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The influence of some metabolits addition on fermentation process, during butter milk manufacturing

Marcel Avramiuc, Ana Leahu

Universitatea „Ştefan cel Mare” Suceava, Romania

Abstract

In this work there was studied the addition influence of some minerals (Zn, Mg), folic acid, lactose and/or of various combinations among those biocompounds upon evolutiuon of some fermentation process indices (titratable acidity and free aminoacids content) during the 17 hours of butter milk processing. The tests accomplished at established intervals (4, 12, 15 and 17 hours) have shown an oscillating evolution of free aminoacids content, finding, in all searched variants, decrease of this index at the end of the process (after 17 hours), in comparison with the first 4 hours of thermostating process. The addition of Mg, Zn or Mg, Zn and lactose has determined increase of the free aminoacids percent within analysed samples.

In all variants, the titratable acidity of the butter milk samples has constantly risen once with thermostatic period increasing. Although the evolution of butter milk samples acidity could not be correlated with the amount or type of addition, in the end of the thermostating period (after 17 hours) the variants with folic acid and/or lactose have had higher values of this index, in comparison with the other variants and blank. Recommended as maximum value for the moment of butter milk fermentation processs interruption, the titratable acidity of 90˚T has been reached and even slightly exceeded after only 12 hours of thermostating process.

Since some types and doses of additions used in this work have made possible the reduction of thermostating period, those additions could be used in milk acid products industry with this end in view, but also to turn to good account light raw material, having less content of some biocompounds.

Keywords: free aminoacids, titratable acidity, pH, biocompounds, butter milk

1. Introduction

The introduction of some active biological compounds into the food produce, lack in nutritive substances, are the methods to obtain fortified food, needing a nutritional association, physiological adapted, and having as result the ensurance of a maximum protection of body (Nicol, 1995; Nagy, 1999). Those methods are used to prevent food lack of balance, caused by various deficiency state, being an efficacious way to ensure an optimum healthy state of people (Segal et al., 1987; Mincu et al., 1989; Juillet and Bornet, 1998).

The dairy products can be supplemented, in need, with liposoluble vitamins (derivative of provitamins, respectively vitamins A and/or vitamins D), with hydrosoluble vitamine (tiamine, riboflavin, niacine, ascorbic acid) or with biominerals, such as: iodine, iron, fluor etc. (Segal, 1999; Banu et al., 2003).

In this work, the introduction of some biocompounds (folic acid, zinc, magnesium and lactose) at butter milk processing has made to evidence the effect of these additions on biochemical processes, leading to the obtaining of the above mentioned product, having, indirectly, in view the changes occured within transformations of carbohydrates and proteins made by lactic bacteria.

The exploration of the sugar compounds transformation during milk acid products processing can be made by means of lactose and/or lactic acid content determination, or

* Corresponding author: *e-mail address*: avramiucm@usv.ro

indirectly through titratable acidity and pH values evolution studying. Also, it can explore the proteolysis process using either laborious methods, evaluating proteins and casein contents, casein/proteins ratio, or caseino-macropeptides content (Fukada, Roig and Prata, 2004; Elgar et al., 2000; De Noni and Resmini, 2005; Mollé and Léonil, 2005; Haasnoot, 2005; Haasnoot, Marchesini and Koopal, 2006), or it can determine the evolution of free aminoacids content, as acted in this work.

2. Materials and Methods

In this work the materials has included: the raw material represented by cow milk, with a certain content of fat, lyophilized starter cultures produced by CHR HANSEN firm, and additions, in some proportions, of folic acid, zinc, magnesium and lactose.

In order to get butter milk, it was used the classic technique, which included the following operations: milk reception and cleaning - pasteurization - cooling - inoculation - allocation in small packings - fermentation - precooling - cooling - storage.

The butter milk was made according to technological directions, observing effective health and health-veterinary measures, and the starter cultures have been lactical bacteria belonging to *Lactococcus* genus.

Tables 1 and 2 reproduce the features of the raw material (milk) and the manufacturing recipe used for butter milk.

Table 1. Features of the raw material used for butter milk obtaining

Biochemical indices	Values
Acidity (ºT)	15
pH	6.6
Total nitrogen (%)	0.53
Crude protein (%)	3.38
Free aminoacids (%)	0.77
Fat (%)	2
Density (g/cm^3)	1.03

Table 2. Manufacturing recipe for butter milk

Compounds	Quantities
Cow milk (fat min. 2%)	250-300 litres
Lyophilized starter cultures type XPL-1 (CHR HANSEN)	50 units

The cow milk, after qualitative and quantitative reception, has been cleaned, normalized to a fat content of minimum 2%, then pasteurized at 85-95°C, 20-30 minute and cooled to 30-32°C. The inoculation was made directly into the vane, under permanent homogenization, with a lyophilized cultures type XPL-1 (CHR HANSEN), containing *Lactococcus lactis ssp. lactis, Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. diacetilactis*. After inoculation, there were introduced biocompounds whose quantities are rendered in the table 3.

Table 3. Vitamins, mineral compounds and lactose additions established for analysed variants

Analysed product	**BUTTER MILK**					
Sampleaddition*	PII	PIII	PIV	PV	PVI	PVII
Mg (mg/l)	47,8	-	47,8	47,8	-	47,8
Zn (mg/l)	10	-	10	10	-	10
FA (mg/l)	-	30	-	30	30	30
Lactose (g/l)	-		1	-	1	1

*The addition doses have been calculated depending on recommended daily dose, so that should not negatively affect the human body as result of consumption.
FA = folic acid; PI (blank sample) does not appear within the table.

The milk, inoculated and enriched with additions, according to each variant of research, has been distributed in packaging sales, prior properly sterilized and dried. The product, packed in vessels of 250 g, has been subdued to thermostatic process, at a temperature between 24 and 28°C for 12-17 hours, then precooled at 18-20°C, cooled at 2-8°C and stored at 2-4°C.

The samples for analysis have been taken at 4, 12, 15 and 17 hours since the beginning of thermostatic process, determining the values of the following indices: titratable acidity, pH and free aminoacids.

It also has been carried out a blank, in the same work conditions, but without additions.

In this work, it has determined the titratable acidity, expressed as Thőrner degrees (˚T), according to AOAC standard (Cunniff, 1995), and the pH values, according to STAS 8201/82 (Costin et al., 2003).

The total nitrogen (%) and the crude protein (%) were determined according to Kjeldahl method (Costin et.al., 2003), and for free aminoacids dosing (%) it was used Sőrensen method (Vâță et al., 2000).

The density (g/cm^3) was determined using a lacto-thermo-density device, and fat (%) was determined by means of acid-butirometric method (Costin et.al., 2003).

3. Results and Discussion

The results of determinations are reproduced in the table 4.

Table 4. The evolution of acidity and free aminaocids values in butter milk samples

Sample number/ compounds added	**Analysed parameter**	**Thermostating length**			
		4 hours	**12 hours**	**15 hours**	**17 hours**
PI (blank)	*FAA (%)	2.620	2.200	2.092	2.140
	Acidity (°T)	40	100	106	109
	pH	5.58	4.78	4.70	4.61
PII (Mg, Zn)	FAA (%)	2.930	2.850	2.850	2.742
	Acidity (°T)	40	93	112	107
	pH	5.60	4.71	4.55	4.52
PIII (folic acid)	FAA (%)	2.400	2.720	2.550	2.250
	Acidity (°T)	35	102	114	120
	pH	5.7	4.66	4.52	4.47
PIV (Mg, Zn, lactose)	FAA (%)	2.850	3.045	2.705	2.705
	Acidity (°T)	40	95	112	116
	pH	5.60	4.70	4.54	4.49
PV (Mg, Zn, folic acid)	FAA (%)	2.930	2.834	2.653	2.780
	Acidity (°T)	37	102	110	115
	pH	5.66	4.67	4.59	4.53
PVI (folic acid, lactose)	FAA (%)	2.780	2.577	2.630	2.630
	Acidity (°T)	35	106	110	112
	pH	5.68	4.71	4.58	4.51
PVII (Mg, Zn, folic acid, lactose)	FAA (%)	2.930	2.125	2.580	2.667
	Acidity (°T)	40	106	110	126
	pH	5.62	4.70	4.58	4.54

*FAA = Free aminoacids

Analysing the free aminoacids content, it can observe that, after the first 4 hours of thermostating process, the highest values have registered the samples PII, PV and PVII, and the least one the sample PIII. After 12 hours, except PIII and PIV samples, in all other samples the free aminoacids values have decreased comparatively with the first 4 hours. In the other analysed intervals the percent content of free aminoacids has registered evolution more or less oscillating, depending on sample, but in the end of the analysed period the values of all samples have been lower, beside the values determined after the first 4 hours of thermostating process.

After 17 thermostating hours, the highest free aminoacids values have been registered in samples PV, PII and PIV, and the least value in sample PI (blank). The fig. 1 reproduces the values of free aminoacids content in butter milk samples at the end of thermostating process as compared to raw material (milk).

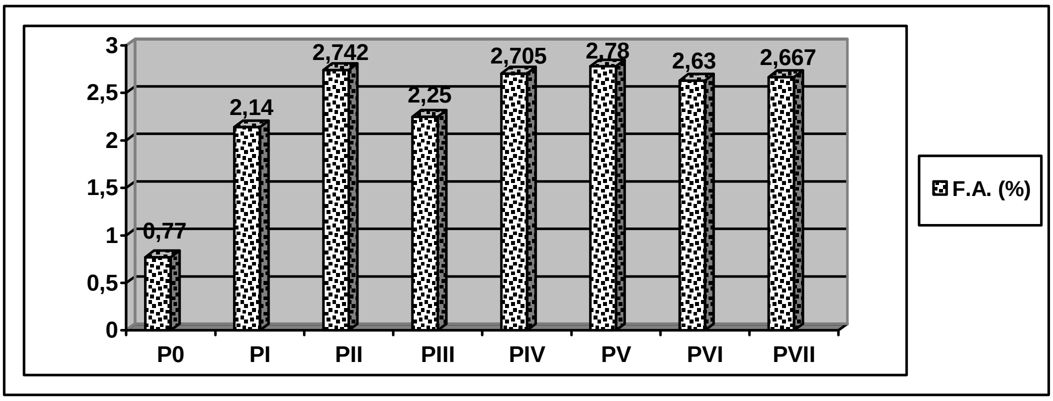

Figure 1. Free aminoacids (F.A.) percentage values of raw material (milk) and of butter milk samples, at the end of thermostating period
P0 = milk sample; PI = blank (butter milk); PII...PVII = work samples (butter milk)

The variation of these protein compounds it can explain through two processes, having influence on features of finished product namely: a) increasing, on the one hand, of the free aminoacids and peptides percentage within analysed product, as a result of hydrolysis process of serum proteins and of caseine (in a small extent) and b) the decrease, on the other hand, of free aminoacids percentage, through their transformation in aroma compounds, which accumulate within the final product, influencing its sensory properties.

It seems that, in the case of samples having aminoacids content superior to the blank, the respective additions have stimulated (intensified) the proteolytic enzymes or have promoted the synthesis of increased amount of these enzymes in lactic bacteria cells. The folic acid and its derivatives represent increase factors for micro-organisms, zinc and magnesium are co-factors of some important enzymes participating in sugar and protein metabolism of organisms, and lactose is a lactic fermentation substrate.

As to titratable acidity, its values have registered constant increase along with thermostating period increase. After 4 hours of thermostating process, the most increase of the titratable acidity were at samples PI, PII, PIV and PVII, after 12 hours at samples PVI, PIII and PVII, after 15 hours at samples PIII, PVI and PV, and after 17 hours at samples PIV, PIII and PV.

At the end of thermostating period, the highest value of titratable acidity has registered the sample PIV and PIII. In the fig. 2 are reproduced the titratable acidity values of butter milk samples at the end of thermostating period, comparatively with raw material.

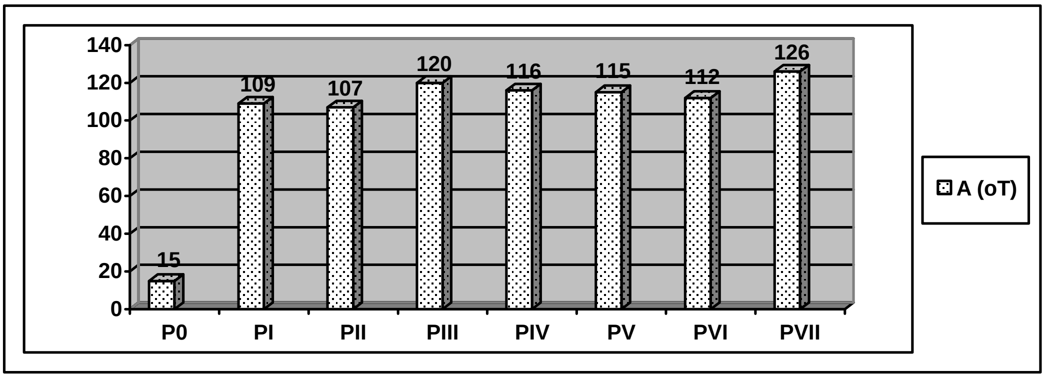

Figure 2. Titratable acidity (˚T) values of raw material (milk) and of butter milk samples, at the end of thermostating period
P0 = milk sample; PI = blank (butter milk); PII…PVII = work samples (butter milk)

The pH values have registered, in all cases, constant decrease once with increase of the thermostating period. At the end of analysed period, the most reduced values were found in samples PIII and PIV.

4. Conclusion

The introduction of some biocompounds (folic acid, zinc, magnesium and lactose) within raw material, destined to butter milk obtaining, has led to modifications of the acidity and of the free aminoacids content of this product, comparatively with blank (obtaining from milk without additions).

During butter milk processing, the free aminoacids content has had an oscillating evolution, evidencing, in all searched variants, decrease of this index at the end of the processing period (after 17 hours), as compared to the first 4 hours of thermostating period. The addition of some biocompounds (Mg, Zn or Mg, Zn and lactose) has determined higher values of free aminoacids percent within analysed samples.

The values of titratable acidity of butter milk samples have registered, in all cases, constant increase once with thermostating period increase. The evolution of the acidity could not be correlated with amount or type of addition, but in the end of the thermostating period (after 17 hours) the variants with folic acid and/or lactose addition have had higher values of this index, comparatively with the other variants and with blank. The maximum value of the titratable acidity (90°T), recommended for the break moment of butter milk fermentation, has been reached and even lightly exceeded after only 12 thermostating hours.

Because some types and doses of additions used in this work have led, comparatively with blank, to important increase of free aminoacids percentage and titratable acidity, doing possible the thermostating length reduction, these additions could be used within acid dairy produce industry to turn to good account light raw materials, having less content of lactose and proteins.

References

1. Banu C. et al., - *Procesarea materiilor prime alimentare şi pierderile de substanţe biologic active*. Ed. „TEHNICA" UTM, Chişinău, 2003,138.
2. Costin G.M. (editor) et..al., - *Ştiinţa şi ingineria fabricării brânzeturilor*. Ed. Academica, Galaţi, 2003, pp 502, 503, 504, 510.
3. Cunniff P. (Ed), - *Official methods of analysis of AOAC International* (16th ed., vol 1,2), Association of Officinal Analytical Chemists International, Arlington, VA, 1995.
4. De Noni, Resmini P., - *Identification of rennet-whey solids in "traditional butter" by means of HPLC/ESI-MS of non-glycosilated caseinomacropeptide A*, *Food Chemistry* 93 (2005), pp 65–72.
5. Elgar D.F., Norris C.S., Ayers J.S., Pritchard M., Otter D.E. and Palmano K.P., - *Simultaneous separation and quantitation of the major bovine whey proteins including proteose peptone and*

caseinomacropeptide by reversed-phase high-performance liquid chromatography on polystyrene-divinylbenzene, Journal of Chromatography A 878 (2000),183–196.

6. Fukada S.P., Roig S.M. and Prata L.F., - *Correlation between acidic ninhydrin and HPLC methods to evaluate fraudulent addition of whey in milk*, Lait 84 (2004), 501–512.
7. Haasnoot W., 2005 - *Detection of adulterants in milk products*, Biacore Journal 5 (2005), 12–15.
8. Haasnoot W., Marchesini G.R.and Koopal K.,- *Spreeta-based biosensor immunoassays to detect fraudulent adulteration in milk and milk powder*, Journal of the AOAC International 89 (2006), 849–855.
9. Juillet M.T., Bornet F., - *Assurer une alimentation équilibrée popur maintenir la santé*. Ind. Alim. Agric., 1998, 115, 12, 19.
10. Mincu I. et.al., - *Orientări actuale în nutriție*. Ed. Medicală, Bucureşti, 1989.
11. Mollé D., Léonil J., - *Quantitative determination of bovine κ-casein macropeptide in dairy products by liquid chromatography/electrospray coupled to mass spectrometry (LC-ESI/MS) and liquid chromatography/electrospray coupled to tandem mass spectrometry (LC-ESI/MS/MS*), International Dairy Journal 15 (2005), 419–428.
12. Nagy K.,1999 - *The role of Food Fortification in Combating Micronutrient Deficiences*. F Hoffmann-La Roche Basle-Switzerland, 1999.
13. Nicol M., - *Vitamines antioxydantés et bêta-caroténe: activités prevéentives en pathologie humaine.* Réunion commentée, Mèd. et Nutr., 31, 1,1995, 41-46.
14. Segal B. et al., - *Metode moderne privind îmbogățirea valorii nutritive a produselor alimentare.* Ed. Ceres, Bucureşti, 1987.
15. Segal Rodica, - *Alimente fortifiate*, in Alimente funcționale, editori: G.M. Costin şi Rodica Segal, Ed. Academica, Galați, 1998,308.
16. Vîță C. et al., - *Îndrumar de lucrări practice pentru biochimia produselor alimentare*. Univ.„Dunărea de Jos”Galați, 2000