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Summary

Supplementation is becoming an increasingly popularpracticeacross various agegroups, aimed at complementing daily diets with concentrated vitamins and/or minerals inthe form of medications or dietary supplements exhibiting nutritional or physiological effects. Increasing attention is given to collagen supplementation dueto its declinewith age. Thebody's ability to producecollagen starts to decreasebetween theages of18 and29, with an averagedeclineof 1%per year after the ageof 40 and a75%reduction in collagenproduction by the ageof80. Therefore, collagen supplementation is beneficial starting as early as 25 or 30 years old. Thenutritional and functional properties of collagen and itshydrolysateareutilized in the dairy sector, among others. Adding bioactiveingredients to dairy products, particularly fermented milk, results in products with highernutritional valueand bioavailability of components. To increasethecontent of well-absorbed protein in milk,collagen orits hydrolysatecanbeapplied, yieldingmilk with potential therapeuticproperties and tailored textureparameters. Dairy products arecurrently at the forefront of probioticfood development. Thetrend of increasing consumption of fermentedproducts stems notonly from the widerangeof products availableon the market but alsofrom theirhigh nutritional and dietaryvalues andtheirtherapeuticand preventiveproperties. Duetotheirspecificproperties, fermented dairy products also serveas an excellent matrix forincorporating nutrients that impart functional properties to the final product.Considering thegrowing interest in health-promoting foods and the occurrenceof allergies to the αs1casein fraction in cow's milk, thereis an increasing demand formilk and dairy products derivedfrom othermammalian species. Asidefrom cow's milk, sheep's and goat's milk arealso used in processing in Poland and globally. Thesearebecoming morepopular as alternatives to cow's milk products dueto theirnutritional valuesand potential assubstitutes forcow's milk.

This studyaimed to determinethe impact ofadding different types ofcollagens (hydrolysateand bovine collagen) at two concentrations (1.5%and 3.0%),storageduration (1 day and 21 days), and probioticstrains(*Lacticaseibacillus casei, Lactobacillusacidophilus, Lacticaseibacillus paracasei* and*Lacticaseibacillus rhamnosus*) on the quality of fermented sheep and goat milk, as wellas the survival of thesestrainsin the digestivesystem.

Inthe initial phaseof theresearch, thetechnological suitability of rawsheepand goatmilk was assessed. The quality evaluation of sheep and goat milk indicated the high quality of the raw milk and appropriate processing properties, enabling its use in the production of probioticfermented milk. Upon analyzing the total microbial count, itwas determined thatboth raw sheep and goat milk intended forthe production of fermented milk requirethermaltreatment (pasteurization) to ensurethe resulting milk's high quality andmicrobiological safety.

In thesubsequent phaseof the study, the feasibility of addingcollageninto theproduction of probioticfermentedsheep and goat milk was evaluated by assessing the resulting milk's physicochemical, organoleptic, and microbiological properties. Inallgroupsof sheep and goat milk, itwas foundthat fermented milk with added collagen exhibitedahigherpH value afterthe first day ofstoragecompared tothe control milk withoutcollagen.It was demonstrated thatthe type of collagen participatesin differentiating pH values, as sheep andgoat milk samples with hydrolysatehad ahigher pH onthe first day of storagethan those with bovine collagen, although thesedifferenceswerenot always statistically significant. Additionally, therewas atendency for increasing the collagen concentration from 1.5%to 3.0%to resultin higherpH values, regardless of whether hydrolysateor bovine collagen was added.

Depending onthe typeand dose, collagen addition also influencedthe lacticacidcontent in the analyzed samples. Fermented sheep milk with 1.5% hydrolysate had a lower lacticacid content than the control counterparts. Conversely, in fermented goat milk, higherlacticacid content was observed in milk withbovine collagen compared tohydrolysate. Anexception was the KLP1.5H sample, which had thesame amountof lacticacid as KLP. Increasing the collagen dose from 1.5% to 3.0%increased lactic acid content in fermented goat milk. On analyzing the results on the 21stday of storage, itwas found that a1.5% addition of bovine collagen favorably impacted the lacticacid content in fermented goatmilk, with allmilk groups showing significantly higherlacticacid content than theircontrol counterparts. Similarly, increasingthe dosefrom 1.5%to 3.0%in milk withbovine collagen significantly increased the lactic acid content.

Theaddition of hydrolysate mosteffectively limited the syneresisof sheep milk, bothon the 1st and 21stday of storage. Increasing the hydrolysatedosefrom1.5%to 3.0% significantly reduced syneresiscomparedto thecontrol sheep milk counterparts and sheep milk with a1.5%hydrolysate addition. Theaddition of bovine collagen was not as effectivein reducing whey separation. In fermented goat milk, whether the type and dose of collagen influenced the increaseor reduction ofsyneresiswas not unequivocally determined. Adding bovine collagen and hydrolysatereduced whey separation only in goat milk fermented by *L. rhamnosus*. Increasing the dosefrom 1.5%to 3.0% enhanced syneresisin goat milk samples only by adding bovine collagen.In goat milk with hydrolysate, theserelationshipswerenot conclusive. Inthis study, the analysis of lightness (L^*) values revealed that on thefirst day of storage, theaddition of collagen, both in the form ofbovine collagen andhydrolysate, caused darkening of the color in allgroups of fermented sheep and goat milkcompared to the controls. Fermented sheep andgoat milk exhibited hues of green and yellow. In probioticsheep milk, alower gel hardness was observed in samples with bovinecollagen compared to their hydrolysatecounterparts. Conversely, when analyzing the hardness results forgoat milk, itwas foundthat only in goat milk with hydrolysateKLP3.0H and KLR3.0H was a significant increase in hardness compared to the controls.

For the other groups, nosignificant effect on thegel hardness of goat milk was noted.Afteroneday of storage, only onecaseshowed that adding hydrolysatereduced milk's springiness (OLA3.0H).Thetype and doseof collagen did not affect the springiness of the othersheep milk samples or any goat milkgroups on the first day of storage. Similarly, after 21 days of storage, therewas no observed effect on the springiness of both sheep and goat milk, except forOLA1.5Wsheep milk, wheregel springiness increased compared to the control group.

Throughout the entirestorageperiod, thenumber of viableprobioticbacteria cells inallgroups of fermented milk remained higherthan 8 log CFU g^{-1}. Addinghydrolysateand bovine collagen to goat milk significantly improved the survival of bacteria, particularlynoted on the 21stday of storagein milk fermented by *L. casei*and *L. acidophilus*, compared to theircontrol counterparts. In sheep milk fermented by *L. acidophilus*and *L. casei*, morebeneficial bacterial survival was observed over the 21-day storage period, with the collagen dosehaving no impact on the growth and survival of both strains. Moreover, itwas demonstrated thatsheepmilk containing hydrolysateprovided favorableconditions forthegrowth of *L. casei*even during refrigerated storage.

Theaddition of collagen to sheep milk enhanced theintensity of the sweet tastedueto the presenceof sweetglycine. Themostintensemilky-creamy tastewas in sheep milkwith hydrolysatefermented by *L. casei*. However, adding collagen (both bovine and hydrolysate) caused aslight off-tasteand off-odorin fermented sheep milk. Theintensity of the additive and off-tastes in goat milk was influenced by the bacterial strain conducting thefermentation and the storagetime. Theprimary issueassociated withusinggoat milk in dairyproduct production is its

characteristic goat tasteand odour. However, adding collagen didnot intensify the perception of the goaty tastebutinstead intensified the milky-creamy and sweet taste.

Theeffect of milk type and storagetime on fermented milk's physicochemical and organoleptic propertieswas assessed. Inallgroups of fermentedmilk, storagetime ledto anincrease in the acidity of the fermented milk. Additionally, higher pH values were observed on the 21stday of storagein allcontrol groups of fermented goat milk compared to fermentedsheep milk. Theanalysis indicated that theproduction of lacticacid by probiotic bacteriawas influenced byboth storagetime and milk type, as confirmedby ANOVAanalysis ($p=0.0000$). On the first day of storage, allcontrolgoat milk samples exhibited lower lacticacid content than sheep milk. On the 21stday of storage, only the goat milk samples KLA, KLP, and KLRshowed lower lacticacid content compared to theirsheep milk counterparts.Thesyneresisof milk was influenced by the type of milk used inthe study. A higherlevelof syneresiswas observed in fermented goat milk than in sheep milk samples. Thehighest level of syneresiswas found in milk fermented by*L. acidophilus*forsheep and goat milk on both testingdays. Conversely, storagetime differentiated the level of syneresisin the OLC,OLP, and OLRsamples, whereless whey separation was observed, whereas in the KLA and KLRsamples, an increasein whey separation wasnoted. No significant differences dueto storage time on the level of syneresis were observed in the other groups.

Theanalysis oflightness (L^*) values showed thaton both the 1st and 21stdays ofstorage, fermented goat milk was darker compared to sheep milk. Additionally, extendingthe storagetime resulted in furtherdarkening of the color of both fermentedsheep and goat milk, indicating asignificant effect of milk typeand storagetime on thelightness (L^*). Analyzing the b^*color component concerning milk type revealed asignificantly greater presenceof yellow color in sheep milk than ingoat milk throughout thestudyperiod.However, extending the storagetime from 1 to 21 days intensified the yellow color only in sheep milk. In contrast, the yellow color in goat milk decreased with storagetime. Sheep milk showed a more profound and purer color than goat milk.

An analysis was conducted on how milk typeand storagetime affect selected texturecomponents (hardness, cohesiveness, and springiness). Thedata analysis indicated that thegel hardness of fermented milkdepends on the milk type. In this case, sheep milkalwaysexhibited aharder gel than goat milk, which is related to the dry mattercontent. Moreover,storagetime didnot significantly affectthe gel hardness of goatmilk. Theopposite trendwas observed forsheepmilk,whereextending the storagetime to 21days resulted in asignificant increasein the hardness of thefermented milk gel. Goat milk showed higher cohesiveness between particles, except forthe OLC and KLCgroups, wherethe opposite correlation was observed. With extended storagetime, the cohesiveness of the milk gel did not changesignificantly in goat milk. Higher springiness values characterized sheep milk. Inmostcases, storagetime increasedthe springiness of thefermentedmilk gel, except forKLC milk, where a decrease in springiness was noted.

Thetype of milk mainly affectedthe tasteof thefermented milk. Off-tastes weremoreintensein fermented goat milk. In mostcases, storagetime contributed to an increasein the intensity of sour taste and odor and a decrease in the perception of sweet taste in both types of milk.

Inthe final stageofthe research, *in vitro*digestion was conducted to determinethe viability of probioticbacterial cells undersimulated gastrointestinal conditions. Thestudyrevealed the lowest survival rate for the *L. rhamnosus*strain during digestion within themodel gastrointestinal system, which also exhibited the greatest reduction in the numberof viablecells. Theaddition of bovine collagen hadabeneficial effect on thesurvival of thisstrain, particularly in goat milk.Furthermore, the survival rate of *L. rhamnosus*varied depending on the type ofmilk.In goat milk, it ranged from

45.29%in KLRto 64.23%inKLR3.0H, whereasin sheep milk, itranged from 41.05%inOLR3.0Wto 48.49%in OLR1.5H. Goat milk also provided morefavorable conditions forthe survival of

L. paracasei, especially with the addition of hydrolysate, compared to sheep milk. Thehighest survival rate in simulated *in vitro*digestionconditions, relative tothenumberof cells beforedigestion, was observed in milk fermented by *L. casei*and *L. paracasei*. Results showed that, in the small intestinephase, milk fermented by *L. casei*and *L. paracasei*hadprobioticcountshigherthan 5log $CFUg^{-1}$,indicating asurvival rateabove50%. Meanwhile, *L. rhamnosus*and *L. acidophilus*strains wereidentifiedat cell countsgreaterthan 4 log $CFU\ g^{-1}$. Theaddition ofcollagenand hydrolysateto sheepmilk resulted in decreased survival of *L. acidophilus*, whereas the addition of hydrolysate to goat milk increased the survival of this strain. Bovine collagen addition positively influenced thesurvival of *L. casei*and *L. rhamnosus*under *in vitro*digestion conditions.