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Influence of lupin-based milk alternative heat treatment and exopolysaccharide-producing lactic acid bacteria on the physical characteristics of lupin-based yogurt alternatives

A. Hickisch [a,*], R. Beer [a], R.F. Vogel [b], S. Toelstede [a]

[a] Fraunhofer Institute for Process Engineering and Packaging IVV, Giggenhauser Str. 35, 85354 Freising, Germany
[b] Technische Universität München, Lehrstuhl für Technische Mikrobiologie, Gregor-Mendel-Straße 4, 85354 Freising, Germany

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ABSTRACT

In the present study we investigated the influence of heat treatment of lupin-based (LB) milk alternatives and different exopolysaccharide (EPS)-producing lactic acid bacteria on the physical characteristics of set-type LB yogurt alternatives. LB milk alternatives, obtained from protein isolate of *Lupinus angustifolius* cv. Boregine, were either pasteurized at 80 °C for 60 s or ultra-high temperature (UHT) heated at 140 °C for 10 s and was fermented with *Lactobacillus plantarum* TMW 1.460 and 1.1468, *Pediococcus pentosaceus* BGT B34 and *Lactobacillus brevis* BGT L150. Fermentation duration was strongly affected by heat treatment: different strains needed between 25 to 35 h in UHT LB milk alternative to reach a pH of 4.5 compared to 14 to 24 h in pasteurized LB milk alternative. EPS extraction revealed slightly higher amounts of EPS for UHT LB yogurt alternatives (~0.5–0.9 g/l; pasteurized: ~0.4–0.7 g/l). The more intensive heat treatment (UHT) resulted also in better rheological (apparent viscosity, hysteresis loop area, flow point, elastic, viscous and complex modulus) and textural properties (firmness, consistency, cohesiveness and index of viscosity) of the investigated LB yogurt alternatives. Furthermore, LB yogurt alternatives out of UHT milk alternative revealed a lower tendency to syneresis, measured with siphon and centrifugation method. This work contributes to the fundamental knowledge of the textural properties of LB yogurt alternatives.

1. Introduction

Interest in vegetarian or vegan nutrition has been rising in recent years. Health related reasons (*e.g.* lactose intolerance or allergy), ethical and sustainability issues or simply a lifestyle choice leads to diets out of which meat or milk products are more often excluded. An increase of the consumption of plant-derived products *e.g.* dairy alternatives should be promoted, but can only be achieved if these products meet consumer's acceptance regarding taste and texture.

So far, the main research of dairy alternatives has been focused on soy based products like milk and yogurt alternatives (Ferragut, Cruz, Trujillo, Guamis, & Capellas, 2009; Li et al., 2014; Yang, Fu, & Li, 2012). However, other plants merit greater attention like lupin which belongs to the genus *Lupinus* in the legume family (*Fabaceae*). In contrast to soy, no genetically modified varieties exist, which is especially in Europe a big consumer concern. Moreover, lupin seeds are rich in protein (about 35%) of high nutritional value and they lack anti-nutritional factors like trypsin inhibitors that are present in soy (Duranti, Consonni, Magni, Sessa, & Scarafoni, 2008). Further, lupin proteins exhibit good techno-functional properties like protein solubility and emulsification (Wäsche, Müller, & Knauf, 2001) which makes them promising for application in dairy substitutes.

Up to now, only preliminary investigations were performed regarding the development of fermented lupin-based (LB) yogurt alternatives. As fermentation substrate lupin flour (Jimenez-Martinez, Hernandez-Sanchez, & Davila-Ortiz, 2003; Snowden, Sipsas, & John, 2007) or enzymatic hydrolyzed protein concentrate (Kuznetsova, Zabodalova, & Baranenko, 2014) was applied. Products were characterized poorly in terms of structural and sensory properties. Further, non-vegan substances like lactose were added to promote the fermentation power of lactic acid bacteria (LAB) (Jimenez-Martinez et al., 2003).

Dairy yogurt alternatives can comprise structural disadvantages: soy protein isolates have promising gelling abilities (Batista, Portugal, Sousa, Crespo, & Raymundo, 2005; Berghout, Boom, & van der Goot, 2015), but resulting yogurt alternatives are described as firm and brittle with dense and close meshed networks caused by a high number of physiochemical bonds (Yang et al., 2012). In contrast lupin protein isolates are associated with weak gelling properties resulting in weak gels with a low number of bonds (Batista et al., 2005; Berghout et al., 2015). It was shown for yogurt out of cow milk that gel firmness can be strengthened through thermal denaturation of proteins causing higher

* Corresponding author.
E-mail address: andrea.hickisch@ivv.fraunhofer.de (A. Hickisch).

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network density and hence a higher capability of water-binding through colloidal linkages (Lucey, Munro, & Singh, 1999; Remeuf, Mohammed, Sodini, & Tissier, 2003).

Another approach to modify the texture of yogurt is the application of exopolysaccharide (EPS)-producing bacteria. Certain strains of LAB are able to express EPS, which were recognized to affect yogurt texture in terms of consistency and rheology (De Vuyst & Degeest, 1999). Heteropolysaccharides were found especially in small amounts in fermented milk ranging from 50 to 600 mg/l (Cerning, 1995). Effects like improved mouth feeling, ropiness, higher creaminess or limited syneresis and increased gel firmness were described (De Vuyst & Degeest, 1999; Folkenberg, Dejmek, Skriver, Skov Guldager, & Ipsen, 2006). The relationship between the structure and function of EPS in yogurt is still unclear, due to a wide range of different heteropolysaccharides. Their functionality is based on their sugar monomers, chain length, degree of branching, molecular size, amount, charge and interaction with milk constituents (De Vuyst & Degeest, 1999). So far, only one study exists reporting about the influence of the EPS-producing LAB with *Lactobacillus* (*L.*) *plantarum* 70810 on the texture of soy-based yogurt alternatives (Li et al., 2014).

To our knowledge, no comprehensive study about the application of lupin protein for dairy yogurt alternatives and, in particular, on its physical characteristics has been performed so far. Therefore, the objectives of this work were to develop and characterize a lupin protein based yogurt alternative in terms of its rheological properties and its susceptibility to syneresis. Thereby, the impact of the heat treatment of the LB milk alternative as well as EPS-producing bacteria was studied in detail.

2. Material and methods

2.1. Preliminary screening of strains and growth conditions

Upon a preliminary screening 30 different LAB which are listed in Table 1 were evaluated towards their yogurt-like texture and yogurt-like odor on lupin protein isolate and their ability to produce EPS. Most of the strains were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Other bacterial isolates were from the collection of the Lehrstuhl für Technische Mikrobiologie Weihenstephan (TMW) or provided by the Brau- und Getränketechnologie (BGT, Technische Universität München, Germany). Strains were propagated under optimal growth conditions on their recommended media according to Fritsch, Vogel, and Toelstede (2015). Additionally some other strains were evaluated and were grown anaerobically on de Man Rogosa and Sharpe broth (MRS, Merck, Darmstadt, Germany) anaerobic at 37 °C unless stated otherwise: *L. delbrueckii* subsp. *bulgaricus* DSM 20080, *Streptococcus salivarius* subsp. *thermophilus* DSM 20259 (Trypticase Soy Yeast Extract broth, 37 °C, anaerobic), *Bifidobacterium bifidum* DSM 20239 (MRS with 0.05% cysteine hydrochloride (Sigma-Aldrich, Munich, Germany), 37 °C, anaerobic), *L. pontis* TMW 1.1086, *L. brevis* BGT L150 (MRS, 30 °C, anaerobic) and *Pediococcus* (*P.*) *pentosaceus* BGT B34 (MRS, 30 °C, aerobic). Total cell counts of the bacterial suspensions were determined by a spiral plating method on MRS agar with an Eddy-Jet spiral plater (IUL Instruments, Königswinter, Germany). The plates were incubated at strain specific conditions and the number of colony-forming units per ml (CFU/ml) was determined with the Countermat Flash and Grow Software (IUL Instruments). Long-term storage of stock cultures was maintained at −18 °C in 10% (*w*/*w*) sterile reconstituted skim milk powder and on angular tubes at 4 °C for a short time (half a year).

Suspensions of 5% (*w*/*v*) lupin protein isolate and 2% (*w*/*v*) glucose were prepared and were heat treated at 65 °C for 30 min in a water bath. Two technical replicates of these suspensions (50 ml) were inoculated with each bacterial strain from precultures with 7.0 ± 0.1 $\log_{10}$ CFU/ml. The suspensions were incubated under optimal conditions of the strains. After 48 h, fermentation was stopped and samples were examined with simple descriptive tests according to their textural appearance and their odor with "–" (not yogurt-like), "+" (adequate yogurt-like) and "++" (strong yogurt-like). Yogurt-like gels with or without syneresis were referred as "strong yogurt-like". Gels with rather weak networks or porous gels embedding bubbles, as well as aggregated, cottage-cheese-like formations were attributed as "not yogurt-like". Yogurt-like odors were stated as "milky", "buttery", "sour", "sweet" or "fruity", while attributes like "sulfurous", "metallic" or "faecal" were declared as "not yogurt-like". Besides, strains were evaluated for their EPS-producing ability. Therefore agar plates were prepared out of the recommended media for each strain fortified with high amounts of glucose (80 g/l) (van Geel-Schutten, Flesch, ten Brink, Smith, & Dijkhuizen, 1998) and bacteria were spread on these plates with an inoculation loop. Plates were incubated under strain-specific growth conditions for 24 h. Strains were referred as EPS-producing LAB, when they showed ropy strands by touching with a toothpick

Table 1
Fermentation of different LAB on lupin protein isolate and glucose — evaluation of yogurt-like texture and odor, as well as EPS production.

	Criteria for selection	Yogurt-like texture	Yogurt-like odor	EPS expression
L. delbrueckii subsp. *bulgaricus* DSM 20080	1, 3, 5	+	+	–
Streptococcus salivarius subsp. *thermophilus* DSM 20259	1, 3	++	–	–
L. acidophilus DSM 20079	1, 4, 5	+	+	–
L. casei DSM 20011	1, 3, 4	+	+	–
Lactococcus lactis subsp. *lactis* DSM 20384	1, 7	+	+	–
Lactococcus lactis subsp. *cremoris* DSM 20069	1, 3	+	–	–
Leuconostoc mesenteroides subsp. *cremoris* DSM 20200	1	+	–	–
L. helveticus DSM 20057	1, 4	+	+	–
L. perolens DSM 12744	1	++	+	–
Bifidobacterium bifidum DSM 20239	1, 4	+	++	–
L. plantarum T MW 1.460	3, 4, 5, 7	++	++	++
L. plantarum TMW 1.1468	3, 4, 5	+	+	+
L. fermentum DSM 20391	4, 5	–	–	–
L. pontis TMW 1.1086	5	–	–	–
L. sanfranciscensis DSM 20451	5	+	–	–
Weissella cibaria TMW 2.1333	5	–	–	–
L. brevis TMW 1.1326	4, 5	–	–	–
L. brevis BGT L150	4, 5, 6	+	+	++
L. amylolyticus BGT TL3	6	–	–	–
L. amylolyticus BGT TL5	6	+	–	–
L. species BGT TL11	6	–	–	–
L. species BGT TL13	6	–	–	–
L. rossiae BGT L1202	6	–	–	++
P. pentosaceus BGT B34	6	+	+	++
P. pentosaceus DSM 20336	7	+	+	–
L. curvatus TMW 1.624	3	+	–	–
L. reuteri DSM 20016	4, 5, 7	–	–	–
L. buchneri DSM 20057	7	–	–	–
L. gasseri DSM 20243	7	+	–	–
Bifidobacterium animalis subsp. *lactis* DSM 10140	4, 7	+	–	–

1: Strains of this species are often used for cultured dairy products (Chandan & Kilara, 2013; Leroy & De Vuyst, 2004).
2: Strains of this species are used in soy yogurt (Li et al., 2014).
3: Strains of this species are often EPS-producers (De Vuyst & Degeest, 1999; Palomba et al., 2012).
4: Strains of this species are often probiotic (Chandan & Kilara, 2013).
5: Strains of this species are often used in sourdoughs (Leroy & De Vuyst, 2004; Vogel et al., 1999).
6: Isolates from spoiled beer; some are slime producers.
7: Strains degrade antinutritive substances *e.g.* phytic acid and oligosaccharides (Fritsch et al., 2015).
– no yogurt-like texture and odor impression, as well as no EPS-production.
+ adequate yogurt-like texture and odor impression, as well as adequate EPS-production.
++ strong yogurt-like texture and odor impression, as well as strong EPS-production.