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Dependence between the Rheological Properties of the Initial Raw Material and Whole Muscle Ham Products from Beef

Oksana Savinok[1]

Abstract: The changes in the rheological characteristics of beef ham products were studied using a dynamic penetrometer with a cone angle of 10° and 30°. The ratios of the immersion depths of the cone of a dynamic penetrometer for different muscles (m.Semimembranosus and m. Longissimus dorsi) were calculated. When using different cones, the ratios varied depending on the content of the connective tissue in the meat, as well as in the process. Moreover, the more connective tissue in meat, the less the ratio of strength characteristics of meat along and across its fibers. The stage at which there was significant deterioration in the sensory indices was determined.

Keywords: *Beef, Dynamic penetrometer, Rheological characteristics, Ham products*.

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INTRODUCTION

Each group of meat products is characterized by a complex of sensory indicators, in which consumers note priority. The main sensory traits of ham meat products made of beef are juiciness, tenderness, elasticity. They largely depend on the properties of the initial raw materials, the processing regimes, the prescription composition of the brine and many other factors. Each of these factors can be decisive. However, other things being equal, it is the original rheological properties of the meat that ensure the juiciness, density and solidity of the product. Rheological characteristics of raw materials are due to the anatomical location of muscle tissue, the age of the animals, the conditions of fattening, the breed and the stage of autolytic ripening. Some of these factors can be determined through sensory analysis, for others instrumental express methods are applied. Different methods are used for research and production purposes, such as gravitational impulse method, texture measurement method (Warner-Bratzler method), penetrative method, etc. (Kosoy and Dorohov, 2006; Migdał et al., 2007; Poldvere et al., 2014; Kakimov et al., 2015).

When exploring the resilient-elastic environments concerning whole muscle beef tissue, it is rational to use devices whose principle of action consists of the introduction of working body in the research object. The dynamic penetrometer with a conic indentor as a portable device of the size of the penetration in the whole muscle raw materials has proved to be perfect (Kosoy et al., 2005; Kosoy and Dorohov, 2006, Kakimov et al., 2015). However, depending on the characteristics of the studied object, different cone apex angles may be used. For plastic bodies, in particular minced meat, it is rational to use cones with angles of more than 30° (Kosov et al., 2005; Kakimov et al., 2015), for the elastic-elastic (whole muscle beef tissue) - cones with apex angles less than 30° (Savinok, 2015).

Previous studies have made it possible to determine the optimal cone angle of the indenter of a dynamic penetrometer and to choose the calculation equations with correction factors (Savinok, 2015, 2016) to estimate the rheological characteristics of whole muscle beef. The smallest error is provided by a penetrometer with a cone angle at the apex of 10 º, and with a force of 0.5 kg.

However, for the practical application of the obtained data, it is necessary to establish a relationship between the rheological indexes of the initial raw materials and finished products after the end of heat treatment and during storage.

Material and Methods

The objects of the research were isolated semi-finished products from carcasses of young Simmental cattle at the age of 14-15 months. The slaughter and refrigeration conditions were identical. The beef halves (30 pieces) were received for processing after 3 days of maturation from the moment of slaughter. The temperature in the depth of the muscles when admitted to the meat processing plant did not exceed +4 ºC. For the production of whole muscle products, pieces of muscle tissue weighing

1.8-2 kg, derived from m. Longissimus dorsi and m.Semimembranosus muscles were selected. The pH values in the analyzed muscles were in the range of 5.42-5.46. The salting process included brine injecting, massaging, maturing in a massage machine in a vacuum.

The percentage of brine during the injecting was 75 %. Massaging was carried out for 8 hours, the duration of the active phase was 20 minutes, the duration of the resting phase was 10 minutes, the depth of vacuum – 85%, the drum rotation speed – 8 rp/m. The duration of ripening was 16 hours, the temperature at the salting was +2 ºC, the diameter of the drum of the massager was 1 m. For the injecting, the supplement of the German-Polish company "Fleisch Mannschaft", Shinka FCB was used. The technology of the brine preparation corresponds to the company's instructions. Heat treatment and storage were carried out according to the following regimes: short time smoking– $\tau = 1.5$ h, $t = 70$ ° C; smoking – $\tau = 20$ min, $t = 70$ ° C; cooking – $\tau = 1{,}5$ h, $t = 78$ ° C, to t at the center = 72 ° C; cooling - t = 4 ° C to t at the center ≤ 22 ° C; final cooling – t = 4 ° C to t at the center = ≤ 6 ° C; storage – $\tau = 9$ days, t air = 0-4 °C. The pH of the meat after the injecting was 5.95-6.01.

The optimal angle of the conical indenter for a dynamic penetrometer, which provides a minimum error in calculating the limiting shear stress (10º and 30º) was chosen based on previous studies (Savinok et al., 2014). In order to study the influence of the initial rheological properties of beef on rheological and respectively the sensory indices, the studies were carried out at the main technological stages: after separation of semi-finished products, after salting, after heat treatment and at storage stages.

Results and Discussion

Since the strength properties along and across the fibers differ in absolute value, to determine the absolute values and their ratios, the measurements were made on the cross section of the muscle tissue of the meat into which the indenter was introduced along the fibers and in the longitudinal section where the introduction of the working body occurred across the fibers. In the latter case, the strength of the meat is much larger than in meat, where measurements were made along the fibers. The strength of the meat was estimated by the rheological value which is the limiting shear stress (LSS) measured in Pa. The ratio of the depth of the cone immersion for the two muscles were different. When using different cones, the ratios varied depending on the content of connective tissue in the meat, as well as in the process. Moreover, the more connective tissue in meat, the less the ratio of strength characteristics of meat along and across its fibers (Table 1). When studying the properties of salted meat, it was noted that the destruction of loose connective tissue fiber between the bundles of muscle fibers and the muscle fibers themselves during the injecting and massaging, helped to reduce the LSS, which was clearly demonstrated by the ratio of the values of LSS, this value increased from 0.53 to 0.82 (cone angle 10º) for m. Semimembranosus, while for m. Longissimus dorsi, which refers to a semi-static, the calculated ratio has changed within the error of measurement. This was due to the fact that the analyzed muscle contains a minimal amount of loose connective tissue fiber between the bundles of muscle fibers, which,

in addition, is filled with fat cells. Consequently, the established relationship can be used for dynamostatic muscles, statodynamic, containing a significant amount of connective tissue between muscle fibers and having thick muscle fibers, which causes their stiffness (Cherniavskiy, 2002). The results obtained will make it possible to use the penetration method to assess the quality of meat (by the content of connective tissue) by the ratio of the lateral LSS (across the fibers) to the axial (along the fibers).

Table 1. Change of the rheological characteristics of beef during processing

Properties and type of object	Name of muscles	Cone angle	Direction of introduction of the cone into the muscle tissue	Depth of the introduction (h), cm	Relative penetration voltage ($\Theta_п$), Pa	Limiting shear stress Θ_o, Pa	h_{along} / h_{across}	$\Theta_{п\ along}$ / $\Theta_{п\ across}$
Meat before processing	m.semimembranosus	10	along	2.57	7432.67	5748.95	1.37	0.53
			across	1.87	14087.31	10896.10		
		30	along	1.63	18483.41	8430.70	1.20	0.70
			across	1.36	26530.13	12100.99		
	m. longissimus dorsi	10	along	2.40	8486.35	6563.93	1.01	0.97
			across	2.37	8758.34	6774.31		
		30	along	1.81	15117.55	6895.46	1.25	0.65
			across	1.45	23349.92	10650.42		
Meat after massaging	m.semimembranosus	10	along	2.69	6796.85	5257.15	1.11	0.82
			across	2.43	8270.94	6397.32		
		30	along	1.68	17269.62	7877.06	1.04	0.91
			across	1.61	18903.59	8622.35		
	m. longissimus dorsi	10	along	3.06	5217.86	4035.86	1.06	0.89
			across	2.90	5836.46	4514.32		
		30	along	1.91	13471.92	6144.85	1.06	0.89
			across	1.80	15123.46	6898.15		

Analysis of the rheological characteristics of the finished product (Table 2) showed that the limiting shear stress in the analyzed ham products after heat treatment decreased by 17.83% with longitudinal measurement of the penetration depth (cone angle 10º) and 43.69% at the transverse for m. Semimembranosus; by 61.2% at longitudinal and 39.0% at transverse – for m. Longissimus dorsi. A slight decrease in LSS with longitudinal measurement of the introduction depth in m. Semimembranosus, indicated partial swelling of collagen during heat treatment and simultaneous longitudinal compression of the muscle fibers. On the other hand, significant decrease in LSS in the transverse measurement in this muscle was justified by the destruction of the intermuscular connective tissue due to mechanical action with saline. The decrease in LSS in m. Longissimus dorsi was justified by a more delicate cellular structure of muscles, a loose connective tissue between the fibers.

Table 2. Change of the rheological characteristics of ham products from beef during storage

Duration of storage, days	Name of muscles	Cone angle, degrees	Direction of introduction of the cone into the muscle tissue	Depth of the introduction, h, cm	Relative penetration voltage $\Theta_п$, Pa	Limiting shear stress $\Theta_о$, Pa	h_{along}/h_{across}	$\Theta_{п\ along}/\Theta_{п\ across}$
1 day	m.semimembranosus	10	along	2.83	6107.40	4723.89	1.14	0.77
			across	2.48	7942.92	6135.50		
		30	along	1.56	20134.78	9183.93	0.96	1.09
			across	1.63	18527.70	8450.90		
	m. longissimus dorsi	10	along	3.86	3292.94	2546.99	1.27	0.62
			across	3.04	5317.09	4112.61		
		30	along	2.36	8772.96	4001.54	1.25	0.64
			across	1.89	13782.16	6286.35		
4 days	m.semimembranosus	10	along	2.70	6746.50	5218.21	1.10	0.83
			across	2.46	8097.03	6262.81		
		30	along	1.38	25581.38	11668.24	0.99	1.01
			across	1.39	25224.72	11505.56		
	m. longissimus dorsi	10	along	3.69	3600.85	2785.15	1.24	0.65
			across	2.98	5526.01	4262.92		
		30	along	2.08	11276.47	5143.45	1.32	0.58
			across	1.58	19518.30	8902.74		
6 days	m.semimembranosus	10	along	2.36	8788.45	6797.59	1.00	1.00
			across	2.36	8816.43	6819.24		
		30	along	1.55	20362.57	9287.82	1.01	0.97
			across	1.53	20904.78	9535.14		
	m. longissimus dorsi	10	along	3.24	4664.13	3607.56	1.08	0.86
			across	3.01	5426.34	4197.11		
		30	along	2.51	7808.73	3561.74	1.14	0.77
			across	2.20	10101.00	4607.29		
9 days	m.semimembranosus	10	along	2.41	8436.49	6525.37	1.06	0.89
			across	2.27	9530.19	7371.31		
		30	along	1.58	19711.35	8990.79	1.10	0.82
			across	1.43	23895.17	10899.12		
	m. longissimus dorsi	10	along	3.84	3315.62	2564.53	1.11	0.80
			across	3.45	4119.17	3186.05		
		30	along	2.50	7867.95	3588.75	1.21	0.69
			across	2.07	11484.77	5238.46		

When storing ham products obtained from beef m. Semimembranosus, an increase in the limiting shear stress, characterizing the rigidity and density of the product, was observed. During the storage period from 6 to 9 days, in the products made of this muscle, intensive separation of moisture was observed, the structure becoming friable. This justified the decrease in LSS after 9 days of storage with longitudinal insertion of the cone (cone angle 10º) and the increase in the transverse. Similar trend was observed for the m. Longissimus dorsi products. The separation of moisture could be explained by the composition of the brine. It contains hydrocolloids, from which the separation of the liquid phase occurs due to syneresis. Consequently, starting from 6 days, the products begin to soften the structure due to the syneresis of the colloid structure formed after heat treatment and subsequent cooling, consisting of meat myofibrillar proteins and brine hydrocolloids.

The use of indentors with angles of 10º and 30º showed similar dependencies, however, when using a cone with an angle of 30º, the error percentage in the measurement was larger. Sensory analysis of ham products during the whole storage period also allowed to note a significant deterioration in quality indicators, starting from 6 days. The products had an intensive separation of moisture upon pressing, the cutting was deteriorated, the consistency became friable, and the monolithicity of the product at the cut disappeared.

Conclusion

The ratios of the depths of the cone immersion for m. Longissimus dorsi and m. Semimembranosus allowed to establish that the more connective tissue in meat, the less the ratio of meat strength along and across its fibers. These data will allow us to rationally sort the meat raw materials for the production of whole-muscle beef products. The study of rheological characteristics of beef ham products using a dynamic penetrometer with a cone angle of the working body of 10º allowed us to recommend this method for determining the stage at which a significant deterioration in sensory indices occurs.

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Influence of Maceration and the Addition of Flavoring Enzyme on the Aromatic Profile of Red Wines from the Region of Central Northern Bulgaria

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Abstract: Gas chromatographic study (GC-FID) for determination of the influence of maceration and addition of flavoring enzyme on the aromatic profile of red wines from Central Northern Bulgaria was conducted. The wines were obtained from selected clones (Gamza 52-9-4, Gamza 52-9-5 and Pamid 5/76) and varieties (Kaylashky rubin, Trapezitsa). Nineteen volatile compounds have been identified. Of these, 5 higher alcohols, 9 esters and 4 terpene alcohols affected the aroma of the wines. Methyl alcohol has been found in wines. Its concentrations were normal for red wines. The highest total concentration of volatile compounds was found in the Gamza clone 52-9-5 control (363.10 mg/dm^3). Gamza 52-9-4 clone, Kaylashky rubin and Trapezitsa varieties have been observed to increase the content of higher alcohols after addition of flavoring enzyme, while in the wines from Gamza 52-9-5 clone and Pamid 5/76 clone, the trend was reversed. The ester composition of the experimental samples was diverse. Increased ester content, after the addition of flavoring enzyme was found in Gamza 52-9-4 clone, Kaylashky rubin and Trapezitsa variety. In Gamza 52-9-5 and Pamid 5/76, the trend was reversed. The dominant ester was ethyl acetate. The highest content of terpene alcohols was observed in the wine from the control variant of clone Pamid 52-9-4 (0.69 mg/dm^3).

Keywords: *Maceration, Red wines, Enzyme, Aromatic profile, Methanol, Esters, Aldehydes, Higher alcohols, Terpenes.*

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INTRODUCTION

The varietal and quantitative diversity of aromatic components in wines are the main indicators for their quality. These components are products of a variety of chemical, biochemical and microbiological changes occurring in the grape maturation process and consequently in the production and aging of the wines. A large variety of volatile components, more than 800, has been found in studies on different worldwide wines (Aznar et al., 2001; Marti et al., 2003; Li, 2006; Kobayashi, 2008; Sumby, 2010). A wide concentration range for individual compounds is reported. The compounds are found in different concentrations ranging from mg/dm^3, in μg/dm^3 to ng/dm^3 (Rapp and Manderey, 1986; Ebeler, 2001; Sanchez-Palomo, 2007).

The volatile compounds with the most significant influence on the aromatic matrix of wines are esters, aldehydes, higher alcohols, terpene alcohols (Lambrechts and Pretorius, 2000; Vilanova et al., 2013). The compounds of these groups are present in wines in wide concentration ranges and have different thresholds of aromatic perception. In this way, they reflect in the wide and varied aromatic perception of the wine composition.

The total quantity of volatile compounds, many of which actively influence the wine aromatic sensory activity moves within the range 800.00 - 1200.00 mg/dm^3 (Ebeler, 2001; Lakatosova et al., 2013).

By arranging the aromatic groups of compounds according to their importance for the wine aroma, the best reflection is provided by the esters (Mason and Dufour, 2000; Ivanova et al., 2013). In wines they can be products of the yeast vital activity and the metabolism of other microflora (Swiegers et al., 2005a; Chobanova, 2012). In the wine aging process their quantity increases on the basis of the chemical mechanism of their formation - the interaction between the wine acids and alcohols (Chobanova, 2012). Their total concentration in young wines ranges from 2.00 to 200.00 mg/dm^3 (Yankov et al., 2000), and in the process of wine aging their concentration can significantly increase and may reach 1000.00 mg/dm^3 (Velkov, 1996).

The higher alcohols are aromatic compounds with high thresholds of aromatic perception. They can be products of both carbohydrate and amino acid yeasts metabolism (Etievant, 1991; Bell and Henschke, 2005). At the study of the volatile composition of Macedonian and Hungarian red wines, a variation in the alcohols content of 32.30 - 45.50 mg/dm^3 was found (Ivanova et al., 2013). Velkov (1996) indicates a variation in the content of higher alcohols in wines - 150.00 - 400.00 mg/dm^3. The diversity of higher alcohols is an important parameter, since at the wine aging they take part in the esterification processes and form esters when interacting with the wine acids (Perestrelo et al., 2006).

Terpenes are also a group wine aromatic components. They have a particular influence of the flavor of wines obtained from muscat grape varieties (Mateo and Jimenez, 2000; Fenoll et al., 2009).

The main and most concentrated of this group are terpene alcohols - linalool, α-terpineol, β-citronelol, nerol and geraniol (Wilson et al., 1986; Lee and Noble, 2003; Chobanova, 2012). Terpenes are the products of the vine metabolism from where they pass into the wine. In the strong muscat varieties their quantity ranges from 1.00 to 3.00 mg/dm^3, followed by varieties with average aromaticity (about 0.50 mg/dm^3) and slightly aromatic (0.10 - 0.20 mg/dm^3) (Velkov, 1996).

Compounds which enter into the volatile composition but do not have an aromatic effect can also be found in the wine. Such a compound is the methyl alcohol. It is obtained in the process of maceration on the base of degradation of the pectin in the fruit under the influence of the pectolytic enzyme complex (Marinov, 2005). Its content in red wines ranges from 36.00 - 350.00 mg/dm^3 (Chobanova, 2012).

The maceration (continuous contact of liquid with solids) is a widely applied technological practice in winemaking, in order to increase the extraction, increase of phenolic substances, microelements, vitamins, nitrogen substances in the obtained wines (Velkov, 1996).

The addition of enzyme systems, in particular β-glucosidase, leads to the hydrolysis of the glycosidic aromatic precursors of grapes must and wine, which may reflect in complicating and improving of the wine aromatic profile (Dignum et al., 2001; Wang et al., 2012).

The aim of the present study is to investigate the influence of the addition of flavoring enzyme on the volatile aromatic composition of red wines obtained from newly selected clones and grape varieties grown in the region of Pleven town, Central Northern Bulgaria.

Materials and methods

Grape varieties and vinification

The study was conducted at the Institute of Viticulture and Enology (IVE) - Pleven. The object of the study are red wines from two consecutive vintages (2015 and 2016) obtained from selected clones and newly selected grapevine varieties:

- Gamza clone 52-9-4 (Nakov et al., 2017)
- Gamza clone 52-9-5 (Nakov et al., 2017)
- Pamid clone 5/76 (Nakov et al., 2011)
- Kaylashky rubin (interspecies hybrid, obtained in IVE - Pleven) - Pamid x Hybrid VI 2/15 x Gamay noir x *Vitis amurensis* (Ivanov, 2016)
- Trapezitsa (interspecies hybrid selected in IVE - Pleven) - Danube gamza x Marseilles early (Ivanov et al., 2012)

The grapes were harvested at a technological maturity and were vinified in the Experimental Wine Cellar of IVE. A classic scheme for the production of dry red wines (Yankov et al., 1992) was applied – crushing and destemming, sulphitation (50 mg/kg SO_2), inoculating with pure culture dry yeasts

Saccharomyces cerevisiae Vitilevure CSM - 20 g/hl, temperature of fermentation - 28°C, separation from solids, further sulphitation, storage.

The grapes from the five studied varieties and clones was divided into two technological variants with a quantity of 30 kg grapes for each variant, as follows:

V1 - control variant

V2 – experimental variant with the addition of Zymovarietal Aroma G flavoring enzyme in the amount of 3 g/100kg in the grape pulp before alcoholic fermentation.

Determination of alcohol content of obtained wines

The alcohol content of the obtained wines was defined by specialized equipment with high precision – automatic distillation unit - Gibertiny BEE RV 10326 (Gibertiny Electronics Srl., Milano, Italy) and Gibertiny Densi Mat CE AM 148 (Gibertiny Electronics Srl., Milano, Italy).

Volatile content determination by GC-FID

Gas chromatographic determination of the volatile components in wine distillates was done. The content of major volatile aromatic compounds was determined on the basis of standard solution prepared in accordance with the IS method 3752:2005. The method describes the preparation of standard solution followed by a preparation of a solution with more compounds. The standard solution in this study include the following compounds (purity > 99.0%): acetaldehyde, acetone, ethyl acetate, methanol, isopropyl acetate, 1–propanol, 2– butanol, propyl acetate, 2–methyl-propanol, isobutanol, 1–butanol, isobutyl acetate, ethyl butyrate, butyl acetate, 2–methyl-1-butanol, 3–methyl-1–butanol, ethyl isovalerate, 1–pentanol, pentyl acetate, 1–hexanol, ethyl hexanoate, hexyl acetate, 1–heptanol, linalool oxide, phenyl acetate, ethyl caprylate, α-terpineol, nerol, β-citronellol, geraniol.

The 2 µl of prepared standard solution was injected in gas chromatograph Varian 3900 (Varian Analytical Instruments, Walnut Creek, California, USA) with a capillary column VF max MS (30 m, 0.25 mm ID, DF = 0.25 µm), equipped with a flame ionization detector (FID). The used carrier gas was He. Hydrogen to support combustion was generated and supplied to the chromatograph via a hydrogen generator Parker Chroma Gas: Gas Generator 9200 (Parker, United Kingdom).

The parameters of the gas chromatographic determination were: injector temperature – 220°C; detector temperature – 250°C, initial oven temperature – 35°C/retention 1 min, rise to 55°C with step of 2°C/min for 11 min, rise to 230°C with step of 15°C/min for 3 min. Total time of chromatography analysis – 25.67 min.

Results and discussion

The resulting chromatographic profiles of the wine variants tested are presented in Figures 1-5.

Table 1 presents the quantities of volatile compounds identified in the studied wines.

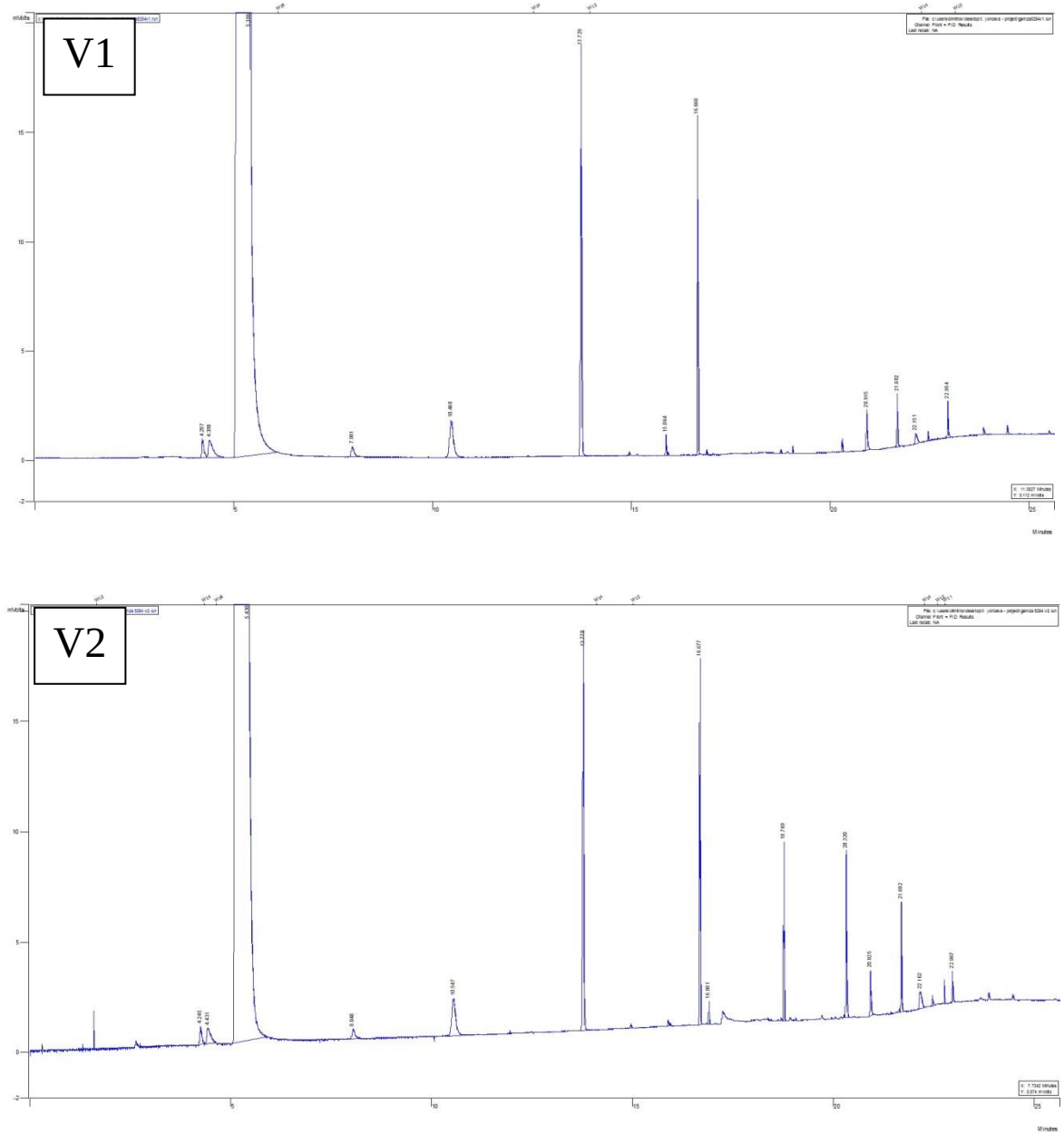

Figure 1. Chromatographic profile of red wine obtained from the selected clone GAMZA 52-9-4; V1 - control variant; V2 - variant with the addition of Zymovarietal Aroma G flavoring enzyme in the amount of 3 g/100kg in the grape pulp before alcoholic fermentation

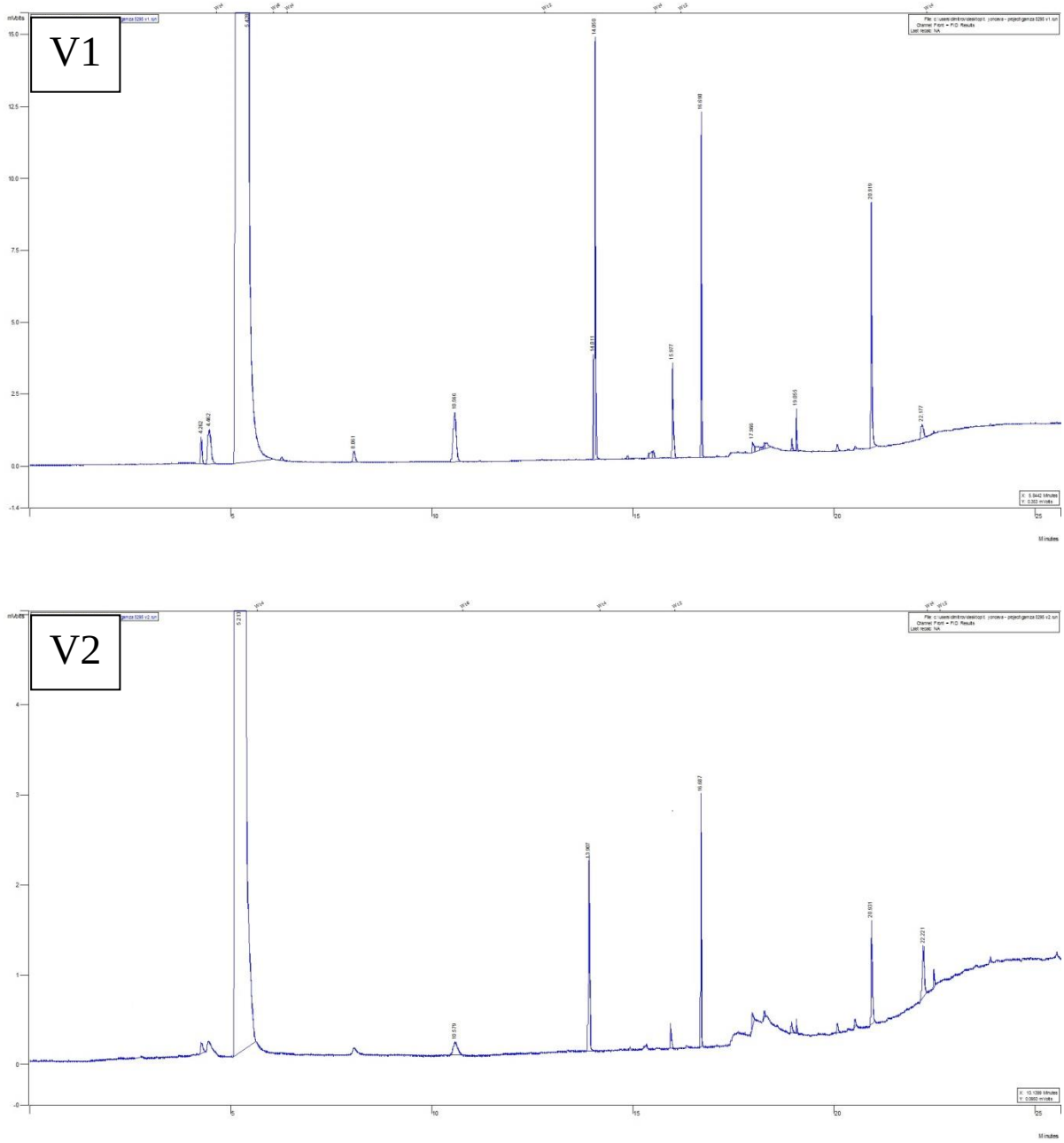

Figure 2. Chromatographic profile of red wine obtained from the selected clone GAMZA 52-9-5; V1 - control variant; V2 - variant with the addition of Zymovarietal Aroma G flavoring enzyme in the amount of 3 g/100kg in the grape pulp before alcoholic fermentation

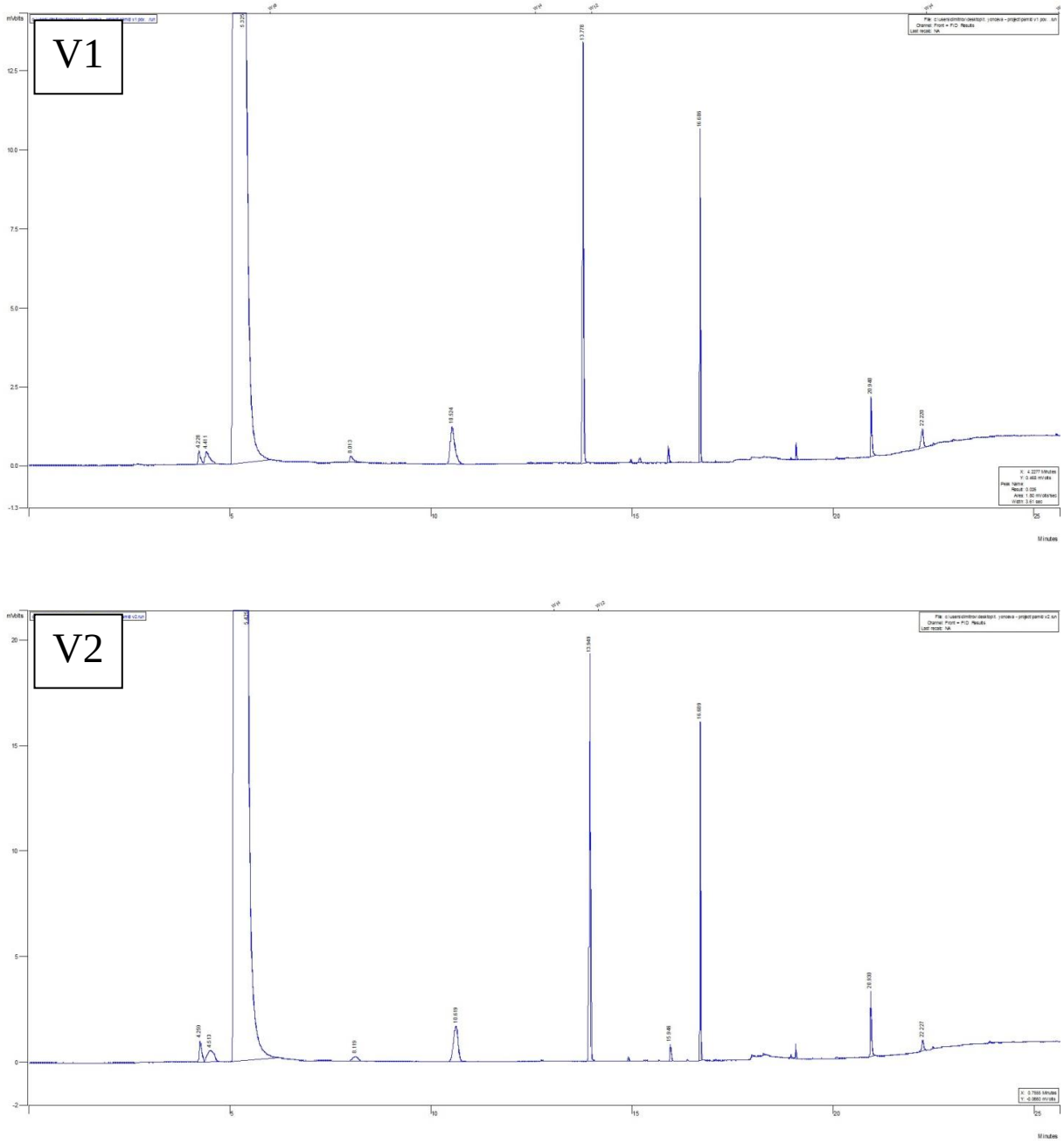

Figure 3. Chromatographic profile of red wine obtained from the selected clone PAMID 5/76, V1 - control variant; V2 - variant with the addition of Zymovarietal Aroma G flavoring enzyme in the amount of 3 g/100kg in the grape pulp before alcoholic fermentation

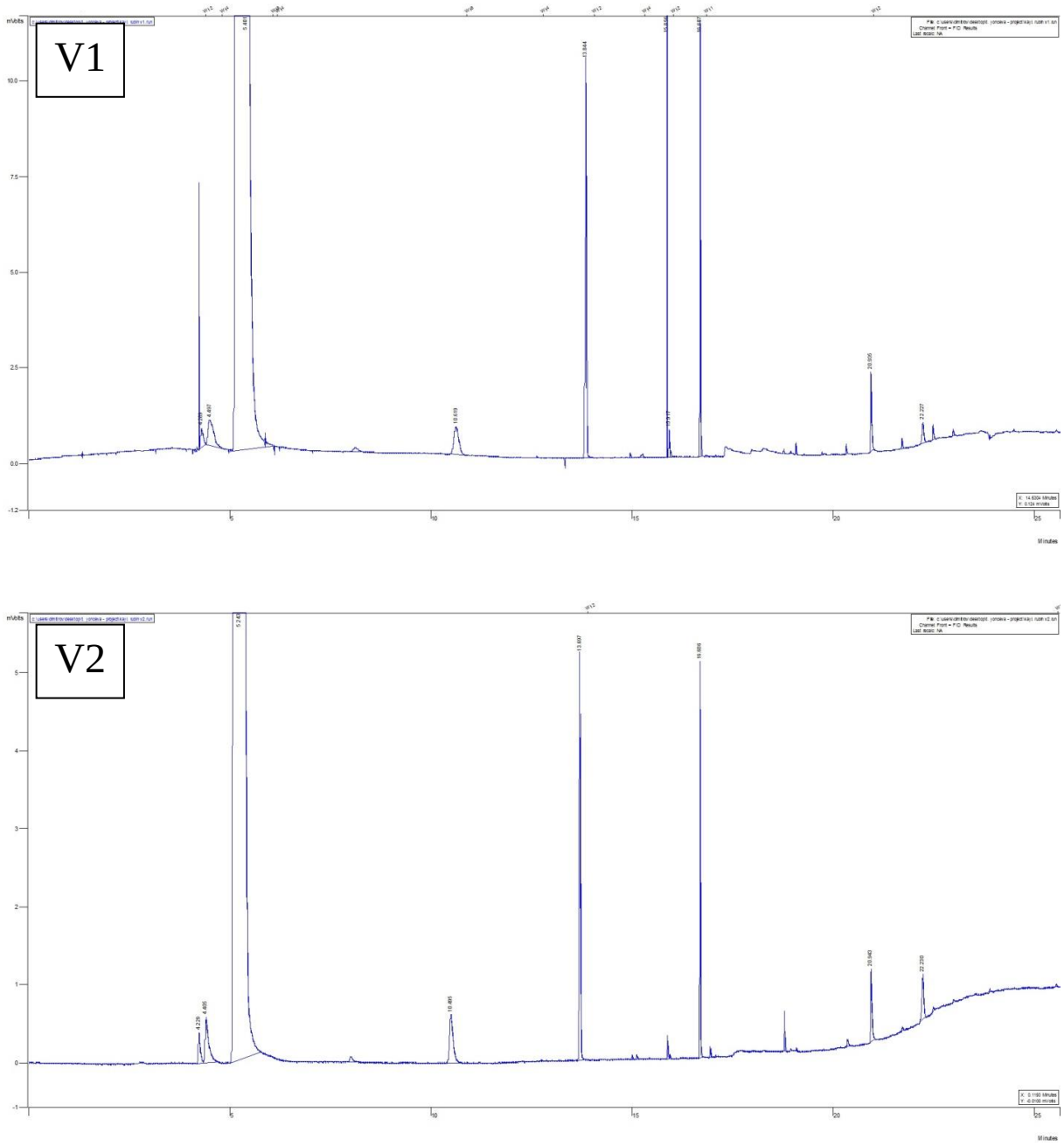

Figure 4. Chromatographic profile of red wine obtained from the selected variety KAYLASHKY RUBIN , V1 - control variant; V2 - variant with the addition of Zymovarietal Aroma G flavoring enzyme in the amount of 3 g/100kg in the grape pulp before alcoholic fermentation

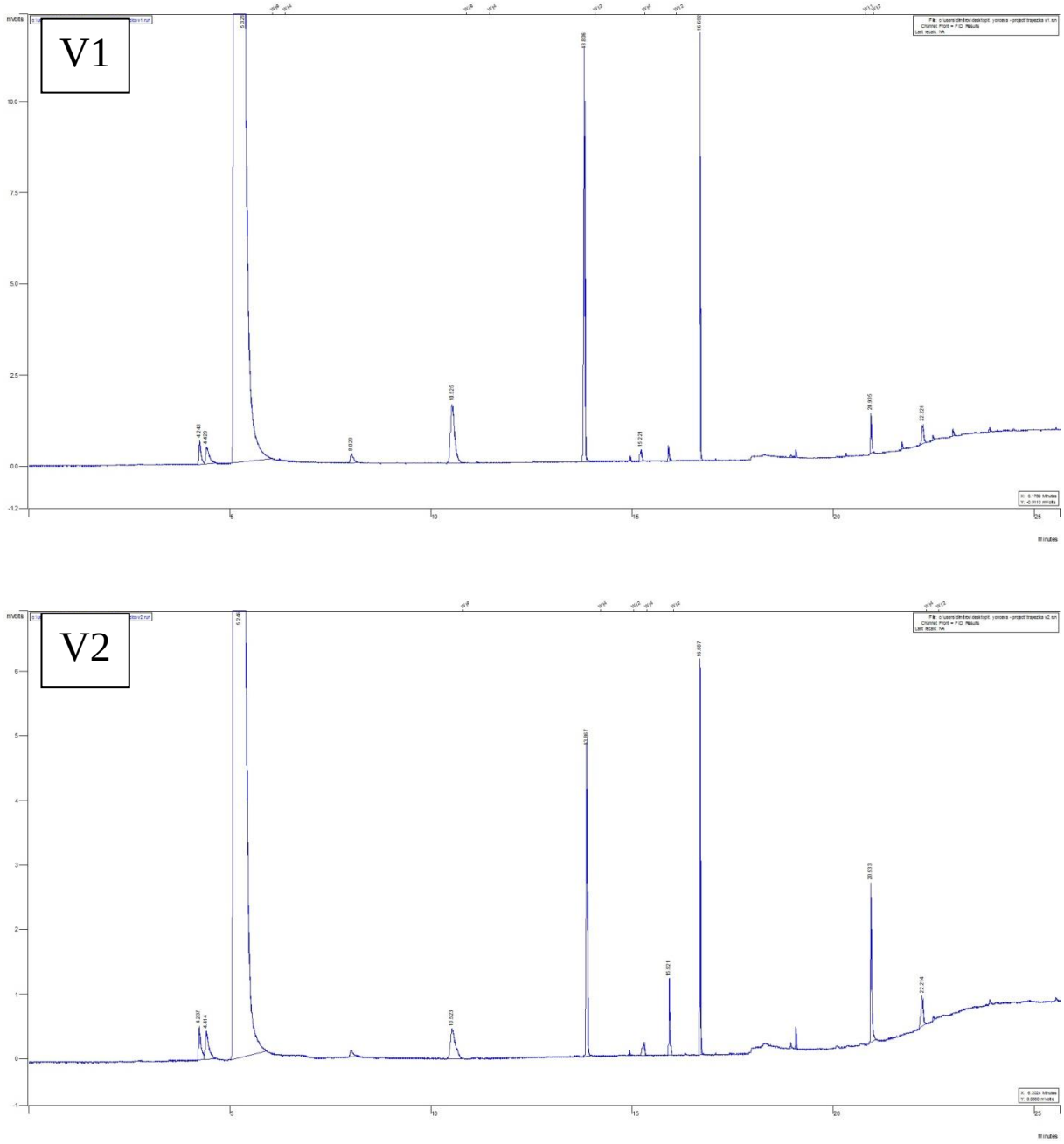

Figure 5. Chromatographic profile of red wine obtained from the selected variety TRAPEZITSA, V1 - control variant; V2 - variant with the addition of Zymovarietal Aroma G flavoring enzyme in the amount of 3 g/100kg in the grape pulp before alcoholic fermentation.

Table 1. Quantity of volatile compounds identified in the red wines examined

IDENTIFIED COMPOUNDS, mg/dm³	**WINES**									
	GAMZA 52-9-4		**GAMZA 52-9-5**		**PAMID 5/76**		**KAYLASHKY RUBIN**		**TRAPEZITSA**	
	V1	V2	V1	V2	V1	V2	V1	V2	V1	V2
Ethyl alcohol, vol.%	15.51	15.56	14.60	14.64	13.39	12.97	13.08	13.29	12.55	12.43
Methanol	52.90	37.20	76.10	≈0.05	43.20	61.40	85.00	105.40	38.40	64.20
2-methyl-1-butanol	ND	ND	72.20	75.35	51.85	53.20	ND	48.25	ND	ND
2-methyl-1-propanol	ND	27.70	ND	ND	26.30	24.10	≈0.05	≈0.05	28.10	≈0.05
3-methyl-1-butanol	ND	ND	26.80	ND	ND	ND	ND	ND	ND	ND
1-pentanol	14.20	≈0.05	97.20	32.25	25.90	21.80	21.70	≈0.05	≈0.05	73.60
1-hexanol	≈0.05	ND	ND	ND	ND	ND	ND	ND	ND	ND
Total higher alcohols	***14.25***	***27.75***	***196.20***	***107.60***	***103.75***	***99.10***	***21.75***	***48.35***	***28.15***	***73.65***
Ethyl acetate	22.50	17.20	21.10	≈0.05	20.00	34.90	25.20	32.50	26.30	44.80
Propyl acetate	36.10	ND	ND	ND	ND	ND	ND	ND	ND	ND
Isopropyl acetate	ND	ND	ND	ND	ND	ND	≈0.05	ND	ND	ND
Isobutyl acetate	63.40	57.90	69.60	36.90	86.90	87.90	53.80	75.30	153.90	97.20
Ethyl butyrate	ND	ND	ND	ND	≈0.05	≈0.05	≈0.05	ND	≈0.05	ND
Ethyl isovalerate	ND	≈0.05	ND	ND	ND	≈0.05	ND	ND	ND	ND
Ethyl hexanoat	ND	22.30	ND	ND	ND	ND	ND	≈0.05	ND	ND
Hexyl acetate	≈0.05	ND	≈0.05	ND	≈0.05	≈0.05	ND	≈0.05	≈0.05	ND
Phenyl acetate	≈0.05	50.30	ND	ND	ND	ND	≈0.05	≈0.05	ND	ND
Total esters	***122.10***	***147.75***	***90.75***	***36.95***	***107.00***	***122.95***	***79.15***	***107.95***	***180.30***	***142.00***
α – terpineol	ND	ND	ND	ND	ND	ND	≈0.05	ND	ND	ND
Nerol	ND	ND	≈0.05	≈0.05	≈0.05	≈0.05	ND	ND	ND	ND
β – citronelol	≈0.05	0.47	ND	ND	ND	ND	≈0.05	≈0.05	≈0.05	ND
Geraniol	0.64	0.12	ND	ND	ND	ND	≈0.05	≈0.05	≈0.05	ND
Total terpenes	**0.69**	**0.59**	**0.05**	**0.05**	**0.05**	**0.05**	**0.15**	**0.1**	**0.1**	**ND**
TOTAL CONTENT OF VOLATILE COMPOUNDS	**189.94**	**213.29**	**363.10**	**144.65**	**254.30**	**283.50**	**186.05**	**261.80**	**246.95**	**279.85**

The ethyl alcohol content of the studied red wines varied in the concentration range of 12.43 vol. % - 15.56 vol. %. This alcohol content meets the requirements for "dry wine" and is an indicator of sufficient and complete metabolic consumption of grapes sugars from yeasts.

Methyl alcohol has been identified practically in all wines. Only in variant V2 of the wine from Gamza clone 52-9-5 it was found in traces. The highest content of methyl alcohol was found in the wine variant V2 from the Kaylashky rubin variety - 105.40 mg/dm^3. Considering its concentrations in the different wine variants it can be observed that in the wines from the selected clones Gamza 52-9-4 and Gamza 52-9-5 there is a tendency of decrease in its levels between the two variants. In the case of Gamza clone 52-9-4 in variant V1 the methanol has been identified at content of 52.90 mg/dm^3 and a lower concentration of 37.20 mg/dm^3 was reported in the wine variant obtained with addition of enzyme - V2. The same trend was observed in the wine obtained from Gamza clone 52-9-5. In the other wines the trend was reverse. In the wine from Pamid clone 5/76, the methanol content in control variant V1 (43.20 mg/dm^3) was lower than the experimental (61.40 mg/dm^3). The same trend was observed in the wines obtained from Kaylashky rubin and Trapezitsa varieties.

The methyl alcohol is a normal product obtained as a result of maceration with solids. The concentrations of methyl alcohol in the wines found in this study were in full correlation with the range of its normal presence (up to 350.00 mg/dm^3) indicated by Chobanova (2012).

The gas chromatographic study identified 19 volatile compounds in the studied wines. There are 5 higher alcohols, 9 esters and 4 terpenes (terpene alcohols), which reflect the aroma of the wine. The highest total concentration of volatile compounds was established in the wine of variant V1 from Gamza clone 52-9-5 (363.10 mg/dm^3), followed by the wine variant V2 from Pamid clone 5/76 (283.50 mg/dm^3). The lowest content of volatile components was found in the wine variant V2 from Gamza clone 52-9-5 (144.65 mg/dm^3).

Regarding the presence of higher alcohols, higher levels of higher alcohols in the variant with added enzyme V2 (27.75 mg/dm^3) were observed in the wine from Gamza clone 52-9-4, compared to the control variant V1 (14.25 mg/dm^3). In the wine from Gamza clone 52-9-5, a reverse trend was observed - the content of higher alcohols was higher in the wine from control variant V1 (196.20 mg/dm^3) compared to the experimental variant V2 (107.60 mg/dm^3). It should be noted that in the wines from this clone was found to have higher quantities of higher alcohols than the other studied wines.

In the wine from clone Pamid 5/76, the total content of higher alcohols was similar, but with slightly higher concentration found in control variant V1 (103.75 mg/dm^3), compared to the experiment variant V2 (99.10 mg/dm^3).

In the case of red wine from the Kaylashky rubin variety, a reverse trend was found with the two-fold higher concentration of higher alcohols in the experimental wine V2 (48.35 mg/dm^3) compared to

the control V1 (21.75 mg/dm^3). The same trend of distinct variations in higher alcohols concentrations of variant V2 (73.65 mg/dm^3) versus control (28.15 mg/dm^3) was observed in Trapezitsa wine.

As can be seen from the results obtained, a positive tendency to increase in the higher alcohols concentrations with the addition of flavoring enzyme in Gamza clone 52-9-4, Kaylashky rubin and Trapezitsa varieties was observed. While in the wines from Gamza clone 52-9-5 and Pamid clone 5/76 the trend was reversed.

The resulting concentrations of higher alcohols are in correlation with the studies and variability ranges represent from other researchers (Ivanova et al., 2013; Velkov, 1996).

The esters have the most significant influence on the aromatic characteristics of the wines. The diversity of higher alcohols and acids in wines leads to a diverse ester composition in the aging process, which reflects in different aromatic nuances.

Considering the total ester content of wine variants from clone Gamza 52-9-4, there was a tendency in increase of esters content after the addition of enzyme in variant V2 (147.75 mg/dm^3) compared to the control sample (122.10 mg/dm^3).

A trend of increased content of esters was also observed in the wine of Pamid 5/76. In this wine variant V2, obtained with the addition of flavoring enzyme (122.95 mg/dm^3) has a higher ester concentration compared with variant V1 (107.00 mg/dm^3). The same trend was observed in the wine of Kaylashky rubin - from 79.15 mg/dm^3 esters in control variant V1 to a significant increase in the experimental variant V2 - 107.95 mg/dm^3.

In the wines from clone Gamza 52-9-5 and Trapezitsa grape variety, a reverse trend was observed. Lower levels of total esters were found in the experimental variants compared to controls.

The highest total concentration of esters was found in the red wine variant V1 of Trapezitsa variety - 180.30 mg/dm^3, followed by the wine variant V2 obtained from clone Gamza 52-9-4 - 147.75 mg/dm^3.

The results for total ester content in wines correlate with the data presented by Yankov et al. (2000).

The main, dominant ester, found in all wines studied was ethyl acetate. This ester of ethanol and acetic acid are present in concentrations of 30 - 300 mg/dm^3, and in young wines in quantities of 50 - 80 mg/dm^3 (Chobanova, 2012). In the present study, the quantities found do not exceed the range indicated for young wines. This reflects on its positive aromatic influence. In the samples of Pamid clone 5/76, Kaylashky rubin and Trapezitsa varieties, its concentration was higher in the V2 variants compared to the control V1. In the wines of clone Gamza 52-9-4 and clone Gamza 52-9-5, this trend was reversed. The highest concentration of this ester is obtained in the experimental variant V2 of the Trapezitsa wine (44.80 mg/dm^3). The lowest was the level of ethyl acetate in the sample of variant V2 of Gamza 52-9-4

clone (17.20 mg/dm^3). At concentrations lower than 150 mg/dm^3, the ethyl acetate contributes to the wine fruit aroma (Tao and Li, 2009).

Ethyl hexanoate was identified in wine of variant V2 of clone Gamza 52-9-4. This ester was also available in variant V2 of the Kaylashky rubin variety, in traces. It is important and contribute to the fruit character of wines (Li et al., 2008).

Hexyl acetate was identified in wines from variants V1 of clone Gamza 52-9-4 and Gamza 52-9-5. Also in the two variants of the wine of the Pamid clone 5/76. It is also identified in the sample of variant V2 of the Kaylashki rubin variety and in variant V1 obtained from Trapezitsa grape variety. This ester gives characteristic pear aroma (Peinado et al., 2004).

Phenylacetate was identified in the wines obtained from Gamza clone 52-9-4 and Kaylashky rubin. This ester gives a floral aroma to the wine (Guth, 1997).

Terpene alcohols were also identified in the studied wines. The wine with the highest total concentration of terpene alcohols was variant V1 of the clone Gamza 52-9-4 (0.69 mg/dm^3), followed by variant V2 (0.59 mg/dm^3) from the same clone. The remaining wines have a very low content of terpene alcohols. With the highest concentration was found geraniol (0.64 mg/dm^3) in variant V1 of Gamza clone 52-9-4. This terpene was also found in the wine samples of the Kaylashky rubin and Trapezitsa varieties. The aroma of rose is the characteristic nuance it gives to (Hoffman et al., 1973).

β-citronelol was found in the highest amount in variant V2 of clone Gamza 52-9-4 (0.47 mg/dm^3). This terpene was also available in the wines of the Kailashky rubin and Trapezitsa varieties. The flavor that gives this terpene are lily and coniferous shrub (Lengyell, 2012).

Nerol was found in traces in wines from Gamza 52-9-5 and Pamid 5/76, and α-terpineol was found only in wine of V1 variant from the Kaylashky rubin variety.

Conclusions

1. The GC-FID study of red wines obtained from newly selected clones and varieties of vines identified a total of 19 volatile compounds. Of these, 5 higher alcohols, 9 esters and 4 terpene alcohols have been found to be aromatic.

2. Methyl alcohol was identified in all wines. It was in quantities typical for red wines.

3. The highest total concentration of volatile compounds was found in wine variant V1 of clone Gamza 52-9-5 (363.10 mg/dm^3) and the lowest in wine variant V2 obtained from the same clone (144.65 mg/dm^3).

4. There was a tendency for increasing of higher alcohols content after addition of flavoring enzyme at the wines from Gamza 52-9-4, Kaylashky rubin variety and Trapezitsa variety. In contrast,

in the samples of Gamza 52-9-5 and Pamid 5/76 a reverse trend was observed - a higher content of higher alcohols in the control variant.

5. The ester composition was diversed. A trend of increased ester content after addition of flavoring enzyme was observed in Gamza 52-9-4 clone, Kaylashky rubin and Trapezitsa varieties. At clones Gamza 52-9-5 and Pamid 5/76, the trend was reversed. The dominant ester was ethyl acetate. Its concentrations were normals for young wines. It exerts a positive influence on the aromatic profile.

6. The highest content of terpene alcohols distinguishes wine of variant V1 from clone Gamza 52-9-4 (0.69 mg/dm^3). With the highest concentration geraniol was identified. The wines of clone Gamza 52-9-4 have the highest total terpenes content compared to the others where they were low.

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Evaluation on Allelopathic Potential of Velvet Bean (*Mucuna cochinchinensis*) on Germination of Goosegrass (*Eleusine indica* L.)

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Abstract: The experiment was conducted at the Toxicology laboratory, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Malaysia in 2013. Allelopathic potential of aqueous methanol and water extracts of *M. cochinchinensis* leaves, seed and root was investigated on seed germination and seedling growth of goosegrass and biotest crop species: lettuce (*Lactuca sativa*). The treatments consisted of five concentrations (100, 75, 50, 25, 0 %); plant parts (leaves, seed, root) and extraction solvents (methanol, water) were replicated three times and arranged as a completely randomized block (CRD) design. Germination, radicle and hypocotyl growth of all test plant species were inhibited at concentrations (100, 75, 50 and 25%). The total germination percentage was lowest with the methanolic extracts of leaf, seed and root of *M. cochinchinensis* at 100 % concentration in the order of 0.00, 30.66 and 4.66%, respectively. Concomitantly, the radicle length inhibition percentages of methanolic extracts at higher concentration of 100% were 100, 88.5 and 94.4% of the leaf, seed and root extracts, respectively. The water extracts recorded the highest germination percentage and lower inhibitory activity of the radicle and hypocotyl length. The study confirmed plant growth-inhibitory compounds of *M. cochinchinensis* is dependent on the extraction solvents and extract concentrations as expressed that methanolic solvent at higher extract concentration had the stronger inhibitory activity.

Keywords: *Allelopathy, Velvet bean, Concentration, Goosegrass, Inhibition, Weed control.*

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INTRODUCTION

The current trend of organic farming and natural agriculture getting a pride-of-place to counteract heavy reliance on agrochemical such as pesticides and inorganic fertilizers in conventional agricultural production has led to the study of allelopathy. Allelopathy is defined as "a beneficial or detrimental effect from a donor plant to the recipient by chemical pathway" (Rice 1984). This development has encouraged the exploitation of phytotoxic activity of allelopathic plants in weed management through naturally occurring plant chemicals as a sustainable strategy that reduced concerns about pesticide residues in food, environmental pollutions, harmful side-effects on human health and the evolution of herbicide resistant weed biotypes. This is particularly important in sustainable agriculture where the use of synthetic herbicides is prohibited.

Several plant based herbicides such as mesotrione and glufosinate were originally derived from allelopathic compounds (Coloquhoun, 2006). The compounds involved in allelopathic interference being secondary plant metabolites include a host of phenolics- flavonoids, coumarins, terpenoids and organic acids from diverse botanical families (Céspedes et al., 2006, 2013; Muñoz et al., 2013), which could be considered to exhibits either inhibitory or stimulatory role in the plant environment depending on the concentration of the compounds. Allelochemicals are typically known to suppress germination, causing dysfunctions of root and seedling growth. More importantly, they alter several plant physiological processes, such as water utilization, mineral uptake, photosynthesis, cell morphology, membrane permeability, protein synthesis and enzyme activities, among countless others (Weir et al., 2004).

Velvet bean (*Mucuna cochinchinensis*), a non-edible and wild tropical legume is grown generally for its beneficial nitrogen-fixing potential with nodulating N_2-fixing rhizobial bacteria. It produces high amount of aromatic non-protein amino acid, L-3,4-dihydroxyphenylalanine (L-Dopa) (Fujii, 1991). Previous studies have demonstrated that intercropping subsistence maize with velvet bean potentially reduces *Striga* parasitism and smothers speargrass (*Imperata cylindrica*) with concomitant yield increases, improved soil nitrogen and reduced soil erosion (Avav et al., 2008; Nwaichi and Ayalogu 2010; Akal et al., 2012). Adaptable strategies using allelopathic plants in crop rotation, as mulches, residues, cover crops and companion crops have been utilized to contained the effects of noxious weeds in the farming systems of the south, south-east Asia and sub-Saharan Africa. *Mucuna, Desmodium, Pueraria,* and *Stylosanthes* species are widely used in between row crops of rubber and oil palm in Malaysia and cereal field crops endemic to *Striga* parasitism in Africa to smother weeds while at the same time fixing nitrogen to the soils (Shaharuddin and Jamaluddin, 2007; Hooper et al., 2010). Besides improving soil fertility by fixing nitrogen and making it available to the main crop and reduce competition from noxious weeds, it also improves palm growth and reduces the immaturity period (Hasnol et al., 2012).

The current level of our understanding of *mucuna* is that it fixes atmospheric N through a symbiotic relationship with soil microorganisms - making the plant an efficient source of N. In addition, it was observed that few other weeds cohabitate with velvetbean in the tropics, and those that grow along with it are usually found in limited amounts and are generally along the fringes of a field (Ibrahim, personal observation). Nowadays research is being geared up to exploit the interface between chemical dynamics in this underutilized legume that exhibits allelopathic potential and biological control of weeds to contribute to sustainable weed management in agro-ecosystem. Therefore, the objective of this research was to utilize the allelochemicals in different plant parts of *Mucuna* - leaf, seed and root for its effects on the germination of common test species (lettuce) and goosegrass assayed under laboratory conditions.

Materials and Methods

Allelopathic effect of methanol and water-soluble extracts from velvet bean on seedling germination and growth

Mature velvetbean plants that were grown in the glass house of Faculti Pertanian, Universiti, Putra Malaysia in 2013 were harvested and separated into leaves, seed and roots. These plant portions were thoroughly washed and rinsed with distilled water, oven-dried at 50 ^{o}C for 72 hours, ground with a Wiley mill in order to pass through a 1-mm screen mesh, and stored in a refrigerator at 4 ^{o}C until required. The dried leaves, seed and roots were extracted by soaking 0.5 kg in 1l of methanol and distilled water to generate two fractions from each part and placed on a shaker for 48 hours at room temperature. The aqueous extracts were filtered through four layers of cheese cloth to remove the fiber debris and then filtered once again through a filter paper (no. 1; Whatman International, Maidstone, UK). Each extract was dried *in vacuo* on a rotary evaporator at 45 ^{o}C and then weighed. The methanol and water-extracted fractions were redissolved with 100 ml of sterile distilled water. The final concentration of each extract was 50 g/l**.** The aqueous solutions were described as 100 % and distilled water was added to the solutions to make different dilution (75, 50 and 25 %). The pH of the extracts ranges from 6.02 to 6.56. Extracts were stored in a refrigerator at 8 ^{o}C until further used for bioassay tests.

Goosegrass (*Eleusine indica*) was used as representative weed species because of its noxious effects in arable crop production. Lettuce (*Lactuca sativa*), was selected as a general biotest specie because it is frequently used as a model specie in allelopathic bioassay (Macìas et al., 2000). The seeds were surface- sterilized with 1.5 % (v/v) sodium hypochloride for 1 minute before they were washed (three times) with sterile distilled water. Empty and undeveloped weed seeds were discarded by floating in tap water. The seeds of goosegrass were treated with solution of KNO_3 (0.2 %) for 48 hours in the dark to break their dormancy and washed many times with distilled water. Glass petri dishes (9 cm diameter) were used and underlain with two sterile filter papers (Whatman No. 2). Ten seeds of lettuce

and fifty seeds of googegrass were placed in the petri dishes to which 4 ml of each extract solutions of varying concentrations were added. Sterile distilled water was used as the control. The petri dishes were sealed with paraffin wrappers to prevent water loss by evaporation and to avoid contamination. The petri dishes were kept in an incubator at 28 ^{o}C for one week. The experiment was laid out as a 2 x 2 x 5 factorial in a completely randomized design with 3 repetitions. Germination was considered to have occurred as the rupture of the seed coat and the radicle protrusion beyond the seed coat by at least 1 mm.

The total germination (TG) was determined, as described by Siddiqui (2007), and the percentage inhibition (1 – Lt/Lc) x 100, Lt = radicle length of the germinated seeds exposed to treatment, and Lc = radicle length of control germinated seed) computed. All data were subjected to ANOVA and statistically analyzed by using a one-way ANOVA in JMP SAS statistical software (v. 9; SAS, Cary, USA) and the Tukey-Kramer HSD test was used to determine the differences between the treatment means at the 5 % probability level.

Results and Discussion

The inhibitory effect of both the methanol and water extracts on the total seed germination and radicle inhibition depended on the extract concentration and the plant species. For *L. sativa*, the seed germination was completely inhibited by the *M. cochinchinensis* root and leaves extracts at 75 and 100 % concentrations with lower inhibition as the concentration decreased (Table 1), which significantly affected the radicle inhibition of the plant. Both seed germination and radicle inhibition were less sensitive to the seed extract at different concentration when compared with the leaves and root extracts.

Table 1. Effects of methanol extract from different parts of on germination and seedling growth of *L. sativa*

Concentration (%)	Total germination (%)	Radicle Length (cm)	% Radicle inhibition	Hypocotyl length (cm)
Leaves				
0	100a	6.66a	0.00	2.49ab
25	63.33b	5.09ab	23.57	3.01a
50	20.00c	3.59b	46.10	2.53ab
75	6.67c	0.32c	95.52	1.30bc
100	0.00c	0.00c	100.00	0.00c
SE±	4.94	0.35		0.33
F-ratio	74.00	68.02		13.85
Prob> F	<.0001	<.0001		0.0004
Seed				
0	100a	6.66a	0.00	2.49ab
25	76.69ab	6.00a	9.91	2.79a
50	53.33bc	3.98b	40.24	2.23abc
75	40.00c	3.69b	44.59	2.04bc
100	30.00c	2.56b	61.56	1.82c
SE±	6.15	0.42		0.14
F-ratio	21.32	16.43		7.82
Prob> F	<.0001	0.0002		0.0040
Root				
0	100.00a	6.66a	0.00	2.49a
25	26.67b	3.43b	48.50	2.22a
50	3.33c	0.27c	95.95	0.17b
75	0.00c	0.00c	100.00	0.00b
100	0.00c	0.00c	100.00	0.00b
SE±	4.22	0.63		0.38
F-ratio	103.25	21.81		11.02
Prob> F	<.0001	<.0001		0.0011

Values in the column with same letter are not significantly different at P<0.05.

Although the aqueous extracts showed lower inhibition of germination and seedling growth when compared to the methanol aqueous extracts, there was significantly lower germination and subsequent inhibition (Table 2). The results of our findings collaborated earlier reports enunciated by Fujii et al. (1991) that velvetbean (Mucuna pruriens) increased the graminaceous crop yield in mixed culture, but smother the growth of noxious weeds such as nutsedge (Cyperus spp.) and cogongrass (Imperata cylindrica).

Table 2. Effects of water extract from different parts of M. cochinchinensis on germination and growth of L. sativa

Concentration (%)	Total germination (%)	Radicle Length (cm)	% Radicle inhibition	Hypocotyl length (cm)
Leaves				
0	100.00a	6.66a	0.00	2.49ab
25	96.97a	5.83ab	12.46	3.94a
50	53.33b	5.07ab	23.87	3.64a
75	50.00b	2.75b	58.71	3.39a
100	20.00c	2.41b	63.81	1.00b
SE±	5.57	0.76		0.42
F-ratio	37.04	6.15		7.99
Prob> F	<.0001	0.0092		0.0037
Seed				
0	100.00a	6.66a	0.00	2.49a
25	96.67a	5.94ab	10.81	2.60a
50	86.67ab	5.33b	19.97	2.92a
75	63.33bc	3.07c	53.90	2.54a
100	50.00c	2.88c	56.76	1.09b
SE±	5.16	0.38		0.20
F-ratio	17.79	20.35		12.18
Prob> F	0.0002	<.0001		0.0007
Root				
0	100.00a	6.66a	0.00	2.49ab
25	86.67a	4.47b	32.88	3.47a
50	56.67b	3.17bc	52.40	4.17a
75	43.33b	2.57bc	61.41	2.40ab
100	13.33c	2.03c	69.52	1.22b
SE±	5.96	0.46		0.47
F-ratio	33.59	16.15		5.76
Prob> F	<.0001	0.0002		0.0114

Values in the column with same letter are not significantly different at P<0.05.

Similarly, the methanol leaf extract (100, 75, 50, and 25 %) significantly suppressed the germination and radicle growth of E. indica (Table 3), while the root extract of M. cochinchinensis significantly decreased the germination at the 100% and 75% concentrations. The radicle and hypocotyl also responded to the different levels of concentration of the extracts of M. cochinchinensis as expressed with corresponding decreased in length. However, aqueous seed extract did not significantly inhibit the germination of E. indica at 0, 25, 50 and 75 % concentration except at 100 % concentration (Table 4). There was significant inhibition of the radicle length at any of the tested concentrations. The two plant parts (seed and root) extracts irrespective of the extraction solvent (methanol and water) did not differed

significantly on the hypocotyl length. However, the leaf extracts of the methanol and water solvent recorded significant difference with respect to the hypocotyl length.

Generally, the level of inhibition of seed germination and radicle length decreased were increased with the increasing concentration of the extracts. At the highest extracts concentration of 100 %, both methanol leaves and root extracts completely inhibited the germination and radicle length of L sativa and E. indica, indicating their suppressive effects on the seed and seedling growth at higher concentration. The increasing inhibitory rate with the increasing concentration was in accordance with previous reports (Fujii, 1991; Chon et al., 2003; Meksawat and Pornprom, 2010; Hussain et al., 2011) for other allelopathic species.

Table 3. Effects of methanol extract from different parts of *M. cochinchinensis* on germination and growth of *E. indica*

Concentration (%)	Total Germination (%)	Radicle Length (mm)	% Radicle inhibition	Hypocotyl length (mm)
Leaves				
0	76.00a	13.90a	0.00	35.00a
25	32.00b	6.43b	53.74	3.67b
50	18.00b	0.25c	98.20	3.00b
75	2.00c	0.00c	100.00	0.00b
100	0.00c	0.00c	100.00	0.00b
SE±	3.35	0.96		1.78
F-ratio	85.96	40.98		71.32
Prob> F	<.0001	<.0001		<.0001
Seed				
0	76.00a	13.90a	0.00	35.00
25	77.33a	9.96ab	28.35	36.00
50	37.33b	4.73bc	65.97	34.33
75	35.33b	2.97bc	78.63	33.33
100	30.66b	1.60c	88.49	36.33
SE±	2.94	1.14		2.57
F-ratio	62.73	20.37		0.23
Prob> F	<.0001	<.0001		0.9169
Root				
0	76.00a	13.90a	0.00	35.00
25	36.00b	2.72b	80.43	32.00
50	24.00b	1.99b	85.68	35.00
75	24.00b	1.57b	88.71	25.33
100	4.66c	1.33b	90.43	28.00
SE±	3.66	0.84		2.80
F-ratio	52.58	41.12		2.37
Prob> F	<.0001	<.0001		0.1226

Values in the column with same letter are not significantly different at P<0.05.

Table 4. Effects of water extracts from different parts of *M. cochinchinensis* on germination and growth of *E. indica*

Concentration (%)	Total germination (%)	Radicle Length (mm)	% Radicle inhibition	Hypocotyl length (mm)
Leaves				
0	76.00a	13.90a	0.00	35.00
25	54.00b	8.00b	42.45	23.33
50	32.67c	7.70b	44.60	30.67
75	11.33d	5.18b	62.73	34.33
100	4.67d	4.37b	68.56	35.00
SE±	3.50	0.91		2.72
F-ratio	71.84	16.78		3.37
Prob> F	<.0001	0.0002		0.0542
Seed				
0	76.00a	13.90a	0.00	35.00
25	74.00ab	11.43ab	17.77	31.67
50	70.00ab	10.03ab	27.84	34.00
75	56.00bc	6.03c	56.62	32.67
100	38.67c	5.50c	60.43	33.00
SE±	3.98	1.44		1.94
F-ratio	15.49	6.17		0.43
Prob> F	0.0003	0.0009		0.7825
Root				
0	76.00a	13.90a	0.00	35.00
25	46.00b	7.47b	46.26	31.00
50	32.67b	3.67b	73.60	30.67
75	14.00c	3.17b	77.19	34.67
100	9.33c	3.03b	78.20	31.67
SE±	3.75	1.17		1.86
F-ratio	51.72	15.76		1.24
Prob> F	<.0001	0.0003		0.3546

Values in the column with same letter are not significantly different at P<0.05.

Conclusion

The results from this study showed that velvet bean possesses a strong allelopathic potential and exhibits strong inhibition of goosegrass germination in the bioassay. It revealed that the inhibition was concentration and extraction solvent-dependent. The inhibitory magnitude of the plant laef was greater than the root and stem and the extent of inhibition of the plant parts was proportional to the increase of applied concentration.

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Evaluation of Different Isolates of Entomopathogenic Fungi against *Metopolophium dirhodum* (Walker) (Homoptera: Aphididea) from Constantine, Algeria

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Abstract: The aim of the present study was to investigate the effect of seven entomopathogenic fungi *(Aspergillus californicus, Beauveria bassiana, Fusarium oxysporium, Metharizium flavoride, Cladosporium cladosporioides, Trichoderma viride and Verticillium alfalfae*) against aphid insects: *Metopolophium dirhodum.* The selected entomopathogenic fungi were isolated from the agricultural soil of the National Institute of Plant Protection of Constantine, Algeria, and were tested against aphid insects that were gathered from the same area. The aphids were exposed to each fungal spore suspension (10^7 conidia/ml) for 10 seconds. The viability/mortality of the insects was evaluated on the 1st, 3rd, 5th, and 7th day after inoculation. After 7 days of inoculation, all the fungi species, except *F. oxysporum,* presented a significant effect ($P < 0.05$) against the studied aphid. The mortality rate was estimated between 21 and 96%. *B. bassaian, C. cladosporioides* and *V. alfalfae* presented the most potent effect on *M. dirhudum* with a percentage above 50% (95.83, 63.98 and 51.83%, respectively). *A. californicus* and *M. flavoride* showed the same effect: 41.97%. *T. viride* and *F. oxysporium* had the lowest effect with 31.44% and 20.83%, respectively. The inter/intra specificity of the fungi was mostly reported, besides other factors, as the modulator of their effectiveness.

Keywords: *Entomopathogenic fungi, M. dirhodum, Mortality rate.*

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INTRODUCTION

The aphids are the most harmful insects affecting wheat plant *Diuraphisnoxia* (Mordvilko), *Sitobionavenea (F.), Schizaphis graminium* (Rondani), *Rhopalosiphumpadi* (L.), *Metoplophium dirhodum* (Walker), and *Siphamydis* (Passerini) are considered as the most important insects that depreciate substantially cereal cultures (Rassipour et al., 1996; Blackman and Esatop, 2000). The rose grain aphid (*M. dirhodum*) is the most important species that could destroy grain worldwide (Dixon,1987), in fact, they choose hazardly a host during the cold season and then emigrate towards drought cereals in summer (Carter et al., 1980). For instance, *M. dirhodum* insect is able to transmit a *Luteovirus*, and it provides a wide resistance against exterminators (Carter et al., 1980). Such situation compels us to focus on methods that can stop these insect pests and consequently disease deployment. The biocontrol using entomopathogenic fungi, has been recently considered the most effective method. Moreover, these fungi are important through their aptitude to fight against exterminators by invading insect crusts. These opportunities make them as the first candidate against this plant culture epidemic (Lacey and Goettel, 1995; Barta and Gagan, 2006).

The most common fungal species belong to the order of Entomophtorales. Entomopathogenic fungi such as *B. bassiana* (Balsamo) and *Peacilomyces ssp*. showed a promising level of activity against aphids (Milner, 1997). The purpose of the present study is to determine the most effective fungi between the studied seven fungal species against *M. dirhodum*, which is the most widely spread aphids in the culture region.

Materials and Methods

Insect rearing

Metopolophium dirhodum (Walker) were collected from a wheat called Cirta HD.122 (*Triticum eastivum* L.) of the National Institute of Plant Protection' suburb (INPV) of Constantine, Algeria.

Fungal isolates

The virulence of seven fungal isolates (Aspergillus californicus, Beauveria bassiana, Fusarium oxysporium, Metharizium flavoride, Cladosporium cladosporioides, Trichoderma viride and Verticillium alfalfae) was tested with a pathogenicity test. The fungi used in this study were obtained from a private collection from agricultural soil of the INPV and then they were isolated.

One gram (1g) of the soil was diluted in 9ml of distilled water. Then 100µL of dilutions which are 10^{-3}, 10^{-4}, 10^{-5} was taken and planted in Potatoes Dextrose Agar (PDA: 200 g potatoes, 20 g D. Glucose, and 20 g Agar) that was supplemented by Chloramphenicol (10mg/l). The dishes were incubated for two weeks at 28°C. The macroscopic and microscopic of any resultant fungal growth were compared in terms of taking into account the standard description of mycelium and spore.

Bioassay

The aphids were attacked for 10 seconds in each fungal suspension prepared at the concentration of 1.10^7 conidia/ml of sterile distilled water and supplemented with a drop of Tween 80 (0.05%). Dishes were kept at room temperature at 25°C and humidity Aw=60%. The viability/mortality of the insects were evaluated on the 1st, 3rd, 5th and 7th day after inoculation day. All the treatments were repeated six times.

Statistical analysis

The calculation of insect mortalities was corrected using Abbot's (1925) calculation method "Formula percent mortality rates". The data were subjected to one-way ANOVA and significant differences were detected. Furthermore, multiple comparisons were made through Tukey's HSD using SPSS ® for data analysis (version 17.00 Software. 2006 SPSS.Inc.Chicago.il, USA).

Results and Discussion

At 7 days after application, the effect of the isolated fungi against the aphid insect *M. dirhodum* varied widely and ranged between 21% and 96%. The results revealed that *B. bassiana* had the most effective toxicity with 95.83%, followed by *C. cladosporioides* with 63.98%, then *V. alfalfae* with 51.83%. *A. californicus* and *M. flavoride* presented approximately the same effect with 41.97%. Compared to the other fungal species, the effect of *T. viride* and *F. oxysporium* against *M. dirhodum* were the lowest with 20.83% and 31.44%, respectively (Fig. 1).

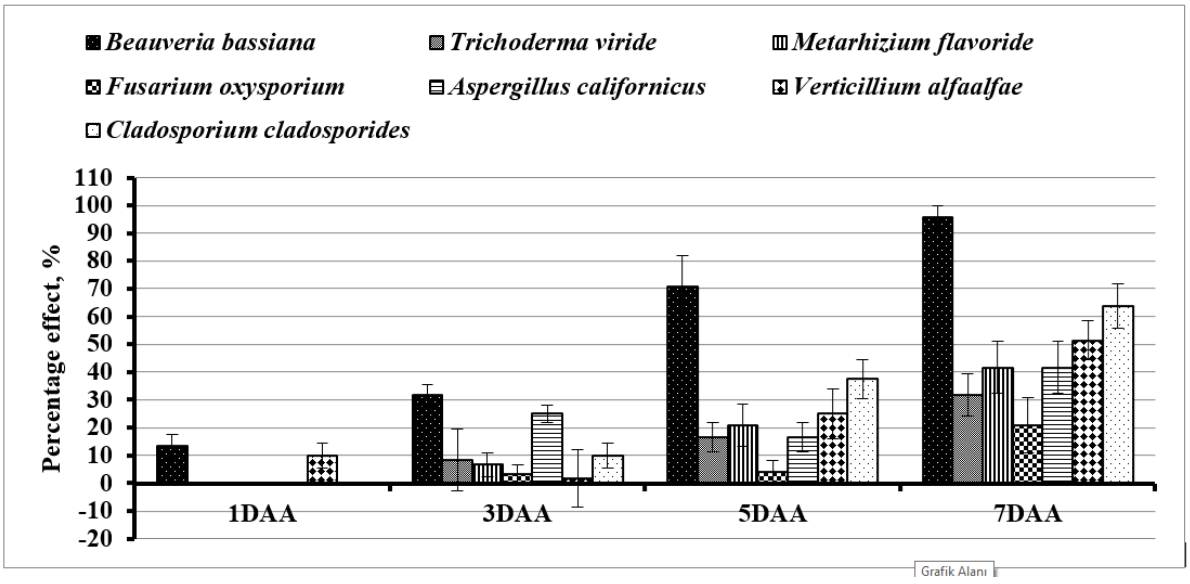

Figure 1. Effect of fungi on *M. dirhudum* (%)

The death rate presented in our study by the density of the aphids after application revealed in the same way, that *B. bassiana* presented the most significant fatal effect at all times of treatment ($P < 0.05$) compared to the control and to the other fungal species. Its effect began on the first day of application

with a death rate of 13.33%. In contrast, *T. viride* had no pronounced effect compared to the control at all times of incubation, it did not affect the aphids at the third day after application with only 6.6% of death rate. In addition to *B. bassiana, A. californium* presented significant toxicity against *M. dirhodum* (3.50; $P < 0.05$) on the third day of application, thereby *C. caladosporioides* had exercised significant fatal effect beside *B. bassiana* with 2.33 ($P < 0.05$) on the fifth day after application. However, after the seventh day, all fungal species, except *F. oxysporium* presented significant fatal effect against the aphid compared to the control (Table 1).

Table 1. Effect of *M. dirhodum* (Walker) population density before and after the fungal suspension application

	1DAA	**3DAA**	**5DAA**	**7DAA**
Aspergillus californicus	0.00 ^{b}	25.00 ± 3.16 ^{ab}	16.67 ± 5.27 ^{bc}	41.67 ± 9.38 ^{bc}
Beauveria bassiana	13.33 ± 4.22 ^{a}	31.67 ± 3.80 ^{a}	70.83 ± 10.92 ^{a}	95.83 ± 4.17 ^{a}
Fusarium oxysporium	0.00 ^{b}	**0.83** ± 5.83 ^{b}	4.17 ± 4.17 ^{c}	20.83 ± 10.03 ^{c}
Metharizium flavoride	0.00 ^{b}	4.33±0.21 ^{ab}	(2.83±0.17^{ab}	2.00±0.26^{bc}
Cladosporium cladosporioides	0.00 ^{b}	10.00 ± 4.47 ^{ab}	37.50 ± 7.05 ^{b}	63.89 ± 7.59 ^{ab}
Trichoderma viride	0.00 ^{b}	6.67 ± 4.22 ^{ab}	20.83 ± 7.68 ^{bc}	41.67 ± 9.38^{bc}
Verticillium alfalfae	10.00 ± 4.47 ^{ab}	1.67 ±10.38 ^{ab}	37.50 ± 7.05 ^{bc}	63.89 ± 7.95^{bc}
P.value (P ≤ 0.05)	0.000	0.000	0.000	0.000
S.E.M	0.062	0.23	0.21	0.26

1DAA: 1st day after application; **3DAA**: 3rd day after application; **5DAA**: 5th day after application; **7DAA**: 7th day after application; **P**: Probability; a. b, c, means with different superscripts letters within the same line are significantly different according to Tukey's HSD multiple range test ($P < 0.05$). **S.E.M**: Standard Error of Mean.

The pathogenic effect of fungi against insect's pest populations has been observed for a long time (Butt et al., 1994; Hesketh et al., 2008; Freed et al., 2012). Several fungi species were developed as biological control agents for aphids (Shah and Pell, 2003; De Faria and Wraight, 2007). The rose grain aphids *M. dirhodum* (Walker) (Hemiptera: Aphididea), is one of the most serious species found in almost all grain–producing regions of the world. *M. dirhodum* is a vector of *Luteovirus* and barley yellow dwarf **(**Carter et al., 1980).

The development of aphid colonies is highly threatened by the presence of entomopathogenic fungal species considered as their most important cause of death as reported by Remaudière et al. (1981). Hence, more than 750 fungal species were reported as a potent agents against insects propagation, their ability to modulate the insects population was well discussed in literature (McCoy et al., 1988; Gillespie and Moorhouse, 1989). The most studied fungal species in the biocontrol of aphids are ascomycetes belonging to the order of *Hypocreales* (*Beauveria, Metarhizium, Nomura, Verticillium et Peacilomyces*). *Beauveria bassianan* and *Metarhizium anisopliae* were considered as the most effective ones regarding

the recorded high mortality rate (epizooties) **(**Burges, 1981; Carruthers and Soper, 1987; McCoy et al., 1988).

In our study, *B. bassaian*, *C. cladosporioides* and *V. alfalfae* presented the most potent effect on *M*. *dirhudum* with a percentage above 50% (95.83, 63.98 and 51.83%, respectively).

In contrast, *Aspergillus californicus* and *Metarhizium flavoride* showed an equal effect estimated by 41.97%. Finaly, *Trichoderma viride* and *Fusarium oxysporium* showed the lowest effect with 31.44% and 20.83%, respectively.

B. bassiana have killed 90% of the aphids after 3 days of application and these results agreed with those of Mburu et al. (2009) who reported that *B. bassiana* developed a considerable activity against *M. dirhudum*. It has been shown, in the same context, that this species presented a large spectrum and strong virulence, affecting the host with a simple contact action. Its safety for the vertebrate make it used, in the same case, in classical biocontrol by introduction technique (Meyling and Eilenberg, 2007).

Kim et al. (2013) found that among 47 cultures of *B. bassiana*, Bb08 showed the highest mortality (78%) against green peach aphid three days after treatments.

The significance of *Cladosporium spp*. as one of the effective biological control agents against whiteflies, aphids and scale insects in the world have been reviewed (Roberts and Humber, 1981; Hulden, 1986; Pan et al., 1989; Humber, 1991; Thumar and Kapadia, 1994; Han *et al.*, 1997; Abdel-Baky et al., 1998). *C. cladosporioides* exercised significant fatal effect beside *B. bassiana* with 63.93% ($P < 0.05$) after the seventh day of application.

Saranya et al. (2008) showed that the conidial suspension of *C. oxysporum* generated corrected mortality of 77.5% in *Aphis craccivora* Koch individuals, when the suspension was used at a concentration of 10^8 conidia/ml incorporated in the Teepol solution. However, Bensaci et al. (2015) revealed that maximum mortality of *C. oxysporum* against *A. fabea* (67.96%) was obtained with conidial suspensions at a concentration of 10^8 conidia/ml.

In addition, Abdel-Baky and Abdel-Salam (2003) showed in a laboratory test, that maximum mortalities of *A.gossypii* (37.5%) and *A. craccivora* (38%) were recorded on the third day after application with conidial suspensions of *Cladosporium* spp. at a concentration of 10^6 conidia/ml.

In the present study, the results describing the effect *V. alfalfae* on aphids were similar to those reported by Chavan et al. (2008) who showed significantly higher efficacy controlling aphids with 68.23% to 89.54% mortality of *V. locanii* .

The effect of *M. flavoride*, registered at 7 days after application (41.67%) was inconsistent with the average effect with 60-86% reported by Murerwa et al. (2014) in their study on the effect of *M.*

anispliae on the aphids *M. dirudum* and *R. padii*. Similar results were reported by Won et al. (2015) on green peach aphids (*Mysuspersicae*).

In the same level, *A. californicus* has registered an effect (below 60%) consistent with that reported by Won et al., (2015). *Aspergillus* species displayed a wide diversity of lifestyles including in clinical, industrial and agricultural environments; some of them may be opportunistic pathogens of a wide range of organisms including agricultural pests (Gibbons and Rokas, 2012). It has been reported through toxicity test that *Aspergillus* species could be useful in aphid control as pest control agents. However, these saprophytic fungi are not targeting only insects, but can also affect immune depressed humans, mammals and birds (Tell, 2005). Non-aflatoxin-producing and non-toxigenic *A. flavus* strains are currently studied in biological control to reduce pre-harvest contamination of crops with aflatoxin (Ehrlich, 2014).

As a part of selection process, five different concentrations of *Aspergillus clavatus* (Desmazie`res), *Aspergillus flavus* (Link) and *Metarhizium anisopliae* ((Metschnikoff) Sorokin) spora were tested against the pea aphid, *Acyrthosiphon pisum* (Harris). *Aspergillus* isolates induced higher mortalities than *M. anisopliae,* which is well known as entomopathogen in the literature (Seye et al., 2014).

Our results showed that *T. viride* presented mortality rate estimated at 31.94%. These results were completely different in comparison to those reported by Ganassi et al. (2001) who registered a massive effect (100%) after 3 days of application of *Trichoderma* against three varieties of *Schizaphis graminum* isolated from a rice plant. The same results were reported by Omar et al. (2012) on *Cockrociches periplaneta* (American insect) and *Pestalotia psidii*, respectively. These reports indicated that the effectiveness of the fungi depended on their metabolites capacity to stimulate the aphids infection.

About *Fusarium*, the rate of mortatlity registered in our study was inconsistent with that obtained by Guesmi-Jouani et al. (2010) who reported a fatal rate exceeding 90%. The effectiveness of *Fusarium* species was high but particularly reported on *Aphids gossypii Glover* species (Ganassi et al., 2001). The selection of entomopathogenic fungi for biological control of insects remains difficult. It depends on the insect‘s susceptibility and vulnerability towards fungus and consecutively the inter- and intra specificity of each fungus against the insects (Glare et al., 2012).

Conclusion

Under the present experimental conditions, the results revealed that *B. bassiana*, had infection ability unlike *M. falvoride*, that showed a rate less than 50% even if it was the most important species used in the biocontrol against insects. *C. cladosporides* and *V. alfalfae* encouraging results were obtained, regarding *T. viride* and *F. oxysporium* that had rates with 31.94% and 20.89 %, respectively. Even though, these two species are very important in biocontrol against insects. In conclusion, the

present study revealed that a specialization could be found to every kind of fungi against a specific insect (interspecific and intraspecific).

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Milk Quality and Food Practices in Dairy Cattle Farming in the Semi-arid Region of Setif

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Abstract: The purpose of this work was to bring out the diversity of milks produced in the semi-arid Algerian Setif area and link it to the practices of pastoralists mainly in the food sector. In 24 dairy farms, representing different feeding strategies, a breeding follow-up detailing the ways of driving cows was adopted. In parallel, a seasonal analysis of the physicochemical and microbiological characteristics of 144 mixed milk samples (6 samples per season /spring, summer/ and per farm during two passages) in 24 farms was carried out. Milk quality parameters were highly variable and generally satisfactory. The physicochemical composition of the milks could be described as average for the majority of the samples, and marked a remarkable normativity. The majority of the farm milk samples displayed average fat content compliant. It was below 35 g/l in only 21.52% of the samples and showed significant fluctuations during the summer season, ranging from 31 to 41.7 g/l. Seven farms had average contents of above 35g/l for both periods. Variations in the butter fat between the different farms could be explained by the production and eating behavior strategies adopted by each farm. The protein content recorded in both seasons appeared much more stable than the fat content of all the milk collected. The average protein level for the 24 farms was 34.21g/kg. However, 8.33% of the milk samples in spring and 12.5% of those collected in summer had levels considered insufficient (less than 33g/kg). The microbiological results were highly variable with average counts of total aerobic mesophilic microflora exceeding the maximum standard of 105 CFU/ml. Hygienic quality was a concern for all milk samples despite the variety of situations. The typology of milk samples allowed to describe the diversity in milk quality based on variations in the levels of useful materials and fluctuations in total microflora.

Keywords: *Dairy cattle, Raw milk, Diversity, Physicochemical quality, Hygienic quality, Typology.*

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INTRODUCTION

Milk is a biological food with obvious nutritional value (Faye and Loiseau, 2000). It is considered to be a major animal protein source with a vital role in human nutrition. Due to its high consumption by the Algerian population (more than 147 l /inhabitant/year in 2012), it is highly strategic (Makhlouf et al., 2015). National milk requirements are estimated at 4.5 billion liters, while national production covers only about 40%. Domestic supply is still largely assured by imports (18%) and 63% of the total food bill in 2014, the equivalent of $ 2.05 billion (Makhlouf and Montaigne, 2017).

The interest in the development of local milk production thus becomes a priority for the public authorities. Also, because the milk production in Algeria is 80% assured by the cattle herd (Kacimi El Hassani, 2013), the development of dairy cattle breeding thus constitutes an essential component of the dairy policy of the Ministry of Agriculture. However, in Algeria, this breeding continues to be subject of a set of constraints that hinder its growth.

The production system suffers from the limited technical level of the breeders, associated with the climatic and organizational obstacles. In addition to these aspects, the lack of water resources and land induces a low recourse to fodder crops, which explains the low productivity of livestock (Madani and Mouffok, 2008). Furthermore, the low specialization does not allow the often imported genetic material to express its potentialities.

The low hygienic and sanitary mastery combined with a faulty supply contribute to obtaining a product of average or poor quality which affects the conversion rates by the dairy factories (Aggad et al., 2009) and consequently the rate of integration of raw milk. Among the measures that can boost the development of the dairy sector, the improvement of the raw milk quality is an unavoidable necessity as long as it improves processing yields or milk availability. On the other hand, improving sanitary and nutritional quality strengthens food and safety. Many studies have been done on the factors of variation of milk production. However, little research links the overall quality of raw milks with all production conditions that is to say with all farming practices (Guetarni, 2006; Aggad et al., 2009).

This work aims to highlight the diversity of raw milks produced in the semi-arid Algerian zone defined by their physicochemical and hygienic composition and link them to farming practices. These practices will be analyzed in the general context of farm operation in order to identify the relationships between the milk types and farming systems.

Materials and methods

Sampling and laboratory analysis

Cow's milk samples (a total of 144) were collected from 24 farms located in the Daïr of Ain Arnet (6 samples per farm) and analyzed for physicochemical parameters (pH, fat content, milk protein) and hygienic characteristics (mesophilic total aerobic).

The farms selected corresponded to six production systems, representative of the diversity of the study area, particularly in terms of feeding practices (types of seasonal basic rations, type and duration of grazing, quantity and type of concentrate) (Mansour and Abbas, 2015).

The mixing milk from each farm was subjected to 6 analyzes during two passages - at the rate of 3 samples per season and per farm - the first carried out during the spring, the second during the summer. Controls began in March and ended at the end of August.

The collection of samples for the determination of the milk quality took place just after the morning milking and concerns exclusively the milk of mixture of this milking. The samples were refrigerated through a cooler to avoid the effect of ambient temperature during transport to the laboratory. Two samples of mixing milk from each farm were taken for each analysis: the first of 1 l, for physicochemical analyzes and the second of 90 ml for the determination of the hygienic quality. This required volume was taken by means of a ladle ignited by the alcohol to be burned to avoid any external contamination to the sample, then poured into 100 ml glass bottles previously sterilized. The milk samples were sent directly to the laboratory and the maximum time between sampling and analysis did not exceed 3 hours.

On each milk sample, we made 3 simultaneous determinations and we considered the arithmetic mean of the results. The physicochemical analyzes performed were as follows:

- **The pH,** measured by a digital pH meter type Tacussel;
- **The density** was measured by a Dornic thermo lactodensimeter set at a temperature of 20 °C;
- **The fat content** was evaluated according to the method of Gerber applied to the milk: 10 ml of concentrated sulfuric acid, followed by 11 ml of milk and 1 ml of amyl alcohol were put in a butyrometer. The butyrometer was capped, shaken 3 to 4 times until the casein was completely dissolved, and placed in the centrifuge at 1000-1200 rpm for 5 to 6 minutes. The fat content was read directly on the graduated branch of the inverted butyrometer (AFNOR, 1985).

- **Protein content**: the nitrogen determination in the various fractions was carried out according to the Kjeldahl method (AFNOR, 1977). A quantity of milk was mineralized with sulfuric acid in the presence of mercury oxide acting as a catalyst to convert the nitrogen of the organic compounds into ammonia nitrogen. Ammonia was liberated by the addition of caustic soda, distilled and collected in a solution of boric acid. The ammonia was then titrated with 0.1 N hydrochloric acid.
- **Titratable Acidity** (NaOH titration): it was titrated with sodium hydroxide solution (N/9) in the presence of 1% phenolphthalein as a colored indicator turning pink towards pH 8.4. This acidity was expressed in degree Dornic (decigram of lactic acid per liter). It is equal to the volume of NaOH consumed, multiplied by 10 (Gaursaude, 1985).
- **The total aerobic mesophilic** microflora (FMAT) was counted on a PCA agar (Institut Pasteur, Algeria) in bulk, following a series of 10-fold dilutions and after incubation in the oven for 24 hours at 30 °C. All colonies were enumerated and the results expressed in colony-forming units per ml of milk (CFU/ml) (Guiraud, 1998, Maury, 1987).

Data processing and statistical analysis

The statistical analyzes were carried out in two complementary stages:

Descriptive analysis for the calculation of means and standard deviations, maximum and minimum of the studied parameters. The comparison of the average milk quality parameters with respect to values considered normal by the Student's T test (Schwartz, 1992) on the one hand and between them, on the other hand, by the analysis of the one-way variance (Dagnelie, 1975).

Multi-variety analysis to link the quality of milks and breeding practices. Milk samples were grouped in class using a Principal Component Analysis (ACP) followed by a Hierarchical Classification (CAH). The software SPAD version 5.0, has been used for this.

Results

Main characteristics of the farms studied

Twenty-four farms were selected for quality monitoring reflecting the 6 groups previously identified (Mansour and Abbas, 2015) at the rate of 4 farms per group (Table 1). The management of livestock activities in these farms was the responsibility of farmers whose educational level and social status differed. Only the exploitation 6 of group 2 was led by two officials holding the diploma of agronomist engineer, while the other farmers (87.5%) were owner-breeders breeding from father to son and only two of them have agricultural training (farm 23 and 24 in group 6). The herds were composed on average of 9 to 32 dairy cows, mainly of Montbéliarde breed. Milk production per cow varied (from 3011.12 kg/year /cow to 3298.57 kg/year/cow).

Table 1. Distribution of dairy farms followed by group

Group	Holdings
Group 1 Dairy modules with acceptable feed integration on large cereals units	exp1 exp2 exp3 exp4
Group 2 Dairy modules with high fodder integration on very large cereal farms	exp5 exp6 exp7 exp8
Group 3 "Modules" milk-meat "with low fodder and grass integration on small cereal units	exp9 exp10 exp11 exp12
Group 4 Large modules "milk and meat" with herbal tendency on medium-sized cereal farms	exp13 exp14 exp15 exp16
Group 5 Module "meat" Aboveground on average grain farms	exp17 exp18 exp19 exp20
Group 6 "meat" modules Aboveground on small grain farms	exp21 exp22 exp23 exp24

The six groups monitored had average useful agricultural area (UAA) ranging from 14.33 ha (Group 6 farms) to 30.25 ha (Group 1 farms). Most of these usable areas were reserved by almost all farms for the cultivation of cereals, especially durum wheat and barley. In fact, 83.33% of the farms monitored devoted more than 50% of their agricultural area to this type of crop - up to more than 85% of the UAA. The average areas devoted to fodder crops were low in farms in Group 6 and 3, but larger in farms in Group 2. Thus the density of cows/hectare of fodder reached an average of 0.33 cow/ha with a maximum of 6 cow/ha on farms in Group 6 and a minimum of 1.95 cows/ha on farms in Group 2 (Table 2).

Cows were supplemented with dry fodder and concentrated feeds purchased or produced in farms. Dry forage in winter was similar to that of fall for the majority of farms (83%). Farms 15, 16, 21 and 22 increased the quantities of rations distributed in winter compared to that of autumn. During the fall and winter season, the basic ration was oat hay and/or mixed or non-straw meadow. The quantities of hay and straw distributed in these two seasons at farm level oscillated respectively between 6 kg (exploitation 3, 4 and 17) to 18.75 kg (exploitation 24) and between 4 kg (exploitation 21) to 10 kg (exploitations 3, 8, 11, 13, 14 and 16).

Apart from the three farms (15, 16 and 23), the composition of the basic ration was similar during the spring and summer seasons in all farms (87.5%). During its seasons, straw was the staple food in 54.16% of farms, with a quantity between 6 kg (farm 1) and 13 kg/d/cow. Hay was only used in 4 farms (5, 6, 7 and 8) with varying amounts of 8 kg to 10 kg/day/cow.

All farms completed their ration with concentrate. This supplementary food was procured with a frequency of 2 times per day with a quantity of between 5 kg and 13 kg. Only 20.83% of farms (1, 9, 10, 12 and 18) completed their ration with wheat bran. In the other farms, the breeders provided a concentrate composed of either a mixture of wheat bran, barley and maize crushed (54.16%), or a mixture of barley + bran + but + soya (29.16%) or a mixture of wheat bran, cracked barley and CMV (16.66%).

Table 3 summarizes the composition of the ration distributed on the farms studied during the different seasons. All farms that have been monitored did not graze in winter. Spring grazing was done twice a day on fallow in the morning and on natural grassland in the afternoon in 45.83% farms, while 41.66% of the farms grazed only on meadow. However, the rest, the farms 5 and 23 grazed only the fallow. In the summer, more than 50% of the farms grazed on the stubble in the morning and on the natural meadow in the afternoon.

Table 4 summarizes grazing practices in the different groups for milk quality.

Table 2. Structural characteristics and milk production performance in farms followed

	Exp.	**UAA**	**SF**	Meadow	Number of cows	Density of animals (cows/ ha of fodder)	SF/cows	Dairy yield (kg of milk / cow / year)
Group 1	**exp1**	55	12	5	15	6.66	0.8	2689.12
	exp2	28	3.34	3	8	1.53	0.41	3003.27
	exp3	25	4	5	15	0.65	0.26	3141.50
	exp4	13	8.5	1.5	10	1.17	0.85	3612.73
Group 2	**exp5**	56	26	2	48	1.84	0.54	2827.35
	exp6	873	36	60	66	1.83	0.54	3141.5
	exp7	73	13	45	8	5.07	1.62	3534.19
	exp8	48	10	5	8	1.5	1.25	3455.65
Group 3	**exp9**	25	1.5	1.5	8	4.5	0.18	3062.96
	exp10	10	3	2	13	2.66	0.23	2670.28
	exp11	18	2	3	15	8.66	0.13	3612.73
	exp12	25	3.5	1.5	10	2.85	0.35	3062.96
Group 4	**exp13**	20	3	7	8	3.33	0.37	3220.038
	exp14	15	3	1	12	8	0.25	3141.50
	exp15	15	6	9	17	2	0.35	3298.58
	exp16	20	1	4	25	13	0.04	3377.11
Group 5	**exp17**	20	9	5	13	0.88	0.69	2670.28
	exp18	30	0	10	10	3.4	0	3926.88
	exp19	12	2	6	10	7.5	0.2	3455.65
	exp20	16	1	3	9	4	0.11	3141.50
Group 6	**exp21**	6	1	1	8	1.5	0.12	2748.81
	exp22	7	2	1	9	1.41	0.22	2670.28
	exp23	30	1.5	3	10	1.15	0.15	3308.00
	exp24	75	30.03	12	46	2.6	0.65	3317.42

Exp: exploitation, UAA: Useful Agricultural Area, SF: Forage Area.

Table 3. Seasonal Composition (kg / day / cow) of rations in the farms followed

Groups	**Exp.**	Autumn			Winter			Spring			Summer		
		Hay	Straw	Conc.	Hay	Straw	Conc.	Hay	Straw	Conc.	Hay	Straw	Conc.
Group 1	**exp1**	15	0	13	15	0	13	0	13	13	0	13	13
	exp2	18.75	0	10	18.75	0	10	0	10	8	0	10	8
	exp3	6	10	11	6	10	11	0	6	5	0	6	5
	exp4	6	8	13	6	8	13	6	8	8	6	8	5
Group2	**exp5**	8	0	11	8	0	11	8	0	5	8	0	5
	exp6	8	0	11	8	0	11	8	0	5	8	0	5
	exp7	9	0	10	9	0	10	9	0	5	9	0	5
	exp8	10	0	11	10	0	11	10	0	6	10	0	6
Group 3	**exp9**	0	8	11	0	8	11	0	8	5	0	8	5
	exp10	0	8	11	0	8	11	0	6	11	0	6	11
	exp11	10	10	12	10	10	12	8	0	5	8	0	5
	exp12	0	8	11	0	8	11	0	8	5	0	8	5
Group 4	**exp13**	0	10	13	0	10	13	0	6	0	0	6	5
	exp14	0	10	8	0	10	8	0	6	0	0	6	5
	exp15	0	8	11	8	10	11	8	6	0	0	6	5
	exp16	8	10	10	10	12	10	8	6	0	0	6	5
Group 5	**exp17**	6	8	8	6	8	8	0	8	8	0	8	8
	exp18	10	6	8	10	6	8	10	6	0	10	6	3
	exp19	13	7	8	13	7	8	13	7	8	13	7	8
	exp20	10	9	12	10	9	12	8	9	6	8	9	8
Group 6	**exp21**	12	4	8	15	4	8	0	4	8	9	4	8
	exp22	11.25	5.25	8	13	6	8	0	10	8	10	6	8
	exp23	12.75	8	10	12.75	8	10	0	9	5	12.75	8	8
	exp24	18.75	8	10	18.75	8	10	13	6	5	13	8	8

Conc: Concentrate

Table 4. Overall grazing practice in groups followed for milk quality

Groups	Meadow	Fallow	Stubble
Group 1	++	++	+
Group 2	++	-	+
Group 3	+	-	+
Group 4	++	+	+
Group 5	+	+	+
Group 6	+	+	+

Overall quality of the mixing milk

The fat content of cow's milk varies from 35 to 45 g/l (Alais, 1984). The majority of farm milk samples showed consistent average fat content. The fat content was less than 35 g/l in only 21.52% of our samples. While, respectively, during the first pass and the second passage, 70.83% and 58.33% of the farms showed contents higher than 35 g/l (Figure 1). Only seven farms had average levels greater than 35 g/l for both passes.

In operation 2, 15 and 16 during the second period and to a lesser extent in operation 8 during the first pass, the milk fat content showed the greatest amplitude fluctuations, ranging from 31 at 39, from 32 to 39, from 31 to 38 and from 34 to 38.8 g/kg.

The milk fat content showed significant fluctuations during the summer season, ranging from 31 to 41.7 g/l. Farm 3 had the highest average fat content in both seasons (Table 5 and Table 6). Farms 8 and 16 had the lowest butterfat levels during the second pass and the first pass, respectively. This weakness could only be attributed to rationing errors since there was no dilution effect (annual milk yield in farms 8 and 16 is respectively 3455.65 and 3377.11 kg/cow/year).

The average protein content for the surveyed farms was 34.21g/kg, the maximum was 37.59 g/kg and the minimum - 30.63g/kg (Figure 2). However, 8.33% milk samples in the spring season and 12.5% milk samples collected in the summer season had a protein content of less than 33 g/l. In all the farms, the protein content showed acceptable average levels (35 g/l) indicating the effect of the massive and regular inputs of the concentrate. This observation is in agreement with the results of other studies showing that massive intakes of concentrates constitute a stabilizing factor of the protein content (Coulon and Remond, 1991; Srairi et al., 2005).

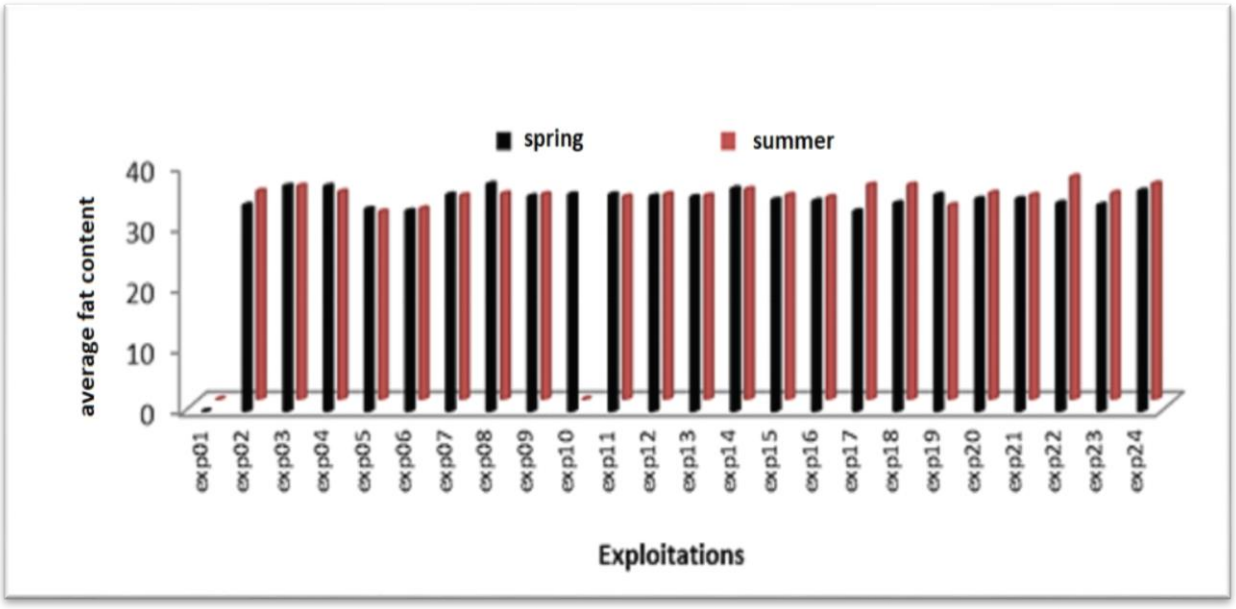

Figure 1. Variation in the average fat content mixing milk collected in two seasons

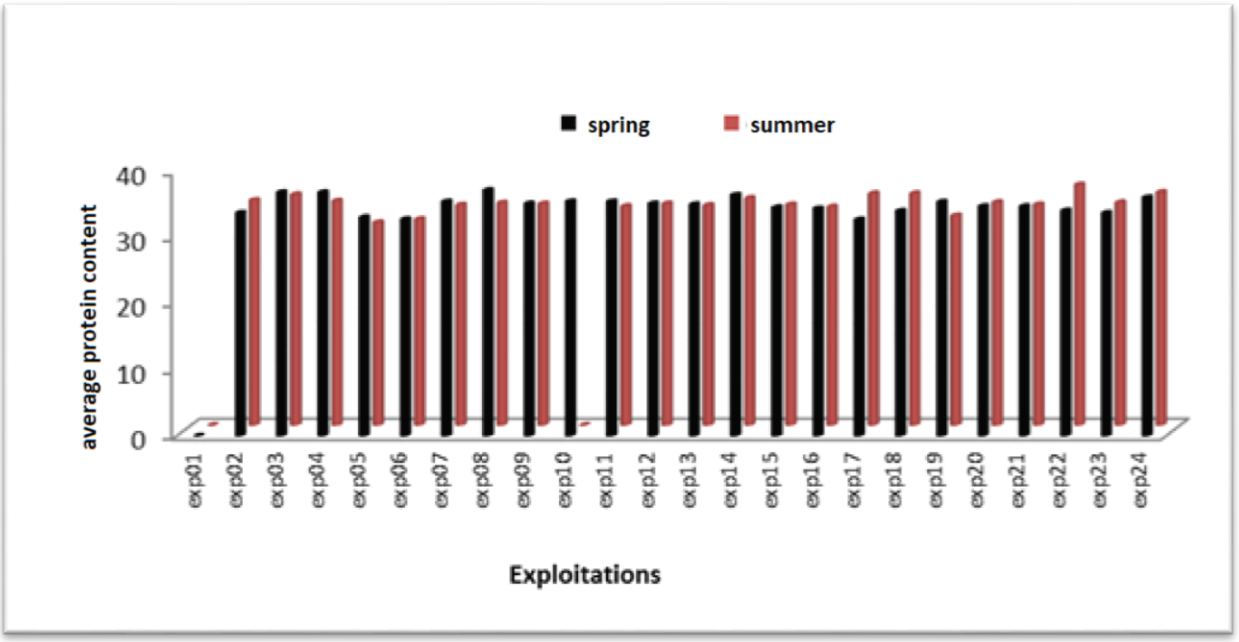

Figure 2. Variation of the average protein content of the mixture milk collected in the two seasons

The mixing milks of the farms monitored in the two passages had an acceptable mean pH (6.62 and 6.64 respectively in the first and second passages). In fact, fresh cow's milk has a pH between 6.6 and 6.8 (Luquet, 1985).

These values can be modified considerably by microbial infections. Acute forms lead to acidification and chronic forms to alkalization (Araba, 2006). This is an important parameter that determines the subsequent destination of the milk, that is, its processability. Indeed, low acidity has

important effects on mineral equilibrium and stability of the colloidal casein suspension (Ramet, 1985; Alais and Linden, 2004).

The density recorded in all farm milks met the standards cited by Alais (1984) (1028-1033). The density of the milk is linked to its high dry matter content, if it is too high, which explains why the milk is skimmed (Luquet, 1985).

The average acidity of the milk collected in the two seasons varied from 16.16 to 18.67° D. The acidity of the milk can be an indicator of the quality of the milk at the time of delivery because it makes it possible to appreciate the quantity of acid produced by bacteria or possible frauds (Joffin and Joffin, 2004). Fresh milk has a titration acidity of 16 to 18° D. Preserved at room temperature, it acidifies spontaneously and gradually (Mathieu, 1998). Most of the samples (95.13%) taken in both seasons were compliant. Acidity and pH depend on casein content, mineral salts and ions (Alais, 1984), hygienic conditions during milking, total microbial flora and metabolic activity (Mathieu, 1998). The aerobic mesophilic flora always informs us about the hygienic quality of raw milk, it is considered as the determining factor of the shelf life of fresh milk (Guinot-Thomas et al., 1995). It is the most sought-after flora in microbiological analyzes.

In general, the total microbial load of the raw milk mixing of the farms followed was very important (on average 2.4 x 10^6 CFU/ml in the first passage and 3.2x 10^6 CFU/ml in the second passage). Several studies (Srairi and Hamama, 2006; Ghazi and al., 2010) as well as national regulations (JORA, 1998) agree that a load greater than 10^5 CFU/ml means a significant contamination. These counts are also greater than the maximum permissible loads by the two French and American regulations, which are respectively 5x10^5 CFU/ml and 3 x 10^5 CFU/ml (Alais, 1984). These high levels of contamination are closely dependent on the general hygiene conditions and the health status of the animal.

Table 5. Results of physicochemical analyzes of mixing milks collected in the spring

	pH	Density	Acidity ° D	Fat content	Protein content
Norms	**6.5-6.8[a]**	**1028-1034[b]**	**16-18[c]**	**35-45[b]**	**33-36[b]**
EXP 1	6.53±0.15	**1032.33±0.58**	18.33±0.58	34.66±0.57	**36.66±0.79**
EXP 2	6.6±0.2	**1028.33±0.57**	16.67±0.58	38.33±1.15	33.66±2.08
EXP 3	6.66±0.06	1029.33±0.58	16.16±0.28	39.1±0.95	36.77±1.30
EXP 4	6.59±0.041	1029.33±0.58	16.66±0.28	39.1±0.95	36.77±1.30
EXP 5	6.62±0.02	1029.33±0.58	16.33±0.28	38.83±1.75	33±1.005
EXP 6	6.59±0.03	1029.33±0.58	**16.16±0.28**	38.93±0.90	32.71±0.62
EXP 7	6.6±0.03	1029.33 ±0.58	16.5±0.87	38±1	35.37±1.19
EXP 8	6.57±0.03	1030.33±0.58	16.5±0.5	**36.6±2.42**	37.10±2.15
EXP 9	6.56±0.06	1028.66±0.57	16.67±2.08	37.13±1.20	35.08±1.12
EXP10	6.61±0.03	**1028.33±0.57**	16.33±0.57	37.7±0.65	**35.4±1.15**
EXP11	6.63±0.02	1028.66±0.57	16.5±0.5	36.06±0.51	35.39±0.84
EXP12	6.58±0.06	**1028.33±0.57**	17.67±0.58	37.13±1.20	35.08±1.12
EXP13	6.53±0.06	1028.66±0.57	16.83±0.76	37.96±0.95	34.96±0.94
EXP14	6.57±0.04	**1028.33±0.57**	17.67±0.58	38.86±0.85	36.37±1.59
EXP15	6.74±0.09	1028.66±0.57	18.33±0.57	34.33±2.30	34.5±1.5
EXP16	6.6±0.34	1029.33±0.57	17.66±0.57	34±1	34.33±1.52
EXP17	6.65±0.35	**1028.33±0.57**	17.67±0.58	37±1	32.66±2.51
EXP18	6.6±0.17	1029.67±0.58	17.66±0.57	36.66±2.08	33.96±1.70
EXP19	6.67±0.12	1029.33±0.58	17.67±0.58	35.66±1.52	35.33±0.57
EXP20	6.50±0.39	1030.67±0.58	17.66±0.57	33.66±0.57	34.66±2.88
EXP21	**6.6±0.43**	**1028.33±0.57**	17.67±0.58	38±1	34.66±4.50
EXP22	**6.8±0.1**	1030.33±0.58	18.16±0.28	37.66±1.52	34±1
EXP23	6.77±0.05	1029.66±0.57	17.33±0.57	34.66±1.15	33.66±1.15
EXP24	6.7±0.1	1030.33±0.58	17.33±0.58	37±2	36±2.64

[a]: Luquet (1985); [b]: Alais (1984); [c]: Mathieu (1998).

Table 6. Results of physico-chemical analyzes of mixing milk collected in summer

	pH	Density	Acidity ° D	Fat content	Protein content
Norms	**6.5-6.8[a]**	**1028-1034[b]**	**16-18[c]**	**35-45[b]**	**33-36[b]**
EXP 1	6.58±0.10	1029.33±0.58	17.67±0.58	37±2	**37.33±0.58**
EXP 2	**6.5±0.3**	1028.66±0.57	18±0.5	**36±3.60**	34±4.35
EXP 3	6.71±0.04	1028.66±0.57	16.67±0.76	40.76±0.86	34.84±1.76
EXP 4	6.71±0.04	**1028.33±0.57**	18±0.5	38.26±0.64	33.88±0.97
EXP 5	6.71±0.07	**1028.33±0.58**	17.33±0.29	38.93±1	30.63±0.54
EXP 6	6.7±0.02	1029.66±0.57	17.5±0.5	34.56±1.69	31.12±0.33
EXP 7	6.71±0.06	1029.33±0.58	17.83±0.76	34.13±1.80	33.28±0.7
EXP 8	6.71±0.07	1028.67±0.58	17.66±0.76	32.95±1.81	33.56±0.51
EXP 9	6.69±0.01	1028.66±0.58	17±0.87	34.33±1.15	33.46±0.4
EXP10	6.72±0.04	1029.66±0.57	18±0.5	33.23±1.66	**37.59±0.79**
EXP11	6.71±0.04	1029.33±0.58	17.5±0.5	34.63±0.40	33.08±0.38
EXP12	6.69±0.015	1029.66±0.57	17.17±0.76	33.5±1.80	33.46±0.4
EXP13	6.70±0.07	1030.33±0.58	17.33±0.58	33.96±1.55	32.91±0.73
EXP14	6.68±0.02	1029.66±0.57	17.5±0.5	35.16±1.04	33.24±0.31
EXP15	6.65±0.35	**1031±2.64**	17.66±0.57	**35.33±3.78**	33.33±0.57
EXP16	6.52±0.33	1029.66±1.15	17.83±0.29	**35±4**	33±1.73
EXP17	6.63±0.37	1029.66±0.57	17.66±0.57	35.5±0.5	35±3
EXP18	6.55±0.31	1028.33±0.57	17.7±0.26	36.33±1.15	35±2.64
EXP19	**6.73±0.20**	1029.33±0.58	17.33±0.58	37.33±1.52	31.66±1.52
EXP20	6.5±0.2	1028.66±0.57	17.83±0.29	37±1.73	33.66±1.15
EXP21	6.55±0.27	1029.66±0.57	17.5±0.5	34.66±0.57	33.33±1.52
EXP22	6.56±0.11	1030.67±0.58	17.66±0.57	38±1	36.33±3.05
EXP23	6.53±0.37	1029.33±0.58	**18.67±0.58**	39.33±1.52	33.66±1.15
EXP24	6.55±0.22	**1028.33±0.57**	17.67±0.58	38.3±1.12	35.2±1.05

[a]: Luquet (1985); [b]: Alais (1984); [c]: Mathieu (1998).

Overall quality of mixing milk and farming practices: towards the establishment of a type of raw milk

The first two factor axes of the PCA on milk quality data yieled 67.47% of the total variability (Figure 3). Axis 1 explains 40.92% of the total variation and is considered to represent the protein level, while axis 2 represents 26.55% of the total variation and is linked to the variables fat content and aerobic germs.

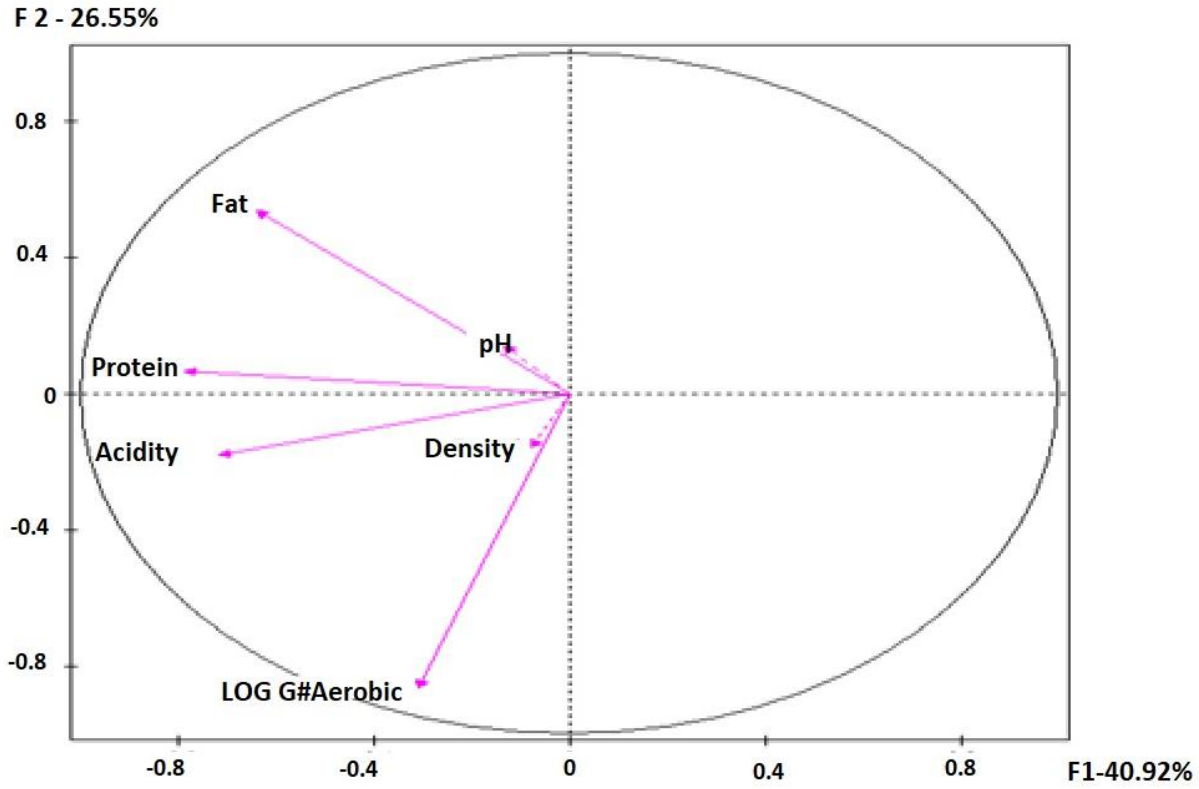

Figure 3. Distribution of milk quality variables on the F1 and F2 axes of the ACP

Classification has identified five distinct classes unequally distributed milk with distinct characteristics (Table 7 and Figure 4).

Table 7. Characteristics of the different classes of milk identified

	Class 1 (n=26)	**Class 2 (n=41)**	**Class 3 (n=22)**	**Class 4 (n=20)**	**Class 5 (n=35)**
pH	6.65±0.18	6.64±0.12	**6.71±0.11**	6.59±0.27	**6.56±0.11**
Density	**1029.61±1.29**	1029.12±0.74	1029.13±1.12	1029.3±0.97	1029.25±1.09
Acidity (°D)	17.75±0.66	17.14±0.62	**17.93±0.51**	17.73±0.52	**16.84**±0.92
Fat (g/l)	35.28±1.95	**38.37±1.30**	**37.55±1.56**	35.72±2.14	**34.73**±1.97
Protein (g/l)	34±1.77	34.17±1.44	**37.3±1.21**	**33.82±1.54**	**33.36±1.94**
FMAT (10^5CFU/ml)	7.01±3.6	7.58±5.65	12.86±17.55	**149.7±15.79**	**7.35±1.29**

n: number of samples**,** FMAT: total aerobic mesophilic flora. The results are expressed as mean ± standard deviation

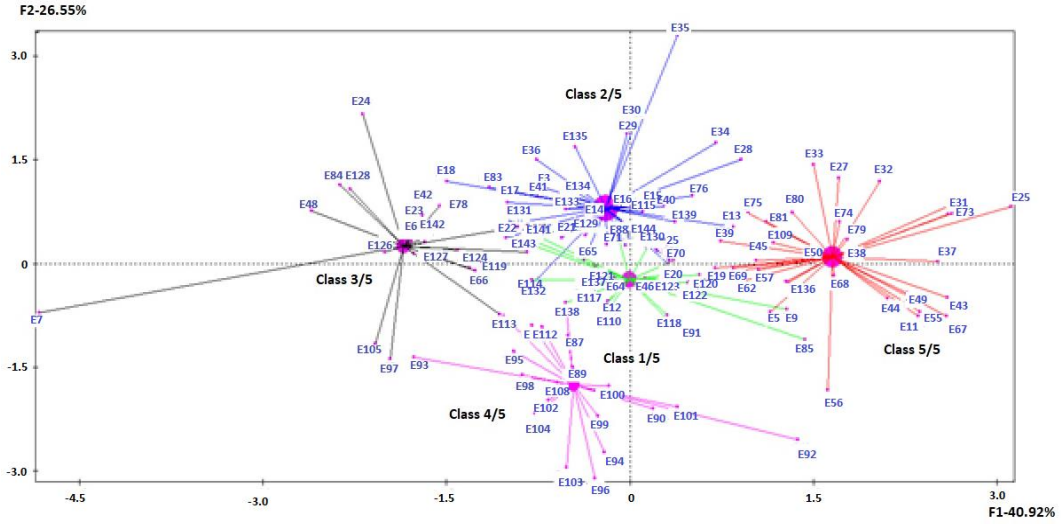

Figure 4. Different classes of milks identified in the study area

The discrimination of the five classes of milk in the study area has led us to look for the factors of variation that influence this distinction. For this purpose, six qualitative physicochemical variables (fat content, protein content, pH, acidity, density) and microbiological (total germ content) quality were retained. The results of the analysis were summarized in Table 8. The analysis of variance showed there are significant variations between classes and are mainly at fat and protein content, acidity, total germs.

Table 8. Results of the inter and intra class analysis of milks

Item	Sources of variation	df	Sum of squares	Average squares	F of Fisher	P
pH	intergroup	4	0.173	0.043	0.762	
	intragroup	139	7.896	0.057		
	Total	143	8.069			0.551
Density	intergroup	4	23.506	5.876	3.035	
	intragroup	139	269.154	1.936		0.020
	Total	143	292.660			
Acidity	intergroup	4	167.266	41.817	52.835	
	intragroup	139	110.013	0.791		**< 0.0001**
	Total	143	277.280			
Fat content	intergroup	4	435.177	108.794	40.343	
	intragroup	139	374.848	2.697		**< 0.0001**
	Total	143	810.025			
Protein content	intergroup	4	360.383	90.096	50.293	
	intragroup	139	249.005	1.791		
	Total	143	609.388			**< 0.0001**
Log FMAT	intergroup	4	27.639	6.910	43.834	
	intragroup	139	21.911	0.158		**< 0.0001**
	Total	143	49.550			

FMAT: total aerobic mesophilic flora

The classes of milk per farm could be described as follows:

Class 1 "class of milk with medium protein and fat content, and the lowest microbial load"

The first class contained 26 of the 144 milk samples (18.05%). They were characterized by medium protein (34 g/l) and fat (35.28 g/l), the highest density 1029.61, an average acidity equal to 17.75°D, associated with the lowest average aerobic count (7.01 x 10^5 CFU/ml).

Class 2 "milks with the highest fat content, a low average protein count and a low germ count"

The second class contained 41 milk samples collected (28.47%). Its main characteristics were the highest fat content 38.37 g/l, the lowest acidity 17.14°D, the lowest density 1029.12 and average protein content 34.17 g/l. The samples of this class were characterized by a means of counting aerobic germs ($7.58x10^5$ CFU/ml)

Class 3 "milks with the highest protein level, a high fat content and a high number of total germs"

The third class included 22 milk samples collected (15.27%). It wss characterized by milks having high fat content 37.55 g/l, the highest protein level (37.3 g/l), the highest acidity17.93 °D, a high count of aerobic germs 12.86 x 10^5CFU/ml and the highest pH (6.71).

Class 4 "milks with low protein, average fat content and highest total germ number"

Twenty milk samples were part of this class (i.e. 13.88% of milk analyzed). They were distinguished by a low protein level (below the standard of 34 g/l), an average fat content slightly higher than the standard of milk, 35 g/l, associated with highest aerobic counting14.96 $x10^6$ CFU/ml.

Class 5 "milks with the lowest protein and butyrous content and low total germ load"

The fifth class had thirty-five samples or 24.30% of the milk collected. Milk samples of this class were characterized by unsatisfactory protein and butyral levels and the lowest, respectively 33.46 g/l and 34.73 g/l, acidity (16.84°D) and pH (6.56) the lowest, average density of 1029.25, associated with low aerobic count ($7.35x10^5$ CFU/ml).

The analysis of the distribution of the different classes of milk per farm (Table 9) clearly revealed that apart from exploitation 3, none of the farms studied produce, throughout the seasons, milk of the same quality belonging to the same class. In fact, only the acceptable forage-integrated group 1 farm 3 with a significant pasture area relative to the data of the study area, can produce milk throughout the seasons with the highest fat content (100%) - milks of class 2.

Table 9. Distribution of milk samples collected by class and farm

		Class 1	**Class 2**	**Class 3**	**Class 4**	**Class 5**
Group 1	**exp 1**	**4**	1	1	0	0
	exp2	3	0	3	0	0
	exp3	0	**6**	0	0	0
	exp4	0	3	3	0	0
Group 2	**exp5**	0	5	0	0	1
	exp6	0	5	0	0	1
	exp7	0	3	1	0	2
	exp8	1	2	2	0	1
Group 3	**exp9**	0	0	1	0	5
	exp10	0	0	1	0	5
	exp11	2	0	1	0	3
	exp12	0	0	1	0	5
Group 4	**exp13**	0	2	1	0	3
	exp14	0	2	1	1	2
	exp15	0	2	1	1	2
	exp16	2	0	0	**4**	0
Group 5	**exp17**	1	0	0	**5**	0
	exp18	0	0	1	**5**	0
	exp19	2	1	1	2	0
	exp20	3	2	1	0	0
Group 6	**exp21**	3	1	1	0	1
	exp22	1	3	0	0	2
	exp23	2	2	0	1	1
	exp24	2	1	1	1	1
	Total	**26**	**41**	**22**	**20**	**35**

Discussion

This study was carried out under the conditions of cattle breeding of the Daïra de Ain-Arnet. It made it possible to quantify the variability of herd characteristics and also to establish a summary characterization of the actual quality of the milk produced.

The results of physicochemical quality follow-up analyzes of 140 samples of raw milk produced in the semi-arid zone revealed an average quality of raw milk. The parameters were very variable and overall satisfactory. As a whole, the physicochemical composition of the milks could be described as average for the majority of the samples, and marked a remarkable normativity.

The majority of the milk samples in the six groups had consistent average fat content. Milk samples from farms in Group 01 showed the highest average fat content in both periods. The farms in this group invested in fodder which explains the high fat of their samples. In fact, the fat content seems to be the most variable, following its very strong correlation with the forage content, the nature of the fibers and concentrates used in rations for dairy cows (Hoden et al., 1988).

Variations in the butterfat between different farms are explained by the production and feeding behavior strategies adopted for each farm. In fact, milk fat consists mainly of volatile fatty acids that are formed from the carbohydrates of fodder (cellulose) and fermentable carbohydrates (starch). As a result, the higher the ration's fibre content, the higher the acetic acid production and the milk content (Stoll, 2002).

Some farms aim for maximum milk production by reducing concentrate expenditure and increasing the use of dry fodder (exploitation 3.4 and 5), which may be favorable for the fat content (fat content greater than 38 g/l). Whereas farms 1 and 10 are more particularly focused on a maximum yield without considerations of the expenses generated by the use of high quantities of concentrate, which may explain the high protein content of their milk sample (protein content greater than 35 g/l).

On average, the fat content was slightly lower than that observed by Bassabasi et al. in 2013 on spring collection samples (0.92 g/l) and (2.41g/l) from that observed by Bony et al. (2005) in Reunion. However, the average value of 72 raw milk samples from five farms recorded by Srairi et al. in 2005 in Morocco is slightly lower than that observed in our study (4.39 g /l).

With the exception of milk from farms 2 and 17, the protein content recorded in both seasons appeared to be much more stable than the fat content of all the milk collected. The protein content, during the two passages, was slightly higher than that obtained for raw milks on the island of Réunion (31 g/l) (Bony et al., 2005) and Morocco (31.31 g/l). (Bassabasi et al., 2013).

One of the commonly-used variation factors in explaining changes in the protein content of milk is the proportion of the concentrate in the diet. Indeed, the incorporation of a large amount of concentrate (> 10 kg/cow/day) in the ration of cows of farm 10 and 1 has led to an increase in the protein content of milk (> 35 g/l). Unlike farm 05, where the energy supply was insufficient (<6 kg/cow/d). This explains the inferiority of the protein content compared to the standard (33-36 g/l) cited by Alais in 1984.

In general, the average density of fresh milk mixes in both seasons was relatively low (1029). As the wettability hypothesis is discarded, it is possible to attribute this significant decrease to the presence of fat with a density of less than 1 (0.93 to 20°D) (Goursaude, 1985).

The majority of the analyzed milk samples had a consistent acidity and were in the range of 16 to 18°D. The titration acidity is the sum of 4 reactions. The first three represent the natural acidity of milk (acidity due to casein, mineral salts and phosphates) and the last is related to the acidity due to lactic acid and other acids from the microbial degradation of lactose and possibly lipids in the process of alteration.

The microbiological results were highly variable with average counts of total aerobic mesophilic flora exceeding the maximum standard of 10^5 CFU/ml. This results in poor hygiene control either during milking or in the overall environment of livestock buildings.

The averages recorded were relatively lower compared to those reported by Karimuribo et al. (2005) in Tanzania (10^7 CFU/ml). The enumeration values were also higher than those obtained for raw milk mixtures collected in France (Desmasures et al., 1997; Michelle et al., 2001), in Denmark (Aagaard et al., 1998) or on American farms (Hogan et al., 1988) in probable relation with more rigorous hygienic conditions. Milk collected in good conditions from a healthy animal, contains little microorganism and is protected against bacteria by inhibiting substances of very short duration (Guiraud and Rose, 2004).

According to the study conducted by Ameur et al. (2011) in the region of Freha (Algeria), raw milk collected has a very high microbial contamination rate (between 10^5 and 10^7 CFU/ml), detrimental both to the transformation in the dairy industry to public health. Our counts were in this contamination interval, they were superior to the results reported by Aggad et al. (2009) in western Algeria where the average level of contamination is close to $83x10^4$ CFU/ml with raw milk samples taken from tank reception. This is explained by a lower degree of cumulative contamination from production to the arrival of milk at the dairy. However, they agree with those found in Morocco by Affif et al. (2008), Labioui et al. (2009), Mennane (2007), Srairi et al. (2005) and enumerated in Mali by Bonfoh et al. (2002).

The multidimensional statistical analyzes made it possible to characterize the milk samples according to the two main groups of variables reflecting the quality of the milk in the semi-arid region of Sétif- the contents of useful materials and the general hygiene.

Class 1 is that of samples with average levels of useful material (fat content and protein content), having a moderate hygienic quality compared to other samples ($7.01x10^5$ CFU /ml), having the highest density 1029.61 and average acidity equal to 17.75 °D. This class mainly includes milk samples collected from farms in group 6 (30.76% of samples), group 5 (23.07% of samples) and group 01 (26.92% of samples). Groups 5 and 6 are modules outside soils, characterized by fairly limited pasture

land but distributing rations of bases without any negligible straw (> 39% of the basic ration). The introduction of a high proportion of straw in the basic ration causes the increase of the fibrosity which can induce at a high butyric rate.

However, cereal straw is a food that is low in soluble sugars, nitrogen, minerals and vitamins. It is a cumbersome fodder and little digestible. It is therefore necessary to have an appropriate complementation to optimize this forage, to allow its digestion and the functioning of the rumen, and finally to ensure the needs of animals. The consumption of high quantities of concentrate in these farms also made it possible to produce milk with average but satisfactory protein levels.

Group 1 farmers' production of 26.92% of class 1 milks is due not only to the introduction of straw at an average rate of 37% in the basic ration of cows but also to their investment in fodder and provision of important grazing surfaces.

Class 2 contains milk samples with the highest butterfat levels and consists mainly of samples of the 02 group farms characterized by a high degree of forage integration with a good basal ration distribution.

Fodders contribute to the increase of milk fatty acids thanks to microorganisms that ferment cellulose and hemicellulose in acetates and butyrates, precursors in the manufacture of milk fat. These fodder, the main source of fiber, are important for the maintenance of a high butyrous rate milk (Sutton, 1989).

Class 3 contains milks with the most favorable characteristics: high levels of fat content and protein content. This class mainly includes milk from Group 1 farms (31.81%). The production of milk with the most favorable levels of useful matter is undoubtedly due to the good feeding practices within this group: satisfactory share of forage in the diet, significant pasture area and an average complementation of concentrate.

The milks of this class are distinguished mainly by the highest protein level and therefore come mainly from breeding (group 01) practicing a medium supplementation in the highest concentration. This is consistent with the work of Coulon et al. (1998) and Bony et al. (2005) arguing that the highest protein levels are generally related to the highest energy intakes. However, these samples have the disadvantage of being very charged with total germs (on average 1.28×10^{6} CFU/ml). This may be the consequence of poor general hygiene, mainly that of the environment (livestock building). Hygiene is a practice that limits microbial teat contamination (Agabriel et al., 1995). This is an important element in a breeding to maintain animal health, product quality and to reduce the cost of breeding.

Hygiene problems are mainly increased by breeders because of:

- lack of milk storage (refrigeration) at 4 ° C prior to collection except in farms 5 and 6 of group
- lack of appropriate means of transport for milk to the farmer.

- milking conditions at the farm level (no milking parlor).

Class 4 consists mainly of milk samples from farms in group 5 (60% of milk collected), having a priori the least favorable characteristics (low protein content and the highest count in total germs). The small portion of forage in the rationing of animals in these groups may be at the origin of the low butyrous rate of these samples. The valorization of the use of concentrate is not reached in this group.

Class 5 mainly includes milk samples collected from farms in group 03 (51.42%). These milk samples are characterized by the lowest protein and butyrous content. Farms in this group are characterized by low forage and grass integration that may be responsible for the low fat content.

Concentrate recovery is optimal in Group 1 farms, resulting in 29.16% of the highest grade milk samples in the third class. The high level of fodder integration in Group 2 farms has resulted in over 60% of their milks having the highest butterfat content in the second class. The fifth class is dominated by milk samples from farms in the third group. These milk samples are characterized by levels below the standard for fat content. The poor forage and grass integration can be at the origin of the weakness of this physicochemical parameter. Group 5 farmers can not produce milk with high protein levels (60% of their milk samples are in the fourth class). Farmers in Group 6, despite their small pasture area, produce more than 70% of the milk samples with satisfactory physicochemical parameters.

Examination of all the characteristics of milk shows that it does not currently exist in the ideal class study area, which combines both high levels of useful matter and good health value. Similarly, the examination of the average farm values, their evolution over the two seasons and the distribution of the milk produced by each farm in the different classes clearly shows that with the exception of farm 3, no of the farms studied produced milk of the same quality throughout the seasons: among the 144 milk samples analyzed, none had at the same time the highest butyrous and protein content with a satisfactory microbial load (<105 CFU/ ml).

The distribution of milk samples in the different classes confirms the irregular quality of the milk produced in the different groups. We also note that a significant proportion of breeders produce milk with very high fat content (28.47% of milk samples are class 2), but fail to produce milk with a high protein content (only 15.27% of the milk samples collected belong to the third class).

Three classes of milk receive special attention: on the one hand, classes 1 and 2 having the most favorable characteristics (satisfactory fat and protein content) associated with the lowest count of total germs. On the other hand, the class 5 which, unlike low-fat, low protein milk combined with a low count of total germs.

- Apart from class 1, there are percentages close to milk samples taken in the spring season and in the summer season, the other classes have an unequal constitution of milks collected in both seasons:

- Class 2 contains 70.73% of the milk collected in the spring,
- Class 3 contains mainly milk in the summer (77.27%),
- Class 4 includes 60% of milk collected in summer,
- almost all the samples taken in class 5 are taken in summer (91.42% of the samples in the class).

The analysis of the distribution of milk samples collected by season in the different classes shows that the majority of milks collected in the spring season have the highest butterfat levels (constituting the major part of the samples in class 2), the production of milks with the highest protein levels is observed in the summer season (+ 70% of samples in class 3), while the microbial load of whole milk milks increases significantly during the summer season (60% of milk of class 4).

The typology of the milk samples made it possible to provide a descriptive framework of the variety of variations that milk can undergo in a breeding environment based on variations in the levels of useful materials and fluctuations in total flora that reflect the general hygiene and storage conditions and also confirms the direct consequences of farming practices (lack of rationing, poor basic ration, feed "with concentrate", overall hygiene in livestock buildings) on the quality of the milk.

The distribution of milk samples in the different classes confirms the irregular quality of the milk produced on the farms. While it is therefore difficult, from these results of this study, to rank the specific effect of each dietary practice, we can nevertheless think that dietary factors in the broadest sense play a predominant role, at least for classes 2 and 3.

Conclusion

This work allowed to establish a summary characterization of the reality of the situation of the overall quality of the milk under the conditions of current cattle breeding of the semi-arid region of Setif. The physicochemical composition of the milk analyzed was satisfactory for all the parameters studied. However, a high total mesophilic flora load characterized all the samples analyzed.

As far as quality is concerned, these results are very important, considering that our work has opened up a fairly new but important field for a greater professionalization of the sector and an improvement in milk collection rates.

Indeed, although the average quality is acceptable, we noted a very interesting variability for the implementation of a program of improvement of physicochemical parameters and their stability between seasons. Of course, at this level there is only one quality food that can ensure quality milk. The development of irrigated and dry forage crops, silage as well as the improvement of rationing by the diversification and improvement of the quality of the resources constituting the basic rations, on the one hand and the use of concentrates little expensive and adequate (compound, balanced), on the other hand, are the guarantors of a nutritionally good quality milk.

In terms of microbiological quality, the situation seems more worrying despite the diversity of situations. It goes without saying that livestock modernization remains to be a leitmotif of development programs. This modernization must be based on the rehabilitation of livestock facilities that are still too old (buildings, equipment, utensils, vats). It must also cover the training component, which is very important to instill in breeders but also to the various agents of the sector the necessary actions to produce and accompany this noble and highly perishable product.

Finally, this work has shown the way for a long-term research program to take place in order to strengthen the results obtained and especially to deepen them in the framework of an observatory allowing a rigorous and individual follow-up (individual performance control). This program could then best guide the development policies of the milk sector.

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