ABSTRACT

Horticultural herbs in cow diet as natural promoters sources of qualitative stability of milk

Horticultural herbs production may be for culinary purposes, for scents and fragrances, for medicinal uses or other. Some of the most popular culinary herbs grown commercially and by home gardeners are: basil, cilantro, dill, oregano, mint, parsley, rosemary, sage, tarragon and thyne. These plants are generally adapted to a wide range of soil type and growing conditions. In the efforts towards healther nutrition a succession of useful plants have been rediscavered in recent years as suppliers of primary products for food stuffs or feedstuffs. There is growing interest to manipulate the dairy cow diets to increase the nutritional quality of milk. The finding feedsheffs, which are rich in antioxidant compaunds, can be a strategy to improve the milk quality. Compared with inorganic compaunds, plant derived products have proven to be natural, less toxic and residue free, and are ideal growth promoters for milk and meat production.

Mixtures of legumes with cereals are cultivated for green forage production, they provide valuable roughage for dairy cows.

The effects of feeding of pea and oat intercropped with cilantro and sage as natural promoteres of milk qualitative stability were evaluated in this study.

The shelf life assessement requires knowledge of the food quality evolution during storage and the quality level corresponding to the acceptability limit. The identification of the acceptability limit is difficult to quantify.

Producers have to identify the acceptability limits of their products according to company policy and quality targets.

Thesis objectives and structure

This study attempts to examine the following hypotheses:

1. The application of intercropping pea and oats with cilantro or sage will produce a valuable roughage for dairy cows with high milk production?

2. The application of intercropping pea and oats with cilantro or sage will produce a high milk production in Holstein cows?

3. The application of intercropping pea and oats with cilantro or sage will improve the qualitative stability of milk and dairy product?

The thesis is structured in 6 chapters, and aims to provide an overall view of the present status of using some horticultural herbs in cows diet as natural promoters sources of qualitative stability of milk and milk products. The assessement process of qualitative stability has been approaced considering a number of sequential steps.

Chapter 1 presents the considerations regarding using horticultural herbs in cow nutrition.

Feed supplements are a group of feed ingrediants that can cause a desired animal response. Common feed additives used in animal diets include immunostimulators, antimicrobials, antioxidants and enzymes. Herbal plants are a new class of growth promoters, and in recent years this feed additives has gained estensive attention in the feed industry.

The definition of high quality milk must be expended: the quality of milk can also be based on the amount of antioxidants that it contains, protecting the characteristics of milk life time by reducing oxidation.

In some studies carried out on milk and a few fractiunui milk products, it was found that these plants have antioxidant. Milk, whey, casein inhibit lipid peroxidation and peroxil/suproxide radicals generation.

The leaf, root, bark, berry, bud, flower and seed used for the purpose of cooking are commonly refurred to as herbs and spices. The herbs and spices were and are primarily used for enhancing the flavor of foods. Nutritional contribution of these dietary plants has been in the past neglected presumably due to low consumption. However, the literature indicates that within the last decade, this view is beginning to change.

There is a growing amount of literature concerning the potential benefits of herbs and spices for their medicinal role in confering protection against cardiovascular and neurodegenerative diseases, cancer and type 2 diabetes.

In defining these benefits, one approach is to investigate the bioactive properties of these foods within a nutritional context. This approach has led to questions about the contribution of a group of phytochemical constituents wich predominate in herbs and spices – the polyphenols. The predominant class of polyphenolic acids are flavonoids.

Chapter 2 presents on overview of research regarding experiments used in the study of shelf life of dairy products. Shelf life is commonly estimated using two types of stability testing: real time stability tests and accelerated stability tests. In real time stability tests a product is started at recommended storage conditions and monitored until it fails the specifications. In accelerated stability tests, a product is stored at elevated stress

conditions (such as temperature, humidity and pH). Degradation at the recommended storage conditions can be predicted using known relationship between the acceleration factor and the degradation rate. Temperature is the most common acceleration factor because its relationship with the degradation rate is characterized by Arrhenius equation.

Chapter 3. The aim and the objectives of the research

The aim of this work was to improve qualitative stability of milk and diary products by including in cow diet some horticultural herbs rich in polyphenols and to estimate qualitative stability using two types of stability testing: real-time and accelerated tests.

The main research objective was to do some experiments necessary to identify elements with semnification regarding introduction in the cow diets of some horticultural herbs (cilantro and sage) and to evaluate effects on feed consumption, milk production and qualitative stability of subsequent dairy products. The specific objectives were:

- cultivation on cilantro and sage in intercropping system and to evaluate prioduction and nutritional potential of the resulted feed;

- introduction in the cow diet of feed obtained in intercropping system and to evaluate the effects on the feed intake;

- to assess the qualitative stability of some milk and milk products compared with experimental milk and milk products.

Chapter 4 includes materials and methods for realization of research activities.

The research methodology used for realization of these objectives were: formulation and selection of research problems, experimental design, sampling plan, measurement techniques, data collection methods, statistical techniques for processing and analysis of data, interpretation and report writing.

All experiments were made at Agricultural Research and Development Station Simnic-Craiova.

Annual pea and oats were incorporated with cilantro and sage (mix of pea with oats as control; mix of pea, oats and cilantro, and a mix of pea, oats and sage). A block design was used, each contained two intercropping treatments and a control.

The efficiency of using feed resulted in cow diet was evaluated on 12 Holstein cows.

- The Dairy products (milk, yogurt, butter, Telemea cheese) samples used in this study were collected from local cold store and from Agricultural Research and Development Station (ARDS) Şimnic-Craiova.

- The storage temperatures were simulated in refrigerated environment or incubation units.

- Quality changes including total germs count, psychrotrofic germs count, pH, chemical compounds, sensory evaluation were monitored.
- Data generated from the chemical kinetics of hexanal development that progressed during storage were modeled using Arrhenius equations to predict the shelf life of UHT milk.
- Data generated from pH, titratable acidity (in % lactic acid), mezophzlic germs count, yeast and moulds count, and sensory evaluation monitored during storage period were used to assess the shelf life of yogurt.
- Data generated from acid values, peroxidic values and senzory analysis monitored during storage period were used to assess shelf life of butter.
- Data generated from total germs count, pH, free whey (%) and sensory analysis monitored during storage period were used to assess ahelf life of Telemea cheese.

Chapter 5 includes Results and discussion regarding:

1) Studies about intercropping pea and oats with cilandro and sage.

In each growing and development stage (boot, heading, milk development and dough development of oats) the production of dry matter (DM) and crude protein do not were different compared with control feed (traditional pea and oats intercropped). The greatest DM yield was obtaind in daugh development stage, 6720 kg/ha for pea and oats, 6700 kg/ha fpor pea and oats plus cilandro, and 6720 kg/ha for pea and oats plus sage.

The percent of crude protein (% from DM) decreased from about 18% in boot stage to 11% in dough development of oats in all intercropped feeds.

The crude protein yield increased from around 620 kg/ha to 779 kg in milk development stage for all feeds.

Natural detergent fiber (NDF) concentration showed an increasing from boot stage to dough development stage from around 49% (of DM) to over 56%.

Acid detergent fiber (ADF) concentrations showed an increasing trend from boot stage to daugh development for all three feeds.

As ADF increase there is a decline in digestibility of organic matter (OM) digestibility of DM, and total digestive nutrients (TDN) for all feeds.

2) Effect of inclusion in cow diet of intercropped feeds on quantitative and qualitative of milk production.

Three cow diets were calculated per this study. Inclusion of pea and oats feed (PO), pea and oats plus cilandro (PO + C) and pea and oats plus sage (PO + S) was 51% of DM in each diet. The rest (49%) was a mix of cereal grains with supplements of protein, macro

and microminerals and vitamins. Not energy for lactation (NEL) of diets was around 1,6 Mcal per kg of DM. Dry matter intake (DMI) was 20,4 kg /day in the control diet (PO), 20,6 kg/day in the diet with PO + C and 20,8 kg/day in the diet witg PO + S. Milk production was 29,0 kg/day for PO; 29,1 kg/day for PO + C and 29,2 kg/day for PO + S. Fat production was around 1 kg/day for all feeds. The differences between intercropping treatments were not significant for milk yield, milk components and DMI. All three intercropping feeds were good to support a high milk production of Holstein cows. The small increasing trends in DMI and milk production were attributed to the higher digestibility of DM in PO + C and PO + S intercropps.

3. The effect of some horticultural herbs in cow diet as natural parameters sources of qualitative stability of milk.

a) The characterstics of milk products taken in study and the conditions of storage.

The level of: total solids, fat, non fat solids, titratable acidity, iodine value and *Enterobacteriaceae* and *Salmonella* count were within the regulatory standard and did not exceed the permissible levels.

Storage conditions: relative humidity 70%, temperature +3 ^{o}C.

b) Storage stability of pasteurized milk (HTST):

The storage stability of pasteurized milk (HTST) stored at 4 ^{o}C, 7 ^{o}C and 14 ^{o}C using total microbial count was 17,4 days, 16,5 days and 5,5 days respectively. For food safety the shelf life period is reduced at 75% (real shelf life of 13,12 and 4 days respectively).

The storage stability of pasteurized milk (HTST) stored at 4 ^{o}C, 7 ^{o}C and 14 ^{o}C using psychrotrofic germs count was 20 days, 18,4 days and 9,7 days respectively. For food safety the shelf life period is reduced at 75% (real shelf life of 15,14 and 7 days respectively).

c) Storage stability of sterilised milk (UHT):

Hexanal formation via lipid oxidation that progressed during storage period at 25, 35 and 45 ^{o}C was monitored.

The mean rate of the hexanal accumulation was 0,0790 μg/kg/day at 25 ^{o}C; 0,3142 μg/kg/day at 35 ^{o}C and 0,8571 μg/kg/day at 45 ^{o}C.

The shelf life of UHT milk was 210 days (real time).

In accelerated tests the UHT milk was stored at 35 ^{o}C and 45 ^{o}C, and the predicted shelf life was made using relationship between the acceleration factor and the degradation rate. Shelf life at 35 ^{o}C was 57 days and at 45 ^{o}C was 16 days (Q10 = 3.66; Ea = 24 kcal/mol).

The high temperature short time (HTST) pasteurised milk from cows fed with intercropped feeds showed the same storage stability as conventional milk. Growing dinamics of total germs and psychrotrophic germs in pasteurised experimental milk, stored at 4 ^{o}C, 7 ^{o}C and 14 ^{o}C was not influenced by the new feeds in cow's diet.

Mean initial concentration of hexanal in milk from cows fed with experimental feeds was 6,66 μg/kg for PO, 5,33 μg/kg for PO + C and 5,00 μg/kg for PO + S respectively. Compared with conventional milk (12 μg/kg) initial concentration of hexanal was reduced with 44,5% to PO diet, 55,6% for PO + C diet and 58,34% for PO + S diet. All experimental feeds improved the oxidative status of the Holstein cows.

d) Storage stability of yogurt

Durind the production of yogurt the starter cultures grow and produce lactic acid. This reduces the pH to below the isoelectric point of the milk proteins and causes coagulation, producing the characteristics gel structure and acid taste of the product.

The storage stability of yogurt determined using sensory changes and microbiological counts as failure criteria was 13 days.

e) Storage stability of butter:

The results show a tendency towards increased acidity from the initial value in refrigerated butter, compared with frozen butter. Refrigerated butter exhibited refrigerator / stale off flavors concurrent with increased levels of oxidation and higher peroxidic value after 12 months. Off flavors in frozen butter were not evident until 18 months.

From the butter made from milk of cows fed with intercropped feeds, the values of acidity do not differ between refrigerated and frozen butter.

Regarding perception of flavor intensity as a function of storage and time, the flavor of initial fresh butter was maintained 18 months in refrigerated conditions for PO diet, 21 months for PO + C and 24 months for PO + S.

Stale of flavors was detected at 9 months in refrigerated butter for PO diet, and at 12 months for PO + C and PO + S diets.

The horticultural plants are promoters of good flavor properties of refrigerated or frozen butter.

f) Storage stability of Telemea cheese

The storage stability of fresh Telemea cheese considering 50% rejection probability was 20 days stored at 4 ^{o}C and 16 days stored at 7 ^{o}C (in conventional telemea cheese).

Using a probability of 50% of the telemea cheese rejection the storage stability was 21,22 and 24 days at 4 ^{o}C for PO diet, PO + C and PO + S diet respectively. At 7 ^{o}C storage temperature the storage stability of telemea cheese was 16, 17 and 18 days for PO diet, PO + C diet and PO + S diet respectively. Using cilantro and sage intercropped with pea and oats the storage stability of telemea cheese was longer with 1-3 days at 4 ^{o}C storage temperature conditions and with 1-2 days at 7 ^{o}C storage temperature conditions.

The following conclusions (Chapter 6) can be drown from the present study:

- The cultivation of the two horticultural herb (cilantro and sage) represents a valuable contribution to the increase of botanical, biodiversity in the area of forage production.
- Mixed cultivation (intercropping) represents a traditional method of cultivation. Whose advantages lie in yield stability. The positive effects of this method are determined by the competitive behaviour of the mixing plants (reciprocal stimulation).
- Cultivation of the 4 plants in the ratio of 70:30 for pea and oat intercropped, and 65:25:10 for pea and oats plus cilantro or sage was a succes solution.
- Vegetation biomass obtained by intercropping was a valuable feed resources for diary cows, and supported a high milk production.
- Storage stability testing of dairy products is an important process used in today's industry as a need to ensure food safety and to protect consumers' health.
- The storage stability of milk products is primarely influenced by the number and type of microorganisms present in the raw milk, state of packaging material, hygienic and sanitary conditions during the production cycle as well as by storage temperatures of the final product.
- The pasteurization process known as HTST wich uses a combination time – temperature (72 ^{o}C for at least 15 seconds) is capable of extending storage stability of milk for up 20 days stored at 4 ^{o}C.
- The freshly packed UHT milk used during this study showed satisfactory milk composition and microbiological quality.
- Accelerated storage stability testing of UHT milk offers a way to estimate storage stability without having to wait a long time for the answer as in real time determination. In this study the measurement method oh hexanal together with Q10 predictive method was the procedure for indirect determination of shelf life of UHT milk. The storage stability was estimated to be 217 days whn is stored at 25 ^{o}C, 81 days at 35 ^{o}C and 35 days at 45 ^{o}C.

- Some determination of the yogurt during storage period due to bacterial action is inevitable: first, due to the continuing action of the yogurt culture bacteria and, second, due to spor-forming bacteria that survive the heat treatment, as the yogurt mix is not usually sterilized.

- Control of storage stability of butter is multifactorial.

Butter is best stored at -20 ^{o}C. After 12 months slightly oxidised flavours are espected.

- Survival analysis was used in sensory storage stability of Telemea cheese. The storage stability of Telemea cheese stored at 4 ^{o}C was 20 days and dropped to 16 days at 7 ^{o}C. The sensitivity of Telemea cheese to temperature highlights the need of maintaining a low chiling temperatures during distribution and storage.

The advantages of using cilantro and sage in intercropping system with pea and oats lie in a longer qualitative stability of some dairy products, in specific temperature storage conditions.