The Impact of Water Activity on Storage Stability of a Newly Reformulated Salami

A pilot scale study

CRISTIAN TUDOSE, CORINA NEAGU, DANIELA BORDA, PETRU ALEXE*

Dunarea de Jos University of Galaþi, Faculty of Food Science and Engineering, 111 Domneasca Str., 800201, Galati, Romania

Two formulations of salami were obtained: a conventional salami (50 % beef, 20 % pork and 30 % animal fat, w/w) and a reformulated one (50 % beef, 20 % pork and 30 % a mixture of olive and palm oil - 2.5:1, w/w). Storage studies were performed by accelerated shelf life testing at 20°C and different relative humidity, in comparison with 4 and 8°C after 22 days of storage. Halsey model was the best one to describe the desorption isotherms. The products stability was evaluated by TBA (thiobarbituric acid) index and colour changes. TBA increased with the increase of the environment relative humidity at 4 and 8°C. Modified Weibull model described the changes in colour as a function of TBA and water activity. Bound water characteristics have markedly changed at 20°C for both products. The results of this work can be used as reference in defining reformulated salami minimal durability.

Keywords: salami, desorption isotherms, water activity, colour, lipid oxidation

Healthier lipid formulation based on processing strategies is one of the most important current trends in reformulating red meat products, designed to reduce the negative impact on consumer‘s health, generally associated with high levels of saturated fats. The replacement of the animal fat with fats of vegetal origin whose characteristics (fatty acids profile) are more in line with health recommendations [1] could favour the widely-consumed meat products to be more closely aligned to WHO (World Health Organization) recommendations for a well balanced food diet and improvement of the eating habits. Specifically, WHO urged for reducing the daily fat intake to less than 30 % of total calories and limit the level of saturated fatty acids to maximum 10 % of the total energy intake [2].

Lately, many studies have been focused on total or partial replacement of animal fat with fats of vegetal origin rich in unsaturated fatty acids [3] and investigated the stability of the products during storage [4]. Employed by food industry as strategy to better cover the dietary and health needs of modern consumers, salami reformulation is hardly a change of the products recipe and requires detailed studies to correctly define the product shelf-life.

The rate and the extent of lipid oxidation which affects the quality characteristics of newly reformulated products should be carefully analyzed together with the water activity changes that are strongly influenced by extrinsic factors such as storage temperature and relative humidity, for the entire shelf-life of the products. Intrinsic factors such as type of meat product (cooked or ready-to-eat), technology employed, preservatives added but also other factors such as size of the membrane pores used for packaging, should also be taken into account.

Desorption isotherms represent powerful tools that provide valuable information on the relationship between the equilibrium moisture content and the relative humidity of the surrounding environment [5] at different temperatures. Knowledge on the rate at which food is approaching the moisture equilibrium [6] as function of storage temperatures in conjunction with changes undergone by food quality characteristics over time, could provide insight on the minimal durability of newly developed meat products.

The aim of the current study was to develop a new formulation for salami and compare it with a conventional one, providing models to explain the influence of dehydration rate on salami quality characteristics (lipid oxidation, colour). The temperatures selected for this study were related with refrigeration storage practices (4°C), technological recommended value for salami storage (8°C) and the case in which no refrigeration practice is employed (20°C) for accelerated shelf life testing, with the aim of providing a scientific base for defining the product minimal durability.

Experimental part

Sample preparation. The beef, pork and fat were purchased refrigerated (4°C) from a local abattoir. The salami was prepared at pilot scale in the meat plant of the Faculty of Food Science and Engineering, Dunarea de Jos University of Galati, Romania, according to industrial procedures. This type of salami has a composition of 50 % beef, 20 % pork and 30 % fat.A conventional formulation used as control (P_1) with animal fat and another one with the complete substitution of the animal fat with vegetal oil mixture (P_2) were produced. The reformulated salami was manufactured with a 2.5:1 mixture of extra virgin olive oil (Emilio Vallejo, Spain) and palm oil (Indochina), considered to be the best ratio as shown by the results obtained in a preliminary study [7]. All the analyses were carried out in the first day of storage and after the samples reached moisture equilibrium in the 22nd day of storage.

Physico-chemical analysis. Proximate composition of meat samples from each batch was analyzed in triplicate. Ash content was determined by calcining the samples at 525 ± 20°C to constant weight [8], protein content by Kjeldhal [9] and fat content by Soxhlet method [10]. Moisture content was determined by AOAC [11]. The values of water activity were measured with Fast Lab meter (GBX Instruments, France) using salt standard solutions for calibration.

* email: petru.alexe@ugal.ro, Phone: +40336130181, +40236460165

Thiobarbituric acid (TBA) analysis

The extent of lipids oxidation was assessed based on a colorimetric method that measures the absorbance correlated with the intensity of a red chromogen formed between TBA and secondary lipid oxidation products as described by Visessanguan *et al.* 2006 [12].

Evaluation of colour

The colour measurement was carried out using a chromameter Hunter Lab Miniscan XEPlus (Hunter Associates, USA). The colour parameters, Luminosity - L*, redness or greenness - a*, and yellowness or blueness- b*, were recorded at the first day and at the last day of storage [13]. Ten measurements for each of the five replicates were taken. The colour difference (ΔE) between the samples was evaluated using the following equation:

$$\Delta E = \sqrt{(L_m^* - L_p^*)^2 + (a_m^* - a_p^*)^2 + (b_m^* - b_p^*)^2} \qquad (1)$$

where, ΔE indicates the degree of overall colour change compared to the initial colour of the sample, L_m^*, a_m^*, b_m^* represent the values of the parameters in the first day of storage and L_p^*, a_p^*, b_p^* represent experimental sample values after 22nd days of storage.

Measurement of desorption equilibrium Desorption isotherms were determined by gravimetric static method. Five saturated saline solutions (LiCl, CH_3COOK, $MgCl_2$, KI, KCl) with a_w values ranging from 0.113 to 0.874 were prepared in hermetically sealed jars and stored 7 days at constant temperature. All measurements were made on triplicate samples.

Data analysis Minitab 16.0 software (Minitab Inc., USA) was used for statistical analysis. Tukey's test and one-way analysis of variance (ANOVA) were applied to evaluate the significant differences ($p<0.05$) between control and experimental samples, and where appropriate, 95% confidence intervals were indicated (95% CI). Data were expressed as mean ± standard deviation (SD). RMSE and R^2 values were calculated to assess the accuracy and correlations [14].

Desorption models

Three widely applied mathematical models that have physical significance attached to model parameters were selected in this study: BET, GAB, and Halsey equations [5,15],

$$\frac{a_w}{(1-a_w)\cdot M} = \frac{1}{C\cdot M_0} + \frac{(C-1)}{C\cdot M_0}\cdot a_w \qquad (2)$$

where, M_0 - monolayer moisture content (%), C - energy constant, a_w - water activity, M - humidity (%)

$$M = \frac{M_0\cdot a_w\cdot C\cdot K}{(1-a_w\cdot K)(1-K\cdot a_w + C\cdot K\cdot a_w)} \qquad (3)$$

where, C - GAB model parameter a_w - water activity, K - energy constant, M_0 - monolayer moisture content (%)

$$a_w = \exp\left[\frac{-k}{M^n}\right] \qquad (4)$$

where, k – constant, M - humidity (%), n – constant

Model for colour changes Power law model was selected to test the correlation between colour variables as a function of water activity and oxidation rate expressed by TBA values calculated as follows:

$$\ln(L^*\cdot a^*) = B\cdot\left[(TBA)^d\cdot(a_w)^e\right] \qquad (5)$$

where, L^*, a^* - colour characteristics for meat samples, B, d and e - model parameters, TBA - malonaldehyde value and a_w - water activity.

Properties of sorbent water

The properties of bound water were determined for a given temperature (4, 8 and 20°C) using Caurie's equation:

$$\ln\left(\frac{1}{M}\right) = -\ln(C\cdot M_0) + \left(\frac{2}{N}\right)\cdot\left(\frac{1-a_w}{a_w}\right) \qquad (6)$$

where, C - bound water density, M_0 - monolayer moisture content (%), N - the number of adsorbed monolayers.

Results and discussions

The average composition of the traditional salami recipe (P_1) and the reformulated one (P_2) are mentioned in table 1. In the current study the storage time corresponds to the time to reach the equilibrium. The salami was stored at 8°C and 75% relative humidity for the entire shelf-life.

It can be noticed that the reformulated salami had slightly higher moisture content, and the substitution of back fat with palm and olive oil (P_2) (95%CI: 13.71, 15.72) determined a lower moisture loss ($p<0.05$ than in the control (P_1) (95%CI: 18.36, 20.64) during storage at 8°C.

Table 1
PROXIMATE COMPOSITION OF P_1 AND P_2 SALAMI

Product	day	Moisture (kg water/kg meat product) (%)	Protein (%)	Fat (%)	Ash (%)	NaCl (%)	TBA mg malonaldehyde/kg
P_1	1st day	56.64±0.97[a]	17.79±1.25	22.44±1.12	3.99±0.02	2.09±0.32	0.59±0.35
	22nd day	37.15±1.26	26.20±1.15	31.48±3.38	5.92±0.03	3.07±0.77	2.04±0.81
P_2	1st day	62.63±1.14	14.65±1.09	19.59±1.85	3.65±0.08	1.89±0.12	0.63±0.03
	22nd day	47.79±1.05	18.02±0.95	26.39±3.94	4.79±0.03	2.21±0.25	2.71±0.47

[a)] *standard deviation (SD*

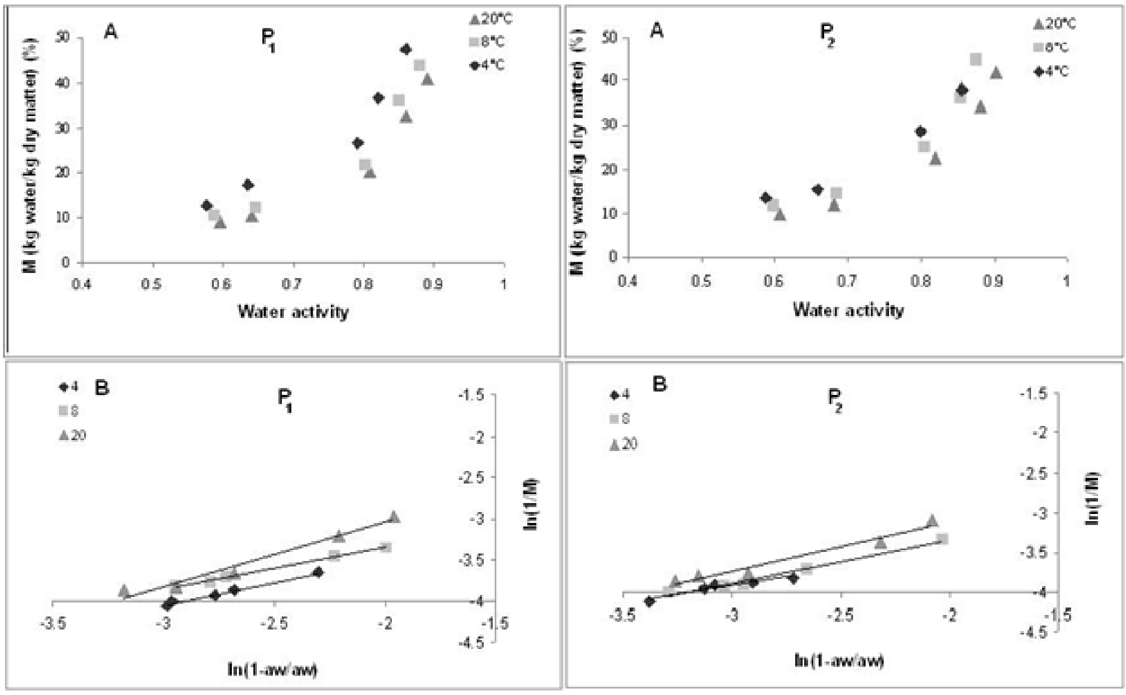

Fig.1. a.Desorption curves for the conventional meat product (P_1) and for the reformulated product (P_2) with vegetal origin oils b.Caurie's linear curves for the conventional meat product (P_1) and the reformulated product (P_2) at different temperatures 4, 8 and 20°C

Table 2
ESTIMATED MODEL PARAMETERS OF SORPTION ISOTHERMS FOR P_1 AND P_2 SALAMI

Model / Sample	Temperature	Parameters						
	°C	M_0	C	K	k	n	RMSE	R^2
BET								
P_1	4	-0.67900	0.4140					
	8	-0.67000	0.4120				0.665	0.359
	20	-0.46800	0.6330					
P_2	4	0.00004	1.0000					
	8	-0.68700	0.3762				0.613	0.681
	20	-0.69300	0.3670					
GAB								
P_1	4	4.987	-8.510E+71	0.959				
	8	4.808	-0.736E+00	0.971			0.481	0.968
	20	3.106	1.300E+71	0.978				
P_2	4	5.378	-0.127E+00	1.004				
	8	4.595	1.590E+66	0.952			1.282	0.873
	20	3.454	3.250E+75	0.964				
Halsey								
P_1	4				50.586	1.713		
	8				41.376	1.486	2.92E-05	0.988
	20				3.865	1.133		
P_2	4				350.200	2.265		
	8				36.164	1.694	6.66E-04	0.973
	20				8.257	1.360		

Table 3
COLOR AND TBA VALUES AT DIFFERENT RELATIVE HUMIDITY

Sample	Temp °C	Salts																			
		LiCl				CH_3COOK				$MgCl_2$				KI				KCl			
		L*	a*	b*	TBA	L*	a*	b*	TBA	L*	a*	b*	TBA	L*	a*	b*	TBA	L*	a*	b*	TBA
P1	4	58.01±0.41ᵃ	9.39±0.08	11.80±0.01	0.90±0.08	59.00±0.53	9.32±0.09	10.81±0.01	0.99±0.09	55.75±0.51	10.40±0.09	11.10±0.09	1.06±0.09	60.60±0.52	8.54±0.08	11.30±0.09	1.17±0.09	59.40±0.40	8.73±0.07	11.00±0.09	1.36±0.01
	8	57.05±0.51	8.33±0.07	11.40±0.01	1.20±0.09	50.10±0.49	11.20±0.10	10.08±0.01	1.25±0.09	49.97±0.41	9.64±0.07	11.50±0.08	1.65±0.10	57.50±0.42	9.43±0.09	11.00±0.08	1.35±0.10	58.60±0.47	7.74±0.05	12.00±0.10	1.44±0.01
	20	50.22±0.39	9.85±0.07	12.80±0.02	1.10±0.01	54.80±0.55	5.56±0.04	11.30±0.02	0.83±0.07	50.36±0.45	10.30±0.09	11.30±0.09	1.04±0.08	58.90±0.46	10.3±0.09	11.50±0.08	0.86±0.07	56.10±0.42	10.00±0.10	13.00±0.10	1.09±0.02
P2	4	56.44±0.47	9.57±0.09	14.80±0.02	1.80±0.02	47.30±0.42	11.70±0.09	17.45±0.02	1.85±0.09	54.87±0.46	9.62±0.08	11.60±0.10	1.87±0.09	57.50±0.41	8.62±0.07	14.40±0.10	2.22±0.18	55.80±0.35	8.37±0.07	12.00±0.09	2.21±0.02
	8	55.68±0.40	9.27±0.08	13.10±0.02	2.00±0.01	48.10±0.48	9.77±0.08	13.19±0.02	1.95±0.09	45.58±0.47	10.30±0.07	12.10±0.09	2.06±0.12	51.50±0.39	2.64±0.18	13.30±0.11	2.25±0.19	55.50±0.48	9.48±0.08	15.00±0.13	2.26±0.02
	20	39.76±0.28	4.25±0.04	13.40±0.03	1.70±0.01	48.50±0.44	5.19±0.05	14.42±0.03	1.71±0.07	36.21±0.29	7.61±0.06	14.50±0.10	1.51±0.11	53.50±0.39	9.15±0.06	15.00±0.12	1.53±0.01	37.20±0.27	4.52±0.03	15.00±0.12	1.55±0.01

ᵃ) *standard deviation (SD)*

The equilibrium moisture content (M) of the samples reached in this desorption experiment at 4, 8 and 20°C (fig. 1A) was influenced by the salami stick size and by the casing pores size. Even though the isotherms are displaying in the upper part the shape of type II isotherms and the results obtained in the current study are in line with other researches [1,16,17], the endpoint of equilibrium reached was higher than in other studies. The differences were caused by the type of synthetic casings used for salami in the current study, while in other studies natural membranes [6] were applied, and, in another study the product was sliced [5] facilitating water removal from the meat product. The desorption curves at 4, 8 and 20°C were very close to each-others, however, it can be noticed (fig. 1a) that at all a_w levels, the equilibrium moisture content decreased with the increase in temperature.

All the experimental measurements of the equilibrium moisture content ranged from 9.08 to 47.53 %, at water activity (a_w) values from 0.577 to 0.880. The equilibrium moisture content at 4, 8 and 20°C of the reformulated salami with vegetal oil (P_2) and the control product (P_1) was similar ($p>0.05$).BET, GAB and Halsey mathematical models were applied to the experimental data at 4, 8 and 20°C. The parameters were estimated using nonlinear regression and are presented in table 2.

Halsey was the model that best fitted the experimental data, with very low RMSE values ranging from 2.92E-05 to 6.66E-04, suggesting a high accuracy in describing the experimental data. Moreover, the correlation coefficient for the model, $R^2_{corrected}$, confirmed the good match between the experimental and predicted values (0.988 for P_1 and 0.973 for P_2).

GAB model was also reliable and its ability to estimate the monolayer moisture content made it adequate for further inference calculations based on Caurie's equation. The values of the monolayer moisture content (M_0) for P_1 and P_2 are in line with the values obtained by Singh et al. (2001) [6] and Pushpadass et al. (2014) [16] but higher than the one reported by Ahmat et al. (2014) [1].

TBA and colour analysis were performed after the 22nd day of storage in saline solutions, when the moisture equilibrium was reached (table 3). The TBA values of the products increased with the increase in relative humidity values at constant temperature (4 and 8°C). The reformulated product (P_2) registered higher TBA values than the conventional ones (P_1) due to the presence of higher concentration of unsaturated fatty acids in the vegetal oils compared with the animal fat.

Temperature influenced the oxidation rate, but, while at 4°C a reduced oxidation rate and implicitly lower TBA values were noticed for both P_1 and P_2 products compared to the samples stored at 8, at 20°C, a slightly lower oxidation rate was noticed compared with 8°C, for the entire domain.

The colour changes were evaluated by two parameters: *L** - luminosity that turned into grey at the end of storage period and *a**- value that expressed the changes from red to green, and expressed as a function of water activity and oxidation intensity.

A modified Weibull model that was adapted for both P_1 and P_2 products (table 4). Good RMSE and $R^2_{corrected}$ values were obtained for both models suggesting a strong correlation between colour, TBA and water activity values. The model shows that the dependant variable that describes the simultaneous variation of the colour parameters L* and a* does not have a significant variation as a function of temperature, for both reformulated and control samples, except for the case of the reformulated sample stored at 20°C ($p<0.05$) (95%CI: 5.519, 5.579). This indicates that P_2 product with replaced fat presents it is more susceptible to changes in colour if there is a temperature abuse during its storage.

Caurie's equation was applied to estimate the characteristics of bound water at (fig. 1b). Steeper slopes

Model / Sample	Temp	Parameters				
WEIBULL	°C	B	d	e	RMSE	$R^2_{corrected}$
P_1	4	6.065	-0.001	-0.603	0.665	0.859
	8	6.966	-0.262	0.537		
	20	6.445	0.009	0.292		
P_2	4	5.821	0.0260	-0.977	0.613	0.891
	8	6.380	-0.019	0.216		
	20	5.868	-0.145	1.075		

Table 4
WEIBULL MODEL PARAMETERS FOR P_1 AND P_2 SALAMI

were registered for the conventional product P_1 compared to the reformulated one P_2. Correlation coefficients were significant (R2>0.97) for both P_1 and P_2 at each experimental temperature and the properties of bound water could be accurately estimated.

Conclusions

Desorption isotherms for reformulated salami and conventional salami were determined at 4, 8 and 20°C to characterize product stability. These isotherms are type II sigmoid in shape. Halsey model was the best model in terms of correlation and accuracy to describe desorption but GAB model also provided reliable estimation of equilibrium moisture content. At all studied temperatures the desorption isotherms for reformulated and conventional salami were similar. Higher TBA values were registered for the reformulated product compared to the conventional one, correlated with the increase in environmental relative humidity at constant temperature (4 and 8°C). The reformulated salami demonstrated a better water holding capacity for the entire experimental domain. A modified Weibull equation was applied for the first time to explain the relationship between the colour changes, TBA and water activity.

Acknowledgement: The work has been funded by the Sectorial Operational Programme Human Resources Development 2007-2013 of the Romanian Ministry of European Funds through the Financial Agreement POSDRU/159/1.5/S/132397. This work benefited from the technical support of the RE-SPIA (695/09.04.2010, SMIS code 11377) project, financed by REGIO (Regional Operational Programme 2007-2013).

References

1. JIMENEZ-COLMENERO, F., Trends Food Sci. Technol., **18**, 2007, p.567-578
2. JIMENEZ-COLMENERO, F., Trends Food Sci. Technol., **11**, 2000, p.56-66
3. YILDIZ-TURP, G., SERDAROGLU, M., Meat Sci., **78**, 2008, p.447-454
4. COFRADES, S., JIMENEZ-COLMENERO, F., HERRERO, A.M., SOLAS, M.T., RUIZ-CAPILLAS, C., Food Hydrocolloids, **30**, 2013, p.351-357
5. AHMAT, T., BRUNEAU, D., KUITCHE, A., AREGBA, A.W., Meat Sci., **96**, 2014, p.1417-1424
6. SINGH, R.R.B., RAO, K.H., ANJANEYULU, A.S.R., PATIL, G.R., Food Res. Int., **34**, 2001, p.143-148
7. TUDOSE, C., PATRASCU, L., ALEXE, P., Studia Universitatis Babes-Bolyai Chemia, **59**, 2014, p.87-102
8. AOAC, **920.153**, 19th ed. Association of Official Analytical Chemists, 2012a
9. AOAC, **928.08**, 19th ed., Association of Official Analytical Chemists, 2012b
10. AOAC, **960.39**, 19th ed., Association of Official Analytical Chemists, 2012c
11. AOAC, **950.46**, 19th ed., Association of Official Analytical Chemists, 2012d
12.VISESSANGUAN, W., BENJAKUL, S., SMITINONT, T., KITTIKUN, C., THEPKASIKUL, P., PANYA, A., LWT- Food Sci. Technol., **39**, 2006, p.814-826
13. JAWORSKA, G., BERNAS, E., Food Chem., **113**, 2009, p.936-943
14. NEAGU C., BORDA D., ERKMEN, O., Food Bioprod. Process., **92(4)**, 2014, p.369-375
15. SINGH, R.R.B., RAO, K.H., ANJANEYULU, A.S.R., PATIL, G.R., J. Food Eng., **75**, 2006, p.228-239
16. FARID, B.C., CHEJNE, F., Meat Sci., **86**, 2010, p.921-925
17. CLEMENTE, G., BON, J., BENEDITO, J. and MULET, A., Meat Sci., **82**, 2009, p.413-418
18. PUSHPADASS, H.A., EMERALD, F.M.E., CHATURVEDI, B., RAO, K.J., Journal of Food Processing and Preservation, **38**, 2014, p.2192-2200

Manuscript received: 18.04.2016