samples were randomly taken from the different desiccators. For the isolation of foodborne pathogens, aliquots of samples were placed on Agar and Sabouraud medium. Cultures were incubated in Petri plates under appropriate rules on culture media indicated pH of 2.5 to 5.5 which were kept in thermostat for 48-72 H at t=32-370C.The colonies observed were counted and sub-cultured for identification.

Humidity, peroxide value determination and microbiological analysis were conducted at 0, 2, 4, 6, 8, 10, 12, 14 and 16 weeks of storage.

Results and discussions

Moisture

The walnut moisture content increased under all storage conditions (Figure 1) and ranged between 0,243-0,874 gH_2O/g product. The moisture was still increasing for all samples even at 16 week of storage. Even at the lowest storage aw (0.15), moisture increased from about 7% (the initial moisture level) to almost 24% by week 16. The obtained results show that walnuts are clearly hygroscopic, and readily pick up moisture even at extrememly low aw, and this can be explained by the chemical composition of walnuts morphological parts (shell and inner membrane).

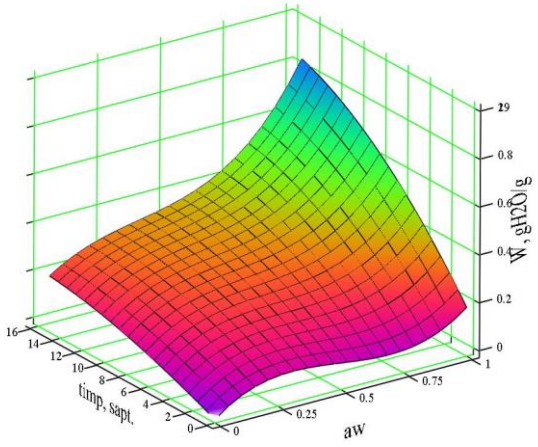

Fig. 1. Moisture Changes During Storage at Various Water Activities

Peroxide Values (PVs)

Lipid oxidation is, in part, dependant on the vapor pressure surrounding a food matrix and tends to be problematic for long-term food storage. Lower (0.0 – 0.2) and higher (0.6 - 1.0) water activities generate oxidative by-products at higher rates than near the monolayer values (0.33 – 0.42) in model systems (Labuza, 1975).

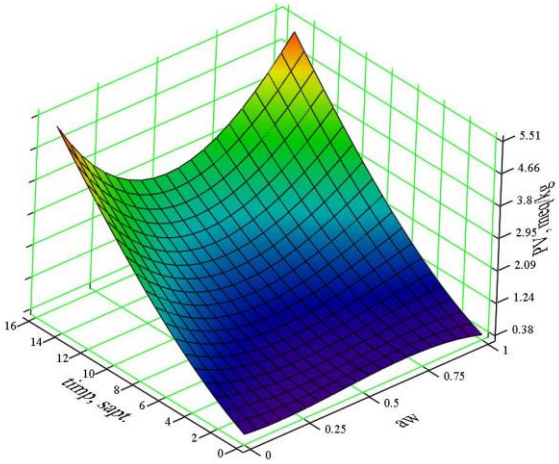

Fig. 2. Peroxide Values During Storage at Various Water Activities

The PVs increased over time for all storage conditions (Figure 2). The range of peroxide values during 16 storage weeks was 2-9 meq/kg. The PV increases were not linear and the humidity of storage affected the PV after about 4 weeks of storage. The PVs increased in a_w order from 0,0- 0,35> 0, 4 - 0,48< 0,52 - 0,92. The effect of water activity on oxidation rates agree with other studies where the lowest rate of oxidation has been found at intermediate water activities (Labuza, 1975).

Microbiological analisys

Fungal isolates were obtained from the various stored samples of walnuts. They were identified as Aspergillus niger, Aspergillus flavus, Fusarium and others, while the bacteria isolates were identified as Bacillius subtilis, Pseudomonas and Staphylococcus sp., respectively. The microorganism counts in walnuts exposed to different water activities at different time intervals are shown in Table 1. At water activity of 0.879 - 1.00 there was an considerable increase in the total number of microorganisms count on the 1st week of storage.

Table 2. Microorganism counts in walnuts exposed to different relative humidity at different time intervals.

Time	**Water activity, fungi counts (cfu/g)**						
(weeks)	**0.157**	**0.344**	**0.559**	**0.749**	**0.879**	**0.955**	**~1**
0	**$0,4*10^3$ cfu/g**						
1	0,4	0,5	0,5	0,8	1,0	1,6	1,8
4	0,2	0,4	0,8	2,0	1,3	2,4	3,0
8	-	0,4	1,3	1,0	3,4	3,8	6,0

Time (weeks)	Water activity, fungi counts (cfu/g)						
	0.157	**0.344**	**0.559**	**0.749**	**0.879**	**0.955**	**~1**
12	0,2	0,35	1,7	2,9	5,2	5,5	9,1
16	0,1	0,2	1,5	6,0	7,0	8,0	14,0

Fungi were the predominant organisms isolated from the walnut with few bacteria isolates at different water activity of storage. Some groups of these fungi have been reported by Riyaz-Ul-Hassan (2003) as the most predominantly encountered species of fungi from deteriorating walnuts. The interacting effect of water activity on the growth of the fungi shows that 0,153-0,344 water activity was the best storage condition as some fungi were eliminated and even where growth occurred, it was in traces that decreased with time.

Conclusion

Walnut storage conditions have significant effects on overall quality, especially if the product is to maintain high quality over long periods of storage. Containing more than 90% unsaturated fatty acids, walnuts maintain best product quality (low oxidation rate, maintenance of desirable flavor and taste) when stored at water activities between 0.35 and 0.412. Above this range oxidation and microorganisms growth increased, whereas below the range, the oxidation rate increased.

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